[Note: A 90-day continuous subcutaneous infusion study of UT-15 (lot numbers LRX-97J01 and LRX-98A01) in rats (conducted at
Study No. 585D-103-434-97), using osmotic pumps, at 0 (vehicle control), 0 (saline control), 50, 150 and 450 ng/kg/min, revealed no significant toxicological findings except for implantation site lesions (swelling, edema and inflammation) and significantly increased absolute and relative spleen weights in all treated female groups (dose dependent) and in the high dose male group. Increased absolute and relative liver (high dose females) and heart (high dose males) weights were also observed.

A 28-day continuous subcutaneous infusion study in rats (conducted at
study number 1458), at targeted doses up to 450 ng/kg/min, compared the toxicological effects of a batch of UT-15 (lot number UT15MIX-99G001) that had a different impurity profile to the lot that was previously tested (lot number UT15RP-98I001) in the 26-week rat toxicity study. No significant differences in the toxicological profiles of these lots were noted.]

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Twenty-six Week Subcutaneous Continuous Infusion Toxicity Study in Dogs Followed by a 4-Week Recovery Period

Testing Facility: [redacted]

Study Number: 2129-98 [redacted] Lab.'s No.)

Study Dates: Initiation of Treatment – November 2, 1998
Terminal Sacrifice – May 3 to June 4, 1999

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

Animals: Beagle dogs [redacted] 4/sex/ group, housed individually in stainless steel cages and fed once daily with 400 g of [redacted] 25% Lab Dog Diet #8727C, were about 8 months old (males weighed 7.1 to 10.6 kg and females 7.0 to 10.5 kg) at the onset of treatment. An additional three dogs/sex/group were assigned to the vehicle control and high dose groups for the recovery phase of the study.

Dose Levels and Mode of Administration: The target dose levels and the concentrations of dosing solutions are given below.

Treatment Groups	Target Dose Levels[1]		*Concentration*
	ng/kg/min	mg/kg/day	mg/ml
1. Saline control[2]	0	0	0
2. Vehicle control[3]	0	0	0
3. Low dose	50	0.072	0.06
4. Mid dose	100	0.144	0.12
5. High dose	200	0.288	0.24

[1.] The test and control articles were administered at a constant rate of 0.05 ml/kg/hour to achieve target dose levels. [2.] Saline control animals received 0.9% sodium chloride for injection USP. [3.] Vehicle control animals received the vehicle containing sodium citrate, citric acid, sodium chloride and meta-cresol dissolved in water for injection USP (pH 6.8 – 7.4).

UT-15 (Lot No. UT15-98H01) solutions were prepared weekly by dilution of appropriate volumes of UT-15 stock solution (10 mg/ml) with the vehicle to achieve the desired concentrations. The dosing solutions were filtered through a [redacted] filter to assure sterility.

Analyses of test article concentrations at three different time points during the study showed that the concentrations of the dosing solutions were about [redacted] (values corrected for volatiles and other impurities) of the nominal values.

It is stated that "the dose levels were selected by the sponsor together with the study director based on available data."

The test and control articles were infused subcutaneously, 24 hours/day, at a rate of 0.05 ml/kg/hour. The infusion site on the dorsal side was surgically prepared, and the catheter was inserted under local anesthesia and secured in place. The catheter was connected to medical grade tubing that was passed through a jacket and tether system to the outside of the cage door. The infusion line was then connected to the reservoir containing the test article solution. The infusion rate was controlled using an infusion pump.

The infusion site was changed approximately every 7 days (to another dorsal site). A topical antiseptic (4% chlorhexidine gluconate) was applied to the surgical sites throughout the treatment period. During the treatment period, animals (both control and treated) exhibiting skin lesions (wound, dermatitis, ulceration, edema and/or erythema) were treated with topical applications of 10% iodine and/or 0.05 or 4% chlorhexidine gluconate.

Observations/Measurements: Animals were observed daily for clinical signs and mortality. A detailed clinical examination was performed one week prior to initiation of dosing and once weekly thereafter, with particular attention paid to surgical sites. Body weights were recorded for all animals one week prior to initiation of treatment, weekly during treatment and recovery periods and at study termination. Food consumption was recorded daily. Indirect ophthalmoscopy and slit lamp examinations were performed for all animals during the pretreatment period and also during weeks 13 and 26. Electrocardiograms (limb leads I, II and III; and augmented leads aVR, aVL and aVF) were recorded for all animals during the pretreatment period and also during treatment weeks 13 and 26. Hematology [RBC, WBC (total and differential), platelet and reticulocyte counts, hemoglobin, hematocrit, MCV, MCH, MCHC, prothrombin time, activated partial thromboplastin time, and blood cell morphology] and serum chemistry (urea nitrogen, creatinine, glucose, cholesterol, triglycerides, total protein, albumin, globulin, ALT, AST, alkaline phosphatase, bilirubin, calcium, sodium, potassium, phosphorus and chloride) evaluations, and urinalysis were performed on all animals pretest and during treatment weeks 13 and 26, and on all recovery animals at the end of the recovery period .

Blood samples were collected from all dogs pretest and on days 1, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168 and 182 for toxicokinetic evaluations. Blood samples were collected 3 hours post start of infusion on day 1, and at the same hour on each of the remaining occasions. (Only blood samples from the first three dogs/sex/group were analyzed; samples collected from the remaining animals were discarded.)

All main phase animals were euthanized after 26 weeks of treatment, and all recovery phase animals were kept for an additional 4 weeks (without dosing) before sacrifice.

Complete necropsies were performed on all animals. Adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, spleen, testes, thymus, thyroids (with para-

thyroids) and uterus were weighed. Representative sections of adrenals, aorta (thoracic), brain, cecum, cervix, colon, epididymides, esophagus, eyes, femoral bone, gallbladder, heart, last infusion site, kidneys, lacrimal glands, liver, lungs, lymph nodes (mesenteric and mandibular), mammary gland, optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, skeletal muscle, skin and subcutis, small intestine (duodenum, ileum and jejunum), spinal cord (cervical), spleen, sternum and marrow, stomach, testes, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus and vagina, and all abnormal tissues were fixed in neutral buffered 10% formalin (except epididymides, eyes, optic nerves and testes which were fixed in Zenker's fluid).

Three femoral bone marrow smears were prepared from each animal, but were not evaluated.

Tissues were processed and stained with hematoxylin and eosin/phloxine for microscopic examination. Histopathological examination was performed on all above listed tissues from all animals.

Data were analyzed for homogeneity of variance using Levene Median and for normality using Kolmorogov-Smirnov tests. Homogeneous data were analyzed using the analysis of variance and the significance of intergroup differences was tested using Dunnett's t test. Heterogeneous data were analyzed using Kruskal-Wallis test, and the significance was tested by Dunn's test.

Results: There were no deaths in this study.

The most frequently observed clinical signs in animals of all dose groups, including controls, included the presence of lumps (firm), swellings (soft) and/or swellings around the lumps at or around the infusion site. The incidence and severity of these clinical signs were higher in drug-treated groups (especially in the high dose group) than in saline or vehicle controls. Generally, the lumps/swellings appeared 1 to 3 days following the start of infusion or the change to a new infusion site and they gradually disappeared 2 to 6 days later.

Dose-dependent erythema around the infusion site was observed in females of all dose groups, including a few saline control animals. In males, erythema was frequently seen in mid and high dose animals.

Animals from all drug-treated groups exhibited pain (sensitivity to touch) when palpated at the infusion site, the incidence being higher in the high dose group than in other groups.

Other clinical signs, including decreased activity, prostration, loose feces and wounds at the infusion sites, were observed sporadically in a few animals of one or more dose groups.

No treatment-related clinical signs were seen at the end of the recovery period.

Dose-dependent decreases in mean body weights (2 to 10%), compared to body weights on Day 1, were noted during the first 1 to 4 weeks of treatment in all dose groups (both sexes). Reductions in body weights (1-3%) were also noted for saline and vehicle controls during this period. No further significant decreases in body weights were noted in any dose groups by about Day 29, and the animals started to gain weight thereafter. By the end of the treatment period (Day 183), the mean body weights of treated and control groups were 2-7% and 5-10% higher, respectively, than corresponding values on day 1.

At the end of the recovery period, the body weight gains were comparable in the high dose and vehicle control groups.

During the first week of treatment, the food consumption of the high dose group (both sexes) was significantly lower than control. The food consumption improved considerably during the second and third weeks of treatment, and by the fourth or fifth week, the food consumption of the high dose group was comparable to that of controls. There were no significant treatment-related effects on food consumption at other dose levels.

During the recovery period, food intake was comparable in treated and control groups.

There were no treatment-related ocular findings in the study.

The test-drug had no effects on electrocardiographic parameters including the morphology of the P, QRS and T complexes, PR and QT intervals, ST segment, mean electrical axis, and heart rate.

Reversible, non dose-related increases in mean white cell counts (9 – 113%; mainly due to an increase in neutrophils), compared to controls, were noted in the mid and high dose groups of both sexes during treatment weeks 13 and 26.

No significant treatment-related findings were seen in the clinical chemistry and urinalysis parameters.

Although not statistically significant, increases in the absolute (14 – 35%) and relative (31%) mean spleen weight, compared to vehicle control, were seen in both sexes of the high dose group. After the recovery period, the mean spleen weight was still higher in the high dose male group than in control.

Macroscopically, thickening of the infusion site and masses around the infusion site were seen in some of the control dogs and in the majority of the drug-treated dogs.

No treatment-related macroscopic findings were noted after 4 weeks of recovery.

Treatment-related histopathological findings observed at the infusion site included cellulitis, edema, fibrosis and hemorrhage. Although the incidence of cellulitis and edema in the treated groups was comparable to that in saline or vehicle controls, the incidence and/or severity of hemorrhage and/or fibrosis was higher in drug-treated groups (not dose-dependent) than in controls.

There were no other significant or otherwise remarkable histopathological findings.

The mean plasma drug concentration (pooled data from males and females) versus study day for each of the three targeted doses is shown in Figure 11. Individual animal data showed that steady-state plasma concentrations were achieved in most animals by three hours on Day 1, and in all animals by Day 7. Moderate to wide variations were observed in steady-state plasma UT-15 concentrations over the 182-day monitoring period. The overall mean plasma steady-state concentrations and the clearance rates are presented below .

The mean Css values increased proportionally with dose. Linear regression analysis of the individual animal Css versus dose data yielded a straight line with a coefficient of determination (r^2) of 0.69. The coefficient increased to 0.93 when one high dose animal (showing aberrant pharmacokinetic pattern with non-detectable drug levels at many time points) was excluded, indicating dose proportional kinetics over the 50 to 200 ng/kg/min dose range.

