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Plasma concentrations of SDZ HTF 919 in rat during a lifespan  
oral (diet) oncogenicity study Week 72

Gender	M	F	M	F	M	F
Dose mg/kg/day	20	20	80	80	180	180
Time (h)	Plasma concentrations (ng/ml) : mean ± SD					
0	4.09 ± 1.56	#	82.05 ± 21.52	83.85 ± 25.35	220.83 ± 38.24	187.45 ± 76.46
6	#	1.16 *	103.94 ± 39.23	66.27 ± 20.45	155.25 ± 23.91	164.01 ± 29.90
12	#	#	86.70 ± 22.54	23.09 ± 3.48	148.23 ± 43.56	81.21 ± 30.56
18	5.49 ± 2.41	#	40.78 ± 12.05	21.74 ± 5.37	119.31 ± 29.49	107.49 ± 42.21
AUC [0-24h] (ng.h/ml)	115.00		1760.76	1049.70	3890.48	3240.90
AUC [0-24h]/Dose (ng.h/ml)/(mg/kg)	5.75		22.01	13.12	21.45	18.01

# : no data  
\* : n=1

Re : Tables A10, A11, A12

Table 3

Plasma concentrations of SDZ HTF 919 in rat during a lifespan  
oral (diet) oncogenicity study Week 104

Gender	M	F	M	F	M	F
Dose mg/kg/day	20	20	80	80	180	180
Time (h)	Plasma concentrations (ng/ml) : mean ± SD					
0	7.36 ± 0.79	2.72 ± 2.02	84.34 ± 21.18	77.48 ± 57.86	233.99 ± 60.88	226.82 ± 80.31
6	10.37 *	1.32 *	104.91 ± 20.17	54.21 ± 26.06	188.59 ± 57.28	138.51 ± 12.71
12	5.83 ± 1.20	3.13 ± 1.55	37.02 ± 16.31	20.86 ± 8.62	112.59 ± 28.43	52.32 ± 5.40
18	3.05 ± 2.70	1.56 ± 0.50	43.03 ± 17.94	28.85 ± 16.08	106.15 ± 51.08	70.03 ± 9.57
AUC [0-24h] (ng.h/ml)	169.81	52.34	1615.74	1067.17	3659.29	2928.65
AUC [0-24h]/Dose (ng.h/ml)/(mg/kg)	7.99	2.62	20.20	13.39	21.44	16.28

\* : n=1

Re : Tables A14, A15, A16

Table 4

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## Plasma concentrations of SDZ HTF 919 in rat during a lifespan

oral (diet) oncogenicity study - Week 111

Gender	F	F	F
Dose mg/kg/day	20	60	180
Time (h)	Plasma concentrations (ng/ml) : mean ± SD		
0	1.57 ± 2.72	41.30 ± 20.64	150.74 ± 31.66
6	2.66 ± 0.66	33.66 ± 25.66	96.35 ± 0.28
12	1.89 ± 0.63	42.72 ± 4.57	49.07 ± 6.58
18	5.55 ± 5.00	28.50 ± 5.21	57.14 ± 19.05
AUC [0-24h] (ng.h/ml)	71.24	678.19	2119.78
AUC [0-24h]/Dose (ng.h/ml)/(mg/kg)	3.56	10.96	11.76

Re : Tables A16, A19, A20

Table 5

## Plasma concentrations of SDZ HTF 919 in rat during a lifespan

oral (diet) oncogenicity study - Week 124

Gender	M	M	M
Dose mg/kg/day	20	60	180
Time (h)	Plasma concentrations (ng/ml) : mean ± SD		
0	4.05 ± 4.02	47.67 ± 23.06	250.74 ± 54.41
6	4.89 *	36.46 ± 19.88	182.88 ± 57.49
12	6.29 ± 5.58	30.56 ± 9.06	90.96 ± 30.42
18	2.84 *	36.66 ± 27.14	115.47 ± 62.93
AUC [0-24h] (ng.h/ml)	107.20	920.18	3720.67
AUC [0-24h]/Dose (ng.h/ml)/(mg/kg)	5.36	11.50	20.67

\* : n=1

Re : Tables : A22, A23, A24

Table 6

The ratio of AUC values of rat (\_\_\_\_\_ ng.h/ml at 180 mg/kg/day at 4 weeks) to human (20.1 ng.h/ml at 12 mg/day) was \_\_\_\_\_

In summary, in the dietary carcinogenicity study in rats, SDZ HTF 919 was given to rats in diet at 0, 20, 80 and 180 mg/kg/day for 110 weeks (females) or 124 weeks (males). The dose selection was adequate based on findings in the 26-week dietary toxicity study in rats (395R). In this study, high dose of 240 mg/kg/day was above MTD and the dose of 180 mg/kg/day was then selected as the high dose in the carcinogenicity study. In the current study, the terminal body weight was 96.5, 88.6 and 75.5% (males) or 93, 86 and 72% (females) of the control in the low, mid and high dose groups, respectively, suggesting that the high dose of 180 mg/kg/day exceeded MTD. The food consumption was slightly lower (11-12%) in the high dose group as compared the control. The mucosal hyperplasia in the small intestine was found in 2 control males and 5 high dose animals (4 males and 1 female). In the original report, the incidence of ovarian cysts (bursal, follicular and luteal) was significantly increased in the treated females as compared to the concurrent control. Subsequent evaluation did not reveal any treatment related increase in the incidence of ovarian cysts. There were no clearly treatment related increases in the tumor incidences. The study is acceptable.

**APPEARS THIS WAY  
ON ORIGINAL**

REPRODUCTIVE TOXICITY:

An Oral Segment I fertility and general reproductive performance  
study in male and female rats  
(Study 3021R)

Testing Laboratory: Sponsor's laboratory

Study Start and Completion Dates: March 2, 1993 and May 20, 1994

GLP and OAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Males (314-393 g, 11-12 weeks old),  
Females (194-266 g, 11-12 weeks old)  
Wistar rats (HanIbm:WIST)

Batch No.: 91903

Methods: To study the potential effects of HTF 919 on the fertility and general reproductive performance in rats, HTF 919 was given by oral gavage to male (15/group) and female (15 or 22/group) rats at 0, 6, 20 and 60 mg/kg/day. The dose selection was based on the findings in a dose ranging study (SANDOZ 6003R). In this study, HTF 919 (Batch #91903) was given to rats by oral gavage at 10, 30 and 100 mg/kg/day. HTF 919 was toxic and lethal to the parental animals at all dose levels. Body weights and food consumption were impaired at 30 and 100 mg/kg/day. The prenatal and postnatal loss were significantly increased (~21-24%) compared to the control (~1-7%). The F1 pup body weight gain was impaired (~20%) in the 100 mg/kg/day group compared to the control. Based on these findings, doses of 6, 20 and 60 mg/kg/day were chosen. Male rats (15/group) were treated for 9 weeks prior to mating and during mating (5 weeks) until all females had delivered. Female rats were treated for 2 weeks prior to mating and the treatment was continued up to gestation day 20 (22 dams/group) or up to lactation day 20 (15 dams/group). Male rats and the dams were necropsied at terminations. All animals were observed daily for clinical signs of toxicity and mortality. Body weights and food consumption were determined. The F1 pups were tested for physical and functional development. One male and one female of the F1 pups per litter were kept and used for the F1 fertility study. The toxicokinetic profile was determined in male rats after 12 weeks of the treatment and in

non-pregnant female rats at the end of the day 14 of pre-mating treatment. In pregnant rats, the toxicokinetic profile was determined on gestation day 20 and in their live fetuses.

Results: HTF 919 was lethal to the parental animals at all dose levels. Total 7, 4 and 21 rats either died or had to be sacrificed in moribund condition in the low, mid and high dose groups, respectively. Among these dead animals, there were 3, 4, and 9 males in the low, mid, and high dose groups, respectively. There were additional 6, 8 and 7 rats that died due to incorrect gastric intubation in the low, mid and high dose groups, respectively. Of these dead animals, there were 3, 1, and 3 males in the low, mid, and high dose groups, respectively. There was a total of 52 animals in each treatment group at start. There were no deaths in the control group. Piloerection, hypersalivation, tremor, swollen abdomens (high dose only) and respiratory difficulties (labored breathing and noisy respiration) were observed mainly in the mid and high dose groups. The total body weight gains of the male rats were -25%, 52% and 56% lower in the low, mid and high dose groups, respectively, than in the control. Food consumption from days 1 to 63 after dosing was significantly reduced in the mid (~7%) and high dose (~15%) male groups compared to the control. In the animals dead or sacrificed, bloody foam, edema, congestion, discoloration and inflammation were found in trachea and lung at necropsy. A distended gastrointestinal track (stomach and intestines) with swollen abdomen was found in the high dose group at necropsy.

Male fertility and reproductive performance were not affected. In the females treated with HTF 919 up to gestation day 20, the mean numbers of corpora lutea, implantation and viable fetuses, pre- and postimplantation loss and fetal body weight were not significantly affected. In the females treated with HTF 919 up to lactation day 20, mean numbers of implantation and body weights at birth and at lactation day 21 were not significantly altered by the treatment of HTF 919. The pregnancy rate was decreased in the high dose group (73%) compared to the control (100%). Due to the mortality in the mid and high dose group, the gestation (females with live born pups/pregnant females) and parturition (females delivering/pregnant females) indices were lower in the mid and high dose groups than those in the control group. The mean numbers of pups delivered were not affected. Pre-perinatal loss was 14, 7, 22, and 12 in the control, low, mid, and high dose groups, respectively. The percentage of pre-perinatal loss was higher in the mid (17%) and

high (26%) dose groups than that in the control (8.5%). However, there were only 4 assessable pregnant females in the high dose group (15 in control group). The high percentage pre-perinatal loss may not have any biological significance. The litter size was slightly reduced in the mid (9.64) and high (8.75) dose groups compared to the control and low dose groups (10.1-12.0). Postnatal loss was comparable up to day 14 post partum in the control and treatment groups. However, all 8 pups (an entire litter) from a dam in high dose group were found dead and cannibalized on day 21 post partum. Due to the loss of these pups, postnatal loss (on day 21) was markedly increased in the high dose group. In the high dose group, gestation length was increased ~10 hours. The percentage of stillborn pups was increased in the high dose (10%) group (0% in the control). The results were summarized in the following table.

