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.

Table 23.

Summary and Statistical Analysis of the F_0 Reproductive and Lactational Indexes for the F_1 Litters (page 1 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
No. of Sperm Positive Females	25	25	25	25
No. of Pregnant Females	25	23	25	25
Fertility Index (no. pregnant females/no. sperm positive females)	100.0	92.0	100.0	100.0
No. of Females with Live Litters (pnd 0)	25[a]	23	25	25
Gestational Index (no. females with live litters/no. females pregnant)	100.0	100.0	100.0	100.0
Gestational Length (days)[b]	22.0 ± 0.1 N=24	21.9 ± 0.1 N=23	22.0 ± 0.1 N=25	22.1 ± 0.1 N=25
No. Implantation Sites per Litter[b]	14.83 ± 0.35 N=24	15.04 ± 0.40 N=23	15.32 ± 0.34 N=25	14.76 ± 0.36 N=25
Percent Postimplantation Loss per Litter[b]	9.35 ± 1.75 N=24	4.37 ± 1.39 N=23	5.14 ± 1.63 N=25	5.21 ± 1.24 N=25
No. of Live Litters:				
Postnatal Day 0	24	23	25	25
Postnatal Day 4	24	23	25	25
Postnatal Day 7	24	23	25	25
Postnatal Day 14	24	23	25	25
Postnatal Day 21	24	23	25	25
Number of Live Pups on Postnatal Day 0[b]	13.5 ± 0.4 N=24	14.6 ± 0.3 N=23	14.6 ± 0.4 N=25	14.2 ± 0.4 N=25

(continued)

Table 23. (continued)

Summary and Statistical Analysis of the F_0 Reproductive and Lactational Indexes for the F_1 Litters (page 2 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
Number of Dead Pups on Postnatal Day 0[b]				
#	0.0 ± 0.0 N=24	0.1 ± 0.1 N=23	0.1 ± 0.1 N=25	0.0 ± 0.0 N=25
Total Number of Pups on Postnatal Day 0[b]				
	13.6 ± 0.4 N=24	14.7 ± 0.4 N=23	14.7 ± 0.4 N=25	14.2 ± 0.4 N=25
Stillbirth Index (no. dead on pnd 0/total no. on pnd 0)[b]				
	0.3 ± 0.3 N=24	0.8 ± 0.6 N=23	0.6 ± 0.6 N=25	0.3 ± 0.3 N=25
Live Birth Index (no. live on pnd 0/total no. on pnd 0)[b]				
	99.7 ± 0.3 N=24	99.2 ± 0.6 N=23	99.4 ± 0.6 N=25	99.8 ± 0.3 N=25
4 Day Survival Index (no. surviving 4 days/no. live on pnd 0)[b]				
	99.7 ± 0.3 N=24	99.4 ± 0.4 N=23	99.5 ± 0.3 N=25	99.7 ± 0.3 N=25
7 Day Survival Index (no. surviving 7 days/no. live on pnd 4)[b]				
#	100.0 ± 0.0 N=24	100.0 ± 0.0 N=23	100.0 ± 0.0 N=25	100.0 ± 0.0 N=25
14 Day Survival Index (no. surviving 14 days/no. live on pnd 7)[b]				
#	99.5 ± 0.5 N=24	100.0 ± 0.0 N=23	100.0 ± 0.0 N=25	100.0 ± 0.0 N=25
21 Day Survival Index (no. surviving 21 days/no. live on pnd 14)[b]				
#	100.0 ± 0.0 N=24	100.0 ± 0.0 N=23	99.5 ± 0.5 N=25	100.0 ± 0.0 N=25
Lactational Index (no. surviving 21 days/no. live on pnd 4)[b]				
#	99.5 ± 0.5 N=24	100.0 ± 0.0 N=23	99.5 ± 0.5 N=25	100.0 ± 0.0 N=25

(continued)

Table 23. (continued)

Summary and Statistical Analysis of the F_0 Reproductive and Lactational Indexes for the F_1 Litters (page 3 of 3)

[a]Female 24 had a live litter, but was included only for the fertility and gestational indexes because she was not weighed on her correct postnatal day 0 and therefore all subsequent data during lactation were not recorded on the correct days.
[b]Reported as the mean ± S.E.M.; pnd=postnatal day. All indexes are the average percent per litter.
[#]Bartlett's test for homogeneity of variances was significant ($p<0.001$) or could not be done because there was zero variance in one or more groups, therefore nonparametric statistical procedures were employed.

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Table 24.

Summary and Statistical Analysis of the F_1 Litter Size and Pup Body Weights During Lactation
(page 1 of 4)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
No. of Live Litters:				
Postnatal Day 0	24	23	25	25
Postnatal Day 4	24	23	25	25
Postnatal Day 7	24	23	25	25
Postnatal Day 14	24	23	25	25
Postnatal Day 21	24	23	25	25
Average Number of Pups per Litter (pnd 0)[a]				
	13.5 ± 0.4 N=24	14.6 ± 0.3 N=23	14.6 ± 0.4 N=25	14.2 ± 0.4 N=25
Average Number of Pups per Litter (pnd 4)[a]				
	13.5 ± 0.4 N=24	14.5 ± 0.3 N=23	14.5 ± 0.4 N=25	14.1 ± 0.4 N=25
Average Number of Pups per Litter (pnd 7)[a]				
#	8.0 ± 0.0 N=24	8.0 ± 0.0 N=23	8.0 ± 0.0 N=25	8.0 ± 0.0 N=25
Average Number of Pups per Litter (pnd 14)[a]				
#	8.0 ± 0.0 N=24	8.0 ± 0.0 N=23	8.0 ± 0.0 N=25	8.0 ± 0.0 N=25
Average Number of Pups per Litter (pnd 21)[a]				
#	8.0 ± 0.0 N=24	8.0 ± 0.0 N=23	8.0 ± 0.0 N=25	8.0 ± 0.0 N=25
Average Pup Body Weight (g) per Litter (pnd 0)[a]				
	6.58 ± 0.09 N=24	6.44 ± 0.10 N=23	6.55 ± 0.13 N=25	6.62 ± 0.11 N=25
Average Male Body Weight (g) per Litter (pnd 0)[a]				
	6.78 ± 0.10 N=24	6.62 ± 0.11 N=23	6.76 ± 0.14 N=25	6.80 ± 0.11 N=25

(continued)

Table 24. (continued)

Summary and Statistical Analysis of the F_1 Litter Size and Pup Body Weights During Lactation
(page 2 of 4)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
Average Female Body Weight (g) per Litter (pnd 0)[a]				
	6.41 ± 0.09 N=24	6.28 ± 0.10 N=23	6.39 ± 0.12 N=25	6.46 ± 0.11 N=25
Average Pup Body Weight (g) per Litter (pnd 4)[a]				
	11.16 ± 0.21 N=24	10.71 ± 0.18 N=23	10.78 ± 0.22 N=25	10.87 ± 0.24 N=25
Average Male Body Weight (g) per Litter (pnd 4)[a]				
	11.42 ± 0.20 N=24	10.94 ± 0.20 N=23	11.01 ± 0.25 N=25	11.16 ± 0.25 N=25
Average Female Body Weight (g) per Litter (pnd 4)[a]				
	10.93 ± 0.22 N=24	10.52 ± 0.18 N=23	10.61 ± 0.21 N=25	10.60 ± 0.24 N=25
Average Pup Body Weight (g) per Litter (pnd 7)[a]				
	17.86 ± 0.26 N=24	17.21 ± 0.27 N=23	17.46 ± 0.29 N=25	17.55 ± 0.28 N=25
Average Male Body Weight (g) per Litter (pnd 7)[a]				
	18.19 ± 0.26 N=24	17.68 ± 0.30 N=23	17.82 ± 0.33 N=25	17.94 ± 0.30 N=25
Average Female Body Weight (g) per Litter (pnd 7)[a]				
	17.54 ± 0.28 N=24	16.74 ± 0.26 N=23	17.17 ± 0.28 N=25	17.18 ± 0.28 N=25
Average Pup Body Weight (g) per Litter (pnd 14)[a]				
	36.98 ± 0.46 N=24	35.62 ± 0.59 N=23	36.44 ± 0.45 N=25	36.46 ± 0.45 N=25
Average Male Body Weight (g) per Litter (pnd 14)[a]				
	37.45 ± 0.47 N=24	36.33 ± 0.62 N=23	37.19 ± 0.50 N=25	36.97 ± 0.49 N=25

(continued)

Table 24 (continued)

Summary and Statistical Analysis of the F_1 Litter Size and Pup Body Weights During Lactation
(page 3 of 4)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
Average Female Body Weight (g) per Litter (pnd 14)[a]				
	36.51 ± 0.47 N=24	34.92 ± 0.58 N=23	35.77 ± 0.44 N=25	35.98 ± 0.44 N=25
Average Pup Body Weight (g) per Litter (pnd 21)[a]				
	60.52 ± 0.82 N=24	57.26 ± 0.84 N=23	59.15 ± 0.88 N=25	59.48 ± 0.77 N=25
Average Male Body Weight (g) per Litter (pnd 21)[a]				
	61.54 ± 0.88 N=24	58.70 ± 0.96 N=23	60.58 ± 1.05 N=25	60.93 ± 0.91 N=25
Average Female Body Weight (g) per Litter (pnd 21)[a]				
	59.52 ‡ ± 0.81 N=24	55.81 ** ± 0.77 N=23	57.89 ± 0.81 N=25	58.20 ± 0.74 N=25
Percent Male Pups per Litter (pnd 0)[a]				
	46.7 ± 2.3 N=24	48.6 ± 2.5 N=23	44.8 ± 2.7 N=25	47.5 ± 2.7 N=25
Percent Male Pups per Litter (pnd 4)[a]				
	47.0 ± 2.3 N=24	48.3 ± 2.4 N=23	45.6 ± 2.7 N=25	46.8 ± 2.8 N=25
Percent Male Pups per Litter (pnd 7)[a]				
#	49.5 ± 0.9 N=24	50.0 ± 0.0 N=23	48.0 ± 1.6 N=25	49.0 ± 1.2 N=25
Percent Male Pups per Litter (pnd 14)[a]				
#	49.8 ± 1.0 N=24	50.0 ± 0.0 N=23	48.0 ± 1.6 N=25	49.0 ± 1.2 N=25
Percent Male Pups per Litter (pnd 21)[a]				
#	49.8 ± 1.0 N=24	50.0 ± 0.0 N=23	48.3 ± 1.6 N=25	49.0 ± 1.2 N=25

