Pre- and Postnatal Developmental Toxicity Evaluation of UT-15 (Administered by Continuous Subcutaneous Infusion) in Rats

Testing Facility: [redacted]

Study Number: 65C-7019-600 ([redacted] No.)

Study Dates: Initiation Date – November 10, 1998
Completion Date – May 23, 1999

GLP Compliance: The study was conducted in compliance with GLP regulations.

Animals: One hundred and fifty female Sprague-Dawley rats, 56 days old and weighing about 200-225 g, were obtained from [redacted]
After a week of quarantine, the females were mated with male Sprague-Dawley rats (previously received from the same supplier) from the [redacted] breeding colony. One hundred sperm-positive female rats [ranging in body weights from 211 to 269 g on gestational day (gd) 0], designated as F0 generation, were assigned to four groups of 25 rats each. (The day on which vaginal sperm or plugs were found was designated as gd 0).

The animals were individually housed, except during mating periods, in solid bottom, polycarbonate cages with stainless steel wire lids and [redacted] cage litter. Certified Rodent Diet No. 5002 and tap water were available *ad libitum*.

Dose Levels, Mode of Administration and Treatment Regimen: The target doses were 0, 50, 150 and 450 ng/kg/min.

Stock solutions (10 mg/ml) of UT-15 (Lot No. UT15-98H01), formulated in vehicle (containing sodium citrate, citric acid and sodium chloride dissolved in sterile water for injection) and adjusted to pH 7.4, were diluted (with vehicle) to achieve the desired final concentrations.

All formulations and stock solutions were determined to be within 97–104% of target concentrations and were found to be stable at 25 and 40°C for four weeks.

The test and vehicle solutions were administered by continuous subcutaneous infusion using a subcutaneously implanted [redacted] osmotic pump ([redacted] which delivers [redacted] hour for a nominal duration of 28 days.

On the morning of gd 5, each F0 female was anesthetized with isoflurane inhalation. The dorsal subscapular area was surgically prepared and an incision about 1.5 cm long was made. The osmotic pump, preloaded with appropriate dosing solution, was inserted into the subcutaneous pocket and the incision was closed with wound clips.

The osmotic pump model used for the study requires about [redacted] to reach steady state infusion rate once it is implanted. Therefore, the pump that was implanted on gd 5 would reach the steady state by the morning of gd 6.

On the morning of postnatal day 4, the animals were anesthetized and a new incision was made adjacent to the initial incision. The original osmotic pump was removed, inspected, and then replaced with a primed [redacted] second osmotic pump. The incision was closed with wound clips.

The duration of exposure for F0 females was about 36 to 38 days, from gestational day 6 to postnatal day 21 (gestational period = 21-23 days).

It is stated that the top dose (450 ng/kg/min) was chosen to induce maternal toxicity or low levels of lethality (≤ 10%), and was based on the results of the Segment II study. The lower doses were assigned as fractions of the high dose.

Observations and Measurements

F0 Maternal Animals

Animals were checked for clinical signs of toxicity and mortality at least twice daily. The body weights were recorded on gd 0, 5, 9, 12, 15, 18 and 20, and on postnatal day (pnd) 0, 4, 7, 14 and 21. Food consumption was recorded for gd 0-5, 5-9, 9-12, 12-15, 15-18, 18-20, pnd 0-4, 4-7, 7-14 and 14-21.

Beginning on gd 20, all females were examined twice daily for evidence of littering, or signs of dystocia. The F0 dams were allowed to rear their F1 young to pnd 21.

F1 Progeny

All F1 pups/litter were counted, weighed, sexed and examined externally for malformations on the day of birth (designated pnd 0). Pups that were stillborn or died before pnd 4 were examined externally and viscerally, and any abnormal tissues or specimens were kept in buffered neutral 10% formalin. On pnd 4, the size of each litter was adjusted to eight (four per sex, if possible). Culled pups were decapitated and discarded. Litters with eight or fewer pups were not culled. Pups were counted, weighed individually, sexed, and examined externally on pnd 4, 7, 14 and 21.

Pups that died or were sacrificed moribund on pnd 5-21 were necropsied, and any abnormal tissues were preserved. Survival indices were calculated on pnd 0, 4, 7, 14 and 21.

During the preweaning period (up to pnd 21), F1 pups were observed daily for developmental landmarks, including pinna detachment (pnd 1-4), incisor eruption (pnd 8-13) and eye opening (pnd 11-16).

At weaning on pnd 21, 20 F1 pups/sex/group were randomly selected from the maximum number of litters for generating the F2 animals. The selected animals were held for a minimum of 49 days until all selected F1 pups were at least 70 days old. Following this selection, the remaining offspring were examined for gross external abnormalities, euthanized and discarded.

The selected pups were examined daily for clinical signs, and weighed weekly. Pups were assessed for vaginal opening (pnd 22-36), cleavage of the balanopreputial gland (preputial separation; pnd 35-44), and neurobehavioral development (auditory startle reflex on pnd 21-34, motor activity on pnd 35-45, and learning and memory on pnd 41-55). All F1 females were evaluated for estrous cyclicity during the last 14 days of the postwean holding period (just before mating).

F1 males and females were then mated (1:1) for a period of 14 days. Females were examined daily during cohabitation for the presence of vaginal sperm or plug. Once vaginal sperms or plugs were found, the mating pairs were separated and individually caged. The F1 pregnant females were weighed on gd 0, 6, 9, 12, 15, 18 and 20, and on pnd 0 and 4, while the F1 males were weighed weekly.