Parameters	Control	6 mg/kg/day	20 mg/kg/day	60 mg/kg/day
Male mating index (%) <sup>1</sup>	100	100	100	100
Male fertility index(%) <sup>2</sup>	100	91	91	100

Females treated up to gestation day 20

Female mating index(%) <sup>3</sup>	100	89	90	67
Pregnant rate (%) <sup>4</sup>	86	76	89	92
# Corpora lutea	13.3	12.7	13.5	13.0
# Implantation	10.9	10.6	11.8	10.6
Viable fetuses	10.5	10.5	11.5	10.0
Preimplantation loss %	17.3	16.6	12.6	21.0
Postimplantation loss %	4.0	1.3	1.9	4.9
Fetal body weight(g)	5.0	5.0	4.8	4.6

Females treated up to lactation day 20

Female mating index(%) <sup>3</sup>	100	92	87	85
Pregnant rate (%) <sup>4</sup>	100	92	92	73
gestation index (%) <sup>5</sup>	100	100	92	50
Parturition index (%) <sup>6</sup>	100	100	92	50
# Implantation	11.0	12.6	11.6	11.8
Litter size	10.1	12.0	9.6	8.8
Still born %	0.0	2.2	2.8	10
Prenatal loss (%)	14 (8.5)	7 (5.0)	22 (17.0)*	12 (26.0)**

Postnatal loss (%) <sup>7</sup>	2 (1.8)	0 (0.0)	1 (1.2)	8 (28.0)***
Body weight (g):				
at birth	6.3	6.1	6.3	5.9
at 21 days	50.4	48.3	50.2	49.7

\* = P < 0.05, \*\* = P < 0.01, \*\*\* = P < 0.001

1 = males inseminated females/males placed with females

2 = females pregnant/males placed with females

3 = females inseminated/females placed with males

4 = females pregnant/females inseminated

5 = females with live born pups/pregnant females

6 = females delivering/pregnant females

7 = the increased postnatal loss was due to loss (dead and cannibalized) of an entire litter (5 pups) from a dam in high dose group on day 21.

F1 sex ratio, mean placental weights and F1 pup malformations, body weight gain and morphological and functional and behavioral development were not significantly affected in any of the groups. In the F1 fertility study, there was no parental toxicity observed in all treatment groups and the fertility and reproductive performance were not affected. There were no treatment related changes in F2 offspring.

In the male rats,  $C_{max}$ s of HTF 919 were 5.1, 29.3 and 29.4 ng/ml in the low, mid and high dose groups, respectively after 12 weeks of treatment.  $T_{max}$ s were  $1.1 \pm 1.0$  to  $2.8 \pm 1.5$  hours.  $AUC_{0-24hours}$  were  $169.1 \pm 85.6$  and  $396.6 \pm 202.7$  ng.h/ml in the mid and high dose groups, respectively. In the non-pregnant female rats,  $C_{max}$ s were 9.2, 15.4 and 24.5 ng/ml in the low, mid and high dose groups, respectively at 2.5 hours after dosing on day 14 of treatment. In the pregnant rats,  $C_{max}$ s were 6.8, 15.5, and 46.3 ng/ml in the low, mid and high dose groups, respectively on day 20 post coitus.  $T_{max}$ s were  $2.2 \pm 1.8$  to  $5.0 \pm 1.4$  hours.  $AUC_{0-6hours}$  were  $62 \pm 42.9$  and  $176.3 \pm 23.8$  ng.h/ml in the mid and high dose groups, respectively. Ratios of drug concentrations of the embryo to plasma at 6 hours after dosing were 0.3 to 0.7 in the mid and high dose groups, suggesting that this drug can be transferred through placenta.

In summary, in the Segment I fertility and reproductive performance study in rats, HTF 919 was given to rats by oral gavage at 0, 6, 20 and 60 mg/kg/day. Male rats were treated for 9 weeks prior to mating and during mating (5 weeks) until all females had delivered. Female rats were treated for 2 weeks prior to mating and the treatment was continued up to gestation day 20 or up to lactation day 20. Mortality to the parental animals following treatment with HTF 919 was unusually high at all dose levels. Both male and female fertility and reproductive

performance were not adversely affected. F1 fertility and reproductive performance were not impaired and there were no treatment related changes in F2 offspring.

Addendum: In a special toxicity study (study 7041R), the plasma levels of progesterone, estradiol, corticosterone, and prolactin were determined on day 21 post coitum in female rats following oral dose (gavage) of HTF 919 at 0, 6, 20, and 60 mg/kg/day from days 0 to 21 post coitum. HTF 919 was lethal at doses of 20 (1/15) and 60 (2/15) mg/kg/day. The plasma levels of progesterone, corticosterone, and prolactin were not affected. The plasma level of estradiol were slightly lower in the treatment groups ( $8.58 \pm 8.58$ ,  $5.89 \pm 4.73$ , and  $4.60 \pm 4.21$  pg/ml for the low, mid, and high dose groups, respectively) than that in the control group ( $14.94 \pm 11.35$  pg/ml).

In a separate study in female rats (7074R), the plasma levels of progesterone, estradiol, corticosterone, and prolactin were determined on days 3 and 10 post coitum following oral dose (gavage) of HTF 919 at 0, 6, 20, and 60 mg/kg/day 2 weeks before mating until days 10 post coitum (part 1) or from day 0 to day 10 post coitum (part 2). HTF 919 was lethal at 60 mg/kg/day (10/25). The plasma levels of progesterone, corticosterone, and estradiol were not affected. However, the plasma levels of prolactin were reduced on day 3 post coitum in all treatment groups but not on day 10 post coitum in part 1. In part 2, the plasma levels of prolactin were reduced on day 3 post coitum in all treatment groups and on day 10 post coitum in high dose group only. The plasma levels of prolactin on day 3 post coitum (part 1) were  $68.48 \pm 78.18$ ,  $33.58 \pm 56.09$ ,  $45.14 \pm 56.05$ , and  $42.55 \pm 39.48$  ng/ml for the control, low, mid, and high dose groups, respectively. The plasma levels of prolactin on day 3 post coitum (part 2) were  $85.41 \pm 69.87$ ,  $45.27 \pm 57.23$ ,  $25.54 \pm 26.28$ , and  $20.20 \pm 41.16$  ng/ml for the control, low, mid, and high dose groups, respectively. The plasma levels of prolactin on day 10 post coitum (part 2) were  $9.64 \pm 16.72$ ,  $17.14 \pm 34.87$ ,  $10.53 \pm 21.52$ , and  $4.37 \pm 6.18$  ng/ml for the control, low, mid, and high dose groups, respectively.

An Oral (in feed) Segment I fertility and general reproductive performance study in rats  
(Study 3043R)

Testing Laboratory: Sponsor's laboratory

Study Start and Completion Dates: June 23, 1994 and June 8, 1995

GLP and OAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Males (287-398 g, ~11 weeks old),  
Females (184-232 g, ~11 weeks old)  
Wistar rats (HanIbm:WIST)

Batch no: 94905

Methods: To study the potential effects of HTF 919 on the fertility and general reproductive performance in rats, HTF 919 was given in feed to male rats at 0, 60, 120, and 240 mg/kg/day or female rats at 0, 75, 150, and 300 mg/kg/day. Male rats (15/group) were treated for 9 weeks prior to mating and during mating until all females had delivered. Female rats (15/group) were treated for 2 weeks prior to mating and the treatment was continued up to gestation day 21 (prenatal part) or up to lactation day 21 (postnatal part). Male rats and the dams were necropsied at terminations. All animals were observed daily for clinical signs of toxicity and mortality. Body weight and food consumption were determined. The F1 pups were tested for physical and functional development. One male and one female of the F1 pups per litter were kept and used for the F1 fertility study. Blood samples were collected from F0 males at the end of mating period and satellite females on day 14 of treatment, day 20 post coitum, and day 14 post partum for determination of plasma level of the test drug.

Results: There were no deaths in this study. Piloerection was observed in one male at 120 mg/kg and all males at 240 mg/kg and one female at 300 mg/kg. Body weight gain was significantly decreased mainly in males. These results were summarized in tables on page 5-23 in volume 1.40. These tables are attached below.

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## RESULTS IN F0 MALES

Dose (mg/kg/d)	No. of males	Clinical signs	No. of males died	Food consumption (g) days 1 - 63	Body Weight gain (%)	
					days 1-8	days 1-64
Controls	15	0	0	1515	1.7	23.2
60	15	0	0	1530	2.9	26.2
120	15	1	0	1434	- 0.9	20.2
240	15	14#	0	1284#	-10.2#	11.5#

## RESULTS IN F0 FEMALES (Prenatal part)

Dose (mg/kg/d)	No. of females	Clinical signs	No. of females died	Body Weight gain in (%)	
				pre-mating days 1-15	pregnancy days 0-20
Controls	15	0	0	8.3	52.2
75	15	0	0	9.0	50.1
150	15	0	0	8.1	46.0
300	15	0	0	5.0	32.9#

## RESULTS IN F0 FEMALES (Postnatal part)

Dose (mg/kg/d)	No. of females	Clinical signs	No. of females died	Body Weight gain in (%)		
				pre-mating days 1-15	pregnancy days 0-20	lactation days 0-21
Controls	15	0	0	8.2	48.6	12.7
75	15	0	0	7.8	46.7	8.2
150	15	0	0	5.7	46.4	17.1
300	15	1	0	3.7*	37.4#	19.5

Food consumption was significantly reduced in high dose males (~15%) from days 1 to 63 after dosing and (5.5-23%) from days 85-113 as compared to the control. Food consumption were reduced in the mid (~7%) and high (21-25%) dose females as compared to the control during pre-mating and gestation.

Male fertility and reproductive performance were not affected. In the females of prenatal part, mean number of implantation was slightly reduced in the low and mid dose groups as compared to the control. However, mean number of implantation was comparable in the control, low, and mid dose groups in the postnatal part.

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Therefore, the reduced mean number of implantation in the low and mid dose groups in the prenatal part is not considered biologically significant. However, numbers of corpora lutea, implantations, and litter size were decreased and pre-implantation loss was increased in the high dose group. These results were summarized in tables on pages 5-23 and 5-24 in volume 1.40 and these tables are attached below.