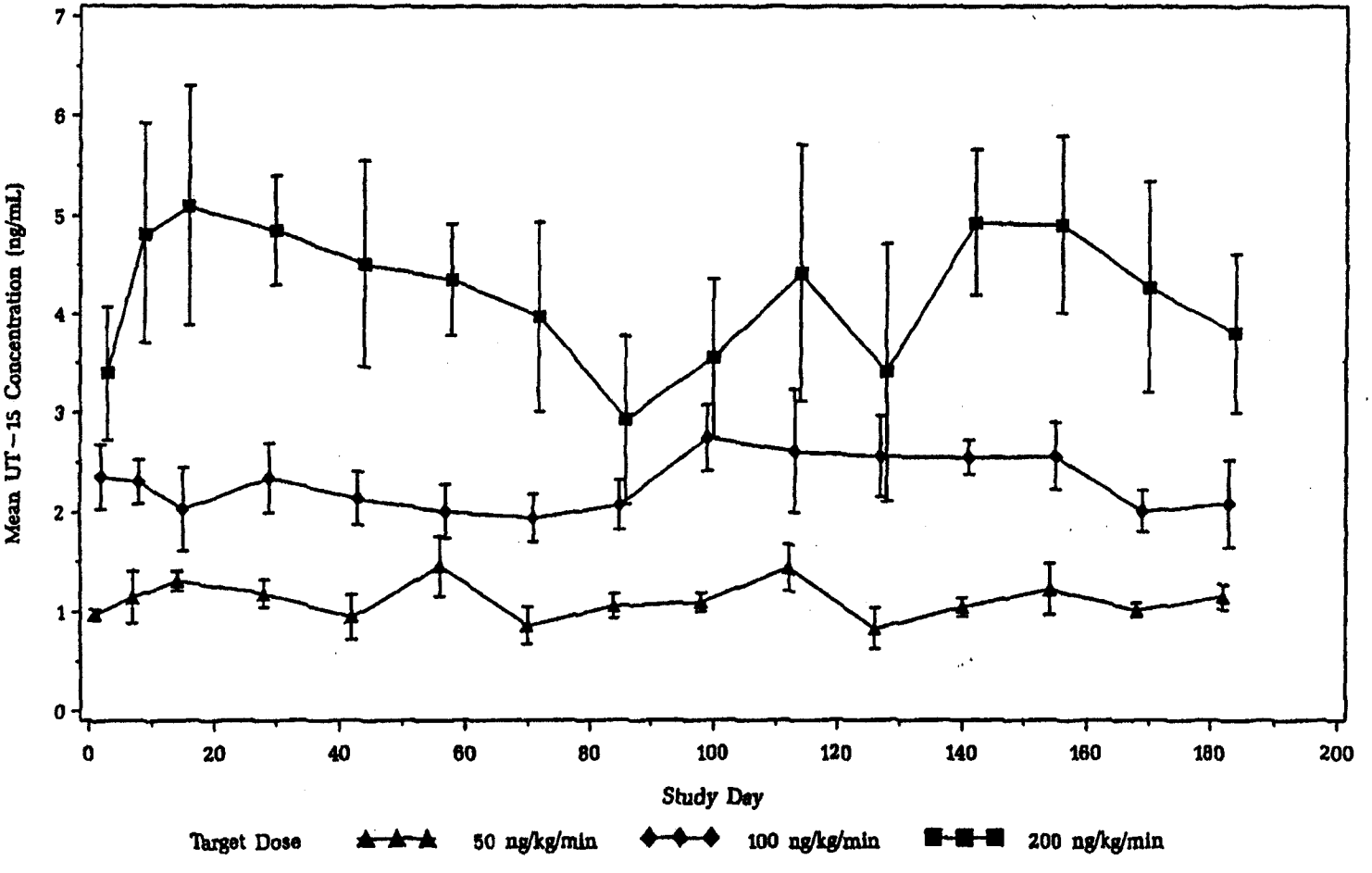
STEADY-STATE CONCENTRATIONS AND CLEARANCE *RATES IN DOGS ADMINISTERED* UT-15 IN A 26-WEEK TOXICOKINETIC STUDY

Parameter	Dose (ng/kg/min)	Female Dogs N = 3	Male Dogs N = 3	Mean
C_{ss} (ng/mL)	50	1.2	1.0	1.1
	100	2.2	2.4	2.3
	200	5.3	3.3*	4.3*
CL/F (mL/kg/min)	50	41.4	49.5	45.4
	100	47.1	47.2	47.1
	200	53.1	69.7*	61.4*

* One male beagle dog at the 200-ng/kg/min dose showed non-detectable UT-15 levels at many time points over the 180-day study and therefore lowered the mean C_{ss} values while at the same time raised the mean CL/F values.

Figure 11.

Plots of Mean UT−15 Concentration (±SEM) versus Study Day by Target Dose



The steady-state concentrations achieved after continuous subcutaneous infusion of UT-15 for 26 weeks in rats and dogs or 12 weeks in human volunteers are compared below. The highest infusion rates achieved in rats and dogs (450 and 200 ng/kg/min, respectively) represent UT-15 doses that produced minimal to moderate toxicological effects, whereas in humans, the highest infusion rate (15 ng/kg/min) was at the upper end of the tolerated dose range.

COMPARISON OF STEADY-STATE CONCENTRATIONS FOR UT-15 IN RAT, DOG, AND MAN ADMINISTERED UT-15 BY SUBCUTANEOUS INFUSION

Dose (ng/kg/min)	Rat C_{ss} (ng/mL)	Dose (ng/kg/min)	Dog C_{ss} (ng/mL)	Dose (ng/kg/min)	Man C_{ss} (ng/mL)
50	3.0	50	1.1	2.5	0.26
150	8.3	100	2.3	5.0	0.51
450	19.7	200	4.3	10.0	0.99
-	-	-	-	15.0	1.56

Human data are obtained from Protocol P01:09 (Reference 8)

[Note: In a 13-week continuous subcutaneous infusion study of UT-15 (lot number LRX-98A01) in dogs (conducted at [redacted] study number 2131-98) at target doses of 50, 150 and 300 ng/kg/min, a high dose female dog was sacrificed on day 13 due to moribund condition with a rectal prolapse. Pathology findings in this dog included multiple intestinal intussusceptions, intestinal hemorrhage, and a severe wound and ulceration at the infusion site. Because of these adverse findings, the high dose was lowered to 200 ng/kg/min on day 15, and to allow a wider spread between mid and high dose levels, the mid dose was lowered to 100 ng/kg/day. Other treatment-related findings included edema and/or erythema at the infusion site, dose-related reduction in body weights during the first 3 weeks of treatment, and dose-related increases in WBC counts in treated female groups during treatment weeks 6 and 13 (except in low dose females during week 6). There were no treatment-related findings at the end of a 2-week recovery period.]

——— osmotic pump was inserted into the subcutaneous pocket. The incision was closed with wound clips.

Males were treated for 10 weeks prior to mating and continued through the 2 weeks mating period. Females were dosed for 2 weeks prior to mating and continued through the mating period till gestational day (gd) 6. (The confirmed day of copulation, evidenced by the presence of vaginal sperm or vaginal copulation plug, was designated as gd 0.)

[According to the manufacturer's specifications, the osmotic pump used in this study delivers 2.5 µl/hour (nominal flow rate) for a nominal duration of 28 days. For F0 males, the pumps were implanted four times during the study (at about 3-week intervals, in weeks 0, 3, 6 and 9, for a total 12 weeks of exposure). Females that were found sperm or plug positive during the first week of mating did not receive a second pump for the remainder of the exposure period. For majority of the F0 females (that successfully mated during the first week of mating), the original osmotic pump implanted at the start of the study remained in place till its removal on gd 6. A few F0 females, that failed to mate within the first week of mating, received a second osmotic pump.]

It is stated that "the highest dose level (450 ng/kg/min) administered was selected to induce maternal toxicity or low levels of lethality (≤ 10%), and was based on the results from a previous Segment II study performed" at ——— The lower doses (50 and 150 ng/kg/min) were chosen as fractions of the high dose.

Observations and Measurements: Animals were checked daily for changes in general condition and behavior. Observations for mortality were made twice daily. Body weights of F0 males were recorded prior to the beginning of treatment and weekly thereafter. F0 females were weighed weekly until confirmation of mating, and during gestation on gd 0, 3, 6, 9, 12 and 15. Food consumption was determined weekly for all animals during the pre-mating period. During pregnancy, food consumption was recorded for gd 0-3, 3-6, 6-9, 9-12, and 12-15. (Food consumption was not determined during the mating period.)

For the females, during the two-week premating exposure period, estrous cyclicity was assessed by daily vaginal lavage. The slides of the 14-day vaginal lavage were fixed, stained with toluidine blue, and read for the stages of the estrous cycle.

After mating, all F0 males were sacrificed by CO_2 asphyxiation, and subjected to a complete gross necropsy. Testes and epididymides were weighed. Seminal fluid from the right cauda epididymis was evaluated for sperm number, motility and morphology. Sperm motility was evaluated immediately after necropsy; sperm number was determined using fixed, stained sperm specimen. The motility and number of sperm were evaluated using an ——— Automated Sperm Analysis System, while sperm morphology was evaluated manually. The testes were fixed in Bouin's solution and changed into 70% ethanol after about 24 hours. The left epididymis, and any gross lesions were fixed in neutral buffered 10% formalin for possible histopathologic examination. (Histopathologic examination was not performed.)

On gd 15, all surviving F0 females were sacrificed, a complete necropsy was performed, and the pregnancy status was confirmed by uterine examination. Numbers of corpora lutea, implantation sites, resorptions, and dead and live conceptuses were recorded. Ovaries and uterus were weighed, and these organs and any gross lesions were fixed in buffered neutral 10% formalin. For confirmation of pregnancy status, fixed uteri, that showed no visible implantation sites, were stained with potassium ferricyanide to visualize any implantation sites which may have undergone very early resorption. (Histopathologic examination of fixed tissues was not performed.)

The indices for reproduction (mating, fertility and pregnancy indices) and gestational parameters (gestational index and pre-and post-implantation losses) 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) were employed. If Bartlett's test indicated homogeneous variances, then parametric statistical tests [General Linear Models procedures for the Analysis of Variance (ANOVA)] were used. All indices were analyzed by Chi-Square test and by the Cochran-Armitage test for linear trend on proportions.

Results: There was no F0 parental mortality or morbidity.

Males

Treatment-related clinical signs for males were limited to swelling at the pump site and flushed appearance of ears, paws, and/or tail. Swelling at the pump site was noted in both control and drug-treated animals, with higher incidences seen in treated groups (no dose relationship). Flushed ears, paws, and/or tail were seen mostly in high dose animals, with only few animals affected in the mid dose group and none in the low dose group.

Body weights of high dose male animals were significantly lower (5 to 6%) than concurrent control weights during the first 3 weeks of the study. No significant differences in body weights were noted between treated (any group) and concurrent control animals thereafter. During the first week of treatment, the food consumption for the mid and high dose groups was significantly lower than control. There were no significant treatment-related effects on food consumption thereafter.

The mating, fertility and pregnancy indices were generally similar across control and treatment groups.

There were no treatment-related effects on absolute and relative testes and epididymides weights and sperm evaluation parameters (epididymal sperm concentration, percent motile sperm, percent progressively motile sperm, and percent abnormal sperm).

Females

Consistent with the findings in males, treatment-related clinical signs in females were limited to swelling at the pump site and flushed ears and paws. Swelling at the pump site was observed both in control and treated rats, with higher incidences seen in treated groups (no dose relationship). Flushed ears and paws were seen mostly at the high dose.

Estrous cyclicity during the 14-day premating period showed no differences across control and treatment groups.

There were no treatment-related effects on body weights during the premating period and throughout the gestation period. During the premating period, the food consumption in treated groups was lower than control (no dose relationship). There were no treatment-related effects on food consumption during the gestation period.