(continued)

Table 24. (continued)

Summary and Statistical Analysis of the F_1 Litter Size and Pup Body Weights During Lactation
(page 4 of 4)

[a]Reported as the mean ± S.E.M.; pnd=postnatal day.
[#]Bartlett's test for homogeneity of variances was significant ($p<0.001$) or could not be done because there was zero variance in one or more groups, therefore nonparametric statistical procedures were employed.
[‡]$p<0.05$; ANOVA Test.
[‡‡]$p<0.01$; Dunnett's Test.

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Table 25.

Summary and Statistical Analysis of the F_1 Reproductive and Lactational Indexes for the F_2 Litters (page 1 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
No. Animals Started on Study				
Males	20	20	20	20
Females	20	20	20	20
No. Females Paired				
	20	20	20	19[a]
No. of Females that Mated				
	19 £ Ψ	19	20	14
Mating Index (no. females that mated/no. females paired)				
	95.0	95.0	100.0	73.7
No. of Pregnant Females				
	19	18	18	12
Fertility Index (no. pregnant females/no. females that mated)				
	100.0	94.7	90.0	85.7
No. of Females with Live Litters (pnd 0)				
	19	18	18	12
Gestational Index (no. females with live litters/no. females pregnant)				
	100.0	100.0	100.0	100.0
No. Males Paired				
	20	20	20	19
No. of Males that Mated				
	19 £ Ψ	19	20	14
Mating Index (no males that mated/no. males paired)				
	95.0	95.0	100.0	73.7
No. Males Siring Litters				
	19	18	18	12
Fertility Index (no. males siring litters/no. males that mated)				
	100.0	94.7	90.0	85.7
Pregnancy Index (no. pregnant females/no. males that mated)				
	100.0	94.7	90.0	85.7

(continued)

Table 25 (continued)

Summary and Statistical Analysis of the F_1 Reproductive and Lactational Indexes for the F_2 Litters (page 2 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
Days until Sperm Positive (days)[b,c]				
	3.7 ± 0.6 N=19	3.4 ± 0.6 N=17	4.0 ± 0.5 N=19	3.7 ± 0.8 N=12
Gestational Length (days)[b,d]				
	21.9 ± 0.1 N=19	22.4 ± 0.1 N=16	22.1 ± 0.1 N=17	22.2 ± 0.2 N=10
No. of Live Litters:				
Postnatal Day 0	19	18	18	12
Postatal Day 4	19	17[e]	18	12
No. Implantation Sites per Litter[b]				
	14.84 ± 0.81 N=19	16.72 ± 0.83 N=18	17.00 ± 0.54 N=18	17.17 ± 1.11 N=12
Percent Postimplantation Loss per Litter[b]				
	7.29 ± 1.51 N=19	8.75 ± 2.00 N=18	7.36 ± 1.70 N=18	11.90 ± 2.81 N=12
Number of Live Pups on Postnatal Day 0[b]				
	13.8 ± 0.7 N=19	15.2 ± 0.7 N=18	15.8 ± 0.5 N=18	15.0 ± 1.0 N=12
Number of Dead Pups on Postnatal Day 0[b]				
#	0.3 ± 0.1 N=19	0.4 ± 0.3 N=18	0.2 ± 0.1 N=18	0.4 ± 0.3 N=12
Total Number of Pups on Postnatal Day 0[b]				
	14.1 ± 0.8 N=19	15.6 ± 0.7 N=18	15.9 ± 0.6 N=18	15.4 ± 1.0 N=12
Stillbirth Index (no. dead on pnd 0/total no. on pnd 0)[b]				
	1.6 ± 0.6 N=19	2.1 ± 1.6 N=18	1.0 ± 0.5 N=18	2.6 ± 1.6 N=12

(continued)

Table 25 (continued)

Summary and Statistical Analysis of the F_1 Reproductive and Lactational Indexes for the F_2 Litters (page 3 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
Live Birth Index (no. live on pnd 0/total no. on pnd 0)[b]				
	98.4 ± 0.6 N=19	97.9 ± 1.6 N=18	99.0 ± 0.5 N=18	97.4 ± 1.6 N=12
4 Day Survival Index (no. surviving 4 days/no. live on pnd 0)[b]				
	99.0 ± 0.5 N=19	96.8 ± 1.5 N=17[e]	99.1 ± 0.5 N=18	95.5 ± 2.8 N=12

[a]Female 324 was euthanized on study day 36 due to a mass in the left inguinal area that was displacing the urethra and vagina to the right.
[b]Reported as the mean ± S.E.M.; pnd=postnatal day. All indexes are the average percent per litter.
[c]Days until sperm positive could only be calculated for those females for which sperm were detected in the vaginal smear.
[d]Gestational length could not be calculated for females that were pregnant, but for which sperm were never detected in the vaginal smear.
[e]Female 256 was euthanized moribund on postnatal day 0 after delivering her litter. Her pups were also euthanized on postnatal day 0 and therefore this litter is not included in the calculation of the 4 day survival index.
[#]Bartlett's test for homogeneity of variances was significant ($p<0.001$) or could not be done because there was zero variance in one or more groups, therefore nonparametric statistical procedures were employed.
[£]$p<0.05$; Chi-Square Test.
[¥]$p<0.05$; Cochran-Armitage Test.

Table 26.

Summary and Statistical Analysis of the F_2 Litter Size, F_2 Pup Body Weights and Percent F_2 Males During Lactation (page 1 of 2)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
No. of Live Litters:				
Postnatal Day 0	19	18	18	12
Postatal Day 4	19	17[a]	18	12
Average Number of Pups per Litter (pnd 0)[b]				
	13.8 ± 0.7 N=19	15.2 ± 0.7 N=18	15.8 ± 0.5 N=18	15.0 ± 1.0 N=12
Average Number of Pups per Litter (pnd 4)[b]				
	13.6 ± 0.7 N=19	14.7 ± 0.7 N=17[a]	15.6 ± 0.5 N=18	14.3 ± 1.0 N=12
Average Pup Body Weight (g) per Litter (pnd 0)[b]				
	6.65 ± 0.11 N=19	6.37 ± 0.15 N=18	6.39 ± 0.14 N=18	6.35 ± 0.17 N=12
Average Male Body Weight (g) per Litter (pnd 0)[b]				
	6.79 ± 0.13 N=19	6.52 ± 0.15 N=18	6.54 ± 0.14 N=18	6.54 ± 0.20 N=12
Average Female Body Weight (g) per Litter (pnd 0)[b]				
	6.53 ± 0.10 N=19	6.12 ± 0.14 N=17[c]	6.25 ± 0.14 N=18	6.13 ± 0.14 N=12
Average Pup Body Weight (g) per Litter (pnd 4)[b]				
	10.12 ± 0.32 N=19	9.80 ± 0.39 N=17[a]	9.83 ± 0.35 N=18	9.42 ± 0.44 N=12
Average Male Body Weight (g) per Litter (pnd 4)[b]				
	10.26 ± 0.35 N=19	10.00 ± 0.40 N=17[a]	10.12 ± 0.35 N=18	9.71 ± 0.44 N=12
Average Female Body Weight (g) per Litter (pnd 4)[b]				
	9.98 ± 0.30 N=19	9.34 ± 0.28 N=16[c]	9.56 ± 0.34 N=18	9.10 ± 0.42 N=12

(continued)

Table 26 (continued)

Summary and Statistical Analysis of the F_2 Litter Size, F_2 Pup Body Weights and Percent F_2 Males During Lactation (page 2 of 2)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
Percent Male Pups per Litter (pnd 0)[b]				
	48.8 ± 2.6 N=19	54.3 ± 4.1 N=18	47.9 ± 2.7 N=18	52.4 ± 3.6 N=12
Percent Male Pups per Litter (pnd 4)[b]				
	49.5 ± 2.6 N=19	55.5 ± 4.4 N=17[a]	47.7 ± 2.6 N=18	53.2 ± 3.8 N=12

[a]Female 256 was euthanized moribund on postnatal day 0 after delivering her litter. Her pups were also euthanized on postnatal day 0.
[b]Reported as the mean ± S.E.M.; pnd=postnatal day.
[c]One litter had male pups only.

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SUMMARY OF GENETIC TOXICITY STUDIES

Bacterial Reverse Mutation Assay

[This assay uses tester strains of Salmonella typhimurium and Escherichia coli that contain mutations causing them to require amino acids histidine and tryptophan, respectively, in order to grow. The principle of this bacterial reverse mutation test is that it detects chemicals that induce mutations which revert mutations already present in the tester strains, thus restoring the functional capability of the bacteria to synthesize the essential amino acid. The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent tester strain.]