## REPRODUCTIVE PERFORMANCE (Prenatal part)

Dose (mg/kg/d)	No. of males mating	No. of females mated	No. of females insemin.	No. of females pregnant	Mating period (days)
Controls	15	15	15	10	2.33
75	15	15	15	14	2.60
150	15	15	15	14	2.13
300	15	15	14	13	3.64

#= p<0.001

## REPRODUCTIVE PERFORMANCE (Postnatal part)

Dose (mg/kg/d)	No. of males mating	No. of females mated	No. of females insemin.	No. of females pregnant	Mating period (days)	Pregnancy length (days)
Controls	15	15	15	13	2.20	22.31
75	15	15	15	14	2.86	22.07
150	15	15	15	15	2.67	22.00
300	15	15	15	13	2.93	22.08

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## REPRODUCTIVE OUTCOME AND LITTER DATA (Prenatal part)

Dose (mg/kg/d)	No. of Corpora Lutea	No. of Implan- tations	Litter size	Pre- impl. loss (%)	Post- impl. loss (%)	Fetal body weight <sup>1</sup> (g)	Fetal incidences of	
							Retar- dations	Malfor- mations
mean values								
Controls	13.90	13.50	12.50	2.80	7.27	4.67	12/126	4/126
75	12.86	11.43	11.43	11.56	0.0*	4.89	14/160	5/160
150	13.21	10.86*	10.57	16.18	2.93	4.87	15/148	4/148
300	10.77**	8.54#	8.00#	20.53	4.80	4.76	17/104	5/104

In females of postnatal part, the postnatal loss was higher in the mid (7) and high (9) dose groups than that in the control and low dose groups (0). In the mid dose group, 5 pups were missing or cannibalized during the first 4 days post partum and 2 pups were lost during day 8 and 14 post partum. In the high dose group, all 8 pups from a dam were found dead, cannibalized, or missing during days 19-21 post partum. Therefore, the increased postnatal loss was not due to reproductive toxicity of the test drug. The number of implantation and litter size were slightly lower in the high dose group than those in the control. The mean fetal body weight at birth was not affected. The mean fetal body weight on 21 post partum was decreased in the mid and high dose groups. These results were presented in a table on page 5-25 in volume 1.40 and this table is attached below.

## REPRODUCTIVE OUTCOME AND LITTER DATA (Postnatal part)

Dose (mg/kg/d)	No. of Implan- tations	Litter size day 0 p.p.	Pre- and peri- natal Loss in %	Post- natal Loss in (%)	Body <sup>1</sup> weight (g) day 0 p.p. <sup>2</sup>	Body <sup>1</sup> weight (g) day 21 p.p.	Pup necropsy observ.	Phys., func- tional effects
Controls	12.00	11.08	7.7	0.0	6.11	45.74	1/121	40/98
75	11.29	10.29	8.9	0.0	5.94	43.57	0/117	40/94
150	12.80	11.60	9.4	5.9*	5.39*	40.23*	9/145	78/111
300	9.00*	9.42	3.4	9.9**	5.76	33.06#	5/87	54/82

<sup>1</sup> combined results of males and females

<sup>2</sup> after standardization

\* = p < 0.05

\*\* = p < 0.01

# = p < 0.001

F1 sex ratio, mean placental weight, F1 pup malformations, body weight gain and morphological and functional and behavioral development were not significantly affected in any of the groups. F1 fertility and reproductive performance were not affected. There were no treatment related changes in F2 offspring.

In male rats,  $AUC_{0-24\text{hours}}$  were 193, 612, and 1145 ng.h/ml in the low, mid, and high dose groups, respectively (after 12 weeks of treatment). In female rats,  $AUC_{0-24\text{hours}}$  were 210, 842, and 1645 ng.h/ml in the low, mid, and high dose groups, respectively (on day 14 of treatment).

In summary, in the oral (in feed) Segment I fertility and reproductive performance study in rats, HTF 919 was given in feed to male rats at 0, 60, 120, and 240 mg/kg/day or female rats at 0, 75, 150, and 300 mg/kg/day. Male rats were treated for 9 weeks prior to mating and during mating until all females had delivered. Female rats were treated for 2 weeks prior to mating and the treatment was continued up to gestation day 21 (prenatal part) or up to lactation day 21 (postnatal part). HTF 919 produced maternal toxicity at mid and high doses as evidenced by decreased body weight (male) and retarded body weight gain. Male fertility and reproductive performance were not impaired. Female fertility and reproductive performance were not adversely affected at doses up to 150 mg/kg/day. Decrease in the mean number of implantation, litter size, and fetal body weight and increase in the pre-implantation loss were noted at maternal toxic dose of 300 mg/kg/day. HTF 919 was also toxic even at doses lower than 300 mg/kg/day as evidenced by decreased body weight gain at 240 mg/kg/day in the 26-week dietary toxicity study in rats and at 180 mg/kg/day in the 2-year dietary carcinogenicity study in rats. F1 fertility and reproductive performance were not affected and there were no treatment related changes in F2 offspring.

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A Segment II embryotoxicity study in rats with toxicokinetics and placental transfer (prenatal part)  
(Study 4001R)

Testing Laboratory: Sponsor's laboratory

Study Start and Completion Dates: March 3, 1992 and  
December 16, 1992

GLP and OAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Females (201-202 g, 12-13 weeks old)  
Wistar rats (HanIbm:WIST)

Methods: To determine the potential teratological effects of HTF 919 in rats, HTF 919 was given by oral gavage to pregnant rats at 10, 30 and 100 mg/kg/day during days 6 to 15 post coitus. All animals were observed daily for clinical signs of toxicity and mortality. Body weights and food consumption were determined. The pregnant rats were sacrificed on day 21 post coitus and examined together with their fetuses. The toxicokinetic profile was determined on the first and last days of treatment in the dam's plasma and placenta, and their live fetuses.

Results: HTF 919 was lethal to the dams at 30 and 100 mg/kg/day. Total 1 and 7 rats either died or had to be sacrificed in moribund condition in the 30 and 100 mg/kg group, respectively. Piloerection and respiratory distress (labored breathing, gasping, slowed breathing and noisy respiration) were observed mainly in the mid and high dose groups. Total body weight gain was lower in the mid (~35%) and high (~53%) dose groups than in the control group. Bleeding from eye (mid dose), nose and mouth (high dose) was observed. In the animals dead or sacrificed, liver and/or lung congestion and edema and a distended gastrointestinal track were found at necropsy. Histological examination revealed erosions/ulcers in the stomach wall in one animal in the mid dose group. The laryngitis was diagnosed in one animal in the high dose group.

Pregnancy rates were 100, 92, 100 and 88% in control, low, mid and high dose groups, respectively. Mean number of corpora lutea, implantation sites, pre- and postimplantation losses were normal. Fetal weight was not significantly altered.

Placental weight was significantly reduced in the mid (~14%) and high (~16%) dose groups. The results were summarized in the following table.

Parameters	Control	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Pregnant rate (%) <sup>1</sup>	100	92	100	88
# Corpora lutea	12.8	12.8	13.1	12.8
# Implantation	11.5	11.5	11.5	11.3
Viable fetuses	11.3	11.0	11.4	10.7
Preimplantation loss %	10.2	9.9	12.0	11.4
Postimplantation loss %	2.1	7.1	1.4	5.6
Fetal body weight (g)	4.8	4.8	4.5	4.6
Placental weight (g)	0.71	0.67	0.61**	0.59*

\* = P < 0.05, \*\* = P < 0.01

1 = females pregnant/females inseminated

There were no findings in the fetal visceral examinations. Cleft palate was identified in one fetus in the mid dose group. In the high dose group, 8 fetuses from same litter had fusion of vertebrae. Since it was found in one litter and at lethal dose (to dams), it is not considered to have any biological significance. Retardation of ossification was found in all groups including control. The frequencies were higher in the mid and high dose groups as compared to the control. The results were presented in Tables 10 and 12 on pages 215, 217, 218, 219, 220, 221, 222, 223, and 224 in volume 1.35. These tables are attached below.

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TABLE 10

SDZ MTP 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF FETAL EXTERNAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	274	192
Live Fet.	N	283	252	273	192
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	1	0
<b>WHOLE BODY</b>					
Litter Incidence	N	0	0	1	0
Fetal Incidence	N	0	0	1	0
<b>M RUST</b>					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.4	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	4.2	0.0
Affected Fetuses/Litter	MEAN <sup>†</sup>	0.00	0.00	0.32	0.00
	S.D.	0.00	0.00	1.57	0.00
<b>PALATE</b>					
Litter Incidence	N	0	0	1	0
Fetal Incidence	N	0	0	1	0
<b>M CLEFT</b>					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.4	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	4.2	0.0
Affected Fetuses/Litter	MEAN <sup>†</sup>	0.00	0.00	0.28	0.00
	S.D.	0.00	0.00	1.36	0.00

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U  
CLASS CODES: M=Malformation R=Retardation

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TABLE 12

SDX RTF 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	273	192
Live Fet.	N	283	252	273	192
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>SKELETON</b>					
Litter Incidence	N	3	2	2	4
Fetal Incidence	N	3	2	2	5
<b>M MULTIPLE DEFECTS: STERNEBRAE &amp; RIB CARTILAGES ASYMMETRICAL</b>					
Fetal Incidence	N	2 f	2	2	3
	%	0.7	0.8	0.7	1.6
Litter Incidence	N	2 f	2	2	2
	%	0.0	8.7	8.3	11
Affected Fetuses/Litter	MEAN <sup>u</sup>	0.64 u	0.88	0.84	1.70
	S.D.	2.22	3.00	2.94	5.06
<b>M MULTIPLE DEFECTS: VERTEBRAE RIB STERNEBRAE</b>					
Fetal Incidence	N	1 f	0	0	0
	%	0.4	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	4.0	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN <sup>u</sup>	0.36 u	0.00	0.00	0.00
	S.D.	1.82	0.00	0.00	0.00
<b>M MULTIPLE DEFECTS: VERTEBRAE RIB</b>					
Fetal Incidence	N	0 f	0	0	2
	%	0.0	0.0	0.0	1.0
Litter Incidence	N	0 f	0	0	2
	%	0.0	0.0	0.0	11
Affected Fetuses/Litter	MEAN <sup>u</sup>	0.00 u	0.00	0.00	1.02
	S.D.	0.00	0.00	0.00	2.98

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U  
CLASS CODES: N=Malformation R=Retardation

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TABLE  
PAGE

SDZ NTP 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	273	192
Live Fet.	N	283	252	273	192
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>HEAD</b>					
Litter Incidence	N	2	1	4	5
Fetal Incidence	N	4	2	4	11
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	4 f	2	4	11*
	%	1.4	0.8	1.5	5.7
Litter Incidence	N	2 f	1	4	5
	%	8.0	4.3	17	28
Affected Fetuses/Litter	MEAN†	1.40 u	0.87	1.46	5.71
	S.D.	5.61	4.17	3.36	11.01
<b>CERV. VERTEBRA</b>					
Litter Incidence	N	0	0	1	0
Fetal Incidence	N	0	0	1	0
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.4	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	4.2	0.0
Affected Fetuses/Litter	MEAN†	0.00 u	0.00	0.35	0.00
	S.D.	0.00	0.00	1.70	0.00
<b>CERV. VERT. BODY</b>					
Litter Incidence	N	6	4	9	8
Fetal Incidence	N	7	4	22	20

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U \* = p<0.05  
CLASS CODES: M=Malformation R=Retardation