The F0 female terminal body weights, gravid uterine and ovary weights, female reproductive indices, numbers of corpora lutea and implantation sites and litter incidences of resorptions, live and nonlive (resorbed plus dead) implants, and pre- and post-implantation losses are presented in Table 16.

UT-15 treatment had no effect on mating, fertility and gestational indices. There were no significant differences in maternal body weights and organ weights between control and treated groups. There were no treatment-related effects on the numbers of corpora lutea or total implantation sites per dam, resorptions per litter, litters with resorptions or nonlive implants, live and nonlive implants per litter, and pre- and post-implantation losses per litter.

Under the conditions of this study, the reproductive and developmental NOAELs are at or above 450 ng/kg/min.

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Table 16.

Summary and Statistical Analysis of the Female Reproductive Indexes, Necropsy Weights and Uterine Contents (page 1 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
No. of Males Paired	25	25	25	25
No. of Females Paired	25	25	25	25
No. of Females that Mated	25	24	24	23
Mating Index (%) (no. females that mated/no. females paired)	100.0	96.0	96.0	92.0
No. of Pregnant Females	20	23	20	22
Fertility Index (%) (no. pregnant females/no. females that mated)	80.0	95.8	83.3	95.7
No. of Females with Live Litters	19	23	20	22
Gestational Index (%) (no. females with live litters/no. pregnant females)	95.0	100.0	100.0	100.0
Pregnancy Index (%) (no. pregnant females/no. males that mated)	80.0	95.8	83.3	95.7
Days Until Sperm Positive (days)[a,b]	2.0 ± 0.2 N=21	2.1 ± 0.5 N=20	2.4 ± 0.5 N=23	2.5 ± 0.5 N=22
Maternal Body Weight at Sacrifice (g)[b,c]	347.93 ± 6.25 N=15[d]	332.46 ± 6.41 N=19	333.47 ± 5.13 N=19	343.46 ± 5.75 N=21
Gravid Uterine Weight (g)[b,c]	19.270 ±1.480 N=16	18.644 ±1.087 N=19	17.356 ±1.111 N=19	18.815 ±1.088 N=21
Paired Ovary Weight (g)[b,c]	0.2444 ± 0.0129 N=16	0.2087 ± 0.0082 N=19	0.2273 ± 0.0114 N=19	0.2115 ± 0.0088 N=21

(continued)

Table 16. (continued)

Summary and Statistical Analysis of the Female Reproductive Indexes, Necropsy Weights and Uterine Contents (page 2 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
No. Corpora Lutea per Dam[b]				
	16.30 ± 0.57 N=20	15.30 ± 0.49 N=23	15.25 ± 0.56 N=20	15.41 ± 0.42 N=22
No. Implantation Sites per Litter[b]				
	14.95 ± 0.92 N=20	13.43 ± 0.84 N=23	13.15 ± 0.86 N=20	14.55 ± 0.91 N=22
Percent Preimplantation Loss per Litter[b]				
	11.54 ± 4.24 N=20	14.37 ± 4.61 N=23	15.31 ± 4.21 N=20	10.51 ± 4.55 N=22
No. Resorptions per Litter[b]				
	1.50 ± 0.51 N=20	0.83 ± 0.29 N=23	1.05 ± 0.28 N=20	0.77 ± 0.17 N=22
Percent Resorptions per Litter[b]				
	12.51 ± 4.93 N=20	6.23 ± 2.33 N=23	7.36 ± 1.95 N=20	5.67 ± 1.41 N=22
No. Litters with Resorptions				
	14	8	11	12
% Litters with Resorptions				
	70.00	34.78	55.00	54.55
No. Late Fetal Deaths per Litter[b]				
	0.00 ± 0.00 N=20	0.00 ± 0.00 N=23	0.00 ± 0.00 N=20	0.00 ± 0.00 N=22
Percent Late Fetal Deaths per Litter[b]				
#	0.00 ± 0.00 N=20	0.00 ± 0.00 N=23	0.00 ± 0.00 N=20	0.00 ± 0.00 N=22
No. Litters with Late Fetal Deaths				
	0	0	0	0
% Litters with Late Fetal Deaths				
	0.00	0.00	0.00	0.00

(continued)

Table 16. (continued)

Summary and Statistical Analysis of the Female Reproductive Indexes, Necropsy Weights and Uterine Contents (page 3 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	450
No. Nonlive Implants per Litter[b,e]				
	1.50 ± 0.51 N=20	0.83 ± 0.29 N=23	1.05 ± 0.28 N=20	0.77 ± 0.17 N=22
Percent Nonlive Implants per Litter[b,e]				
	12.51 ± 4.93 N=20	6.23 ± 2.33 N=23	7.36 ± 1.95 N=20	5.67 ± 1.41 N=22
No. Litters with Nonlive Implants[e]				
	14	8	11	12
% *Litters* with Nonlive Implants[e]				
	70.00	34.78	55.00	54.55
No. Live Implants per Litter[b]				
	13.45 ± 1.16 N=20	12.61 ± 0.88 N=23	12.10 ± 0.80 N=20	13.77 ± 0.88 N=22
Percent Live Implants per Litter[b]				
	87.49 ± 4.93 N=20	93.77 ± 2.33 N=23	92.64 ± 1.95 N=20	94.33 ± 1.41 N=22
Percent Postimplantation Loss per Litter[b]				
	12.51 ± 4.93 N=20	6.23 ± 2.33 N=23	7.36 ± 1.95 N=20	5.67 ± 1.41 N=22

[a]Days until sperm positive could only be calculated for those females for which sperm were detected in the vaginal smear.
[b]Includes all pregnant dams, reported as the mean ± S.E.M.
[c]Does not include the pregnant dams for which sperm were never detected since the actual gestational day 0 was not known.
[d]Decrease in *N* is due to one sacrifice weight inadvertently not being recorded.
[e]Nonlive=resorptions plus dead.
[#]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.

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

Testing Facility [redacted]

Study Number: 65C-7019-200 ([redacted] No.)

Study Dates: Initiation Date – January 20, 1998
Completion Date – April 8, 1998

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

Animals: One hundred seventy female Sprague-Dawley rats, 56 days old (201-225 g body weight), were obtained from [redacted]. After a week of quarantine, females were mated with male Sprague-Dawley rats that were obtained earlier from the same supplier and kept for breeding purposes. One hundred twenty-five sperm-positive females [about nine weeks of age and 216-275 g body weight on gestational day (gd) 0], were assigned to five groups of 25 rats each. (The confirmed day of copulation, evidenced by the presence of vaginal sperm or vaginal plug, was designated as gd 0.)

Non-mated females were group housed (maximum 3 per cage), and mated females were individually housed in solid bottom polycarbonate cages with stainless steel wire lids and [redacted] cage bedding. Males were housed individually. Food ([redacted] certified rodent chow – No. 5002) and tap water were available *ad libitum*.

Dose Levels, Mode of Administration and Treatment Regimen: The target doses were 0, 50, 150, 450 and 900 ng/kg/min.

Stock solutions (10 mg/ml) of UT-15 (Lot No.LRX-98A01), formulated in vehicle (containing sodium citrate, citric acid, sodium chloride dissolved in sterile water for injection) and adjusted to pH 7.4, were diluted (with vehicle) to achieve the desired final concentrations.

Analyses of the test solutions indicated that dosing formulations were 92-96% of target concentrations and the stock solution was 95% of target. It was shown that dosing formulations were stable at 25 and 40° C for about four weeks.

The test and vehicle solutions were administered by continuous subcutaneous infusion using subcutaneously implanted [redacted] osmotic pumps [redacted] at a nominal flow rate of [redacted] to achieve the target dose levels.

On the morning of gd 5 (between 0700 and 1100 hours), each study female rat was anesthetized by isoflurane inhalation. The dorsal subscapular area was surgically

prepared and an incision about 1.5 cm in diameter was made. The osmotic pump, preloaded with appropriate dosing solution, was inserted into the subcutaneous pocket and the incision was closed with wound clips.

According to the manufacturer's specifications, the osmotic pump model used in the study requires [redacted] to reach the steady state infusion rate once it is implanted. Therefore, the pump that was implanted on the morning of gd 5 would be at steady state by the morning of gd 6. The pump was removed at scheduled necropsy on gd 20 at about the same time that the pump was inserted on gd 5 (for a duration of 14 days of exposure at steady state).

[It is stated that the doses were selected based on the results of a dose range-finding study (continuous sc infusion of UT-15 at 0, 50, 150 and 450 ng/kg/min on days 6 through 20 of gestation) in rats. In that study, there was only minimal maternal toxicity and no evidence of developmental toxicity at the high dose. Therefore, 900 ng/kg/min was selected as the top dose for the definitive study to provide maternal and/or developmental toxicity. The remaining doses were the same as that used in the range-finding study.]

Observations and Measurements: Animals were checked at least once daily for clinical signs during gestational days 0-4 (prior to pump implant), and twice daily during gd 5-20 (pump implant period). Body weights were recorded on gd 0, 5, 6, 9, 12, 15, 18 and 20. Food consumption was determined for gd 0-5, 5-6, 6-9, 9-12, 12-15, 15-18 and 18-20.

Blood samples from five dams per dose group were collected from the lateral tail vein on gd 15 at time 0 (at about the same time the osmotic pump was inserted on gd 5), and 5 different dams per group were bled at 4 hours after time 0, for maternal toxicokinetic evaluations.

On gestational day 20, all surviving females were killed by CO_2 asphyxiation at the approximate time (within 2 to 3 hours) the osmotic pump was implanted on gd 5. The wound clips were removed, the incision opened and the pump was removed, examined grossly and discarded. Animals were weighed and a complete necropsy was performed. Liver and uterus were weighed. Pregnancy status was confirmed by uterine examination. Uteri which showed no visible implantation sites were stained with ammonium sulfide (10%) in order to visualize any implantation sites which may have undergone very early resorption. Numbers of corpora lutea, implantation sites, early and late resorptions, and dead and live fetuses were recorded. Live fetuses were dissected out, counted, weighed, sexed and examined for external morphological abnormalities, including cleft palate. Approximately one-half of the live fetuses in each litter were examined for visceral malformations, and their sex confirmed. These fetuses were decapitated, the heads fixed in Bouin's solution and serial free-hand sections of the head were examined for soft tissue craniofacial malformations and variations. Although all fetuses in each litter were evicerated, fixed in ethanol, cleaned of tissues with KOH and stained with alizarin red S/alcian blue, only intact fetuses in each litter (not decapitated) were examined for skeletal malformations and variations. All fetal carcasses were stored in glycerin: 70%

ethanol (1:1; including those examined and not examined for skeletal malformations). Fetal head sections were stored in 70% ethanol after examination.

Developmental toxicity parameters were calculated for each litter (dam) and then the mean was calculated using the litter (dam) values.

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. Nominal scale measures 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 two-tailed Fisher's Exact Probability test was used for pairwise comparisons. A test for statistical outliers was performed on maternal body weights and feed consumption.

Results

Maternal Toxicity

Treatment-related maternal clinical observations, which were limited to dams at 450 and 900 ng/kg/min, included flushed ears and/or feet, piloerection, rough coat and chromodacryorrhea. Other clinical observations, which were related to surgical procedures (swelling around implant and sores/scabs at or near incision/implant site), were seen in all groups including controls.

No dams died, aborted, delivered early or were removed from the study.