Testing Facility : [redacted]

Study Number: AA20YG.502.BTL [redacted]
UT-15 (Sponsor's project No.)

Study Dates: September 20 to October 21, 1999

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

Lot No. of the Test Compound: UT15MIX-99G001

Concentrations Tested: 0, 100, 333, 1000, 3333 and 5000 µg/plate. (The doses were selected based on the results of a preliminary assay, in which doses up to 5000 µg per plate did not produce any precipitation or significant cytotoxicity.)

Vehicle: Sodium citrate formulation – pH 7.0 [prepared by mixing sodium citrate, citric acid and sodium chloride in sterile water for injection (sponsor-specified vehicle)]

Tester Strains: Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and Escherichia coli WP2 uvrA.

Metabolic Activation System: Aroclor 1254-induced rat liver S-9 fraction in a cofactor pool containing glucose-6-phosphate, β-nicotinamide adenine dinucleotide phosphate and magnesium and potassium chlorides in phosphate buffer (pH 7.4)

Positive Control Compounds Not Requiring S-9 Activation: 2-nitrofluorene (TA98), sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537) and methyl methane-sulfonate (WP2 uvrA)

Positive Control Compound Requiring S-9 Activation : 2-aminoantracene (all strains – to test the activity of S-9 mix)

Test Procedure: The test article was exposed to the test system using the plate incorporation assay procedure. The vehicle or solutions of test article or reference compounds, S-9 or sham mix and cultures of tester strains were added into respective tubes containing molten minimal top agar at 45 ± 2^0 C . After mixing, the mixture was overlaid onto the surface of Vogel-Bonner minimal agar plates. When the overlay had solidified, the plates were inverted and incubated for about 48 to 72 hours at 37 ± 2^0 C. Revertant colonies were counted manually or by an automated colony counter. For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated. (All concentrations of test article and vehicle and positive controls were plated in triplicate.)

The growth of the background lawn was evaluated for evidence of test article toxicity using a dissecting microscope. The precipitate was evaluated by visual examination. Test article toxicity and the degree of precipitation were scored relative to the vehicle control plates.

A test compound is considered to be positive for mutagenic effect if it produces a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article. [The increase in mean revertants at the peak of the dose response should be equal to or greater than two (for strains TA98, TA100 and WP2uvrA) or three (for strains TA1535 and TA1537) times the mean vehicle control value.]

A dose level is considered toxic if one or both of the following criteria are met: 1. greater than 50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value, and 2. a reduction in the background lawn.

An assay is considered to be valid if the following criteria are met:

1. each tester strain must have appropriate genotypic characterics. 2. all cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle control as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2uvrA, 10-60. 3. tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/ml. 4. each positive control must have a three-fold increase in the number of revertants over the respective vehicle control value, and 5. a minimum of three non-toxic levels are required for evaluation.

Results: The results are summarized in Table 27. The test compound, under the conditions of the study, did not produce any significant dose-related increase in revertant colonies with any tester strains with or without metabolic activation. Respective positive control compounds produced significant increases in mutant frequencies, demonstrating the sensitivity of the tester strains as well as the activity of the S-9 mix.

No precipitation was observed at any concentrations in this study. Evidence of toxicity was observed at 5000 µg/plate with tester strains TA100, TA1535 and TA1537 in the absence of metabolic activation.

Historical negative and positive control data are presented in Table 28.

The present study is considered to be a valid study since all criteria for an acceptable study are met.

Table 27

Salmonella/E. coli Mutagenicity Assay
Summary of Results

Test Article Id : UT-15
Study Number : AA20YG.502.BTL Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	17 ± 3	104 ± 10	14 ± 2	5 ± 1	14 ± 2
100	17 ± 2	97 ± 17	13 ± 5	6 ± 3	13 ± 3
333	21 ± 3	83 ± 5	14 ± 3	5 ± 2	18 ± 2
1000	19 ± 4	105 ± 6	10 ± 1	4 ± 2	14 ± 3
3333	14 ± 5	77 ± 24	12 ± 2	4 ± 2	6 ± 3
5000	10 ± 1	46 ± 8	4 ± 3	2 ± 1	9 ± 4
Pos	257 ± 36	419 ± 58	143 ± 26	730 ± 171	72 ± 7

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	21 ± 3	115 ± 29	9 ± 2	5 ± 2	13 ± 2
100	29 ± 3	124 ± 8	10 ± 2	5 ± 4	14 ± 6
333	22 ± 1	87 ± 19	13 ± 3	4 ± 2	14 ± 2
1000	22 ± 4	104 ± 2	12 ± 4	7 ± 3	12 ± 1
3333	21 ± 2	86 ± 12	12 ± 2	4 ± 3	15 ± 2
5000	21 ± 4	78 ± 20	10 ± 5	4 ± 3	13 ± 6
Pos	355 ± 115	491 ± 47	69 ± 9	31 ± 13	312 ± 141

0.0 = Vehicle plating aliquot of 500 μL
Pos = Positive Control concentrations as specified in Materials and Methods section.

Table 28.

Historical Negative and Positive Control Values
1996 - 1998

revertants per plate

Strain	Control	Activation							
		None				Rat Liver			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA98	Neg	17	7			22	7		
	Pos	326	220			763	450		
TA100	Neg	119	22			138	24		
	Pos	579	157			889	438		
TA1535	Neg	10	4			11	4		
	Pos	433	136			107	78		
TA1537	Neg	5	3			7	3		
	Pos	769	477			134	153		
WP2 *uvrA*	Neg	16	5			17	5		
	Pos	200	132			294	159		

SD = standard deviation; Min = minimum value; Max = maximum value; Neg = negative control (including but not limited to deionized water, dimethyl sulfoxide, ethanol and acetone); Pos = positive control

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Mouse Lymphoma L5178Y Cell Assay

[The objective of this assay was to evaluate the ability of UT-15 to induce forward mutations at the thymidine kinase locus in the mouse lymphoma L5178Y cell line. These cells are heterozygous for the enzyme thymidine kinase ($TK^{+/-}$). Chemical-induced mutations at the TK locus result in the loss of thymidine kinase activity with the formation of a homozygous strain ($TK^{-/-}$). Both $TK^{+/-}$ and $TK^{-/-}$ strains can grow in normal medium, but the incorporation of 5-trifluorothymidine (TFT) into medium results in cytotoxicity to the $TK^{+/-}$ cells, with growth and replication occurring only in mutant $TK^{-/-}$ cells. Thus, resistance to TFT indicates mutation, induced by the test compound, at the TK locus. In this assay system, both gene mutations (large colonies) as well as chromosome aberrations (small colonies) can be detected.]

Testing Facility: (

Study Number: 19159-0-431 ICH (

Study Dates: January 22 to March 11, 1998

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

Lot No. of the Test Compound: LRX-98A01

Concentrations Tested: 1. without metabolic activation – 15.7, 31.3, 62.5, 125, 250, 300, 400, 500 and 600 µg/ml for the first trial (4-hour treatment period) and 15.7, 25.0, 31.3, 37.5, 50, 62.5, 75, 87.5, 100, 125 and 150 µg/ml for the confirmatory trial (24-hour treatment period). 2. with metabolic activation – 15.7, 31.3, 62.5, 125, 250, 300, 400, 500 and 600 µg/ml for the first trial (4-hour treatment period) and 15.7, 31.3, 62.5, 125, 200, 250, 300, 350, 400, 450 and 500 µg/ml for the confirmatory trial (4-hour treatment period).

[In preliminary dose range finding studies (dose levels ranging from 4.93 to 2500 µg/ml), doses of 250 µg/ml and above with 24 hour treatment period, or 625 µg/ml and above with 4 hour treatment period were observed to be highly cytotoxic. Doses for the present study were selected based on these results to cover a toxicity range from 10 to 20% survival to no apparent effect on growth relative to the vehicle control.]

The test article remained in solution in culture medium at all dose levels tested.

Solvent: DMSO

Test Culture: The mouse lymphoma L5178Y cell line, heterozygous at the TK locus and designated as clone 3.7.2C, was used for the assay. Tests for mycoplasma contamination

and karyotype stability evaluations, as measured by mean chromosomal number, were routinely performed on stock cultures.

Metabolic Activation System: Aroclor 1254 induced rat liver S9 fraction and an energy producing system comprised of nicotinamide adenine dinucleotide phosphate (NADP, sodium salt) and isocitrate.

Positive Control Compounds: Methyl methanesulfonate (MMS, without S9) and methyl-cholanthrene (MCA, with S9)

Test Procedure: Logarithmically growing laboratory stock cultures of L5178Y cells were seeded into a series of tubes at 6 x 10^6 cells per tube. The cells were pelleted by centrifugation, the culture medium removed, and the cells resuspended in a final volume of 10 ml treatment medium containing test compound or positive or vehicle control solutions with or without S9 reaction mix. The tubes were placed in an ——— incubator at about 37°C and rotated at 80 ± 10 orbits per minute. After an exposure period of about four hours, the cells were washed twice, resuspended in 20 ml of culture medium and returned to the incubator for growth and expression of the $TK^{-/-}$ phenotype (expression period of 2 days). Cell densities were determined on day one and were adjusted to 3 x 10^5 cells/ml in 20 ml of growth medium. If the cells in a culture failed to multiply to a density of 4 x 10^5 on the first day after treatment, the culture was not subcultured. On day two, cell counts were again determined, and appropriate cultures were selected for cloning and mutant selection.