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TABLE 12  
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SDS BTF 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	273	192
Live Fet.	N	283	252	273	192
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>R NOT OSSIFIED</b>					
Fetal Incidence	N	7 f	3	22**	20†
	%	2.5	1.2	8.1	10
Litter Incidence	N	6 f	3	9	8
	%	24	13	38	44
Affected Fetuses/Litter	MEAN%	2.12 u	1.37	8.17	10.10
	S.D.	4.14	3.66	19.49	17.29
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	0.4	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	4.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.00 u	0.33	0.00	0.00
	S.D.	0.00	1.60	0.00	0.00
<b>CERV. VERT. ARCH</b>					
Litter Incidence	N	0	0	2	0
Fetal Incidence	N	0	0	3	0
<b>M DISPLACED</b>					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.4	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	4.2	0.0
Affected Fetuses/Litter	MEAN%	0.00 u	0.00	0.35	0.00
	S.D.	0.00	0.00	1.70	0.00

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U \* = p<0.05 \*\* = p<0.01 † = p<0.001  
CLASS CODES: M=Malformation R=Retardation

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TABLE 12  
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SDZ NTF 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF PATAL SKELETAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	273	192
Live fet.	N	283	252	273	192
Dead fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.4	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	4.2	0.0
Affected Fetuses/Litter	MEAN <sup>u</sup>	0.00	0.00	0.32	0.00
	S.D.	0.00	0.00	1.57	0.00
<b>M FUSED</b>					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.4	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	4.2	0.0
Affected Fetuses/Litter	MEAN <sup>u</sup>	0.00	0.00	0.32	0.00
	S.D.	0.00	0.00	1.57	0.00
<b>THORACIC RIBS</b>					
Litter Incidence	N	6	3	7	6
Fetal Incidence	N	12	3	18	24
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	9 f	2	16	21 <sup>†</sup>
	%	3.2	0.8	5.9	11
Litter Incidence	N	5 f	2	6	6
	%	20	8.7	25	22
Affected Fetuses/Litter	MEAN <sup>u</sup>	3.04	0.73	7.15	9.48
	S.D.	6.94	2.43	16.25	21.97

Statistical key: f=Chi-square + Fishers exact test    u=Kruskal-Wallis + Mann-Whitney U    \* = p<0.05    † = p<0.001  
CLASS CODES: M=Malformation R=Retardation

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TABLE 12  
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SDE HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	273	192
Live Fet.	N	283	252	273	192
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>M FLYING</b>					
Fetal Incidence	N	0 f	0	1	1
	%	0.0	0.0	0.4	0.5
Litter Incidence	N	0 f	0	1	1
	%	0.0	0.0	4.2	5.6
Affected Fetuses/Litter	MEAN% S.D.	0.00 u 0.00	0.00 0.00	0.39 1.70	0.51 2.14
<b>M CARTILAGES ASYMMETRICAL</b>					
Fetal Incidence	N	3 f	1	1	2
	%	1.1	0.4	0.4	1.0
Litter Incidence	N	1 f	1	1	2
	%	4.0	4.3	4.2	11
Affected Fetuses/Litter	MEAN% S.D.	1.00 u 5.00	0.43 2.09	0.35 1.70	1.06 3.09
<b>TR. VERT. BODY</b>					
Litter Incidence	N	6	4	7	3
Fetal Incidence	N	11	4	8	3
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	11 f	4	8	3
	%	3.9	1.6	2.9	1.6
Litter Incidence	N	6 f	4	7	3
	%	24	17	29	17
Affected Fetuses/Litter	MEAN% S.D.	3.57 u 7.06	1.39 3.15	2.73 4.61	1.62 3.86

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U  
CLASS CODES: M=Malformation R=Retardation

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SDX MTF 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	273	192
Live Fet.	N	283	252	273	192
Dead Fet.	N	0	0	0	0
Lata. Res.	N	0	0	0	0
<b>TH. VERT. ARCH</b>					
Litter Incidence	N	0	0	1	0
Fetal Incidence	N	0	0	1	0
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.4	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	4.2	0.0
Affected Fetuses/Litter	MEAN± S.D.	0.00 u 0.00	0.00 0.00	0.32 1.57	0.00 0.00
<b>STERNUM</b>					
Litter Incidence	N	1	0	2	2
Fetal Incidence	N	1	0	2	8
<b>R STERNEBRA(E) RETARDED OSSIFICATION</b>					
Fetal Incidence	N	1 f	0	1	6*
	%	0.4	0.0	0.4	3.1
Litter Incidence	N	1 f	0	1	2
	%	4.0	0.0	4.2	11
Affected Fetuses/Litter	MEAN± S.D.	0.36 u 1.82	0.00 0.00	0.32 1.57	3.03 9.35
<b>M STERNEBRAE FUSED</b>					
Fetal Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	0.5
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	5.6
Affected Fetuses/Litter	MEAN± S.D.	0.00 u 0.00	0.00 0.00	0.00 0.00	0.51 2.14

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U \* = p<0.05  
CLASS CODES: M=Malformation R=Retardation

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TABLE 12  
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SDZ HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	273	192
Live Fet.	N	283	252	273	192
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>M STERNESRA(E) NOT OSSIFIED</b>					
Fetal Incidence	N	0 f	0	1	1
	%	0.0	0.0	0.4	0.5
Litter Incidence	N	0 f	0	1	1
	%	0.0	0.0	4.2	5.6
Affected Fetuses/Litter	MEAN	0.00 u	0.00	0.32	0.51
	S.D.	0.00	0.00	1.57	2.14
<b>LUMB. VERT. BODY</b>					
Litter Incidence	N	0	0	0	2
Fetal Incidence	N	0	0	0	5
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	0.5
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	5.6
Affected Fetuses/Litter	MEAN	0.00 u	0.00	0.00	0.69
	S.D.	0.00	0.00	0.00	1.95
<b>M FUSSES</b>					
Fetal Incidence	N	0 f	0	0	4*
	%	0.0	0.0	0.0	2.1
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	5.6
Affected Fetuses/Litter	MEAN	0.00 u	0.00	0.00	2.02
	S.D.	0.00	0.00	0.00	8.57

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U \* = p<0.05  
CLASS CODES: M=Malformation R=Retardation

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TABLE 12  
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SDS HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RAT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	10 MG/KG	30 MG/KG	100 MG/KG
Litters Evaluated	N	25	23	24	18
Fetuses Evaluated	N	283	252	273	192
Live Fet.	N	283	252	273	192
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>SAC. VERT. BODY</b>					
Litter Incidence	N	0	0	0	1
Fetal Incidence	N	0	0	0	3
<b>M FUSED</b>					
Fetal Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	1.6
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	5.6
Affected Fetuses/Litter	MEANS	0.00 u	0.00	0.00	1.52
	S.D.	0.00	0.00	0.00	6.43
<b>FORELIMB</b>					
Litter Incidence	N	11	7	12	10
Fetal Incidence	N	36	20	33	37
<b>M DIGITS RETARDED OSSIFICATION</b>					
Fetal Incidence	N	36 f	20	33	37
	%	13	7.9	12	19
Litter Incidence	N	11 f	7	12	10
	%	44	30	50	56
Affected Fetuses/Litter	MEANS	12.41 u	8.47	12.26	19.48
	S.D.	19.35	15.74	17.38	23.22

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U  
CLASS CODES: M=Malformation R=Retardation

On the first day of treatment,  $C_{max}$ s of HTF 919 were 23.5, 24 and 26.4 ng/ml in the low, mid and high dose groups, respectively.  $C_{max}$ s were 15.2, 51.7 and 48.2 ng/ml in the low, mid and high dose groups, respectively on day 15 post coitus.  $T_{max}$ s were 2.3 to 4.5 hours.  $AUC_{0-24hours}$  were 64.5, 202.6 and 294.2 ng.h/ml in the low, mid, and high dose groups, respectively on the first day of the treatment.  $AUC_{0-6hours}$  were 38.3, 76.7, and 114.2 ng.h/ml in the low, mid, and high dose groups, respectively on the first day of the treatment.  $AUC_{0-6hour}$  were 37.6, 149.1 and 210.9 ng.h/ml in the low, mid, and high dose groups, respectively on the last day of the treatment. Ratios of drug concentrations of the embryo to plasma at 6 hours after dosing were 0.3 and 0.9 in the mid and high dose groups, respectively, suggesting that this drug can be transferred through placenta.

In brief, in the Segment II reproductive study in rats, HTF 919 was given by oral gavage to pregnant rats at 0, 10, 30 and 100 mg/kg/day during days 6 to 15 post coitus. HTF 919 was lethal to the dams at  $\geq 30$  mg/kg/day. HTF 919 was not teratological in this study.

Addendum:

(1) In an embryotoxicity dose ranging study in rats (study #204/91), female rats (HanIbm:WIST) were treated with HTF 919 (batch # 91903) by oral gavage at 0, 30, 100, and 300 mg/kg/day from pregnant days 6 to 15. The results indicated that HTF 919 was lethal at high dose. Clinical signs of toxicity (dyspnea, piloerection, red/brown discoloration and/or wet fur of the nose, throat, and forepaws, weight loss, and pulmonary congestion) were noted in the mid and high dose groups. There were no treatment related changes observed in the low dose group.

(2) HTF 919 and its metabolites were determined in fetuses in pregnant rats following oral dose of <sup>14</sup>C-HTF 919 at 20 mg/kg given on day 13 or 17 of gestation (report R98-263). The results indicated that the maximal level of radioactivity in fetuses was 0.17 nmol/g detected at 6 hours after dosing and this was much lower than those identified in the maternal blood (1.53 nmol/g), kidney and intestine (15 nmol/g). The major metabolites identified in the fetal tissue were M38 and M29 which represents ~30% and 9% of the total radioactivity in the fetus, respectively. Another unknown metabolite accounted for 13% and the parent drug only for ~5% of the total radioactivity.

A Segment II embryotoxicity study in rabbits with toxicokinetics and placental transfer  
(Study 4007K)

Testing Laboratory: Sponsor's laboratory

Study Start and Completion Dates: May 5, 1992 and May 24, 1993

GLP and OAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Females (3638 - 3897 g, 18-24 weeks old)  
Chinchilla hybrid rabbits (chbb:CH)

Methods: To determine the potential teratological effects of HTF 919, HTF 919 was given by oral gavage to artificially inseminated rabbits at 30, 60 and 120 mg/kg/day during days 6 to 18 post insemination. The dose selection was based on a dose ranging study (SANDOZ 30291) in which HTF 919 at 200 mg/kg/day produced a continuous body weight loss and was lethal to the dams. All

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animals were observed daily for clinical signs of toxicity and mortality. Body weights were determined. The pregnant rabbits were sacrificed on day 29 post insemination and examined together with their fetuses. The toxicokinetic profile was determined on the first and last days of treatment in the pregnant rabbits and their live fetuses.

Results: There were no treatment related deaths. There were no treatment related clinical signs of toxicity. Body weight gains were about the same in the control and treatment groups. There were no treatment related pathological alterations.