Beginning on gd 20, all F1 pregnant females were monitored twice daily for parturition.

F2 Progeny

All F2 pups/litter were counted, sexed, weighed and examined grossly as soon as possible on the day of birth (pnd 0) and on pnd 4. Pups that were stillborn or died before pnd 4 were examined externally, viscerally, and preserved in buffered neutral 10% formalin. Grossly malformed pups were sacrificed and examined. All F2 pups were decapitated and discarded on pnd 4.

Necropsy of F0 Females and F1 Parental Males and Females

On pnd 21, all surviving F0 dams were necropsied and the thoracic and abdominal organs were examined grossly. The implantation sites were counted. Organs or tissues showing any abnormalities were preserved. Uteri from any F0 females that appeared nonpregnant were stained with 10% ammonium sulfide for confirmation of pregnancy status.

On pnd 4 of F2 litter, F1 dams and nonpregnant F1 females were necropsied and examined as described above. Paired ovarian and uterine weights were recorded.

At or after the pnd 4 date of their F2 litter, F1 males were necropsied and organs were examined grossly. Paired testes and epididymides weights were recorded.

The indices for reproductive performance, gestational parameters and offspring parameters were calculated.

Quantitative continuous data were statistically analyzed using Bartlett's test for homogeneity of variances. If Bartlett's test indicated lack of homogeneity of variances,

then nonparametric statistical tests (Kruskal-Wallis test followed by Mann-Whitney U test for pairwise comparisons; Jonckheere's test to identify dose-response trends) were employed. If Bartlett's test indicated homogeneous variances, then parametric statistical tests [appropriate General Linear Models (GLM) procedures for the Analyses of Variance (ANOVA)] were used. Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data to allow use of parametric methods. All indices were analyzed by Chi-Square test and by the Cochran-Armitage test for linear trend on proportions. When Chi-Square revealed significant differences among groups, then a Fisher's Exact Probability test was used for pairwise comparisons. A test for statistical outliers was performed on parental body weights and F0 maternal feed consumption.

Results

F0 Maternal Animals

No F0 females died during the study.

There were more incidences of swelling at the implantation site noted in drug treated animals (not dose-related) than in controls during gestation and lactation periods.

Mean maternal body weights were similar across control and treatment groups during the gestation period except on gd 9, when the mean body weight of the high dose group was significantly lower than the control value. Dose-related reductions in food consumption were noted in treated groups on gd 5-9, 5-20 (gestational treatment period) and 0-20 (entire gestational period).

During the lactation period, there were no statistically significant body weight differences among groups with the exception that the mean body weights of the mid and high dose groups were significantly lower than control on pnd 7. The food consumption was reduced at all dose levels (not dose-related) on pnd 4-7, corresponding to the time of implantation of the second osmotic pump on pnd 4. No treatment-related effects were observed for food consumption prior to or after pnd 4-7.

F0 reproductive and lactation indices are presented in Table 23. The fertility and gestation indices, the length of gestation period, the number of implantation sites per litter or the percent postimplantation loss per litter were similar across control and treated groups. There were no treatment-related effects on the number of live, dead or total pups at birth, stillbirth or livebirth indices, and lactation index.

The necropsy of the F0 females showed no treatment-related findings except for "serum pockets" surrounding the pump, the incidence of this observation being higher in treated groups (3 to 6 animals/group; not dose related) than in the control group (2 animals).

F1 Progeny

F1 litter size and pup body weights during lactation are presented in Table 24. There were no treatment-related effects on litter size, survival indices, pup body weights and sex ratio (percent male pups per litter) of pups.

There were no treatment-related necropsy findings in pups that died or were sacrificed moribund on pnd 0 through 21.

F0 maternal treatment had no effect on eye opening, vaginal patency and preputial separation in F1 offspring. Pinna detachment was significantly delayed at low dose (by 0.26 days compared to control), but unaffected at mid and high doses. Incisor eruption was significantly accelerated (by 0.47 days) at the high dose. Dose-related effects of treatment were not seen in auditory startle behavior, motor activity, and learning and memory assessments.

F1 male and female body weights during prebreed, mating and holding periods until sacrifice were unaffected by the F0 maternal treatment with UT-15. There were no dose-related clinical signs in F1 animals; the estrous cyclicity, evaluated during the final 14 days of prebreed period, was similar across all groups.

F1 female and male reproductive indices are presented in Table 25. The mating index for the high dose group (73.7%) was significantly lower than that for the control group (95%). No significant differences from control were noted for this parameter at lower doses. Although not statistically significant, dose-related decreases in fertility and pregnancy indices were seen in treated groups (for both indices, control 100%, low dose 94.7%, mid dose 90.0% and high dose 85.7%). There were no treatment-related effects on gestational length, number of implantation sites per litter, or the percent post-implantation loss per litter.

F1 maternal body weights during pregnancy and lactation were unaffected by treatment.

There were no unscheduled deaths among F1 males. One F1 female at 450 ng/kg/min was euthanized on day 36 during the prebreed period because of an inguinal mass, and one F1 female at 50 ng/kg/min was euthanized moribund on pnd 0. No treatment-related findings were seen on necropsy. Absolute or relative reproductive organ weights were unaffected by drug treatment.

F2 Progeny

F0 treatment had no effects on the numbers of live, dead or total F2 pups, live or still-birth indices (Table 25), average number of pups per litter, average pup body weights per litter, and percent male pups per litter on pnd 0 and 4 (Table 26).

There were no treatment-related findings at the F2 necropsy on pnd 0 through 4.

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