**A 4-Week Repeated Oral Dose Toxicity Study of PROJECT O in Beagle Dogs Followed by a 4-Week Reversibility Study**

**11 SUMMARY**

PROJECT O dissolved in the vehicle, 10% hydroxypropyl-β-cyclodextrin (HP-β-CD) containing propylene glycol (PG) solvent, was administered orally once daily for 4 weeks at dose levels of 3, 10, 30, 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 100 mg/kg group in order to assess the reversibility of toxicity during a subsequent 4-week recovery period. The animals in the control groups received water for injection (negative control) or the vehicle (vehicle control). Systemic exposure to PROJECT O was also evaluated. The following observations and examinations were performed: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, gross pathology, organ weights, histopathology, electron microscopy, and parathyroid hormone analysis.

Soft stool was observed in all test article groups at the same frequency as in the vehicle control group, and this symptom was considered an effect of the vehicle.

No test article-related changes were noted at 3 mg/kg.

At 10 mg/kg, increased alkaline phosphatase was noted in 1 male. Hypertrophy and proliferation of the Kupffer cells in the liver (slight or very slight) were observed in males and females.

At higher dose levels (30 and 100 mg/kg), vomiting was observed in all males and all females, mainly 1 hour after dosing. Slight decreases were noted in body weight in males and females and in food consumption in females at 100 mg/kg. The following blood chemistry and histopathological changes were observed in a dose-dependent manner: increased alkaline phosphatase and total bile acid in males at 30 mg/kg and greater and in females at 100 mg/kg; increased total bilirubin (mainly due to increased direct bilirubin) and decreased albumin in males and females at 30 mg/kg and greater; decreased calcium in males at 30 mg/kg and greater; decreased total protein in males and females at 100 mg/kg; and hypertrophy and proliferation of the Kupffer cells (marked or moderate), mononuclear cell infiltration (slight or very slight), and/or clear cell change in the centrilobular hepatocytes (very slight) in the liver in males and females at 30 mg/kg and greater. In the Kupffer cells, cytoplasm was positive for PAS stain, and enlarged irregularly shapeed lysosomes including amorphous/fibrillary/laminated structures were observed.

At the end of the 4-week recovery period, hypertrophy and proliferation of the Kupffer cells in the liver remained, but the incidence and severity of these changes were lower than those at the end of the dosing period. Other changes which had been caused by 4-week treatment recovered.

In toxicokinetics, on Day 1, tmax did not differ between dose levels. Cmax and AUC24 increased dose-dependently, but less than dose proportionally, at all dose levels except in females at 100 mg/kg. Cmax and AUC24 in females at 100 mg/kg were lower than those at 30 mg/kg, and the vomiting observed in many animals at 100 mg/kg was considered one of the reasons for the low exposure. After repeated dosing, Cmax and AUC24 did not change at 3 to 30 mg/kg. At 100 mg/kg, these parameters increased with repeated dosing, and a dose-proportional or greater than dose-proportional increase in exposure from that at 3 mg/kg was noted at

100 mg/kg in males on Days 14 and 28 and in females at Day 28. tmax did not differ after repeated dosing. There were no clear sex differences in any parameter.

It was concluded that, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was 3 mg/kg/day for males and females. Reversibility of the test article-related changes was indicated at the end of the 4-week recovery period.