There were no adverse effects on the mean numbers of corpora lutea, implantation sites and live embryos. The embryonic development was not impaired. The results were summarized in Tables 6 and 7 on pages 28, 29, and 30 in volume 1.36. These tables are attached below.

4007K

TABLE 6

SDZ HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RABBIT  
SUMMARY OF REPRODUCTION DATA

		CONTROL	30 MG/KG	60 MG/KG	120 MG/KG
No. of Assessable Females	N	12	12	9	12
- with viable fetuses	N	11	12	9	12
- with total fetal death	N	1	0	0	0
Corpora Lutea	TOTAL	148	178	112	154
no. per animal	MEAN	12.3 u	14.8	12.4	12.8
	S.D.	1.6	4.3	2.5	1.6
Implantation Sites	TOTAL	95	105	73	104
no. per animal	MEAN	7.9 u	8.8	8.1	8.7
	S.D.	4.1	3.6	1.7	3.4
Preimplantation Loss	TOTAL	53	73	39	50
% per animal	MEAN	36.8 u	40.3	32.1	30.0
	S.D.	30.2	25.8	20.6	29.7
Live Fetuses	TOTAL	81	36	65	93
no. per animal	MEAN	6.8 u	8.0	7.2	7.8
	S.D.	3.6	3.5	2.6	3.0
	MEAN	80.6 u	92.1	86.1	91.7
	S.D.	29.1	10.7	25.4	13.8
Males	TOTAL	36	46	35	41
no. per animal	MEAN	3.3 u	3.8	3.9	3.4
	S.D.	2.1	2.1	1.6	1.4
	MEAN	49.6 u	45.1	58.5	48.9
	S.D.	28.6	20.3	19.9	19.1
Females	TOTAL	38	50	30	52
no. per animal	MEAN	3.5 u	4.2	3.3	4.3
	S.D.	2.3	2.3	1.7	2.2
	MEAN	50.4 u	54.9	41.5	51.1
	S.D.	28.6	20.3	19.9	19.1

Statistical key: d-ANOVA + Dunnett-test u-Kruskal-Wallis + Mann-Whitney U

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TABLE 5  
PAGE 2

SDZ HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RABBIT  
SUMMARY OF REPRODUCTION DATA

		CONTROL	30 MG/KG	60 MG/KG	120 MG/KG
Postimplantation Loss no. per animal	TOTAL	14	9	8	11
	MEAN	1.2 u	0.8	0.9	0.9
	S.D.	1.3	1.0	1.3	1.5
% impl. per animal	MEAN%	19.4 u	7.9	13.9	8.3
	S.D.	29.1	10.7	25.4	13.8
Dead Fetuses no. per animal	TOTAL	0	0	1	0
	MEAN	0.0 u	0.0	0.1	0.0
	S.D.	0.0	0.0	0.3	0.0
% of impl. per animal	MEAN%	0.0 u	0.0	1.4	0.0
	S.D.	0.0	0.0	4.2	0.0
Resorptions: Early+Late no. per animal	TOTAL	14	9	7	11
	MEAN	1.2 u	0.8	0.8	0.9
	S.D.	1.3	1.0	1.3	1.5
% of impl. per animal	MEAN%	19.4 u	7.9	12.5	8.3
	S.D.	29.1	10.7	25.9	13.8
Resorptions: Early no. per animal	TOTAL	10	6	6	6
	MEAN	0.8 u	0.5	0.7	0.5
	S.D.	1.0	1.0	1.3	1.2
% of impl. per animal	MEAN%	16.0 u	5.3	11.1	4.9
	S.D.	28.2	11.0	26.2	11.5
Resorptions: Late no. per animal	TOTAL	4	3	1	5
	MEAN	0.3 u	0.3	0.1	0.4
	S.D.	0.7	0.5	0.3	1.2
% of impl. per animal	MEAN%	3.5 u	2.6	1.4	3.5
	S.D.	7.5	5.0	4.2	9.7

Statistical key: d-ANOVA + Dunnett-test u-Kruskal-Wallis + Mann-Whitney U

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TABLE 7

SDZ HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RABBIT  
SUMMARY OF PLACENTAL AND FETAL WEIGHTS

		CONTROL	30 MG/KG	60 MG/KG	120 MG/KG
<b>PLACENTAL WEIGHT UNITS: GRAM</b>					
of all Viable Fetuses	MEAN	8.3 u	7.7	8.0	7.7
	S.D.	1.2	1.4	1.7	1.5
	N	11	12	9	12
of Male Fetuses	MEAN	8.2 u	7.2	8.0	7.7
	S.D.	1.4	1.1	1.7	1.6
	N	10	11	9	12
of Female Fetuses	MEAN	8.1 u	7.7	7.5	7.3
	S.D.	1.0	1.5	0.7	0.9
	N	10	12	8	11
<b>FETAL WEIGHTS UNITS: GRAMS</b>					
of all Viable Fetuses	MEAN	44.2 u	41.1	42.3	41.8
	S.D.	6.0	7.2	6.0	7.0
	N	11	12	9	12
of Male Fetuses	MEAN	44.8 u	38.5	42.4	42.2
	S.D.	6.3	5.4	6.0	7.5
	N	10	11	9	12
of Female Fetuses	MEAN	43.1 u	41.3	40.4	40.1
	S.D.	4.6	7.5	3.1	4.4
	N	10	12	8	11

Statistical key: d-ANOVA + Dunnett-test u-Kruskal-Wallis + Mann-Whitney U

There were no findings in external and visceral examinations. There were no treatment related skeletal malformations. The results of fetal skeletal examination were presented in Table 11 on pages 34, 35, 36, and 37 in volume 1.36. These tables are attached below.

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TABLE 11

SDZ HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RABBIT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	30 MG/KG	60 MG/KG	120 MG/KG
Litters Evaluated	N	11	12	9	12
Fetuses Evaluated	N	81	96	65	93
Live Fet.	N	81	96	65	93
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>SKELETON</b>					
Litter Incidence	N	0	2	0	2
Fetal Incidence	N	0	2	0	2
<b>M MULTIPLE DEFECTS: VERTEBRAE RIB</b>					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	8.3	0.0	0.0
Affected Fetuses/Litter	MEAN <sup>†</sup>	0.0 u	1.4	0.0	0.0
	S.D.	0.0	4.8	0.0	0.0
<b>M MULTIPLE DEFECTS: STERNEBRAE &amp; RIB CARTILAGES ASYMMETRICAL</b>					
Fetal Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	1.1
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	8.3
Affected Fetuses/Litter	MEAN <sup>†</sup>	0.0 u	0.0	0.0	1.4
	S.D.	0.0	0.0	0.0	4.8
<b>M SCOLIOSIS</b>					
Fetal Incidence	N	0 f	1	0	1
	%	0.0	1.0	0.0	1.1
Litter Incidence	N	0 f	1	0	1
	%	0.0	8.3	0.0	8.3
Affected Fetuses/Litter	MEAN <sup>†</sup>	0.0 u	1.7	0.0	1.0
	S.D.	0.0	5.8	0.0	3.6

Statistical key: f=Chi-square + Fishers exact test u-Kruskal-Wallis + Mann-Whitney U  
CLASS CODES: M=Malformation R=Retardation

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TABLE 11  
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SDZ HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RABBIT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	30 MG/KG	60 MG/KG	120 MG/KG
Litters Evaluated	N	11	12	9	12
Fetuses Evaluated	N	81	96	65	93
Live Fet.	N	81	96	65	93
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>CERV. VERT. BODY</b>					
Litter Incidence	N	0	1	0	0
Fetal Incidence	N	0	2	0	0
<b>R RETARDED OSSIFICATION</b>					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	8.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0 u	0.7	0.0	0.0
	S.D.	0.0	2.4	0.0	0.0
<b>R NOT OSSIFIED</b>					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	8.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0 u	0.7	0.0	0.0
	S.D.	0.0	2.4	0.0	0.0
<b>STERNUM</b>					
Litter Incidence	N	1	3	3	4
Fetal Incidence	N	3	5	5	5
<b>R STERNEBRA(E) RETARDED OSSIFICATION</b>					
Fetal Incidence	N	3 f	5	5	5
	%	3.7	5.2	7.7	5.4
Litter Incidence	N	1 f	3	3	4
	%	9.1	25	33	33
Affected Fetuses/Litter	MEAN%	2.5 u	6.8	6.6	5.6
	S.D.	8.2	17.3	10.2	10.1

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U  
CLASS CODES: M-Malformation R-Retardation

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TABLE 11  
PAGE 3

SDZ HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RABBIT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	30 MG/KG	60 MG/KG	120 MG/KG
Litters Evaluated	N	11	12	9	12
Fetuses Evaluated	N	81	96	65	93
Live Fet.	N	81	96	65	93
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>CAUDAL VERTEBRA</b>					
Litter Incidence	N	2	0	0	0
Fetal Incidence	N	2	0	0	0
<b>M DISPLACED</b>					
Fetal Incidence	N	1 f	0	0	0
	%	1.2	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	9.1	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN <sup>u</sup>	0.8 u	0.0	0.0	0.0
	S.D.	2.7	0.0	0.0	0.0
<b>M HYPOPLASIA</b>					
Fetal Incidence	N	1 f	0	0	0
	%	1.2	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	9.1	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN <sup>u</sup>	1.0 u	0.0	0.0	0.0
	S.D.	3.4	0.0	0.0	0.0
<b>PELVIS</b>					
Litter Incidence	N	0	1	0	0
Fetal Incidence	N	0	1	0	0
<b>R OS PUBIS RETARDED OSSIFICATION</b>					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	8.3	0.0	0.0
Affected Fetuses/Litter	MEAN <sup>u</sup>	0.0 u	0.7	0.0	0.0
	S.D.	0.0	2.4	0.0	0.0

Statistical key: f-Chi-square + Fishers exact test u-Kruskal-Wallis + Mann-Whitney U  
CLASS CODES: M-Malformation R-Retardation

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TABLE 11  
PAGE 4

SDZ HTF 919  
ORAL EMBRYOTOXICITY STUDY  
RABBIT  
SUMMARY OF FETAL SKELETAL OBSERVATIONS

		CONTROL	30 MG/KG	60 MG/KG	120 MG/KG
Litters Evaluated	N	11	12	9	12
Fetuses Evaluated	N	81	96	65	93
Live Fet.	N	81	96	65	93
Dead Fet.	N	0	0	0	0
Late Res.	N	0	0	0	0
<b>HINDLIMB</b>					
Litter Incidence	N	0	1	0	0
Fetal Incidence	N	0	1	0	0
<b>R DIGITS RETARDED OSSIFICATION</b>					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	8.3	0.0	0.0
Affected Fetuses/Litter	MEAN <sup>±</sup> S.D.	0.0 u 0.0	0.7 2.4	0.0 0.0	0.0 0.3

Statistical key: f=Chi-square + Fishers exact test u=Kruskal-Wallis + Mann-Whitney U  
CLASS CODES: M=Malformation R=Retardation

On the first day of treatment (day 6 post insemination), the maximum plasma concentrations ( $C_{max}$ ) of HTF 919 were 16.3, 77.8 and 139.3 ng/ml in the low, mid and high dose groups, respectively.  $C_{max}$ s were 10.8, 74.8 and 214.9 ng/ml in the low, mid and high dose groups, respectively on the last day of treatment (day 18 post insemination).  $T_{max}$ s were 0.8 to 1.6 hours. Area under the curves ( $AUC_{0-6 \text{ hours}}$ ) were 61.3, 342.5 and 1014.5 ng.h/ml in the low, mid and high dose groups, respectively on the first day of the treatment.  $AUC_{0-6 \text{ hours}}$  were 32.0, 189.4, and 719.1 ng.h/ml in the low, mid and high dose groups, respectively on the last day of treatment. Ratios of drug concentrations of the embryo to plasma at 6 hours after dosing were 1.5 and 0.4 in the mid and high dose groups, respectively, suggesting that this drug can be transferred through placenta. The results were summarized in the following table.