A total of 4 sperm-positive females out of 125 were found to be not pregnant at scheduled sacrifice, two at 0 ng/kg/min and one each at 150 and 900 ng/kg/min.

[All pregnant dams had one or more live fetuses at scheduled sacrifice. The number of litters (fetuses) evaluated were 23 (338), 25 (347), 24 (337), 25 (339) and 24 (318) at 0, 50, 150, 450 and 900 ng/kg/min, respectively.]

Mean maternal body weights were mostly similar across control and treatment groups on gd 0 and 5 (prior to implantation of pumps). Beginning gd 6, and for all subsequent time points till terminal sacrifice (gd 9, 12, 15, 18 and 20), mean maternal body weights were significantly reduced (7 to 12%) at 900 ng/kg/min. Mean maternal body weights were also significantly reduced (5 to 7%) at 450 ng/kg/min on gd 9 and 12. No significant differences in body weights were noted between control and other treatment groups. Maternal food consumption was significantly reduced during the entire dosing period (gd

6-20) and throughout the gestation period (gd 0-20) at 450 and 900 ng/kg/min. A dose-related reduction in food consumption was noted in treated groups on gd 5-6 and 6-9.

There were no drug-related effects on maternal gravid uterine or liver weights.

Developmental Toxicity

The numbers of corpora lutea (per dam) and implantation sites (per litter), percent pre-implantation loss per litter, and the incidences (per litter and the number of litters affected) of resorption, late fetal death, and nonlive (dead plus resorbed) or affected (nonlive plus malformed) implants are presented in Table 17. There were no treatment-related effects on the numbers of corpora lutea and total implantation sites or on the percent pre-implantation loss per litter. There were no significant effects of drug treatment on the incidence of resorptions, late fetal deaths, and nonlive or affected implants. Although percent resorptions and nonlive implants per litter showed dose-related positive trends, pairwise comparisons revealed no significant differences between treated and control groups. There were no treatment-related effects on live litter size, sex ratio or fetal body weight per litter (Table 17).

The fetal and the litter incidences of malformations and variations are presented in Table 18. There were no statistically significant treatment-related effects on the incidence of fetal external, visceral, skeletal or total malformations or variations (results expressed as percent affected litters, percent affected fetuses per litter or percent of total fetuses affected).

The specific types of fetal malformations and variations observed in the present study are presented in Table 19. External malformations were limited to one control fetus with cleft palate and another fetus at 450 ng/kg/min with anasarca and micromelia. Visceral malformations included mild hydrocephaly (only in treated groups; not dose-proportional), hydronephrosis (all groups including control; not dose-related) and hydroureter (one fetus each from the control and 900 ng/kg/min groups). Skeletal malformations were limited to the presence of a hole in the sternal cartilage (one fetus at 150 ng/kg/min), fused rib cartilage (one fetus at 450 ng/ kg/min) and bipartite cartilage of the thoracic centrum (one fetus each from the control and low dose groups). No skeletal malformations were seen at 900 ng/kg/min.

Fetal external variations were limited to hematomas (various locations; all groups including control; not dose-proportional), and clubbed limb without bone change in one fetus at 450 ng/kg/min. Visceral variations included enlarged nasal sinus (1 fetus at 150, 4 at 450, and 3 at 900 ng/kg/min), agenesis of the innominate artery (3 control fetuses and 1 fetus at 900 ng/kg/min), enlarged lateral ventricles (all groups; not dose-proportional) and distended ureter (all groups; not dose-related). Fetal skeletal variations included extra rib (rudimentary) on lumbar 1 (all groups; not dose-proportional), short thirteenth rib (one fetus each at 0 and 450 ng/kg/min), wavy ribs (1 fetus at 900 ng/kg/min), unossified sternebrae (all groups; not dose-related), misaligned sternebrae (1 fetus at 50 ng/kg/min) and reduced ossification of the thoracic centra (all groups; not dose-related).

[Note: Abnormalities observed only in UT-15 treated groups included mild hydrocephaly (dilation of the dorsal midline position of the lateral ventricles of the brain, a visceral malformation) and enlarged nasal sinus (a visceral variation). The fetal incidence of hyrocephaly was as follows: 6/174 @ 50 ng/kg/min, 7/167 @ 150 ng/kg/min, 3/167 @ 450 ng/kg/min, and 1/158 @ 900 ng/kg/min. The litter incidences were 4/25, 4/24, 2/25 and 1/24 at 50, 150, 450 and 900 ng/kg/min, respectively.

The sponsor states that there were no incidences of hydrocephaly in over 6000 laboratory control fetuses of this rat strain ([redacted] Sprague-Dawley). Historical control data from [redacted] showed an average fetal incidence rate of 0.027% for hydrocephaly (7 of the 24,340 fetuses examined), with a maximum incidence rate of 1.43% for any given study. Although the incidence rates (1-4%) observed in the present study exceed the historic control rate, no dose relationship was noted for this finding.

The fetal incidence of enlarged nasal sinus was as follows: 0/174 @ 50 ng/kg/min, 1/167 @ 150 ng/kg/min, 4/167 @ 450 ng/kg/min and 3/158 @ 900 ng/kg/min. The litter incidences were 0/25, 1/24, 3/25 and 2/24 at 50, 150, 450 and 900 ng/kg/min, respectively. Sponsor's historical control data showed that, of the 21 studies examined (more than 4000 fetuses), only one study (1 of 153 fetuses) had the above visceral variation. No dose relationship was noted for this finding in the present study.]

The steady-state plasma concentrations of UT-15 are summarized below. Samples collected from control rats were free of drug. At 50 ng/kg/min, 3 of 5 animals assigned to the 0 hr time point and all 5 animals at the 4 hr time point had drug concentrations below the limit of quantitation of the assay. Mean plasma drug concentrations at both time points increased in a dose-related manner; however, the variability around these mean values was found to be high (coefficient of variation as high as 137%). Additionally, steady-state concentrations generally were lower at 4 hr than at 0 hr, the cause of which is stated to be unknown. The results of the linear regression analysis of the individual animal data showed that the pharmacokinetic linearity was more apparent based on the hour 4 steady-state concentrations (r^2=0.73) than on the 0 hour levels (r^2=0.40).

STEADY-STATE CONCENTRATIONS OF UT-15 IN SPERM-POSITIVE RATS ADMINISTERED UT-15 BY SUBCUTANEOUS INFUSION OVER GESTATION DAYS 5-20

Dose (ng/kg/min)	Day 15 C_{ss} at 0 hr (ng/mL)	Day 15 C_{ss} at 4 hr (ng/mL)
Animals	N = 5	N = 5
Vehicle	0	0
50	0.9*	0*
150	5.3	3.0
450	24.9	7.8
900	30.3	16.1

* At the 0 hour (3 of 5) and 4 hour (5 of 5) sampling times, some concentration values were below the LOQ of the assay [redacted] and were assigned values of 0.

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Table 17.

Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights
(page 1 of 3)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
ALL LITTERS[a]	23	25	24	25	24
No. Corpora Lutea per Dam[b]					
	15.43 ± 0.39 N=23	15.24 ± 0.44 N=25	15.63 ± 0.42 N=24	15.71 ± 0.48 N=24[c]	15.21 ± 0.39 N=24
No. Implantation Sites per Litter[b]					
	15.17 ± 0.34 N=23	14.56 ± 0.64 N=25	14.54 ± 0.60 N=24	14.36 ± 0.54 N=25	14.58 ± 0.46 N=24
Percent Preimplantation Loss per Litter[b]					
	2.49 ± 1.29 N=23	6.58 ± 3.86 N=25	7.35 ± 2.90 N=24	9.15 ± 2.82 N=24[c]	4.50 ± 1.91 N=24
No. Resorptions per Litter[b]					
	0.48 ± 0.15 N=23	0.68 ± 0.19 N=25	0.50 ± 0.16 N=24	0.80 ± 0.24 N=25	1.33 ± 0.35 N=24
Percent Resorptions per Litter[b]					
	3.24 ± 1.08 § N=23	4.46 ± 1.20 N=25	3.50 ± 1.13 N=24	5.28 ± 1.57 N=25	9.02 ± 2.46 N=24
No. Litters with Resorptions					
	8	12	8	11	12
% Litters with Resorptions					
	34.78	48.00	33.33	44.00	50.00
No. Late Fetal Deaths per Litter[b]					
	0.00 ± 0.00 N=23	0.00 ± 0.00 N=25	0.00 ± 0.00 N=24	0.00 ± 0.00 N=25	0.00 ± 0.00 N=24
Percent Late Fetal Deaths per Litter[b]					
#	0.00 ± 0.00 N=23	0.00 ± 0.00 N=25	0.00 ± 0.00 N=24	0.00 ± 0.00 N=25	0.00 ± 0.00 N=24
No. Litters with Late Fetal Deaths					
	0	0	0	0	0
% Litters with Late Fetal Deaths					
	0.00	0.00	0.00	0.00	0.00

(continued)

Table 17. (continued)

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Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights
(page 2 of 3)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
No. Nonlive Implants per Litter[b,d]					
	0.48 ± 0.15 N=23	0.68 ± 0.19 N=25	0.50 ± 0.16 N=24	0.80 ± 0.24 N=25	1.33 ± 0.35 N=24
Percent Nonlive Implants per Litter[b,d]					
	3.24 ± 1.08 § N=23	4.46 ± 1.20 N=25	3.50 ± 1.13 N=24	5.26 ± 1.57 N=25	9.02 ± 2.46 N=24
No. Litters with Nonlive Implants[d]					
	8	12	8	11	12
% Litters with Nonlive Implants[d]					
	34.78	48.00	33.33	44.00	50.00
No. Adversely Affected Implants per Litter[b,e]					
	0.70 ± 0.20 N=23	1.00 ± 0.22 N=25	0.88 ± 0.23 N=24	1.04 ± 0.27 N=25	1.42 ± 0.34 N=24
Percent Adversely Affected Implants per Litter[b,e]					
	4.60 ± 1.32 N=23	6.67 ± 1.50 N=25	5.87 ± 1.52 N=24	6.84 ± 1.72 N=25	9.60 ± 2.40 N=24
No. Litters with Adversely Affected Implants[e]					
	9	15	11	14	14
% Litters with Adversely Affected Implants[e]					
	39.13	60.00	45.83	56.00	58.33
LIVE LITTERS[f]	23	25	24	25	24
No. Live Fetuses per Litter[b]					
	14.70 ± 0.38 N=23	13.88 ± 0.63 N=25	14.04 ± 0.62 N=24	13.56 ± 0.53 N=25	13.25 ± 0.52 N=24
Percent Male Fetuses per Litter[b]					
	50.39 ± 3.02 N=23	45.54 ± 3.54 N=25	50.46 ± 3.14 N=24	49.28 ± 2.78 N=25	48.15 ± 2.86 N=24
No. Male Fetuses per Litter[b]					
	7.48 ± 0.53 N=23	6.52 ± 0.52 N=25	6.92 ± 0.45 N=24	6.76 ± 0.47 N=25	6.46 ± 0.45 N=24

(continued)

Table 17. (continued)

Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights
(page 3 of 3)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
No. Female Fetuses per Litter[b]					
	7.22 ± 0.43 N=23	7.36 ± 0.57 N=25	7.13 ± 0.62 N=24	6.80 ± 0.41 N=25	6.79 ± 0.41 N=24
Average Fetal Body Weight (g) per Litter[b]					
	3.430 ± 0.050 N=23	3.584 ± 0.041 N=25	3.522 ± 0.040 N=24	3.534 ± 0.056 N=25	3.513 ± 0.057 N=24
Average Male Fetal Body Weight (g) per Litter[b]					
	3.519 ± 0.050 N=23	3.657 ± 0.050 N=24	3.631 ± 0.040 N=24	3.627 ± 0.058 N=25	3.616 ± 0.067 N=24
Average Female Fetal Body Weight (g) per Litter[b]					
	3.344 ± 0.050 N=23	3.510 ± 0.041 N=25	3.413 ± 0.040 N=24	3.447 ± 0.054 N=25	3.417 ± 0.053 N=24
Repeated Measures for Average Fetal Body Weight per Litter: Bartlett's (p=0.2432); DOSE (p=0.2423); SEX (p≤0.0001); DOSExSEX (p=0.7789).					

