SUMMARY

A. Objective

The objective of this study was to provide information on the effects of the test substance, H-28548, on male and female reproduction. This encompassed gonadal function, mating behavior, conception, parturition and lactation of the F_0 generation, and the development of offspring from conception through day 40 of postnatal life. In addition, a toxicokinetic assessment of plasma levels of the test substance was performed in the F_0 females on lactation day 21 and the F_1 pups at culling (PND 4), PND 21, and PND 40.

B. Test Guidelines

The protocol was designed to be in general accordance with the U.S. EPA Health Effects Test Guidelines, OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test, July 2000, and the OECD Guidelines for Testing of Chemicals, Guideline 421, Reproduction/Development Toxicity Screening Test, 27 July 1995.

C. Study Design

The test substance, H-28548, in the vehicle, deionized water, was administered orally by gavage to 3 groups of Crl:CD1(ICR) mice, each group consisting of 25 males and 25 females. Dosage levels were 0.1, 0.5, and 5 mg/kg/day administered at a dosage volume of 10 mL/kg. A concurrent control group composed of 25 mice/sex received the vehicle (deionized water) on a comparable regimen. Analyses of samples from the dosing formulations verified that the formulations were at targeted concentrations, homogeneous, and stable under the conditions of use for the current study. F_0 males were approximately 6 weeks and F_0 females were approximately 11 weeks of age at the beginning of test substance administration. F_0 males received 70 daily doses prior to mating. F_0 males were dosed throughout the mating period through 1 day prior to euthanasia for a total of 84 to 85 doses. F_0 females received 14 daily doses prior to pairing and were dosed through lactation day 20 (lactation day 21 for the 5 females/group that were selected for blood collection) for a total of 53 to 65 doses; females with no evidence of mating or those that failed to deliver were dosed through the day prior to euthanasia (post-cohabitation day 23 or post-mating day 23) for a total of 37 to 50 doses.

All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. All F_0 females were allowed to deliver and rear their pups until lactation day 21. Maternal blood samples were collected from 5 females/group at approximately 2 hours following dose administration on lactation day 21 for determination of test substance concentration in the plasma. Clinical observations, body weights, and sexes were recorded for F_1 pups at appropriate intervals. To reduced variability among the litters, 8 pups per litter, 4 per sex when possible, were randomly selected on PND 4 to continue on study and the remaining pups were culled. Blood samples (pooled by litter) were collected for toxicokinetic evaluation from the culled offspring of 10 litters/group. On PND 21, 1 male and 1 female pup per litter were selected for the F_1

generation. Nonselected pups were euthanized and necropsied; blood samples were collected from 5 pups/sex/group for toxicokinetic evaluation. F_0 males were euthanized following the completion of the mating period, and F_0 females were euthanized on post-mating day 23 (females with evidence of mating), post-cohabitation day 23 (females with no evidence of mating), or lactation day 21. Complete necropsies were conducted on all F_0 animals, and selected organs were weighed. Selected tissues of the reproductive system were examined microscopically from all F_0 animals in the control and high-dose groups, all animals dying spontaneously or euthanized *in extremis*, and from any animals in the low- and mid-dose groups with impaired fertility (males that did not sire a litter or females that did not deliver a litter). In addition, the liver and kidneys from all F_0 animals were examined microscopically. All F_1 pups selected for study were observed twice daily for mortality and moribundity following weaning (PND 21) through study termination. F_1 males and females were dosed daily beginning on PND 21 through PND 40. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. Indicators of sexual development (balanopreputial separation and vaginal patency) were evaluated for all surviving F_1 animals. All surviving F_1 animals were euthanized and necropsied on PND 40; blood samples for toxicokinetic evaluation were collected from 5 pups/sex/group at approximately 2 hours following dose administration.

D. Results

F_0 survival was unaffected by test substance administration at all dosage levels. One F_0 male each in the 0.1, 0.5, and 5 mg/kg/day groups was found dead or euthanized *in extremis* on study day 4, 55, or 56. One, 3, 1, and 1 F_0 females in the control, 0.1, 0.5, and 5 mg/kg/day groups, respectively, were found dead or euthanized *in extremis* during lactation days 13-16. In the absence of a dose response and because mortality was also noted for 1 female in the control group, none of the moribundity or mortality noted in the test substance-treated F_0 animals was attributed to test substance administration.

A test substance-related increase in the incidence of unkempt appearance was observed in the majority of F_0 females in the 5 mg/kg/day group at the detailed physical examinations and/or following dose administration during lactation days 4-10. This finding was only noted on 1-2 occasions per animal and therefore was not considered to be adverse. No other test substance-related clinical findings were noted at the detailed physical examinations or approximately 1-2 hours following dosing at any dosage level in either generation.