A total of 3 x 10^6 cells from each selected tube was suspended in agar cloning medium containing TFT (3μg/ml) to recover mutants. This sample was distributed into three 100 mm dishes. The absolute selection cloning efficiency was determined by seeding three dishes with a total of about 600 cells in agar cloning medium. All dishes were incubated at 37°C for 10 to 14 days and the colonies were counted with an automated colony counter.

Both the small and large colonies were quantified for the positive and negative controls. (Note: It is stated that since the test article was found to be negative in this test system, colony sizing was not performed with test article-treated cultures.)

The mutant frequency was calculated as the ratio of the total number of mutant colonies found in each set of three mutant selection dishes to the total number of cells seeded, adjusted by the absolute selection cloning efficiency.

The relative total growth (RTG, also known as the percent relative growth), a measurement of cytotoxicity, was determined by multiplying the relative suspension growth of the cells over the two-day expression period by the relative cloning efficiency at the time of selection.

A confirmatory assay was also conducted after the results of the first trial were known. In the confirmatory assay, a 24-hour treatment period (instead of the 4 hour treatment period

used in the first trial) was used with the nonactivation system (without S9 mix). All other assay procedures were similar to the first trial. The test drug concentrations were slightly modified for the confirmatory assay based on the results from the first trial.

An assay was considered acceptable if the following criteria were satisfied.

1. The average absolute cloning efficiency of the vehicle controls should be between 60 and 130%. (A value greater than 100% is possible because of errors in cell counts and variations in cell division.)
2. A minimum acceptable value for the average suspension growth of the vehicle controls for two days is an 8.0 fold increase over the original cell numbers.
3. The background mutant frequency should be within the normal range of 30×10^{-6} to 120×10^{-6}.
4. At least one of the positive control cultures in each trial should induce a mutant frequency of at least 200×10^{-6}.
5. For test compounds with weak or no mutagenic activity, the assay should include cytotoxic concentrations that reduce the relative growth to 10 or 20% of the vehicle controls.
6. An experimental mutant frequency will be considered acceptable only if the relative cloning efficiency is 10% or greater and the total number of viable clones exceeds about 60 and
7. Mutant frequencies for at least 5 different dose levels are normally determined in each assay, although a minimum of four doses (analyzed cultures) are considered necessary for evaluation.

The test compound is considered as positive if:

1. dose-dependent increases in mutant frequency of 2-fold or greater are obtained over the concurrent vehicle control mutant frequency and 2. the findings are reproducible.

Results: In the first study without metabolic activation (Table 29), although nine treatments (15.7, 31.3, 62.5, 125, 250, 300, 400, 500 and 600 µg/ml) were initiated, doses above 400 µg/ml were terminated because of excessive cytotoxicity, and the lowest dose (15.7 µg/ml) was terminated because sufficient higher doses were available for analysis. Cultures with the remaining six dose levels were cloned for mutant analysis, and a wide range of cytotoxicity (112.2 to 13.3% relative growth) was seen at these dose levels. The test drug did not induce an increase in mutant frequency equal to twice the average mutant frequency of the concurrent vehicle controls at any dose level tested in the study.

In the confirmatory nonactivation assay using a 24-hour treatment period, no evidence of mutagenicity was observed (Table 30).

In the presence of metabolic activation, both the initial and the confirmatory trials showed no evidence of mutagenicity (mutant frequency that equaled or exceeded the minimum criterion for a positive response; Tables 31 & 32).

In all the above assays, both with and without metabolic activation, significant increases in mutant frequencies were induced by the respective positive control compounds.

Colony sizing data for vehicle and positive controls are presented in Table 33. As noted above, since the test article was negative, no colony sizing was performed with test article-treated cultures.

Historical control mutant frequency data are given in Table 34.

Since all the criteria for an acceptable study are met, the present study is considered to be a valid study.

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Table 29.

MUTATION ASSAY WITHOUT ACTIVATION - TRIAL 1

A. TEST ARTICLE: UT-15
B. GENETICS ASSAY NO: 19159
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/ml
E. TEST DATE: 02/03/98

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML.10ES UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
NONACTIVATION CONTROLS[e]			AVG VEHICLE CONTROL				AVG VEHICLE CONTROL			
VEHICLE CONTROL	13.3	14.3	21.1		92	503	83.8		100.0	36.6
VEHICLE CONTROL	16.2	11.6	20.9		98	571	95.2		100.0	34.3
VEHICLE CONTROL	13.2	10.0	14.7	18.9	144	668	111.3	96.8	100.0	43.1
MMS 5 nl/ml	12.5	13.1	18.2		592	386	64.2		63.9	307.5[f]
MMS 10 nl/ml	8.4	9.9	9.2		692	253	42.2		21.2	547.0[f]
TEST COMPOUND			RELATIVE TO VEHICLE CONTROL (%)				RELATIVE TO VEHICLE CONTROL (%)			
31.3 µg/ml	11.6	13.9	94.8		116	631	108.6		103.0	36.8
62.5 µg/ml	13.3	12.8	100.1		104	651	112.1		112.2	32.0
125 µg/ml	11.2	17.5	115.2		106	487	83.8		96.5	43.5
250 µg/ml	10.6	12.8	79.8		101	543	93.5		74.6	37.2
300 µg/ml	7.5	10.8	47.6		91	582	100.2		47.7	31.3
400 µg/ml	1.8*	9.4	16.6		88	464	79.9		13.3	37.9

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]VEHICLE CONTROL = 1% DMSO; MMS = METHYL METHANESULFONATE POSITIVE CONTROL

[f]MUTAGENIC. EXCEEDS MINIMUM CRITERION OF 76.0 X 10E-6

* NOT SPLIT BACK

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Table 30.

MUTATION ASSAY WITHOUT ACTIVATION - TRIAL 2
24-HOUR TREATMENT

A. TEST ARTICLE: UT-15
B. GENETICS ASSAY NO: 19159
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3 µg/ml
E. TEST DATE: 02/24/98

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML..10ES UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (2)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
NONACTIVATION CONTROLS[e]			AVG VEHICLE CONTROL				AVG VEHICLE CONTROL			
VEHICLE CONTROL	10.6	19.5	23.0		50	337	56.2		100.0	29.7
VEHICLE CONTROL	8.4	20.1	18.8		78	362	60.3		100.0	38.7
VEHICLE CONTROL	10.7	19.5	23.2	21.7	55	387	64.5	60.3	100.0	28.4
MMS 2.5 ml/ml	5.8	15.8	10.2		463	342	57.0		44.4	270.8[f]
MMS 2.5 ml/ml	7.9	19.0	16.7		451	221	36.8		47.0	408.1[f]
TEST COMPOUND			RELATIVE TO VEHICLE CONTROL (%)				RELATIVE TO VEHICLE CONTROL (%)			
37.5 µg/ml	13.0	16.3	108.5		71	449	124.1		134.6	31.6
50.0 µg/ml	11.8	16.5	99.7		76	457	126.3		125.9	33.3
62.5 µg/ml	10.8	17.7	97.9		63	399	110.3		108.0	31.6
75.0 µg/ml	11.7	16.4	92.3		57	497	137.4		126.8	22.9
87.5 µg/ml	6.4	17.4	57.0		54	330	91.2		52.0	32.7
100 µg/ml	4.9	14.6	36.6		64	442	122.2		44.7	29.0
125 µg/ml	2.5*	12.6	19.4		87	538	148.7		28.8	32.3
150 µg/ml	1.3*	7.5	11.5		142	788	195.7		22.5	40.1

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]VEHICLE CONTROL = 0.5% DMSO: MMS = METHYL METHANESULFONATE POSITIVE CONTROL

[f]MUTAGENIC. EXCEEDS MINIMUM CRITERION OF 64.5 X 10E-6

* NOT SPLIT BACK

Table 31.

MUTATION ASSAY WITH ACTIVATION - TRIAL 1

A. TEST ARTICLE: UT-15
B. GENETICS ASSAY NO: 19159
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/ml
E. TEST DATE: 02/03/98

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML.10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
S9 ACTIVATION INDUCED[e]	S9 BATCH NO: 0797		AVG VEHICLE CONTROL				AVG VEHICLE CONTROL			
VEHICLE CONTROL	11.9	15.0	19.8		315	504	84.0		100.0	125.0
VEHICLE CONTROL	11.6	13.3	17.1		308	676	112.7		100.0	91.1
VEHICLE CONTROL	11.9	12.8	16.9	17.9	355	582	97.0	97.9	100.0	122.0
MCA 2 µg/ml	8.4	11.9	11.1		820	363	60.5		38.3	451.8[f]
MCA 4 µg/ml	4.0	12.9	5.7		824	247	41.2		13.4	667.2[f]
TEST COMPOUND			RELATIVE TO VEHICLE CONTROL (%)				RELATIVE TO VEHICLE CONTROL (%)			
15.7 µg/ml	11.3	13.9	97.5		306	611	104.0		101.4	100.2
31.3 µg/ml	11.2	14.3	99.4		348	511	87.0		86.5	136.2
62.5 µg/ml	12.0	11.7	87.2		412	502	85.5		74.6	164.1
125 µg/ml	9.6	15.9	94.7		351	522	88.9		84.2	134.5
250 µg/ml	5.4	13.9	46.6		330	472	80.4		37.5	139.8
300 µg/ml	4.6	8.3	23.7		357	555	94.5		22.4	128.6

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]VEHICLE CONTROL = 1% DMSO; MCA = METHYLCHOLANTHRENE POSITIVE CONTROL

[f]MUTAGENIC. EXCEEDS MINIMUM CRITERION OF 225.4 X 10E-6

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Table 32.