[a]Includes all dams pregnant at terminal sacrifice on gestational day 20; litter size = no. implantation sites per dam.
[b]Reported as the mean ± S.E.M.
[c]Decrease in N is due to one ovary inadvertently being lost prior to the corpora lutea count being done.
[d]Nonlive = late fetal deaths plus resorptions.
[e]Adversely affected = nonlive plus malformed.
[f]Includes only dams with live fetuses; litter size = no. live fetuses per dam.
[#]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; Test for Linear Trend.

Table 18.

Summary and Statistical Analysis of Malformations and Variations (page 1 of 4)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
No. Fetuses Examined[a]	338	347	337	339	318
No. Litters Examined[b]	23	25	24	25	24
No. Fetuses with External Malformations[c]	1	0	0	1	0
% Fetuses with External Malformations[c]	0.30	0.00	0.00	0.29	0.00
No. Litters with External Malformations[d]	1	0	0	1	0
% Litters with External Malformations[d]	4.35	0.00	0.00	4.00	0.00
No. Fetuses with Visceral Malformations[c]	3	7	8	5	2
% Fetuses with Visceral Malformations[c]	1.78	4.02	4.79	2.99	1.27
No. Litters with Visceral Malformations[d]	2	5	4	4	2
% Litters with Visceral Malformations[d]	8.70	20.00	16.67	16.00	8.33
No. Fetuses with Skeletal Malformations[c]	1	1	1	1	0
% Fetuses with Skeletal Malformations[c]	0.59	0.57	0.59	0.58	0.00
No. Litters with Skeletal Malformations[d]	1	1	1	1	0
% Litters with Skeletal Malformations[d]	4.35	4.00	4.17	4.00	0.00

(continued)

Table 18. (continued)

Summary and Statistical Analysis of Malformations and Variations (page 2 of 4)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
No. Fetuses with Malformations per Litter[c,e]					
	0.22 ± 0.13 N=23	0.32 ± 0.13 N=25	0.38 ± 0.18 N=24	0.24 ± 0.10 N=25	0.08 ± 0.06 N=24
No. Male Fetuses with Malformations per Litter[c,e]					
	0.13 ± 0.10 N=23	0.21 ± 0.10 N=24	0.29 ± 0.14 N=24	0.08 ± 0.06 N=25	0.00 ± 0.00 N=24
No. Female Fetuses with Malformations per Litter[c,e]					
	0.09 ± 0.06 N=23	0.12 ± 0.09 N=25	0.08 ± 0.08 N=24	0.16 ± 0.07 N=25	0.08 ± 0.06 N=24
Percent Fetuses with Malformations per Litter[c,e]					
	1.39 ± 0.80 N=23	2.32 ± 0.92 N=25	2.45 ± 1.10 N=24	1.69 ± 0.76 N=25	0.58 ± 0.40 N=24
Percent Male Fetuses with Malformations per Litter[c,e]					
	1.88 ± 1.49 N=23	3.06 ± 1.64 N=24	4.70 ± 2.19 N=24	1.16 ± 0.86 N=25	0.00 ± 0.00 N=24
Percent Female Fetuses with Malformations per Litter[c,e]					
	1.09 ± 0.75 N=23	1.24 ± 0.86 N=25	0.83 ± 0.83 N=24	2.07 ± 0.97 N=25	1.19 ± 0.82 N=24
Repeated Measures for Percent Fetuses with Malformations per Litter: Bartlett's ($\dagger$); DOSE (p=0.5888); SEX (p=0.3793); DOSExSEX (p=0.1175).					
No. Fetuses with Malformations[c]	5	8	9	6	2
% Fetuses with Malformations[c]	1.48	2.31	2.67	1.77	0.63
No. Litters with Malformations[d]	3	6	5	5	2
% Litters with Malformations[d]	13.04	24.00	20.83	20.00	8.33

(continued)

Table 18. (continued)

Summary and Statistical Analysis of Malformations and Variations (page 3 of 4)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
No. Fetuses with External Variations[c]					
	3	1	2	2	1
% Fetuses with External Variations[c]					
	0.89	0.29	0.59	0.59	0.31
No. Litters with External Variations[d]					
	2	1	1	2	1
% Litters with External Variations[d]					
	8.70	4.00	4.17	8.00	4.17
No. Fetuses with Visceral Variations[c]					
	12	10	9	15	9
% Fetuses with Visceral Variations[c]					
	7.10	5.75	5.39	8.98	5.70
No. Litters with Visceral Variations[d]					
	9	6	8	10	7
% Litters with Visceral Variations[d]					
	39.13	24.00	33.33	40.00	29.17
No. Fetuses with Skeletal Variations[c]					
	32	32	25	28	21
% Fetuses with Skeletal Variations[c]					
	19.05	18.39	14.79	16.28	13.13
No. Litters with Skeletal Variations[d]					
	18	14	14	18	11
% Litters with Skeletal Variations[d]					
	78.26	56.00	58.33	72.00	45.83
No. Fetuses with Variations per Litter[c,e]					
	2.04 ± 0.32 N=23	1.72 ± 0.36 N=25	1.50 ± 0.27 N=24	1.76 ± 0.25 N=25	1.25 ± 0.28 N=24
No. Male Fetuses with Variations per Litter[c,e]					
	0.74 ± 0.22 N=23	1.00 ± 0.28 N=24	0.92 ± 0.22 N=24	0.92 ± 0.20 N=25	0.63 ± 0.20 N=24
No. Female Fetuses with Variations per Litter[c,e]					
	1.30 ± 0.23 N=23	0.76 ± 0.14 N=25	0.58 ± 0.17 N=24	0.84 ± 0.17 N=25	0.63 ± 0.17 N=24

(continued)

Table 18. (continued)

Summary and Statistical Analysis of Malformations and Variations (page 4 of 4)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
Percent Fetuses with Variations per Litter[c,e]					
	14.16 ± 2.23 N=23	12.15 ± 2.63 N=25	10.51 ± 1.86 N=24	13.33 ± 1.86 N=25	9.17 ± 2.04 N=24
Percent Male Fetuses with Variations per Litter[c,e]					
	10.34 ± 3.04 N=23	15.04 ± 4.16 N=24	11.69 ± 2.67 N=24	16.67 ± 4.13 N=25	9.28 ± 2.90 N=24
Percent Female Fetuses with Variations per Litter[c,e]					
	17.43 ‡ ± 2.96 N=23	10.67 ± 2.13 N=25	7.46 ± 2.03 N=24	11.16 ± 2.27 N=25	9.19 ± 2.79 N=24
Repeated Measures for Percent Fetuses with Variations per Litter: Bartlett's (p=0.5190); DOSE (p=0.3985); SEX (p=0.8847); DOSExSEX (p=0.1559).					
No. Fetuses with Variations[c]	47	43	36	44	30
% Fetuses with Variations[c]	13.91	12.39	10.68	12.98	9.43
No. Litters with Variations[d]	19	18	16	22	14
% Litters with Variations[d]	82.61	72.00	66.67	88.00	58.33

[a]Only live fetuses were examined for malformations and variations.
[b]Includes only litters with live fetuses.
[c]Fetuses with one or more malformations or variations.
[d]Litters with one or more fetuses with malformations or variations.
[e]Reported as the mean ± S.E.M.
[f]Zero variance in one or more groups - test not done.
[#]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.

Table 19.

Summary of Morphological Abnormalities in CD Rat Fetuses: Listing by Defect Type[a] (page 1 of 3)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
ANY MALFORMATIONS					
Total No. of Fetuses Examined for Any Malformations[b]	338	347	337	339	318
No. of Fetuses with Any Malformations[c]	5	8	9	6	2
% Fetuses with Any Malformations	1.5%	2.3%	2.7%	1.8%	0.6%
Total No. of Litters Examined for Any Malformations[d]	23	25	24	25	24
No. of Litters with Any Malformations[e]	3	6	5	5	2
% Litters with Any Malformations	13.0%	24.0%	20.8%	20.0%	8.3%
EXTERNAL MALFORMATIONS					
Total No. of Fetuses Examined for External Malformations[b]	338	347	337	339	318
No. of Fetuses with External Malformations[c]	1	0	0	1	0
% Fetuses with External Malformations	0.3%	0.0%	0.0%	0.3%	0.0%
Total No. of Litters Examined for External Malformations[d]	23	25	24	25	24
No. of Litters with External Malformations[e]	1	0	0	1	0
% Litters with External Malformations	4.3%	0.0%	0.0%	4.0%	0.0%
Anasarca				1(1)	
Cleft Palate	1(1)				
Micromelia				1(1)	
VISCERAL MALFORMATIONS					
Total No. of Fetuses Examined for Visceral Malformations[b]	169	174	167	167	158
No. of Fetuses with Visceral Malformations[c]	3	7	8	5	2
% Fetuses with Visceral Malformations	1.8%	4.0%	4.8%	3.0%	1.3%
Total No. of Litters Examined for Visceral Malformations[d]	23	25	24	25	24
No. of Litters with Visceral Malformations[e]	2	5	4	4	2
% Litters with Visceral Malformations	8.7%	20.0%	16.7%	16.0%	8.3%
Hydrocephaly: Mild		6(4)	7(4)	3(2)	1(1)
Situs Inversus Abdominal Viscera	1(1)				
Hydronephrosis: Bilateral	1(1)			1(1)	
Left	1(1)	1(1)			
Right			1(1)	1(1)	1(1)
Hydroureter: Bilateral				1(1)	
Right	1(1)				1(1)
SKELETAL MALFORMATIONS					
Total No. of Fetuses Examined for Skeletal Malformations[b]	168[f]	174	169[f]	172	160
No. of Fetuses with Skeletal Malformations[c]	1	1	1	1	0
% Fetuses with Skeletal Malformations	0.6%	0.6%	0.6%	0.6%	0.0%
Total No. of Litters Examined for Skeletal Malformations[d]	23	25	24	25	24
No. of Litters with Skeletal Malformations[e]	1	1	1	1	0
% Litters with Skeletal Malformations	4.3%	4.0%	4.2%	4.0%	0.0%
Hole in Cartilage of Sternum			1(1)		
Fused Rib Cartilage				1(1)	
Bipartite Cartilage, Normal Ossification Center: Thoracic Centrum	1(1)				
Bipartite Cartilage, Bipartite Ossification Center: Thoracic Centrum		1(1)			

(continued)

Table 19. (continued)