Test substance-related, higher mean F_0 body weight gains were noted in the 5 mg/kg/day males for the first 7 weeks of dose administration. As a result, a higher mean body weight gain was noted in this group when the overall pre-mating (study days 0-69) and treatment (study days 0-84) periods were evaluated; the difference during the pre-mating period corresponded to increased mean food consumption during this same interval. Consequently, mean F_0 body weights in the 5 mg/kg/day group males were 6.1% to 9.1% higher than the control group during study days 21-84. These test substance-related increases in body weight parameters for F_0 males at 5 mg/kg/day were not considered adverse because they were of relatively minimal magnitude and were consistent with increases in food consumption and increased liver weights. In the 0.5 mg/kg/day group, a test substance-related higher mean body weight gain was observed during study days 0-7. However, mean body weight gains in this group were similar to the control group throughout the remainder of the treatment period and there were no corresponding

effects on mean body weight or mean food consumption in this group; therefore this transient higher mean body weight gain was not considered to be adverse. Mean male F_0 body weights, body weight gains, and food consumption in the 0.1 mg/kg/day group were unaffected by test substance administration.

Test substance-related higher mean body weights (2% to 3% higher) and body weight gains were noted in the 0.5 and 5 mg/kg/day group F_0 females during the pre-mating period. Mean F_0 female body weights and body weight gains at 0.1 mg/kg/day and mean F_0 female food consumption at 0.1, 0.5, and 5 mg/kg/day were unaffected by test substance administration during the pre-mating period. Test substance-related higher mean body weights and body weight gains with corresponding increased food consumption were noted in the 0.5 and 5 mg/kg/day groups compared to the control group during gestation. During lactation days 1-7, slightly lower mean body weight gains with corresponding reductions in mean food consumption were noted for the F_0 females in the 5 mg/kg/day group. Mean body weights, body weight gains, and food consumption in this group were generally similar to the control group throughout the remainder of lactation. In the 0.5 mg/kg/day group, mean body weights, body weight changes, and food consumption were unaffected by test substance administration during lactation. Mean body weight in the 0.1 mg/kg/day group was slightly higher than the control group at the end of gestation on gestation day 18. This small increase was considered a possible consequence of the slightly larger litter sizes (mean of 12 pups per litter as compared to 11 pups per litter for the controls). The lack of any differences in body weight parameters immediately following delivery at the onset of lactation confirms the likelihood that the slight increase in body weight on gestation day 18 was related to litter size. Mean food consumption in the 0.1 mg/kg/day group was unaffected by test substance administration during gestation and lactation. Based on the magnitude of change and/or consistency with food consumption data, and/or increased liver weights and/or relationship to litter size, these test substance-related changes in body weight parameters noted in the F_0 females were not considered adverse.

A slight increase in the incidence of gross white areas on the liver in the 5 mg/kg/day females correlated with microscopic focal necrosis. There were no test substance-related gross findings in the F_0 males and females in the 0.1 and 0.5 mg/kg/day groups or in the F_0 males in the 5 mg/kg/day group.

Microscopic examination of the reproductive organs of both males and females revealed no test substance-related effects at any dose level tested.

Microscopically, minimal to moderate hepatocellular hypertrophy was present in both sexes of F_0 adults at dose levels of 0.5 and 5 mg/kg/day. A corresponding increase in the liver weight parameters was observed at both dose levels. Hepatocellular hypertrophy was characterized by cytoplasmic eosinophilic stippling that is consistent with peroxisome proliferation. In the 5 mg/kg/day F_0 males and females, other liver lesions included increases in single cell necrosis, mitotic figures, lipofuscin pigment, and focal necrosis (females only). A low incidence of single cell necrosis was also present in the 0.5 mg/kg/day male group. Microscopic examination of the kidneys of all F_0 adults revealed a minimal increase in non-adverse tubular cell hypertrophy in males given 0.5 and 5 mg/kg/day. This finding correlated with an increase in mean absolute kidney weight in both sexes given 5 mg/kg/day.

There were no test substance-related causes of death observed in the F_0 adult mice. The failure of eleven F_0 adult pairs to produce litters was not related to test substance administration.

No test substance-related effects were observed on F_0 reproductive performance (mating, fertility, or copulation indices, and mean days between pairing and coitus), mean gestation length, the process of parturition, mean numbers of implantation sites, or unaccounted-for sites.

Mean numbers of F_1 pups born, live litter size, percentage of males at birth, postnatal survival, and the general physical condition of the F_1 pups were unaffected by test substance administration at all dosage levels. Test substance-related lower mean body weights and body weight gains were noted for F_1 males and females in the 5 mg/kg/day group throughout the pre-weaning period. There were no test substance-related body weight or body weight gain differences from the control group in F_1 males and females in the 0.1 and 0.5 mg/kg/day groups.

There were no gross findings for F_1 pups that died, were euthanized *in extremis*, or that were euthanized on PND 21 that were considered to be related to maternal test substance administration.

Delays in the attainment of balanopreputial separation and vaginal patency were noted in the F_1 males and females in the 5 mg/kg/day group when compared to the control group. However, these delays were attributed to the effects on mean body weight noted in this group during the pre-weaning period and not considered to be a direct effect of test substance administration.