MUTATION ASSAY WITH ACTIVATION - TRIAL 2

A. TEST ARTICLE: UT-15
B. GENETICS ASSAY NO: 19159
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/ml
E. TEST DATE: 02/24/98

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
	S9 BATCH NO: 0797		AVG VEHICLE CONTROL				AVG VEHICLE CONTROL			
VEHICLE CONTROL	15.6	15.5	26.9		116	490	81.7		100.0	47.3
VEHICLE CONTROL	16.6	14.7	27.1		116	600	100.0		100.0	38.7
VEHICLE CONTROL	16.4	15.9	29.0	27.7	114	608	101.3	94.3	100.0	37.5
MCA 2 µg/ml	13.1	12.8	18.6		529	590	98.3		70.0	179.3[e]
MCA 4 µg/ml	11.0	11.3	13.8		614	639	106.5		56.3	192.2[e]
TEST COMPOUND			RELATIVE TO VEHICLE CONTROL (%)				RELATIVE TO VEHICLE CONTROL (%)			
15.6 µg/ml	14.6	14.9	87.3		97	716	126.5		110.4	27.1
31.3 µg/ml	13.3	14.1	75.2		132	708	125.1		94.1	37.3
62.5 µg/ml	15.1	16.7	101.2		104	665	117.5		118.9	31.3
125 µg/ml	16.2	16.5	107.2		104	643	113.6		121.8	32.3
200 µg/ml	13.5	13.7	74.2		121	642	113.5		84.2	37.7
250 µg/ml	11.7	12.9	60.5		119	684	120.9		73.1	34.8
300 µg/ml	8.6	14.1	48.6		118	634	112.1		54.5	37.2
350 µg/ml	1.9*	9.4	11.3		122	652	115.2		13.0	37.4

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]VEHICLE CONTROL = 1% DMSO; MCA = METHYLCHOLANTHRENE POSITIVE CONTROL

[f]MUTAGENIC. EXCEEDS MINIMUM CRITERION OF 82.3 X 10E-6

* NOT SPLIT BACK

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Table 33.

ANALYSIS OF COLONY SIZE WITH UT-15 [redacted] ASSAY NUMBER 19159-0-431 ICH					
Dose (µg/ml)	S9	Mutant Frequency ($x 10^{-6}$)*	% Large Colonies	% Small Colonies	Ratio S/L*
Trial 1					
VC[b]	-	29.4	47.5	52.5	1.11
PC[c]	-	265.0	23.6	76.4	3.25
PC[d]	-	495.4	36.0	64.0	1.77
VC[b]	+	88.6	24.1	75.9	3.15
PC[e]	+	388.4	54.8	45.2	0.83
PC[f]	+	577.3	38.4	61.6	1.60
Trial 2					
VC[b]	-	27.8	39.1	60.9	1.56
PC[c]	-	360.3	20.4	79.6	3.91
PC[c]	-	231.7	23.7	76.3	3.21
VC[b]	+	32.0	61.8	38.2	0.62
PC[e]	+	141.3	60.4	39.6	0.65
PC[f]	+	153.0	58.1	41.9	0.72

[a]ratio of small colonies to large colonies
[b]VC= vehicle control
[c] PC= methyl methanesulfonate (5 nl/ml)
[d] PC= methyl methanesulfonate (10 nl/ml)
[e] PC= methylcholanthrene (2 µg/ml)
[f] PC= methylcholanthrene (4 µg/ml)

* Entire plate cannot be sized due to interference from sides of plate. Mutant frequencies will therefore appear different from Tables 4 through 7.

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Table 34.

APPENDIX A HISTORICAL MOUSE LYMPHOMA FORWARD MUTATION ASSAY CONTROL MUTANT FREQUENCY DATA

A. Nonactivation Studies

1. Pooled negative and vehicle controls

Mean (± SD)	$53.0 \pm 22.0 \times 10^{-6}$
Range	
Number of experiments	52
Number of controls	156

2. Positive controls (5.0 nl/ml methyl methanesulfonate)

Mean (± SD)	$272.7 \pm 135.7 \times 10^{-6}$
Range	
Number of experiments	50
Number of controls	50

3. Positive controls (10.0 nl/ml methyl methanesulfonate)

Mean (± SD)	$483.9 \pm 315.2 \times 10^{-6}$
Range	
Number of experiments	52
Number of controls	52

B. Activation Studies

1. Pooled negative and vehicle controls

Mean (± SD)	$65.3 \pm 27.1 \times 10^{-6}$
Range	
Number of experiments	55
Number of controls	162

2. Positive controls (2.0 µg/ml 3-methylcholanthrene)

Mean (± SD)	$454.5 \pm 166.2 \times 10^{-6}$
Range	
Number of experiments	54
Number of controls	54

3. Positive controls (4.0 µg/ml 3-methylcholanthrene)

Mean (± SD)	$567.0 \pm 248.3 \times 10^{-6}$
Range	
Number of experiments	54
Number of controls	54

The historical control data was compiled from fifty experiments. The mean (± one standard deviation) and the range of the mutant frequencies were reported for each control condition. Because some experiments contained multiple controls, the number of independent control cultures exceeded the number of experiments.

Mammalian Erythrocyte Micronucleus Test

(The objective of the study was to assess the clastogenic potential of the test drug when given as a continuous subcutaneous infusion, as measured by the ability of the drug to induce micronucleated polychromatic erythrocytes in the rat bone marrow.

Micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. It has been established that agents that cause chromosome breaks or spindle disruption induce micronuclei formation. When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded, and micronuclei that have been formed may remain behind in otherwise enucleated cytoplasm. An increase in the frequency of micronucleated polychromatic erythrocytes is an indication of test article-induced chromosome damage.)

Testing Facility: [redacted]

Study Number: G98AZ30.125 [redacted]

Study Dates: October 6, 1998 to January 29, 1999

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

Animals: Seven to nine-week-old Sprague-Dawley rats [redacted], Body weight – males 259.0 – 283.4 g ; females 175.4 – 225.1 g

Lot No. of the Test Compound: UT15-98H01

Doses Tested: 0 (vehicle control), 500, 1000 and 1500 ng/kg/minute (administered by continuous subcutaneous infusion using [redacted] osmotic pumps)

[The doses were selected based on a previous toxicity study in which male and female rats were given continuous subcutaneous infusion of the test article (using [redacted] pumps) at 500, 1000 or 1500 ng/kg/minute for three days. No mortality was observed in this study. Clinical signs observed within four hours of drug administration included staggering walk and lethargy in all animals at all dose levels. On the second day of the drug administration, lethargy was noted only in high dose animals. It is stated that "the high dose for the micronucleus test was set at 1500 ng/kg/minute for male and female rats in consultation with the sponsor."]

Vehicle: Sodium citrate formulation pH 7.0 (prepared by mixing sodium citrate, citric acid and sodium chloride in sterile water; sponsor-specified vehicle)

Positive Control: Cyclophosphamide – 50 mg/kg

Test Procedure: Rats were assigned to different groups (5/sex/group except in the vehicle control and high dose groups which had 10 rats/sex/group) using a computer-generated program based on body weight distribution. The animals were anesthetized, and the [redacted] osmotic pumps [redacted] were surgically implanted subcutaneously on the dorsal side of the animals to deliver the test article at appropriate doses or the vehicle at an infusion rate of 10.0 µl per hour. The positive control was administered by intraperitoneal injection 24 hours before sacrifice. All rats were observed for clinical signs and mortality.

Rats were sacrificed 48 (5/sex from all groups) and 72 (5/sex from the vehicle control and high dose groups) hours after the initiation of treatment, femurs were exposed, cut just above the knee, and the bone marrow was aspirated into a syringe containing fetal bovine serum. The bone marrow cells were centrifuged, resuspended in serum and smears were prepared (two to four slides per rat). The slides were fixed in methanol and stained with acridine orange.

The slides were scored blindly. Two thousand polychromatic erythrocytes (PCE) were scored for each rat for the presence of micronuclei which were defined as round, fluorescent green staining nuclear fragments, having a sharp contour with diameters usually from 1/20 to 1/5 of the erythrocyte. The number of micronucleated normochromatic erythrocytes in the field of 2000 PCEs was counted. The proportion of PCEs to total erythrocytes was also recorded per 1000 erythrocytes in order to evaluate the proliferation state of the bone marrow as an indicator of bone marrow toxicity.

Statistical significance was determined using the Kastenbaum-Bowman tables which are based on the binomial distribution.

A test article was considered to induce a positive response if a dose-responsive increase in micronucleated PCEs was observed, and one or more doses produced a statistically significant increase in micronucleated PCEs relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables) at any sampling time. If a single treatment group was significantly elevated at one sacrifice time with no evidence of a dose-response, the assay was considered a suspect or unconfirmed positive and a repeat assay was recommended.

For an assay to be considered valid, the mean incidence of micronucleated PCEs must not exceed 0.3% (3/1000 PCEs) in the vehicle control, and the incidence of micronucleated PCEs in the positive control group must be significantly increased relative to the vehicle control group ($p \leq 0.05$, Kastenbaum-Bowman Tables).

Results: No mortality occurred in the study. Clinical signs, which were noted within three hours of implantation, included staggering walk and lethargy in test article and vehicle treated rats. Diarrhea was seen in high dose males (4/10) two days after the initiation of treatment. Rats treated with positive control compound appeared normal.