Summary of Morphological Abnormalities in CD Rat Fetuses: Listing by Defect Type[a] (page 2 of 3)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
ANY VARIATIONS					
Total No. of Fetuses Examined for Any Variations[b]	338	347	337	339	318
No. of Fetuses with Any Variations[c]	47	43	36	44	30
% Fetuses with Any Variations	13.9%	12.4%	10.7%	13.0%	9.4%
Total No. of Litters Examined for Any Variations[d]	23	25	24	25	24
No. of Litters with Any Variations[e]	19	18	16	22	14
% Litters with Any Variations	82.6%	72.0%	66.7%	88.0%	58.3%
EXTERNAL VARIATIONS					
Total No. of Fetuses Examined for External Variations[b]	338	347	337	339	318
No. of Fetuses with External Variations[c]	3	1	2	2	1
% Fetuses with External Variations	0.9%	0.3%	0.6%	0.6%	0.3%
Total No. of Litters Examined for External Variations[d]	23	25	24	25	24
No. of Litters with External Variations[e]	2	1	1	2	1
% Litters with External Variations	8.7%	4.0%	4.2%	8.0%	4.2%
Hematoma: Head					1(1)
Neck			1(1)	1(1)	
Jaw		1(1)			
Thorax	1(1)				
Back	1(1)		1(1)		
Forelimb	1(1)				
Clubbed Limb without Bone Change				1(1)	
VISCERAL VARIATIONS					
Total No. of Fetuses Examined for Visceral Variations[b]	169	174	167	167	158
No. of Fetuses with Visceral Variations[c]	12	10	9	15	9
% Fetuses with Visceral Variations	7.1%	5.7%	5.4%	9.0%	5.7%
Total No. of Litters Examined for Visceral Variations[d]	23	25	24	25	24
No. of Litters with Visceral Variations[e]	9	6	8	10	7
% Litters with Visceral Variations	39.1%	24.0%	33.3%	40.0%	29.2%
Enlarged Lateral Ventricle (Full): Bilateral			2(2)		
Left		1(1)		1(1)	1(1)
Right					1(1)
Enlarged Lateral Ventricle (Half): Bilateral	1(1)	2(2)	2(2)		1(1)
Left	1(1)	1(1)			1(1)
Right	3(3)			1(1)	
Enlarged Nasal Sinus			1(1)	4(3)	3(2)
Agenesis of the Innominate Artery	3(3)				1(1)
Distended Ureter: Bilateral	1(1)	3(1)		4(4)	
Left	2(1)	2(2)	4(3)	5(3)	2(2)
Right	1(1)	1(1)		3(2)	1(1)

(continued)

Table 19. (continued)

Summary of Morphological Abnormalities in CD Rat Fetuses: Listing by Defect Type[a] (page 3 of 3)

	UT-15 (ng/kg/min, subcutaneous)				
	0	50	150	450	900
SKELETAL VARIATIONS					
Total No. of Fetuses Examined for Skeletal Variations[b]	168[f]	174	169[f]	172	160
No. of Fetuses with Skeletal Variations[c]	32	32	25	28	21
% Fetuses with Skeletal Variations	19.0%	18.4%	14.8%	16.3%	13.1%
Total No. of Litters Examined for Skeletal Variations[d]	23	25	24	25	24
No. of Litters with Skeletal Variations[e]	18	14	14	18	11
% Litters with Skeletal Variations	78.3%	56.0%	58.3%	72.0%	45.8%
Misaligned Sternebrae		1(1)			
Unossified Sternebra(e) (I, II, III and/or IV only)	6(5)	7(4)	4(4)	9(8)	3(3)
Rib on Lumbar I: Bilateral Rudimentary			2(2)	1(1)	
Left Rudimentary	1(1)	1(1)	1(1)	1(1)	
Short Rib: XIII	1(1)			1(1)	
Wavy Rib					1(1)
Normal Cartilage, Bipartite Ossification Center: Thoracic Centrum	8(7)	14(8)	11(8)	11(8)	8(6)
Dumbbell Cartilage, Dumbbell Ossification Center: Thoracic Centrum	2(2)	3(3)			2(2)
Dumbbell Cartilage, Bipartite Ossification Center: Thoracic Centrum	16(11)	10(5)	9(6)	5(5)	8(7)

[a]A single fetus may be represented more than once in listing individual defects. Data are presented as the number of fetuses (number of litters).
[b]Only live fetuses were examined.
[c]Fetuses with one or more external malformations/variations.
[d]Includes only litters with live fetuses.
[e]Litters with one or more externally malformed/variant fetuses.
[f]One fetus did not stain properly and therefore a skeletal evaluation could not be done.

APPEARS THIS WAY
ON ORIGINAL

Developmental Toxicity Evaluation of UT-15 (Administered by Continuous Subcutaneous Infusion) in Rabbits

Testing Facility: [redacted]

Study Number: 65C-7019-400

Study Dates: Initiation Date – April 9, 1998
Completion Date – July 14, 1998

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

Animals: Timed-mated New Zealand White rabbits, 2.7 to 4.0 kg body weight and about 5 to 5.5 months old, were obtained from [redacted] Eighty female rabbits, which were at gestational day (gd) 1, 2 or 3 on arrival, were randomly assigned to four groups of 20 animals each. Quarantine for these rabbits were limited to two to four days prior to implantation of osmotic pumps on gd 5. Females were housed individually in stainless steel cages with mesh flooring. Food (No.5322 [redacted] certified rabbit chow) was available *ad libitum* from gd 3 to scheduled sacrifice. (It is stated that food was restricted on gestational days 1 and 2.)

Dose Levels, Mode of Administration and Treatment Regimen: The target doses were 0, 50, 150 and 300 ng/kg/min.

Stock solutions (10 mg/ml) of UT-15 (Lot No. LRX-98B01), formulated in vehicle (containing sodium citrate, citric acid, sodium chloride dissolved in sterile water for injection) and adjusted to pH 7.4, were diluted (with vehicle) to achieve the desired final concentrations.

Analyses of the test solutions indicated that dosing formulations were 94 to 96% of target concentrations, and the stock solution was 97% of target. It was shown that the dosing formulations were stable at 25 and 40°C for four weeks.

The test and vehicle solutions were administered by continuous subcutaneous infusion using subcutaneously implanted [redacted] osmotic pumps (one to four pumps per animal depending on the dose group, control - 4, low dose – 1, mid dose – 2, and high dose – 4). According to the supplier, the osmotic pump model [redacted] chosen has a nominal delivery rate of [redacted] hour with a nominal duration of 28 days.

On the morning of gd 5 (between 0700-1100 hours), each study female rabbit was anesthetized by isoflurane inhalation. The dorsal subscapular area was surgically prepared and an incision about 1.5 cm in diameter was made. One to four osmotic pumps,

preloaded with appropriate dosing solutions, were inserted into the subcutaneous pockets and the incision was closed with wound clips.

According to the supplier, the osmotic pump model chosen requires ——— ; to reach steady state infusion rate once it is implanted. Therefore, the pump that was implanted on the morning of gd 5 would be at steady state by the morning of gd 6. The pumps were removed (under isoflurane inhalation anesthesia) on gd 19 at about the same time they were inserted on gd 5 (for a duration of 13 days of exposure at steady state) and the incision was reclosed.

[It is stated that the doses were selected based on the results of a dose range-finding study (continuous sc infusion of UT-15 at 0, 100, 300 and 1000 ng/kg/min on days 5 through 19 of gestation) in rabbits. The high dose produced obvious maternal (reduction in body weight, weight gain and food consumption) and developmental (3 of 6 does had fully resorbed litters) toxicities. At 300 ng/kg/min, there was evidence of some maternal (reduced body weight and weight gain) and developmental (presence of dead fetuses in one of six litters) toxicities. Therefore, 300 ng/kg/min was selected as the top dose, and 150 and 50 ng/kg/min (fractions of the high dose) were chosen as mid and low doses, respectively.

Observations and Measurements: Animals were checked at least once daily for clinical signs on gd 1-4 (prior to pump implant), twice daily on gd 5-19 (pump implant period) and at least once daily on gd 20-30 (postexposure period). Body weights were recorded on gd 0, 3, 5, 6, 9, 12, 15, 18, 19, 21, 24, 27, and 30. Food consumption was measured for gd 3-5, 5-6, 6-9, 9-12, 12-15, 15-18, 18-19, 19-21, 21-24, 24-27, , and 27-30.

Blood samples from six does per group were collected from central ear artery on gd 17 (gd 18 for the 24-hour time point) at time 0 (at about the same time the osmotic pump was inserted on gd 5) and at 2, 4, 8 and 24 hours after time 0, for maternal toxicokinetic evaluations. (Same animals were bled at each time point. After pregnancy was confirmed at necropsy, plasma samples from the first five pregnant does per group were analyzed for drug levels.)

On gestational day 30, all surviving females were killed by intravenous administration of sodium pentobarbital. Animals were weighed. The incision site was examined and the wound clips were removed. The thoracic and abdominal cavities and all organs were examined. Liver and uterus were weighed. The pregnancy status was confirmed by uterine examination. Uteri which showed no visible implantation sites were stained with ammonium sulfide (10%), in order to visualize any implantation sites which may have undergone very early resorption. Numbers of corpora lutea, implantation sites, early and late resorptions, and dead and live fetuses were recorded. Dead fetuses were retained in fixative.

All live fetuses were euthanized, counted, weighed, and examined for external malformations (including cleft palate) and variations. All live fetuses were examined for visceral malformations, and their sex determined. Approximately half of the fetuses per

litter were decapitated, and heads were fixed in Bouin's solution for decalcification and subsequent sectioning and examination. All fetal carcasses were evicerated and stained with alizarin red S/alcian blue, and all fetuses (half intact and half decapitated) were examined for skeletal malformations and variations. All maternal carcasses were discarded. All fetal carcasses were stored in glycerin:70% ethanol (1:1) and fetal head sections were stored in 70% ethanol.

Developmental toxicity parameters were calculated for each litter (dam) and then the mean was calculated using the litter (dam) values.

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. Nominal scale measures 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 two-tailed Fisher's Exact Probability test was used for pairwise comparisons. A test for statistical outliers was performed on maternal body weights and feed consumption.

Results

Maternal Toxicity

Treatment-related clinical observations, limited to mid and high dose animals, included flushed ears, erythema on the shaved area of the skin, serum pockets at/near the incision site and mild swelling around pumps. Clinical observations related to surgical procedures (missing wound clips and sores/scabs at or near the incision/implant site) were seen in all groups.

No does died or delivered early. One doe from the mid dose group was removed from study because of an injury during blood collection. One doe from the high dose group aborted on gd 21. One doe each from the control and mid dose groups was found to be not pregnant at necropsy.

[All pregnant does had one or more live fetuses at scheduled sacrifice. The numbers of litters (fetuses) evaluated were 19 (144), 20 (154), 18 (138), and 19 (161) at 0, 50, 150, and 300 ng/kg/min, respectively.]

Mean maternal body weights were mostly similar across all groups on gestational days 0, 3 and 5 (prior to pump implantation). On gd 9, maternal body weights were significantly reduced (7-8%) in all treated groups, and on gd 12, body weights were significantly

reduced (7-8%) at mid and high doses. For all subsequent time points (gd 15, 18, 19,21, 24, 27 and 30), no significant differences in body weights were noted between control and treatment groups.