F_1 survival was unaffected by test substance administration at all dosage levels following weaning. One F_1 male in the 5 mg/kg/day group and 1 female in the control group were euthanized i*n extremis* on PND 22 and 24, respectively. Because the male pup in the 5 mg/kg/day group was extremely small in size, was the only surviving pup in that litter, and there was no other mortality noted in the F_1 pups in this group; the moribundity in the 5 mg/kg/day group was not attributed to test substance administration. No test substance-related clinical findings were noted for the surviving animals at the detailed physical examinations or approximately 1-2 hours following dose administration at any dosage level. A lower mean body weight gain was noted in the 5 mg/kg/day group males during PND 21-28 and attributed to the lower body weights noted in this group during the pre-weaning period. Higher mean body weight gains were noted in the 5 mg/kg/day group males and females during PND 28-35 followed by body weight gains similar to the control group during the remainder of the treatment period. The decrements in mean body weight gains noted it the 5 mg/kg/day group during the pre-weaning period for both sexes, and the post-weaning decreased body weight gain noted for the males, resulted in lower mean body weights during PND 21-40 for the males and on PND 21 and 28 for the females. However, mean body weights in the 5 mg/kg/day females were similar to the control group by PND 35 and the differences in body weight observed in the males were progressively less from PND 21-40. There were no effects on F_1 body weights, body weight gains, or food consumption in the 0.1 and 0.5 mg/kg/day groups during the post-weaning period. F_1 necropsy findings did not indicate any correlation to test substance administration.

For both maternal animals and their offspring, male and female mice behaved in a kinetically similar manner, with an approximately linear relationship between dose and blood levels of the test substance. The mean maternal plasma concentration of H-28548 measured 2 hours after

dosing on day 21 of lactation were 903, 4966, and 36420 ng/mL in the 0.1, 0.5, and 5 mg/kg/day dose groups, respectively. In PND 4 F_1 pups, mean plasma levels were lower (approximately 2- to 4-fold) than the lactation day 21 maternal values. In PND 21 F_1 pups, mean plasma levels of H-28548 in all dose groups were markedly less (approximately 40-to 60-fold lower) than the respective lactation day 21 maternal values. In the F_1 offspring samples on PND 40 that had been directly dosed since weaning on PND 21, mean plasma levels of H-28548 in all dose groups were similar to those of the respective maternal dose groups sampled at PND 21. Evaluation of plasma levels of H-28548 in dams and their offspring indicate that in mice, *in utero* exposure of fetuses to H-28548 that are less than those of the orally dosed dam (PND 4 plasma levels) and that little or no exposure to nursing pups occurs during lactation (based on PND 21 plasma levels). In addition, plasma kinetics in juvenile mice directly dosed beginning at weaning appear to be equivalent to those of adult mice.

E. Conclusions

There were no effects on reproduction (mating, fertility, or copulation indices, number of days between pairing and coitus, and gestation length). Test substance-related higher mean body weights, body weight gains, and food consumption were observed in the 5 mg/kg/day F_0 males throughout the treatment period and 0.5 and 5 mg/kg/day group F_0 females generally during gestation and lactation. These changes in body weight parameters in F_0 males and females were not considered adverse. Increased liver weights and microscopic hepatocellular hypertrophy, consistent with peroxisome proliferation, were present in male and female F_0 adults administered 0.5 or 5 mg/kg/day. Single cell necrosis of hepatocytes was also present in male mice in the 0.5 mg/kg/day dose group and in the males and females of the 5 mg/kg/day dose group. All other test substance-related microscopic changes in the livers of F_0 male and female mice occurred at the 5 mg/kg/day dose level and included increased mitotic figures, lipofuscin pigment, and focal necrosis (females only). Microscopic renal tubular cell hypertrophy was present in F_0 males given 0.5 and 5 mg/kg/day. An increase in mean absolute kidney weight was also present in body sexes given 5 mg/kg/day. No test substance-related effects were noted on postnatal survival. Lower mean offspring body weights and body weight gains were noted in the F_1 males and females in the 5 mg/kg/day group during the pre-weaning period. Evaluation of the plasma levels of H-28548 in dams and their offspring indicate that in mice, *in utero* exposure of fetuses to H-28548 results in plasma concentrations that are less than those of the orally dosed dam, and that little or no exposure to nursing pups occurs during lactation. Also, the plasma kinetics of H-28548 in juvenile mice directly dosed beginning at weaning appeared to be equivalent to those of adult mice.

Based on these results, the no-observed-adverse-effect level (NOAEL) for reproductive toxicity was 5 mg/kg/day, as no effects on reproduction were observed at any of the doses levels tested. The NOAEL for systemic toxicity in F_0 male mice was 0.1 mg/kg/day based on the low incidences of single cell necrosis observed in the liver at 0.5 mg/kg/day. The NOAEL for systemic toxicity in both maternal animals and their offspring was 0.5 mg/kg/day. In maternal animals, this NOAEL was based on microscopic changes in the liver at 5 mg/kg/day. In the offspring, the NOAEL was based on body weight decrements in the F_1 males and females in the 5 mg/kg/day group during the pre-weaning period.