The number of micronucleated PCEs per 10,000 PCEs scored per treatment and the proportion of PCEs to total erythrocytes counted at 48 and 72 hour sampling intervals are

presented in Table 35. Reductions in the ratio of polychromatic erythrocytes to total erythrocytes were observed in mid (2%) and high (2-5%) dose males and females relative to their respective vehicle controls, suggestive of decreased bone marrow cellular proliferation due to cytotoxicity at these dose levels. No significant differences in the number of micronucleated polychromatic erythrocytes between test article and vehicle treated rats were observed at 48 or 72 hour bone marrow collection times ($p \geq 0.05$, Kastenbaum-Bowman Tables). The positive control induced a significant increase in micronucleated PCEs in both males and females.

The historical control data are presented in Table 36.

Since all criteria for an acceptable study are met, the present study is considered to be a valid study.

Table 35.

Summary of Bone Marrow Micronucleus Study Using UT-15

Treatment	Sex	Time (hr)	Number of Rats	PCE/Total Erythrocytes (Mean +/- sd)	Change From Control (%)	Micronucleated Polychromatic Erythrocytes Number per 1000 PCEs (Mean +/- sd)	Number per PCEs Scored
* Diluent	M	48	5	0.55 ± 0.04	---	0.6 ± 0.22	6 / 10000
	F	48	5	0.56 ± 0.03	---	0.3 ± 0.27	3 / 10000
UT-15							
500 ng/kg/min	M	48	5	0.55 ± 0.03	0	0.8 ± 0.57	8 / 10000
	F	48	5	0.56 ± 0.03	0	0.8 ± 0.57	8 / 10000
1000 ng/kg/min	M	48	5	0.54 ± 0.03	-2	0.5 ± 0.50	5 / 10000
	F	48	5	0.55 ± 0.03	-2	0.7 ± 0.57	7 / 10000
1500 ng/kg/min	M	48	5	0.54 ± 0.07	-2	0.8 ± 0.57	8 / 10000
	F	48	5	0.53 ± 0.03	-5	0.6 ± 0.42	6 / 10000
CP,**							
50 mg/kg	M	24	5	0.48 ± 0.03	-13	16.0 ± 7.37	* 160 / 10000
	F	24	5	0.44 ± 0.02	-21	10.6 ± 1.14	* 106 / 10000
* Diluent	M	72	5	0.56 ± 0.02	---	0.5 ± 0.35	5 / 10000
	F	72	5	0.56 ± 0.04	---	0.4 ± 0.42	4 / 10000
UT-15							
1500 ng/kg/min	M	72	5	0.59 ± 0.03	5	1.1 ± 0.82	11 / 10000
	F	72	5	0.55 ± 0.06	-2	0.4 ± 0.42	4 / 10000

* Diluent = Sponsor specified: sodium citrate, citric acid, sodium chloride, sterile water for injection (please refer to page 7 for the vehicle composition and preparation).
** = Animals dosed with CP, 24 hours before sacrifice

[1]*, p≤0.05 (Kastenbaum-Bowman Tables)

Table 36.

Micronucleus Test Historical Control Data
1995 - 1997

Negative Control Animals[1]

Parameter	Ratio of PCE/Total Erythrocytes		MPCE/1000 PCE Scored	
	Males	Females	Males	Females
Mean	0.54	0.55	0.79	0.82
Standard Deviation	0.08	0.08	0.90	0.94
Range				

Positive Control Animals[2]

Parameter	Ratio of PCE/Total Erythrocytes		MPCE/1000 PCE Scored	
	Males	Females	Males	Females
Mean	0.46	0.48	31.92	28.37
Standard Deviation	0.12	0.10	17.18	14.37
Range				

[1]Negative controls include all vehicles and all routes of administration.
[2]Positive control is cyclophosphamide, 20 to 60 mg/kg, dosed by IV, IP, PO or SC. Bone marrow cells were collected at 24 hours after a single administration.

OVERALL SUMMARY AND EVALUATION

Treprostinol sodium (UT-15), a tricyclic benzindene analogue of prostacyclin (PGI_2) with potent systemic and pulmonary vasodilatory and platelet anti-aggregatory effects, is being developed for chronic administration as a continuous subcutaneous (sc) infusion in treating patients with pulmonary arterial hypertension (PAH). (PGI_2, an endothelial cell-derived substance, is a powerful vasodilator and a potent inhibitor of platelet aggregation.) The hemodynamic properties of UT-15 are shown to be similar to those of PGI_2, but unlike PGI_2, UT-15 is chemically stable.

It is proposed that continuous sc infusions of UT-15 be initiated at a rate ≤ 1.25 ng/kg/min, with upward and downward adjustments based on PAH symptoms and drug-related adverse effects. The product labeling recommends that increments not exceed 1.25 ng/kg/min per week for the first 4 weeks and 2.5 ng/kg/min per week for the remaining duration of infusion.

The nonclinical studies conducted with UT-15 are summarized below.

UT-15 produced a concentration-dependent inhibition of ADP-induced aggregation of rat and human platelets *in vitro* with IC_{50} values of 34.6 nM (13.5 ng/ml) and 28.2 nM (11 ng/ml), respectively. UT-15 was found to be 20-fold less potent than prostacyclin in inhibiting the ADP-induced aggregation of human platelets.

UT-15 (1-1000 nM) produced a concentration-dependent relaxation of isolated rabbit mesenteric artery segments precontracted with the thromboxane mimetic U-46619, the order of potency (when compared to other prostaglandins) being UT-15 > carbacyclin (a stable prostacyclin analogue) > 16-dimethyl PGE_2 > PGE_2. UT-15 was found to be 8 and 45 times more potent in inducing vascular relaxation than carbacyclin and PGE_2, respectively.

UT-15 (30 nM for 48 hours) markedly reduced the proliferation of cultured human pulmonary artery smooth muscle cells *in vitro* while inducing a large elevation (about 120 fold) in intracellular cAMP, suggesting that UT-15 exerts its antiproliferative effect via a cAMP-dependent pathway.

UT-15, administered to anesthetized rats at 100 μg/kg, sc, caused significant inhibition of ADP-induced platelet aggregation *ex vivo*, 20 and 40 minutes after dosing, while 25 μg/kg, sc, did not produce any significant inhibition of platelet aggregation. When administered orally, UT-15 produced significant inhibition of platelet aggregation at 5000 μg/kg, but not at lower doses.

In anesthetized rabbits, 10-minute iv infusions of UT-15 (50-500 ng/kg/min) also caused dose-related inhibition of ADP-induced platelet aggregation, with an ID_{50} of 140 ng/kg/min, as compared to 200 ng/kg/min for prostacyclin. These antiaggregatory doses of UT-15 and prostacyclin produced reductions in mean blood pressure of 10 and 16 mmHg,

respectively, indicating only minimal differences between these agents as inhibitors of platelet aggregation or vasodilators in this model.

In anesthetized rats, UT-15 produced significant dose-related reductions in mean arterial pressure (MAP) when administered by subcutaneous (29-60 mmHg at 25-100 μg/kg) or oral (35-55 mmHg at 1-5 mg/kg) routes. Intravenous infusion of UT at 0.4 μg/kg/min reduced MAP by 31 mmHg. UT-15 was found to be about 10-fold less potent than prostacyclin as a hypotensive agent in this animal model.

In anesthetized rabbits, 10-minute iv infusions of UT-15 at 0.05 to 0.5 μg/kg/min produced dose-related reductions in MAP (8-54 mmHg). There was very little difference in hypotensive potency between UT-15 and prostacyclin.

In anesthetized closed-chest cats, 20 min iv infusions of UT-15 (3 to 30 μg/kg/min) caused dose-dependent reductions in diastolic blood pressure (22-74 mmHg) accompanied by non-dose related tachycardia. The maximum hypotensive responses were evident within 5 minutes of infusion and the values returned to baseline level within 40 minutes of terminating the infusion.

In anesthetized open-chest cats, 20 min iv infusions of UT-15 (0.1 to 3.0 μg/kg/min) produced dose-dependent decreases in mean systemic arterial (6-42%) and mean pulmonary arterial (2-26%) blood pressures with little effect on heart rate or cardiac index. UT-15, at these dose levels, produced dose-dependent reductions in hypoxia-induced increments in pulmonary artery blood pressure and pulmonary vascular resistance. In this study, UT-15 was about 3 and 10 times less potent than prostacyclin as a vasodilator under hypoxic and normoxic conditions, respectively.

In anesthetized newborn piglets, UT-15 at 6 μg/kg (iv bolus) abolished hypoxia-induced increases in pulmonary vascular resistance.

In anesthetized dogs, iv bolus injections (0.32 to 3.2 μg/kg) or 10 min iv infusions (0.1 to 1.0 μg/kg/min) of the test drug produced significant dose-related reductions in MAP. After iv bolus injection, there was a reduction in MAP of 8 to 36 mmHg. Ten-minute iv infusions of UT-15 caused dose-related reductions in MAP (8-62 mmHg) with significant dose-related reductions in total peripheral resistance (0.2-1.4 units), and significant reduction in LVdP/dt (648 mmHg/sec) at 1.0 μg/kg/min. There were no significant heart rate, cardiac index or EKG findings.