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Parameters	30 mg/kg/day	60 mg/kg/day	120 mg/kg/day
C <sub>max</sub> , ng/ml on day 1	16.3	77.8	139.3
C <sub>max</sub> , ng/ml on day 18	10.8	74.8	214.9
T <sub>max</sub> , hr on day 1	0.8	1.4	1.5
T <sub>max</sub> , hr on day 18	1.6	1.3	0.8
AUC <sub>0-6 hr</sub> , ng.hr/ml on day 1	61.3	342.5	1014.5
AUC <sub>0-6 hr</sub> , ng.hr/ml on day 18	32.0	189.4	719.1

In summary, in the Segment II study in rabbits, HTF 919 was given orally to artificially inseminated rabbits at 30, 60 and 120 mg/kg/day during days 6 to 18 post insemination. HTF 919 was not teratologic at doses up to 120 mg/kg/day.

Addendum: The radioactivity was determined in fetuses in pregnant rabbits following oral dose of <sup>14</sup>C-HTF 919 at 120 mg/kg given on day 16 of gestation (report R98-023). The results indicated that the level of radioactivity in fetuses was \_\_\_\_\_ nmol/g detected at 24 hours after dosing and this was much lower than those identified in the maternal plasma (\_\_\_\_\_ nmol/g).

An Oral (in feed) Segment III Pre- and Post-natal Development study in rats  
(Study - 578/972144)

Testing Laboratory: [ ]

Study Start and Completion Dates: January 15, 1997 and - -  
February 16, 1998

GLP and OAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Females (189-238 g, 11-12 weeks old)  
Wistar rats (HanIbm:WIST)

Batch No: 94906

Methods: To determine the potential effects of HTF 919 on the pre- and post-natal development in rats, HTF 919 was given in

feed to female rats at 0, 75, 150, and 300 mg/kg/day from day 15 after mating to day 21 of lactation. The achieved doses were 80, 167, and 350 mg/kg/day from day 15 of gestation to day 13 of lactation. All animals were observed daily for clinical signs of toxicity and mortality. Body weight and food consumption were determined. All female rats were allowed to deliver naturally and to rear their offspring to weaning at day 21 of lactation. The offspring were examined for behavioral responses and neuromuscular function, and their growth. Plasma levels of test drug, estradiol, and prolactin were determined on day 20 after mating, on day 10 of lactation, and on day 21 of lactation. Plasma level of the test drug was also determined in F1 offspring at age of 21 days old.

Results: There were no treatment related clinical signs of toxicity and no treatment related deaths. One low dose and one mid dose animals were found dead and these were not considered treatment related. The body weight gain from gestation day 0 to day 21 was decreased in the treatment groups as compared to the control (-8.6%, -14%, and -34% in the low, mid, and high dose groups, respectively). The food consumption was not clearly difference between control and treatment groups during gestation days 0 to 19.

Gestation indices, general condition of offspring, litter size and survival, and sex ratio were not adversely affected. Higher number of females in high dose group had shorter gestation lengths. The body weight gain of the offspring was lower (10%) in the high dose group as compared to the control during 1 to 28 days old after cull. Physical development (pinna unfolding, hair growth, tooth eruption, eye opening, vaginal opening, balano-preputial separation), auditory and visual functions of the offspring were not adversely affected. The mean locomotor activity scores were slightly lower in the high dose females (1808) than that in the control (1998). This decrease in the locomotor activity scores was not seen in the low and mid dose groups nor in the high dose males. The learning ability (water maze performance) and neuromuscular function were not adversely affected. Mating and fertility performance of F1 generation were not adversely affected.

The results of plasma level were presented in Table 1 on page 381 in volume 1.44 and this table is attached below.

PLASMA CONCENTRATIONS OF SDZ HTF 919 IN FEMALE RATS  
DURING AN ORAL (IN FEED) PERI- AND POST- NATAL STUDY

Report 97 2144

0380

Dose mg/kg/day	75			150			300		
Day of sampling	Day 20 P.C.	Day 10 P.P.	Day 21 P.P.	Day 20 P.C.	Day 10 P.P.	Day 21 P.P.	Day 20 P.C.	Day 10 P.P.	Day 21 P.P.
Time (GMT) Plasma concentrations (ng/ml) : mean ± SD (n = 4)									
6:00 (T <sub>0</sub> )	18.93 ± 4.73	6.73 ± 1.71	5.59 ± 3.02	51.36 ± 13.44	35.22 ± 7.31	8.90 ± 2.87	69.79 ± 24.94	62.87 ± 12.35	46.12 ± 3.45
18:00 (T + 12 h)	12.79 ± 2.89	6.66 ± 1.16	-	36.08 ± 19.21	27.19 ± 15.18	-	42.82 ± 17.55	61.68 ± 23.03	-
AUC(0 - 24 h) (h.ng/ml)	380.6 ± 25.3	160.7 ± 11.9	-	1049.2 ± 345.6	749.0 ± 231.8	-	1351.3 ± 421.1	1494.7 ± 156.5	-
AUC/Dose h.ng/ml/mg	5.1 ± 0.3	2.7 ± 0.2	-	7.0 ± 2.3	5.0 ± 1.6	-	4.5 ± 1.4	5.0 ± 0.5	-

- no sampling  
P.C. : Post-Coitum  
P.P. : Post-Partum

Re: Tables A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>

In summary, in the Segment III study in rats, HTF 919 was given orally in feed to rats at 0, 0, 75, 150, and 300 mg/kg/day from day 15 after mating to day 21 of lactation. These rats were exposed to the test drug as evidenced by the detection of test drug in the plasma. Maternal body weight gain from gestation day 0 to day 21 was decreased in the treatment groups as compared to the control (-8.6%, -14%, and -34% in the low, mid, and high dose groups, respectively). The body weight gain of the offspring was slightly lower (10%) in the high dose group as compared to the control. No other adverse effects were observed.

GENETIC TOXICITY:

Mutagenicity evaluation using Salmonella Typhimurium  
(Mut.Bakt. 51/91)

Testing Laboratory: Sponsor's laboratory  
Dates Started and Completed: December 5, 1991 and August 20, 1992

Compliance with GLP and OAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Methods: To examine the potential mutagenic effects of HTF 919, the reverse mutation assay (Ames test) was conducted using the method in five strains salmonella typhimurium (TA1535, TA1537, TA1538, TA98, TA100) in the presence and absence of metabolic activation, S9 mix from rat liver. The following concentrations of HTF 919 were tested: 0.75, 2.5, 7.5, 12.5, 25, 37.5, 50, 62.5, 75, 87.5, 90, 100, 125 and 150 µg/plate in the presence or absence of S9. HTF 919 did not precipitate on the tester plates at the concentrations up to 150 µg/plate. HTF 919 was bacteriotoxic at 75 µg/plate for all strains except TA1538 in the absence of S9. Solvent and 6 positive controls (2-aminoanthracene, benzo(a)pyrene, nitrofluorene, N-Methyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine and 2-nitrofluorene) were also tested. The result was considered positive if the test substance induced two-fold or more increase in revertant colonies compared to the control.

Results: The results indicated that HTF 919 did not significantly increase the colonies except that HTF 919 at 75 µg/plate increased the colonies in strain of TA1538 in the absence of S9 (up to 2.1 times the control). The two fold increase in colonies in strain of TA1538 in the absence of S9 was found in 3 of the 4 experiments. The results were summarized in the following table.

Counts of colony in strain of TA1538 in the absence of S9

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Control	29 ± 0.7	30 ± 4.0	33 ± 1.0	29 ± 1.2
HTF 919 at 0.75 µg/plate	34 ± 0.0	-----	-----	-----
HTF 919 at 7.5 µg/plate	36 ± 0.7	35 ± 7.5	-----	-----
HTF 919 at 25 µg/plate	-----	36 ± 7.8	-----	30 ± 4.7
HTF 919 at 37.5 µg/plate	-----	-----	-----	26 ± 2.1
HTF 919 at 50 µg/plate	-----	-----	56 ± 11.3	40 ± 4.5
HTF 919 at 62.5 µg/plate	-----	-----	53 ± 9.0	44 ± 11.1
HTF 919 at 75 µg/plate	62 ± 7.1	61 ± 8.0	54 ± 6.7	58 ± 9.5
HTF 919 at 87.5 µg/plate	-----	-----	59 ± 15.3	47 ± 2.5
HTF 919 at 100 µg/plate	-----	-----	42 ± 5.4	57 ± 4.2
Nitrofluorene at 2 µg/plate	220 ± 39.3	223 ± 23.2	238 ± 13.1	200 ± 21.5

----- = The test drug was not tested at that concentration.

The positive control significantly increased the colonies compared to the solvent control. The increases ranged from ~1.9 fold in TA100 with S9 to ~23 fold in TA1538 with S9.

In conclusion, the results suggest that HTF 919 is frame shift mutagenic in this test system.



The positive control significantly increased the colonies compared to the solvent control. The increases ranged from ~4 fold in TA1537 without S9 to ~56 fold in TA98 with S9.

In conclusion, the results suggest that HTF 919 is not mutagenic in this test system.

Addendum: There were three Ames tests (batch control) which were not reviewed previously. These studies were briefly reviewed below.

(1) Ames test (Mut.Bakt.11/94): HTF 919 (Batch 94904) was tested for mutagenic potential in an Ames test at 2.3, 4.7, 9.4, 18.8, 37.6, and 75 µg/plate in the presence and absence of S9.