Maternal food consumption was significantly reduced in all treated groups (dose related) during the drug exposure period (gd 6 to 19) and throughout the gestation period (not dose related).

There were no drug-related effects on maternal gravid uterine or liver weights.

Developmental Toxicity

The numbers of corpora lutea (per dam) and implantation sites (per litter), percent pre-implantation loss (per litter), and the incidences (per litter and the number of litters affected) of resorptions, late fetal death, and nonlive (dead plus resorbed) or adversely affected (nonlive plus malformed) implants are presented in Table 20. There were no significant effects of treatment on the numbers of corpora lutea and total implantation sites, or on the percent preimplantation loss. There were no treatment-related effects on the incidence of resorptions, late fetal deaths, and nonlive or affected implants. Although not statistically significant, the percent of litters with adversely affected implants was higher in the high dose group (74%) than in the concurrent control (42%) or lower dose groups (33 to 40%). There were no significant treatment related effects on live litter size, sex ratio, or fetal body weight per litter (Table 20).

The fetal and the litter incidences of malformations and variations are presented in Table 21. There were no significant treatment-related effects on the incidence of pooled fetal external, visceral, skeletal, or total malformations (results expressed as percent affected litters, percent affected fetuses per litter or percent of total fetuses affected). Dose related increases in the percent of fetuses with variations per litter (due to an increase in skeletal variations) were noted in all treated groups (statistically significant at 300 ng/kg/min), especially male fetuses with variations (statistically significant at 150 and 300 ng/kg/min). The incidences of female fetuses with variations were not significantly different between control and treated groups.

The specific types of fetal malformations and variations observed in the study are presented in Table 22. External malformation was limited to one control fetus with omphalocele and one low dose fetus with anencephaly, anophthalmia, microtia, micromelia and webbing of upper portion of hindlimbs to the body. There were no fetal visceral malformations. Skeletal malformations were limited to effects on the sternebrae, ribs, radius, ulna and thoracic centra, with 4 fetuses at 0, 4 at 50, 0 at 150, and 5 at 300 ng/kg/min affected.

Fetal external variation was limited to clubbed limb without bone change in one low dose fetus. Visceral variations included abnormal number of papillary muscles (19/144, 19/154, 21/138 and 23/161 fetuses at 0, 50, 150 and 300 ng/kg/min, respectively), bifurcated papillary muscles (2/154 low dose fetuses), enlarged papillary muscles (1

fetus each at 0 and 50 ng/kg/ min), and half normal size gall bladder (2 fetuses at 50 and 1 at 300 ng/kg/min). Skeletal varitions (higher incidences in drug-treated groups than in control, but not dose related) were limited to extra rib (uni- and bilateral, full and rudimentary) on the first lumbar vertebra in all groups, and bipartite ossification center in thoracic centra in 2 fetuses at 50 ng/kg/min and 1 at 300 ng/kg/min.

It is noted that the increased incidences of skeletal variations observed in the 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 and mid doses (49-50%) in the present study exceeded the historical control rate, no dose relationship was noted. For the right rudimentary rib, the highest incidence rate observed at the high dose (7.5%) was lower than the highest historical control incidence rate (11%) from the nine previous studies.

The pharmacokinetic parameters are summarized below. Results indicated that steady-state concentrations had been achieved and were maintained throughout the monitoring period on gestation day 17, although there were some variations in the plasma drug concentrations over the 24-hr sampling period with mean peak concentrations (Css_{max}) being about 140% of mean minimum concentrations (Css_{min}).

AUC_{ss} values were found to increase proportionally with dose (r^2=0.9992), indicating that the pharmacokinetics of UT-15 in pregnant rabbits were linear within the dose range evaluated. This finding also was supported by plasma clearance values that were constant at all three dose levels.

PHARMACOKINETIC PARAMETERS IN PREGNANT RABBITS ADMINISTERED UT-15 BY SUBCUTANEOUS INFUSION OVER GESTATION DAYS 6-18

Pharmacokinetic Parameter	Infusion Rate 50 ng/kg/min	Infusion Rate 150 ng/kg/min	Infusion Rate 300 ng/kg/min
Animals	N = 20	N = 20	N = 20
Css_{max} (ng/mL)	3.19	8.36	17.89
T_{max} (hr)	4	24	2
Css_{min} (ng/mL)	2.40	5.98	14.60
T_{min} (hr)	0	0	0
AUC_{ss} (ng.hr/mL)	66.0	189.4	399.1
CL/F (mL/kg/min)	18.2	19.0	18.0

Table 20.

Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights (page 1 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
ALL LITTERS[a]	19	20	18	19
No. Corpora Lutea per Dam[b]				
	9.26 ± 0.46 N=19	9.15 ± 0.37 N=20	8.88 ± 0.30 N=17[c]	9.74 ± 0.47 N=19
No. Implantation Sites per Litter[b]				
	8.32 ± 0.46 § N=19	8.05 ± 0.46 N=20	8.28 ± 0.32 N=18	9.37 ± 0.46 N=19
Percent Preimplantation Loss per Litter[b]				
	10.60 ± 2.47 § N=19	13.07 ± 3.57 N=20	6.60 ± 1.92 N=17[c]	3.68 ± 1.47 N=19
No. Resorptions per Litter[b]				
	0.53 ± 0.21 N=19	0.35 ± 0.13 N=20	0.39 ± 0.20 N=18	0.79 ± 0.18 N=19
Percent Resorptions per Litter[b]				
	6.39 ± 2.47 N=19	4.22 ± 1.56 N=20	5.50 ± 3.03 N=18	8.76 ± 2.28 N=19
No. Litters with Resorptions				
	6	6	4	11
% Litters with Resorptions				
	31.58	30.00	22.22	57.89
No. Late Fetal Deaths per Litter[b]				
	0.21 ± 0.10 N=19	0.00 ± 0.00 N=20	0.22 ± 0.17 N=18	0.11 ± 0.07 N=19
Percent Late Fetal Deaths per Litter[b]				
#	1.92 ± 0.88 N=19	0.00 ± 0.00 N=20	2.78 ± 2.16 N=18	1.06 ± 0.73 N=19
No. Litters with Late Fetal Deaths				
	4	0	2	2
% Litters with Late Fetal Deaths				
	21.05	0.00	11.11	10.53

(continued)

Table 20. (continued)

Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights (page 2 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
No. Nonlive Implants per Litter[b,d]				
	0.74 ±0.25 N=19	0.35 ±0.13 N=20	0.61 ±0.24 N=18	0.89 ±0.21 N=19
Percent Nonlive Implants per Litter[b,d]				
	8.31 ±2.71 N=19	4.22 ±1.56 N=20	8.28 ±3.47 N=18	9.82 ±2.52 N=19
No. Litters with Nonlive Implants[d]				
	8	6	6	11
% Litters with Nonlive Implants[d]				
	42.11	30.00	33.33	57.89
No. Adversely Affected Implants per Litter[b,e]				
	0.95 ±0.32 N=19	0.55 ±0.18 N=20	0.61 ±0.24 N=18	1.16 ±0.21 N=19
Percent Adversely Affected Implants per Litter[b,e]				
	10.45 ±3.29 N=19	7.44 ±2.41 N=20	8.28 ±3.47 N=18	12.98 ±2.43 N=19
No. Litters with Adversely Affected Implants[e]				
	8	8	6	14
% Litters with Adversely Affected Implants[e]				
	42.11	40.00	33.33	73.68
LIVE LITTERS[f]	19	20	18	19
No. Live Fetuses per Litter[b]				
	7.58 ±0.43 N=19	7.70 ±0.47 N=20	7.67 ±0.46 N=18	8.47 ±0.49 N=19
Percent Male Fetuses per Litter[b]				
	49.53 ±4.06 § N=19	38.84 ±4.32 N=20	48.62 ±4.19 N=18	56.43 ±4.81 N=19
No. Male Fetuses per Litter[b]				
	3.89 ±0.31 N=19	3.05 ±0.42 N=20	3.72 ±0.37 N=18	4.68 ±0.41 N=19

(continued)

Table 20. (continued)

Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights (page 3 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
No. Female Fetuses per Litter[b]				
	3.89 ± 0.46 N=19	4.65 ± 0.45 N=20	3.94 ± 0.41 N=18	3.94 ± 0.43 N=18[h]
Average Fetal Body Weight (g) per Litter[b]				
	51.47 ± 1.40 N=19	52.32 ± 1.02 N=20	53.73 ± 1.05 N=18	50.70 ± 1.12 N=19
Average Male Fetal Body Weight (g) per Litter[b]				
	52.83 ± 1.68 N=18[g]	53.56 ± 1.01 N=20	54.58 ± 1.26 N=18	51.55 ± 1.05 N=19
Average Female Fetal Body Weight (g) per Litter[b]				
	50.40 ± 1.23 N=19	51.90 ± 1.17 N=20	52.81 ± 1.27 N=18	49.24 ± 1.40 N=18[h]
Repeated Measures for Average Fetal Body Weight per Litter: Bartlett's (p=0.6063); DOSE (p=0.2321); SEX (p=0.0002); DOSExSEX (p=0.9523).				

a Includes all does pregnant at terminal sacrifice on gestational day 30; litter size = no. implantation sites per doe.
b Reported as the mean ± S.E.M.
c Decrease in N is due to one ovary inadvertently being lost prior to the corpora lutea count being done.
d Nonlive = late fetal deaths plus resorptions.
e Adversely affected = nonlive plus malformed.
f Includes only does with live fetuses; litter size = no. live fetuses per doe.
g One litter had only female fetuses.
h One litter had only male fetuses.
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; Test for Linear Trend.

Table 21.

Summary and Statistical Analysis of Malformations and Variations (page 1 of 4)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
No. Fetuses Examined[a]	144	154	138	161
No. Litters Examined[b]	19	20	18	19
No. Fetuses with External Malformations[c]	1	1	0	0
% Fetuses with External Malformations[c]	0.69	0.65	0.00	0.00
No. Litters with External Malformations[d]	1	1	0	0
% Litters with External Malformations[d]	5.26	5.00	0.00	0.00
No. Fetuses with Visceral Malformations[c]	0	0	0	0
% Fetuses with Visceral Malformations[c]	0.00	0.00	0.00	0.00
No. Litters with Visceral Malformations[d]	0	0	0	0
% Litters with Visceral Malformations[d]	0.00	0.00	0.00	0.00
No. Fetuses with Skeletal Malformations[c]	4	4	0	5
% Fetuses with Skeletal Malformations[c]	2.90	2.72	0.00	3.13
No. Litters with Skeletal Malformations[d]	4	3	0	5
% Litters with Skeletal Malformations[d]	21.05	15.00	0.00	26.32

(continued)

Table 21. (continued)