Four hour iv infusions of UT-15 (0.1, 0.3, 1.0 and 3.0 μg/kg/min) in anesthetized dogs produced dose-dependent decreases in MAP (10-68%) and total peripheral resistance (20-73%). Although not dose-dependent, reductions in pulmonary artery pressure and pulmonary vascular resistance were noted at the above dose levels. The vascular effects were rapid in onset, achieving maximum effect within 5-10 min of infusion, with rapid recovery on termination of infusion. It was determined that the plasma concentrations of UT-15 producing 50% of the maximum effect (EC_{50}) on systemic (total peripheral resistance, TPR) and pulmonary vascular resistances (PVR) were 8.6 ng/ml and 11.3

ng/ml, respectively. The concentration-effect vs time plot indicated a close relationship between plasma drug concentration and the onset of hemodynamic effects. It is noted that although the plasma concentrations of UT-15 did not decrease over the 240-min duration of the infusion, decreases in TPR in dogs infused at 0.3 μg/kg/min and PVR in dogs infused at 0.1 and 0.3 μg/kg/min were not maintained. It is suggested that some tachyphylaxis may have occurred for TPR and PVR at these but not higher infusion rates.

Upon termination of infusion, although plasma drug concentrations dropped close to zero levels, the effects on TPR persisted for about 60 minutes at the 1 and 3 μg/kg/min infusion rates. This is attributed to a delay in the clearance of drug from the active site compared to its clearance from the plasma, and/or due to the presence of active metabolite at the active site.

When the concentration-effect data were fitted to the E_{max} pharmacodynamic model, it was suggested that there might be little or no selectivity of UT-15 for the pulmonary or peripheral circulation in the normotensive anesthetized dog.

In the above dog model, UT-15 produced dose-dependent decreases in left ventricular inotropic (+dP/dt) activity at 1 and 3 μg/kg/min doses, and dose-dependent decreases in left ventricular lusitropic (-dP/dt) activity at doses of 0.3 μg/kg/min and above. Cardiac output was increased at 0.3 μg/kg/min and above and heart rate was increased at 0.3 and 3.0 μg/kg/min (30-68%). UT-15 produced dose-dependent decreases in PR and QRS intervals with no effect on QT_c.

UT-15 infusions (0.1-3.0 μg/kg/min) in dogs produced dose-related increases in plasma angiotensin II concentrations (50-263 pg/ml) which correlated inversely with reduction of mean arterial pressure.

PGI_2, when given iv to anesthetized dogs for 4 hours at 0.01 to 0.3 μg/kg/min, produced vascular and cardiac effects similar to those produced by UT-15; however, PGI_2 was found to be 10 times more potent than UT-15 in these studies. PGI_2 infusions also caused dose-dependent increases in plasma angiotensin II levels.

Safety pharmacology studies showed no significant adverse effects of UT-15 on respiratory, gastrointestinal and autonomic nervous systems.

In summary, studies in several animal models have demonstrated the systemic and pulmonary vasodilatory effects of UT-15. Development of tolerance, as reported in patients, has not been clearly seen in the animal studies, although some tachyphylaxis was observed at lower but not higher infusion rates in anesthetized dogs. Since tolerance usually develops after repeated drug administration, the tachyphylaxis noted in the dog, which occurred after a single 4-hour iv infusion, is not considered related to tolerance development.

In pharmacokinetic studies conducted in male and female Sprague-Dawley rats given single iv doses of 200 μg [^{3}H] UT-15/kg, the plasma clearance of total [^{3}H] was found to

be more rapid in males (440 ml/hr/kg) than in females (276 ml/hr/kg). The distribution half-life was 0.8 hr (both sexes) and the elimination half-lives were 10 hr in males and 14 hr in females.

In anesthetized normotensive beagle dogs given 4 hr iv infusions of UT-15 at 0.1, 0.3, 1.0 or 3.0 μg/kg/min, plasma concentrations of UT-15 increased rapidly and reached steady-state levels within 10-15 min from the onset of infusion at all infusion rates. Analysis of the data indicated a biphasic decay of UT-15 in plasma with an initial half-life of about 2 min and a terminal half of about 20 min.

The tissue distribution of radioactivity was examined in male rats of Sprague-Dawley and Long Evans strains following administration of a single 6-hr subcutaneous infusion of 450 ng [^{14}C]UT-15/kg/min. The distribution of radioactivity was found to be rapid, with most tissues reaching maximum concentration within 2 hr from the end of infusion. The tissues with the highest C_{max} were liver, small intestine, nonpigmented skin, kidneys, pigmented skin and large intestine. The tissues with the lowest C_{max} values were the brain and fat. Blood and tissue concentrations declined with time, but 72 hr post-dose, radioactivity was still detectable in 19 of the 29 tissues examined.

In the above study, it was determined that following sc infusion of [^{14}C] labeled drug, the radioactivity was eliminated from blood and plasma with half-lives of 90.2 and 53.7 hr, respectively. The half-live values in tissues ranged from 1.66 hr (thyroid) to 478 hr (fat)

A single dose oral administration study in rats (200 μg [^{3}H] UT-15/kg) showed that there were no major differences in the distribution of radioactivity between males and females or between albino and pigmented rats.

A rat liver microsomal enzyme induction study showed that continuous sc administration of UT-15 (200 ng/kg/min for 7 days) to rats had no effect on the yield of hepatic microsomal protein, total cytochrome P450 content or isozyme activities of CYP1A, CYP2B and CYP3A (levels of other CYP isozymes not measured). Phenobarbital (positive control) caused significant increases in all the above parameters.

An *in Vitro* study using human liver microsomal preparations showed that UT-15, at concentrations ranging from 0.1 to 1000 ng/ml, did not significantly inhibit the activities of any of the six P450 isozymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A) studied.

The metabolic profile of UT-15 was examined in bile collected from rats given single oral or iv doses of 200 μg [^{3}H]UT-15/kg. Analysis of the bile sample showed little or no unchanged drug, suggesting extensive metabolism of UT-15 to more polar compounds. Incubation of the bile sample with beta-glucuronidase/aryl sulphatase showed presence of glucuronide and/or sulfate metabolites.

No metabolic studies were conducted in the dog.

The metabolic profile of UT-15 was investigated in human urine obtained from healthy male volunteers given sc infusion of [^{14}C]UT-15 at 15 ng/kg/min for 8 hours. Analysis of the urine samples showed that 3.7% of the administered dose was excreted as unchanged drug. There was no single major metabolite. Five urinary metabolites (HU1, HU2, HU3, HU4 and HU5) were found. HU5 was identified as UT-15-glucuronide. HU4, HU3 and HU2 were identified as the products of oxidation of the 3-hydroxyloctyl side chain. The structure of HU1 has not been elucidated.

The *in vitro* binding of [^{14}C]UT-15 to female human plasma protein was found to be 91% at UT-15 concentrations of both 0.33 and 10 µg/ml, suggesting that the plasma protein binding of UT-15 is concentration independent.

No protein binding studies were performed using rat or dog plasma. The sponsor was asked about the protein binding studies in animals at the pre-NDA meeting on November 15, 1999. According to the sponsor, they were unable to determine the protein binding of UT-15 [redacted] was unstable (i.e., self-degraded) even at [redacted] (minutes of the meeting).

The role of biliary excretion in the elimination of UT-15-related material was examined in groups of bile duct intact and bile duct cannulated male Sprague-Dawley rats given single sc infusions of [^{14}C]UT-15 at 450 ng/kg/min for 6 hours. About 88 and 99% of the administered dose was excreted within 24 hours following the termination of infusion in bile duct intact and bile duct cannulated rats, respectively. Feces (82%) was the major route of elimination in bile duct intact animals, with about 14% excreted in urine. In bile duct cannulated animals, bile (89.5%) was the major route of elimination, with about 10.5 and 1.05% excreted in urine and feces, respectively.

Excretion studies in dogs also showed that the fecal route was the major route of elimination of UT-15.

In human volunteers given single sc infusions of [^{14}C]UT-15 at 15 ng/kg/min, 79% of the administered dose was excreted in urine with about 13% excreted in feces. This is in contrast with the fecal (biliary) route being the main route of excretion in rats and dogs.

Due to the absence of metabolism studies in animals, we are unable to compare metabolic profiles in animals and man.

The results of acute and chronic (up to 6 months duration) general toxicity studies that were conducted using the subcutaneous route of administration (to support the intended clinical route of administration) in rats and dogs are summarized below.

In rats continuously infused with UT-15 for 26 weeks (50, 150 and 450 ng/kg/min), the primary treatment-related finding was reversible infusion site lesions, the incidence and severity of which were higher in the high dose group animals than in lower dosage group or control group animals. These lesions included erythema, edema, inflammation of the skin, and nodules/masses and/or thickening of the skin that correlated microscopically

with edema, hemorrhage, cellulitis, abscess and fibrosis. Other treatment-related findings included increased incidences of redness of extremities (nose, pinnae, paws and/or tail) in the high dose animals, reversible increases in white blood cell counts in high dose males and females, increased total bilirubin levels in high dose males and increased spleen and heart weights in high dose males and females. The no observed adverse effect level (NOAEL) in this study was 150 ng/kg/min. Steady state plasma concentrations (C_{ss}) were achieved in most animals by 3 hours on Day 1 and in all animals by Day 7, and these levels were sustained over the 26-week duration of the study. Male rats were found to have higher plasma UT-15 levels and lower plasma clearance values than female rats. For both sexes, the C_{ss} increased in a dose-related manner.

Increased spleen and heart weights were also observed at 450 ng/kg/min in a 13-week continuous sc infusion study in rats (0, 50, 150 and 450 ng/kg/min). Osmotic pump implantation site lesions (swelling, edema and inflammation) were also noted in this study.

A single 3 hour infusion of 490 (but not 400) ng UT-15/kg/min in the rat produced ataxia (doses this high were not administered in longer-term studies).