(2) Ames test (Mut.Bakt.22/94): HTF 919 (Batch 94905) was tested for mutagenic potential in an Ames test at 2.3, 4.7, 9.4, 18.8, 37.6, and 75 µg/plate in the presence and absence of S9.

(3) Ames test (Mut.Bakt.53/94): HTF 919 (Batch 94906) was tested for mutagenic potential in an Ames test at 1.17, 2.3, 4.7, 9.4, 18.8, 37.6, and 75 µg/plate in the presence and absence of S9.

These three Ames tests were conducted using \_\_\_\_\_ method in five strains of salmonella typhimurium (TA97a, TA98, TA100, TA102, and TA1535). The results of these studies indicated that HTF 919 was not mutagenic in these testing systems.

Evaluation of the induction of chromosomal aberrations using V79  
Chinese hamster cells  
(Z.29)

Testing Laboratory: Sponsor's laboratory

Dates Started and Completed: December 16, 1991 and  
September 9, 1992

Compliance with GLP and OAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Methods: To examine the potential induction of chromosomal aberrations by HTF 919, the *in vitro* chromosomal aberration test was conducted in V79 Chinese hamster cells (sponsor did not indicate the origin of the cells) in the presence and absence of metabolic activation, S9 mix from rat liver. The following concentrations of HTF 919 were tested: 3.0, 3.4, 4.0, 4.17, 4.6, 5.22 and 5.33 µg/ml without S9 and 40.0, 47.5, 55.0 and 62.5 µg/ml with S9. Cells were exposed to HTF 919 for ~3 hours. Positive controls (cyclophosphamide, 15 and 17.5 µM and ethyl methane sulphonated, 12.5 and 15 mM) were also tested. DMSO (1%) was served as vehicle control. Cells were arrested in metaphase using colcemid (0.15 µg/ml) ~2 hours before harvest. The chromosomal aberrations were observed and compared between the control and test groups.

Results: HTF 919 did not significantly increase the frequency of the chromosomal aberration in the V79 cells with or without metabolic activation. The positive controls, however, significantly increased the frequency of the chromosomal aberration in these cells.

In conclusion, the results suggest that HTF 919 is not inducer of chromosomal aberrations in this test system.

A forward mutation assay at HGPRT locus in V79 Chinese hamster cells  
(HV 5)

Testing Laboratory: Sponsor's laboratory

Dates Started and Completed: April 2, 1992 and June 24, 1993

Compliance with GLP and OAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Methods: To examine the mutagenic potential of HTF 919, the HGPRT test was conducted in Chinese hamster cells (sponsor did not indicate the origin of the cells) in the presence and absence of metabolic activation, S9 mix from rat liver. The following concentrations of HTF 919 were tested: 2.0, 2.27, 2.96, 3.3, 3.5, 3.85, 4.7, 5.0, 6.0 and 6.5 µg/ml without S9 and 30.0, 36.0, 39.0, 42.0, 45.0 and 48.0 µg/ml with S9. Cells were exposed to HTF 919 for at least 3 hours. Positive (methyl methanesulfonate,

90 µg/ml and aflatoxin B<sub>1</sub>, 0.8 µg/ml) and solvent controls were also tested. After treatment, cells were incubated for 6 days and then the mutant frequency was determined.

Results: HTF 919 did not significantly induce a dose-dependent increase in the mutant frequencies. The positive controls, however, significantly increased the frequency in these cells.

In conclusion, the results suggest that HTF 919 is not mutagenic in this test system.

Test for the induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures  
(UDS 1)

Testing Laboratory: Sponsor's laboratory

Dates Started and Completed: April 2, 1992 and November 19, 1992

Compliance with GLP and OAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Methods: To examine the potential effect of HTF 919 to induce DNA repair synthesis, the UDS test was conducted in rat hepatocyte primary culture. The following concentrations of HTF 919 were tested: 0.0128, 0.064, 0.32, 0.5, 1.0, 1.6, 2.0 and 4.0 µg/ml. Positive (2-acetylaminofluorene, 2.25 µg/ml and aflatoxin B<sub>1</sub>, 0.02 µg/ml) and solvent (DMSO, 1%) controls were also tested. <sup>3</sup>H-thymidine and HTF 919 were added to the hepatocyte cultures and then the cultures were incubated for 18-20 hours. The cells were then fixed, stained and the slides were prepared for latter evaluation. The DNA repair synthesis was quantified by determining the production of silver grains by the decay of <sup>3</sup>H-thymidine incorporated into DNA using \_\_\_\_\_

Results: HTF 919 did not significantly affect the net nuclear counts, nuclear counts and the percentage of nuclei in repair at concentrations up to 4 µg/ml. The positive controls, however, induced DNA repair in these cells.

In conclusion, the results suggest that HTF 919 is not mutagenic in this test system.

Mouse bone marrow micronucleus test by the oral route  
(MK 23)

Testing Laboratory: Sponsor's laboratory

Dates Started and Completed: November 26, 1993 and July 6, 1994

Compliance with GLP and OAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Male (28-35 g, 8 weeks old)  
Females (24-31 g, 8 weeks old)  
CD-1 mice (Cr1:CD-1(ICR)BR)

Methods: To examine the potential mutagenic effect of HTF 919, mouse bone marrow micronucleus test was conducted. HTF 919 was given to mice by oral gavage at 12.5, 40 and 125 mg/kg/day for 2 days. Positive (triethylenemelamine, 0.6 mg/kg) and negative (HPM-Cellulose 4T, 0.5%) controls were also tested. Mice were sacrificed 24 hours after the second dose of HTF 919 and bone marrow was collected. The frequency of micronucleated polychromatic erythrocytes was then determined. There was only one sampling time (24 hours after the second dose) in this study. According to the guideline (Guideline for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. Mutation Research 189:103-112, 1987), a minimum of 2 samples should be obtained between 20 and 48 hours after the last dose if two doses are given.

Results: HTF 919 did not significantly increase the frequency of micronucleated polychromatic erythrocytes compared to the control. The positive control, however, significantly increased it compared to the control.

In conclusion, the results suggest that HTF 919 is not mutagenic in this test system. This study was not adequately designed since there was only one sampling time (a minimum of two required). Whether this deficiency would affect the outcome of this study is not known.

LABELING:

The labeling is according to 21 CFR, Subpart B. The following revisions in the labeling are recommended.

1. Sponsor's Version:

**Carcinogenesis, Mutagenesis, Impairment of Fertility**

[ ]

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Evaluation: Systemic exposure data were not included.

Suggested Version:

[ ]

2. Sponsor's Version:

Pregnancy

*Pregnancy Category B*

[ ]

Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

[ ]

Evaluation: Systemic exposure data were not included. The statement of \_\_\_\_\_ should be removed entirely since Tegaserod hydrogen maleate is not intended to be used \_\_\_\_\_

Suggested Version: Pregnancy Category B: Reproduction studies have been performed in rats at oral doses up to 100 mg/kg/day

[ \_\_\_\_\_ and rabbits at oral doses up to 120 mg/kg/day ]

[ \_\_\_\_\_ and have revealed no evidence of impaired fertility or harm to the fetus due to tegaserod ]

[ \_\_\_\_\_ Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

### 3. Sponsor's Version:

#### Nursing Mothers

Tegaserod \_\_\_\_\_ is excreted in the milk of lactating rats with a high milk to plasma ratio. ]

Evaluation: Statements of the potential for serious adverse reactions in nursing infants and tumorigenic potential should be included.

Suggested Version: Tegaserod \_\_\_\_\_ and its metabolites are excreted in the milk of lactating rats with a high milk to plasma ratio. It is not known whether \_\_\_\_\_ is excreted in human milk. \_\_\_\_\_ many drugs are excreted in human milk, \_\_\_\_\_ the potential for serious adverse reactions in nursing infants \_\_\_\_\_

\_\_\_\_\_ potential for tumorigenicity shown for Tegaserod \_\_\_\_\_ in the mouse carcinogenicity study, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

#### SUMMARY AND EVALUATION:

Predominant symptoms of constipation-prone irritable bowel syndrome (IBS) include abdominal pain and discomfort, and



M29.0 (SDZ 244-120) has been identified in all species including mice, rats, dogs, and humans. M29.0 had very low affinity for 5-HT<sub>4</sub> receptors in calf ( $pK_D = 3.7$ ,  $pK_D$  for HTF 919 = 7.8) and human ( $pK_D = 4.1$ ,  $pK_D$  for HTF 919 = 7.7). The second pathway is the systemic metabolic pathway including a direct glucuronidation of HTF 919 to form three isomeric metabolites (M43.2, M43.8, and M45.3) in both rats and humans. The third pathway involves O-demethylation of HTF 919. The 5-O-demethyl derivatives include M52.8 (unconjugated 5-O-demethyl derivative) and M40.6 (conjugated 5-O-demethyl derivative) identified in mice, rats and dogs but not in humans. M52.8 (SDZ 216-454) is a potent 5-HT<sub>4</sub> receptor partial agonist with intrinsic activity of 0.6 (intrinsic activity of 5-HT and HTF 919 = 1 and 0.22, respectively). M52.8 is also more potent than HTF 919 for stimulating the longitudinal muscle movement in isolated guinea pig ileum ( $pEC_{50} = 9.02$  for SDZ 216-454 and 7.69 for HTF 919). The fourth pathway involves oxidation in the indole substructure followed by conjugation identified in rats only. Protein binding of HTF 919 was very high in all species tested including mice (96%), rats (97.8%), dogs (97.5%), and humans (98.2%). HTF 919 was extensively distributed in the extravascular compartments with volume of distribution of ~13 l/kg in mice, 17.5 l/kg in rats, 12.4 l/kg in dogs, and 7.4 l/kg in humans. HTF 919 was mainly excreted via the bile into feces in mice, rats, and dogs. HTF 919 and its metabolites are excreted into the milk in the lactating rats with a high milk to plasma ratio ranging from — to —

In the acute toxicity studies in mice, the minimal lethal doses were identified at 100 mg/kg (p.o.) in males or 5 mg/kg (i.v.). In the acute toxicity studies in rats, the minimal lethal i.v. dose was 5 mg/kg and there were no deaths at oral doses up to 2000 mg/kg. Sedation, dyspnea, muscular spasm, ventral recumbence and ruffled fur were observed in both mice and rats.

In the 17-day i.v. toxicity study in rats (— 087929), i.v. infusion of HTF 919 was given to rats for a period of 1 hour per day at 0.2 and 2.0 mg/kg/day for 17 days. Swelling, redness, and necrosis were observed at injection site (tail) in the high dose group. There was an increase in the hemopoietic activity in the red pulp in the spleen in the 2.0 mg/kg group. In the 2.0 mg/kg group, hemoglobin (12-20%), hematocrit (14% in males), mean cell hemoglobin concentration (6-7%) were significantly reduced and red blood cell distribution width (31-33%) and platelet counts (38-40%) were significantly increased.