Summary and Statistical Analysis of Malformations and Variations (page 2 of 4)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
No. Fetuses with Malformations per Litter[c,e]				
	0.21 ±0.10 N=19	0.20 ±0.12 N=20	0.00 ±0.00 N=18	0.26 ±0.10 N=19
No. Male Fetuses with Malformations per Litter[c,e]				
	0.17 ±0.09 N=18	0.10 ±0.07 N=20	0.00 ±0.00 N=18	0.21 ±0.10 N=19
No. Female Fetuses with Malformations per Litter[c,e]				
	0.05 ±0.05 N=19	0.10 ±0.07 N=20	0.00 ±0.00 N=18	0.06 ±0.06 N=18
Percent Fetuses with Malformations per Litter[c,e]				
#	2.68 ±1.26 N=19	3.33 ±1.99 N=20	0.00 ±0.00 N=18	3.29 ±1.34 N=19
Percent Male Fetuses with Malformations per Litter[c,e]				
#	5.00 ±3.05 N=18	3.50 ±2.64 N=20	0.00 ±0.00 N=18	4.87 ±2.35 N=19
Percent Female Fetuses with Malformations per Litter[c,e]				
#	0.53 ±0.53 N=19	3.75 ±2.74 N=20	0.00 ±0.00 N=18	1.11 ±1.11 N=18
Repeated Measures for Percent Fetuses with Malformations per Litter: Bartlett's (†); DOSE (p=0.2607); SEX (p=0.0809); DOSExSEX (p=0.3434).				
No. Fetuses with Malformations[c]	4	4	0	5
% Fetuses with Malformations[c]	2.78	2.60	0.00	3.11
No. Litters with Malformations[d]	4	3	0	5
% Litters with Malformations[d]	21.05	15.00	0.00	26.32

(continued)

Table 21. (continued)

Summary and Statistical Analysis of Malformations and Variations (page 3 of 4)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
No. Fetuses with External Variations[c]	0	1	0	0
% Fetuses with External Variations[c]	0.00	0.65	0.00	0.00
No. Litters with External Variations[d]	0	1	0	0
% Litters with External Variations[d]	0.00	5.00	0.00	0.00
No. Fetuses with Visceral Variations[c]	21	24	22	25
% Fetuses with Visceral Variations[c]	14.58	15.58	15.94	15.53
No. Litters with Visceral Variations[d]	15	14	10	15
% Litters with Visceral Variations[d]	78.95	70.00	55.56	78.95
No. Fetuses with Skeletal Variations[c]	52	65	83	100
% Fetuses with Skeletal Variations[c]	37.68	44.22	64.34	62.50
No. Litters with Skeletal Variations[d]	17	15	18	19
% Litters with Skeletal Variations[d]	89.47	75.00	100.00	100.00
No. Fetuses with Variations per Litter[c,e]	3.68 ±0.52 N=19	4.00 ±0.65 N=20	5.00 ±0.51 N=18	5.89 ±0.62 N=19
No. Male Fetuses with Variations per Litter[c,e]	1.56 ±0.26 N=18	1.75 ±0.42 N=20	2.39 ±0.38 N=18	3.26 ±0.36 N=19
No. Female Fetuses with Variations per Litter[c,e]	2.21 ±0.41 N=19	2.25 ±0.45 N=20	2.61 ±0.30 N=18	2.78 ±0.43 N=18

(continued)

Table 21. (continued)

Summary and Statistical Analysis of Malformations and Variations (page 4 of 4)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
Percent Fetuses with Variations per Litter[c,e]				
	47.87 ‡ ± 5.56 §§ N=19	49.29 ± 7.20 N=20	63.81 ± 4.70 N=18	68.10 * ± 4.74 N=19
Percent Male Fetuses with Variations per Litter[c,e]				
	42.70 ‡ ± 7.00 §§ N=18	52.58 ± 7.72 N=20	58.65 * ± 6.88 N=18	71.82 ** ± 5.36 N=19
Percent Female Fetuses with Variations per Litter[c,e]				
	55.16 ± 6.66 N=19	50.78 ± 9.36 N=20	65.83 ± 6.28 N=18	66.15 ± 7.72 N=18
Repeated Measures for Percent Fetuses with Variations per Litter: Bartlett's (p=0.2913); DOSE (p=0.1190); SEX (p=0.5652); DOSExSEX (p=0.5395).				
No. Fetuses with Variations[c]				
	70	80	90	112
% Fetuses with Variations[c]				
	48.61	51.95	65.22	69.57
No. Litters with Variations[d]				
	18	19	18	19
% Litters with Variations[d]				
	94.74	95.00	100.00	100.00

a Only live fetuses were examined for malformations and variations.
b Includes only litters with live fetuses.
c Fetuses with one or more malformations or variations.
d Litters with one or more fetuses with malformations or variations.
e Reported as the mean ± S.E.M.
f Zero variance in one or more groups - test not done.
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.
§§ $p<0.01$; Test for Linear Trend.
* $p<0.05$; Dunnett's Test.
** $p<0.01$; Dunnett's Test.

Table 22.

Summary of Morphological Abnormalities in New Zealand White Rabbit Fetuses: Listing by Defect Type[a] (page 1 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
ANY MALFORMATIONS				
Total No. of Fetuses Examined for Any Malformations[b]	144	154	138	161
No. of Fetuses with Any Malformations[c]	4	4	0	5
% Fetuses with Any Malformations	2.8%	2.6%	0.0%	3.1%
Total No. of Litters Examined for Any Malformations[d]	19	20	18	19
No. of Litters with Any Malformations[e]	4	3	0	5
% Litters with Any Malformations	21.1%	15.0%	0.0%	26.3%
EXTERNAL MALFORMATIONS				
Total No. of Fetuses Examined for External Malformations[b]	144	154	138	161
No. of Fetuses with External Malformations[c]	1	1	0	0
% Fetuses with External Malformations	0.7%	0.6%	0.0%	0.0%
Total No. of Litters Examined for External Malformations[d]	19	20	18	19
No. of Litters with External Malformations[e]	1	1	0	0
% Litters with External Malformations	5.3%	5.0%	0.0%	0.0%
Anencephaly		1(1)		
Anophthalmia: Left		1(1)		
Lower Jaw Misshapened		1(1)		
Microtia		1(1)		
Athinia		1(1)		
Omphalocele	1(1)			
Adactyly: Forepaw and/or Hindpaw		1(1)		
Micromelia		1(1)		
Webbing of Upper Portion of Hindlimbs to Body		1(1)		
VISCERAL MALFORMATIONS				
Total No. of Fetuses Examined for Visceral Malformations[b]	144	154	138	161
No. of Fetuses with Visceral Malformations[c]	0	0	0	0
% Fetuses with Visceral Malformations	0.0%	0.0%	0.0%	0.0%
Total No. of Litters Examined for Visceral Malformations[d]	19	20	18	19
No. of Litters with Visceral Malformations[e]	0	0	0	0
% Litters with Visceral Malformations	0.0%	0.0%	0.0%	0.0%

(continued)

Table 22. (continued)

Summary of Morphological Abnormalities in New Zealand White Rabbit Fetuses: Listing by Defect Type[a] (page 2 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
SKELETAL MALFORMATIONS				
Total No. of Fetuses Examined for Skeletal Malformations[b]	138[f]	147[g]	129[h]	160[i]
No. of Fetuses with Skeletal Malformations[c]	4	4	0	5
% Fetuses with Skeletal Malformations	2.9%	2.7%	0.0%	3.1%
Total No. of Litters Examined for Skeletal Malformations[d]	19	20	18	19
No. of Litters with Skeletal Malformations[e]	4	3	0	5
% Litters with Skeletal Malformations	21.1%	15.0%	0.0%	26.3%
Fused Sternebrae: Cartilage Normal	3(3)	4(3)		5(5)
Branched Rib		1(1)		
Fused Ribs	2(2)			
Fused Centra: Thoracic	1(1)			
Misaligned Centrum: Thoracic	1(1)			
Bipartite Cartilage, Normal Ossification Center: Thoracic Centrum	1(1)			
Unilateral Cartilage, Unilateral Ossification Center: Thoracic Centrum	1(1)			
Fused Cartilage: Thoracic Centrum	1(1)			
Small Radius		1(1)		
Small Ulna		1(1)		
ANY VARIATIONS				
Total No. of Fetuses Examined for Any Variations[b]	144	154	138	161
No. of Fetuses with Any Variations[c]	70	80	90	112
% Fetuses with Any Variations	48.6%	51.9%	65.2%	69.6%
Total No. of Litters Examined for Any Variations[d]	19	20	18	19
No. of Litters with Any Variations[e]	18	19	18	19
% Litters with Any Variations	94.7%	95.0%	100.0%	100.0%
EXTERNAL VARIATIONS				
Total No. of Fetuses Examined for External Variations[b]	144	154	138	161
No. of Fetuses with External Variations[c]	0	1	0	0
% Fetuses with External Variations	0.0%	0.6%	0.0%	0.0%
Total No. of Litters Examined for External Variations[d]	19	20	18	19
No. of Litters with External Variations[e]	0	1	0	0
% Litters with External Variations	0.0%	5.0%	0.0%	0.0%
Clubbed Limb without Bone Change		1(1)		

(continued)

Table 22. (continued)

Summary of Morphological Abnormalities in New Zealand White Rabbit Fetuses: Listing by Defect Type[a] (page 3 of 3)

	UT-15 (ng/kg/min, subcutaneous)			
	0	50	150	300
VISCERAL VARIATIONS				
Total No. of Fetuses Examined for Visceral Variations[b]	144	154	138	161
No. of Fetuses with Visceral Variations[c]	21	24	22	25
% Fetuses with Visceral Variations	14.6%	15.6%	15.9%	15.5%
Total No. of Litters Examined for Visceral Variations[d]	19	20	18	19
No. of Litters with Visceral Variations[e]	15	14	10	15
% Litters with Visceral Variations	78.9%	70.0%	55.6%	78.9%
Abnormal Number of Papillary Muscles	19(14)	19(11)	21(9)	23(14)
Bifurcated Papillary Muscle(s)		2(2)		
Enlarged Papillary Muscles(s)	1(1)	1(1)		
Gall Bladder Half Normal Size		2(2)		1(1)
Round Gall Bladder	1(1)	1(1)	1(1)	1(1)
SKELETAL VARIATIONS				
Total No. of Fetuses Examined for Skeletal Variations[b]	138[f]	147[g]	129[h]	160[i]
No. of Fetuses with Skeletal Variations[c]	52	65	83	100
% Fetuses with Skeletal Variations	37.7%	44.2%	64.3%	62.5%
Total No. of Litters Examined for Skeletal Variations[d]	19	20	18	19
No. of Litters with Skeletal Variations[e]	17	15	18	19
% Litters with Skeletal Variations	89.5%	75.0%	100.0%	100.0%
Rib on Lumbar I: Bilateral Full	30(11)	39(12)	65(17)	79(18)
Left Full	6(4)	6(3)	2(2)	10(8)
Right Full	5(4)	7(3)	4(4)	2(2)
Bilateral Rudimentary	6(5)	11(7)	5(4)	2(2)
Left Rudimentary	5(4)	1(1)	6(5)	3(3)
Right Rudimentary	6(5)	4(3)	2(1)	12(7)
Normal Cartilage, Bipartite Ossification Center: Thoracic Centrum		2(2)		1(1)

[a] A single fetus may be represented more than once in listing individual defects. Data are presented as the number of fetuses (number of litters).
[b] Only live fetuses were examined.
[c] Fetuses with one or more external malformations/variations.
[d] Includes only litters with live fetuses.
[e] Litters with one or more externally malformed/variant fetuses.
[f] Six fetuses did not stain properly and therefore skeletal evaluations could not be done.
[g] Seven fetuses did not stain properly and therefore skeletal evaluations could not be done.
[h] Nine fetuses did not stain properly and therefore skeletal evaluations could not be done.
[i] One fetus did not stain properly and therefore a skeletal evaluation could not be done.