In the dog, continuous infusion of UT-15 for 26 weeks (50, 100 or 200 ng/kg/min) produced swelling, edema, fibrosis and hemorrhage at the infusion site (with incidence and severity being higher in drug-treated groups than in controls), dose-related reductions in body weights during the first 4 weeks of treatment, reversible increases in white blood cell counts at mid and high dose levels (both sexes) and increased spleen weights in high dose males and females. No drug-related clinical chemistry, EKG or histopathological findings (other than infusion site lesions) were noted. The no-observed adverse effect level was found to be 50 ng/kg/min. Steady state plasma drug concentrations were achieved in most animals by three hours on Day 1 and in all animals by Day 7, and these levels were sustained for the duration of the study. There were no gender differences, and the mean Css values increased proportionally with dose.

Infusions of UT-15 in dogs at 300 ng/kg/min in a 13-week study or at rates $\geq$ 500 ng/kg/min in a 2-week study produced mortality or moribund condition (ending in sacrifice). Gross necropsy examination of these animals revealed edema and/or erythema at the infusion site, intestinal intussusception and rectal prolapse. (Infusion site lesions were seen with treatment durations as short as 14 days.) Histologically, inflammation, hemorrhage and necrosis of ileum and rectum were noted. The steady-state AUC values were found to increase proportionally with dose.

All of the above toxicity studies appeared to be adequately performed and maximum tolerated doses were employed for the 26-week studies in both rats and dogs. The NOAELs for the 26-week studies in rats and dogs were about 20 and 22 times the recommended clinical starting dose ($\leq$ 1.25 ng/kg/min), respectively, on a ng/m^2 basis.

Carcinogenicity studies were not conducted with UT-15. According to the ICH Guidance for Industry on the "Need for Long-term Rodent Carcinogenicity Studies of

Pharmaceuticals" (ICH S1A, March 1996), long-term carcinogenicity studies are not required where life expectancy in the indicated population is short (i.e., less than 2 to 3 years). The average life expectancy in patients with PAH is less than 2-3 years from the time of diagnosis. Also, according to the sponsor, conducting valid carcinogenicity studies would not be technically possible due to issues with continuous subcutaneous delivery of UT-15 for the lifetime of rats and mice. In addition, there was no histopathological evidence of proliferative responses to UT-15 in any organ system in the 26-week toxicity studies and UT-15 had no mutagenic or clastogenic effects in the standard ICH genotoxicity test battery. Considering all of the above, the Division granted an exemption from conducting carcinogenicity studies with UT-15 (letter to the sponsor dated April 11, 2000).

A fertility and early embryonic developmental toxicity study in Sprague-Dawley rats (continuous sc infusion at 0, 50, 150 and 450 ng UT-15/kg/min) showed no significant dose-dependent treatment related effects on reproductive parameters. The reproductive and developmental NOAELs, under the conditions of the study, were found to be at least 450 ng/kg/min (which is, on a ng/m^2 basis, about 59 times the recommended starting human dose).

In a developmental toxicity study in Sprague-Dawley rats (continuous sc infusion at 0, 50, 150, 450 and 900 ng UT-15/kg/min), maternal toxicity (piloerection, rough coat, chromodacryorrhea, and reduction in maternal body weight gain and food consumption) was observed at 450 and 900 ng/kg/min. There were no statistically significant treatment-related effects on the incidence of fetal malformations or variations. The no effect level for developmental toxicity was at or above 900 ng/kg/min (about 117 times the starting human rate of infusion on a ng/m^2 basis and about 16 times the average infusion rate achieved in clinical trials). Mean plasma drug concentrations increased in a dose-related manner.

In pregnant rabbits given UT-15 by continuous subcutaneous infusion at doses of 0, 50, 150 and 300 ng/kg/min during organogenesis, maternal toxicity (reduction in maternal body weight gain and food consumption) was seen at all doses. There were no significant treatment-related effects on the incidence of fetal malformations. Dose related increases in the percent of fetuses with variations per litter (due to an increase in skeletal variations) were noted in all UT-15-treated groups (statistically significant at 300 ng/kg/min). The incidence of this variation was higher in male fetuses (statistically significant at 150 and 300 ng/kg/min) than in females. The 150 ng/kg/min infusion rate was, on a ng/m^2 basis, about 41 times the starting human rate of infusion and about 5 times the average rate achieved in clinical trials.

The increased incidences of skeletal variations observed in the above study were mainly due to increases in two rib skeletal variations: bilateral full rib or right rudimentary rib on lumbar 1. The highest historical control incidence rate (from 9 studies) for bilateral full rib on lumbar 1 was 41%. Although the incidence rates observed at the high (49%) and mid (50%) doses in the present study exceeded this historical control rate, no dose relationship was noted. For the right rudimentary rib, the incidence rate observed at the

high dose (7.5%) was lower than the highest historical control incidence rate (11%) from the nine previous studies.

Toxicokinetic evaluation of the above rabbit study showed that steady state concentrations were achieved and sustained during the dosing period, and that systemic exposures were increased proportionally with dose, indicating linearity within the dose range tested.

In a pre- and postnatal developmental toxicity study in rats given continuous sc infusion of UT-15 at 0, 50, 150 or 450 ng/kg/min, there were no statistically significant effects on F1 fertility, mating and pregnancy indices.

All reproductive toxicity studies appeared to be adequately performed.

UT-15 was evaluated for genotoxicity in bacterial [Salmonella (E.coli)/mammalian microsome assay] and mammalian (mouse lymphoma assay) *in vitro* test systems, and also in an *in vivo* (rat erythrocyte micronucleus test) test system. UT-15 was not found to be mutagenic or clastogenic in the above studies. All studies appeared to be adequately performed and positive controls in all tests showed significantly increased incidences of mutation or chromosomal damage.

In conclusion, there are no approvability issues for UT-15 based on the non-clinical toxicity-testing program.

APPEARS THIS WAY
ON ORIGINAL

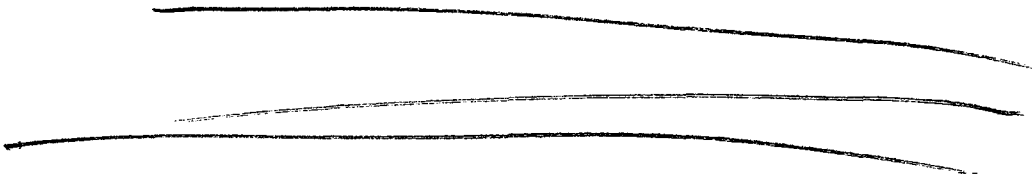
RECOMMENDATION

The NDA is approvable with the following changes to the sponsor's proposed package insert, as revised on February 21, 2001.

The results of the developmental toxicity studies in rats and rabbits, presently described partly under the section **Precautions,** subsection **Carcinogenesis, mutagenesis, impairment of fertility** and partly under the subsection **Pregnancy** should be compiled and presented under the subsection **Pregnancy**. The doses used in animal studies are to be compared, on a body surface area basis, to the human dose. The other changes are considered minor and/or editorial in nature.

Sponsor's proposed text and reviewer's proposed revised text are given below.

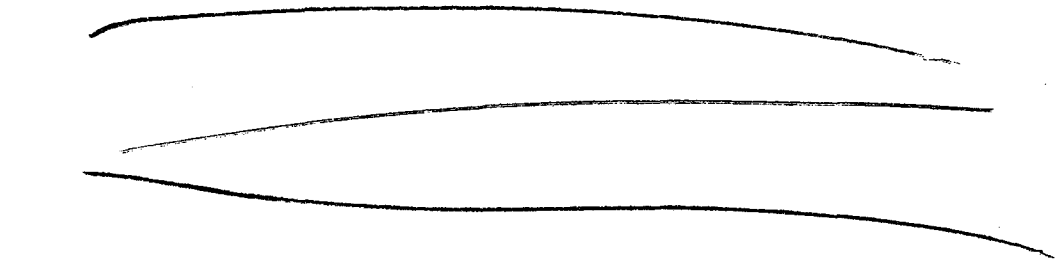
Under the section **Precautions,** the subsection **Carcinogenesis, mutagenesis, impairment of fertility,** the last 3 sentences presently read as follows:



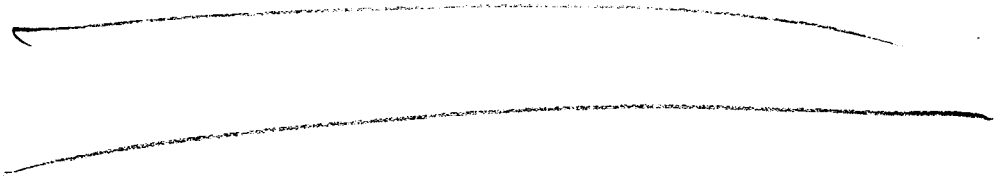
We recommend that the above text be revised to read as follows:

"Treprostinol sodium did not affect fertility or mating performance of male or female rats given continuous subcutaneous infusion at rates of up to 450 ng treprostinol/kg/min [about 59 times the recommended starting human rate of infusion (1.25 ng/kg/min) and about 8 times the average rate (9.3 ng/kg/min) achieved in clinical trials, on a ng/m^2 basis.]"

Under the section **Precautions**, the subsection **Pregnancy** presently reads as follows:



We recommend that the above section be revised to read as follows:



Xavier Joseph, D.V.M.
March 12, 2001

cc.
Orig.NDA
HFD-110
HFD-110/PM

accepted by _____ on _____

APPEARS THIS WAY
ON ORIGINAL