In the 26-week oral toxicity study in rats (395R), HTF 919 was given to rats in feed at 0, 15, 60 and 240 mg/kg/day for 26 weeks. In this study, major treatment related changes were hair loss, decreased terminal body weight gains (22.4-36%), food consumption (11-24%), monocytes (males, 37%) and reticulocytes (males, 28-30%), increased platelet count (males, 20-23%) and histopathological changes (macro and micro vesicular vacuoles in the periportal locations of the liver) mainly in the high dose group. The MTD was identified between doses of 60 and 240 mg/kg/day. The liver was the target organ of toxicity.

In the 2-week i.v. toxicity study in dogs (338422), i.v. infusion of HTF 919 was given to dogs for a period of 1 hour per day at 0.1 and 1.0 mg/kg/day for 2 weeks. Tremor, salivation, and serous rhinorrhea were seen in all groups including control and placebo groups. Inflammation, hemorrhage, phlebitis, and thrombus formation in the subcutis at the infusion sites were observed in all groups with the more severe changes in the high dose group. The target organs of toxicity cannot be established.

In the 26 week oral toxicity study in dogs (SANDOZ 395D), HTF 919 was given orally to dogs at 5, 15, and 50/60 mg/kg/day for 26 weeks. Vomiting, diarrhea, and hypersalivation were observed mainly in the high dose group (50/60 mg/kg). There were no treatment related gross and microscopic pathological changes observed during the study. The gastrointestinal tract is the target organ of toxicity based on the clinical signs of toxicity (vomiting and diarrhea).

In the 52 week oral toxicity study in dogs (95/019/0592), HTF 919 was given to dogs at 0, 5, 15, and 60/70 mg/kg/day for 52 weeks. Major treatment related changes were mainly in the high dose group and these included clinical signs of toxicity (salivation), decreased terminal body weight gains (13-27%), and increased activities of alkaline phosphatase and glutamate dehydrogenase (56-100%).

In the 13-week oral dose ranging study in mice (177DFM), HTF 919 was given to mice in feed at 0, 150, 300, 600, and 900 mg/kg/day for males and 0, 300, 600, 900, and 1200 mg/kg/day for females for 13 weeks. High dose was lethal for both males and females. The major treatment related changes were clinical signs of toxicity (tremor, hunched position, pale extremities and ears, hair loss, rough coat, skin and tail necrosis, wet genital

region, and diarrhea), body weight loss, reduction of food consumption, increase in alanine aminotransferase, and atrophies of thymus and spleen in the high dose group. Since MTD can be identified between 600 and 900 mg/kg/day for males and between 900 and 1200 mg/kg/day for females, selection of doses of 60, 200, and 600 mg/kg/day for the 2-year carcinogenicity study in mice appears adequate. Subsequent evaluation revealed increased mucosal hyperplasia in the small intestine at doses of 300 mg/kg/day or higher.

In the 2-year dietary carcinogenicity study in mice (— 034/970331), CD-1 mice (60/sex/group) were treated with HTF 919 at 0, 60, 200 and 600 mg/kg/day for 2 years. Survival was not affected by the treatment. The terminal body weight was 96, 93 and 81.4% (males) or 94.3, 90.3 and 78% (females) of the control in the low, mid and high dose groups, respectively, suggesting that the high dose of 600 mg/kg/day exceeded MTD. The major treatment related non-neoplastic change was the mucosal hyperplasia in the small intestine in the high dose males (8/60) and females (7/60) (none in the control, low and mid dose groups). The treatment with HTF 919 produced adenocarcinoma in the small intestine in the high dose group (6 males and 2 females) (none in the control, low and mid dose groups). There was no background incidence of this tumor in the historical control data from studies in CD-1 mice conducted at the testing laboratory \_\_\_\_\_ during 1992-1995 and from \_\_\_\_\_ (Spontaneous neoplastic lesions in the Crl:CD-1 (ICR)BR mouse, \_\_\_\_\_ 1987). The high dose (600 mg/kg/day or 1800 mg/m<sup>2</sup>/day) is ~203 times the proposed clinical dose (12 mg/day or 0.24 mg/kg/day if 50 kg body weight assumed or 8.88 mg/m<sup>2</sup>/day). The AUC values (0-24 hours) of HTF 919 at week 4 were 83.2, 701.6, 1659.6 ng.h/ml (males) or 63.3, 481.6, and 2218.2 ng.h/ml (females) for the low, mid, and high dose groups, respectively. The ratio of AUC values (parent compound) of mouse \_\_\_\_\_ ng.h/ml at 200 mg/kg/day at week 4) to human (20.1 ng.h/ml at 12 mg/day) are \_\_\_\_\_. The ratio of AUC values (parent compound) of mouse (\_\_\_\_\_ ng.h/ml at 600 mg/kg/day at week 4) to human (20.1 ng.h/ml at 12 mg/day) are \_\_\_\_\_. This study is acceptable. In conclusion, therefore, HTF 919 produced adenocarcinoma in the small intestine at high dose of 600 mg/kg/day which is ~203 times the proposed clinical dose based on body surface area.

The results of a 13-week oral (in feed) exploratory toxicity study with HTF 919 at 200 and 600 mg/kg/day in male mice indicated that the increased mucosal hyperplasia in the small intestine was associated with decrease in diamine oxidase

activity, which is expected to increase polyamine concentrations. This is supposed to promote cell proliferation (Agents and Actions, 23 (3/4):354-356, 1988).

In the 2-year dietary carcinogenicity study in rats (029/970357), HTF 919 was given to HanIbm Wistar rats (50/sex/group) in diet at 0, 20, 80 and 180 mg/kg/day for 110 weeks (females) or 124 weeks (males). The dose selection was adequate based on findings in the 26-week dietary toxicity study in rats (395R). In this study, high dose of 240 mg/kg/day was above MTD and the dose of 180 mg/kg/day was then selected as the high dose in the carcinogenicity study. In the current study, the terminal body weight was 96.5, 88.6 and 75.5% (males) or 93, 86 and 72% (females) of the control in the low, mid and high dose groups, respectively, suggesting that the high dose of 180 mg/kg/day exceeded MTD. The food consumption was slightly lower (11-12%) in the high dose group as compared the control. The mucosal hyperplasia in the small intestine was found in 2 control males and 5 high dose animals (4 males and 1 female). In the original report, the incidence of ovarian cysts (bursal, follicular and luteal) was significantly increased in the treated females as compared to the concurrent control. Subsequent evaluation did not reveal any treatment related increase in the incidence of ovarian cysts. There were no clearly treatment related increases in the tumor incidences. The high dose of 180 mg/kg/day (1080 mg/m<sup>2</sup>/day) is ~122 times the recommended human dose (12 mg/kg or 0.24 mg/kg/day if 50 kg body weight assumed or 8.88 mg/m<sup>2</sup>/day). The AUC values (0-24 hours) of HTF 919 at week 4 were ~55, 608, 2235 ng.h/ml (males) or 60, 275, and 1869 ng.h/ml (females) for the low, mid, and high dose groups, respectively. The ratio of AUC values (parent compound) of rat            ng.h/ml at 180 mg/kg/day) to human (20.1 ng.h/ml at 12 mg/day) is           . The study is acceptable.

In the oral Segment I fertility and reproductive performance study in rats (3021R), HTF 919 was given to rats by oral gavage at 6, 20 and 60 mg/kg/day. HTF 919 was lethal to the parental animals at all doses. Both male and female fertility and reproductive performance were not adversely affected.

In the oral (in feed) Segment I fertility and reproductive performance study in rats (3043R), HTF 919 was given in feed to male rats at 0, 60, 120, and 240 mg/kg/day or female rats at 0, 75, 150, and 300 mg/kg/day. HTF 919 produced maternal toxicity at mid and high doses as evidenced by decreased body weight (male) and retarded body weight gain. Male fertility and

reproductive performance were not impaired. Female fertility and reproductive performance were not adversely affected at doses up to 150 mg/kg/day. Decrease in the mean number of implantation, litter size, and fetal body weight were noted at maternal toxic dose of 300 mg/kg/day. The  $AUC_{0-24\text{hours}}$  value (1645 ng.h/ml) of the parent compound following dose of 300 mg/kg/day in females is ~82 time the human AUC following the proposed clinical dose of 12 mg/day (20.1 ng.h/ml).

In the oral Segment II reproductive study in rats (4001R), HTF 919 was given by oral gavage to pregnant rats (25/group) at 10, 30 and 100 mg/kg/day during days 6 to 15 post coitus. HTF 919 was not teratological at doses up to high dose of 100 mg/kg/day in this study.

In the oral Segment II study in rabbits (4007K), HTF 919 was given orally to artificially inseminated rabbits at 0, 30, 60 and 120 mg/kg/day during days 6 to 18 post insemination. HTF 919 was not teratologic at doses up to 120 mg/kg/day in this study.

In the oral (in feed) Segment III study in rats (578/972144), HTF 919 was given orally in feed to rats at 0, 75, 150, and 300 mg/kg/day from day 15 after mating to day 21 of lactation. Maternal body weight gain from gestation day 0 to day 21 was decreased in the treatment groups as compared to the control (-8.6%, -14%, and -34% in the low, mid, and high dose groups, respectively). The body weight gain of the offspring was slightly lower (10%) in the high dose group as compared to the control. Physical and functional developments of the offspring were not adversely affected.

HTF 919 was not genotoxic in the *in vitro* chromosomal aberration test in Chinese hamster V79 cells, a forward mutation assay at HGPRT locus in V79 Chinese Hamster cells, mouse bone marrow micronucleus test, and unscheduled DNA synthesis (UDS) test in the rat hepatocytes. However, HTF 919 was positive in strain 1538 in an Ames test, suggesting that HTF 919 is frame shift mutagenic in this test system. However, the result was not reproducible. The result, therefore, is considered "equivocal".

The toxicity profiles of HTF 919 were studied in rats and dogs. Following target organs of toxicity were identified: liver (macro and micro vesicular vacuoles in the periportal location of the liver) in rats and gastrointestinal tract (vomiting and diarrhea) in dogs.



ATTACHMENTS :

APPENDIX I

Tumor and Non-Tumor Data for 2-year Dietary Carcinogenicity Study  
in Mice

APPENDIX II

Tumor and Non-Tumor Data for 2-year Dietary Carcinogenicity Study  
in Rats

CC:  
NDA  
HFD-180  
HFD-181/CSO  
HFD-180/Dr. Choudary  
HFD-180/Dr. Zhang  
HFD-345/Dr. Viswanathan

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APPENDIX I

Tumor and Non-tumor Data for 2-year Dietary Carcinogenicity Study  
in Mice  
— 034/970331)

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