

Fifty cells per treatment per animal are recommended for the UDS assay. The study design meets the standard procedure.

Study outcome: Results of the study showed there was no increase in the net grain in hepatocytes from TMX-67 treated rats. The positive control showed higher numbers of net grain compared to the untreated control. It was concluded that TMX-67 did not increase unscheduled DNA synthesis in the rat liver. Results are shown in the table below.

Group	Dose, mg/kg	Time after dosing, hr	Average net grain per nucleus
1	No treatment	0	-1.76
2	120	2	-1.39
3	120	16	-1.17
4	600	2	-1.14
5	600	16	-1.29
6 (DMN)	10	2	15.93
7 (DMN)	10	16	6.99

Study title: Mammalian bone marrow chromosome aberration test

Key findings: TMX-67 is not mutagenic in bone marrow cells in rats

Study no.: AA21UY.107.BTL

Volume # M4, 4.2.3.3.1.7, and page #: 1

Conducting laboratory and location: _____

b(4)

Date of study initiation: January 10, 2000

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #9128, and % purity: 100%

Methods

Strains/species/cell line: Male and female Sprague Dawley rats were used in the study. Separate groups of rats were used for toxicokinetics. The weight range for male rats was 232-276 g and female rats was 162-200 g. Rats were 6-8 weeks old at the beginning of the experiment.

Doses used in definitive study: The study design is shown in the table below. The drug substance was suspended in 0.5% methylcellulose for oral dosing. Clinical signs were recorded soon after the dosing and on each day till sacrifice.

Treatment	Rats/sex, 18 hr sacrifice	Rats/sex, 42 hr sacrifice	# animals/sex bled for TK
vehicle	5	5	3
TEI-6720, 75 mg/kg	5	5	10
TEI-6720, 150 mg/kg	5	5	10
TEI-6720, 300 mg/kg	5	5	10
TEI-6720, 450 mg/kg	5	5	10
Cyclophosphamide 30 mg/kg	5	5	

One rat/sex in the TK group was used for the replacement of any death. Five animals/sex were designated for replacement at 300 and 450 mg/kg for death before scheduled sacrifice. Blood samples were taken from 3 rats/sex per two time points. Samples were taken at 0.5, 1, 2, 4, 8 and 24 hours for TK analysis. Blood samples were collected from retro-orbital site. Dosing suspensions were within 90% of the nominal value.

Basis of dose selection: Doses were selected on the basis of acute toxicity data. All animals treated at 600 mg/kg in the acute toxicity study died. However, no mortality was noted at 300 mg/kg. However, decreased locomotor activity was observed at 300 mg/kg. Accordingly, 450 mg/kg was selected as the high dose.

Negative controls: 0.5% methylcellulose suspension

Positive controls: Cyclophosphamide at 30 mg/kg/oral

Incubation and sampling times: Animals were treated with colchicines at 1 mg/kg/ip 2-4 hours before the scheduled sacrifice to arrest the cell division at metaphase. Animals were sacrificed by CO₂ asphyxiation. Bone marrow from the femur was aspirated in to Hans' balance salt solution. Cells were treated with 0.075 M KCl (0.5%) for 10 minutes and fixed in methanol: glacial acetic acid at 3:1. Cell suspensions were dropped on to a glass slide, air dried and stained with 5% Giemsa stain before examining aberrations. At least one hundred metaphase cells per animal were scored. Chromatid or isochromatid gaps were recorded, however, these changes were not included in the analysis. Mitotic index was recorded by scoring 1000 cells per animal. The percentage of structural and numerical damage was calculated for each treatment and control groups.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The test article was considered positive if there was a dose dependent and statistically significant increase in the chromosomal aberration. A significant increase at the high dose only was considered to be suspected mutagen without a definitive outcome. However, the sponsor did not indicate whether the study would be repeated if a significant increase was observed only in the high dose. The test article was judged negative if no statistically significant increase in the number of aberrant cells was observed compared to the vehicle control group. The percent of aberrant cells higher than 2% without gap in the vehicle group was considered to be an invalid test. The positive control should have a statistically significant increase in aberration compared to the control.

Study outcome:

No mortality was observed in the TMX-67 treated animals. Lethargy and piloerection were noted in one male and two female rats at 450 mg/kg within 2 hours after dosing. The average body weight of treated rats was comparable to the control. No aberration was noted out of 500 cells scored per treatment group at the end of 18-hour treatment in male and female rats. Cyclophosphamide showed structural aberration in 7.4% and 11.4% cells in male and female rats, respectively at the end of 18 hours of treatment. The control animal also did not show any aberration. The mitotic index of TEI-6720 treated rats was inhibited by 76% and 52% in male and female rats, respectively at the end of 18-hour treatment at 450 mg/kg. The mitotic index was inhibited by 19% and 14% in male and female, respectively at 450 mg/kg at the end of 42 hours. The pharmacokinetic data showed increased exposure with the dose. The maximum concentration in the plasma was achieved within one hour of dosing. However, a secondary peak was observed for the plasma concentrations of TEI-6720 that was possibly due to enterohepatic circulation of the drug. The maximum elimination half life was 21.7 and 16 hours for male and female rats, respectively.

It is concluded that TEI-4720 was not mutagenic in the in vivo chromosomal aberration assay.

The chromosomal aberration data are shown in the table below.

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TABLE 5
Chromosomal Damage in Bone Marrow of Male Rats Following Treatment with TET-6720
Summary - 18 H

Treatment ¹	Time (H)	Total Number of Cells				Struct. Aberr. ² (%)	Number of Aberrations				
		Evaluated	Missed Index (%)	With Num. Aberr.	With Struct. Aberr.		Gaps	Breaks ³	Exchs	Severely Damaged Cells ⁴	Aberrations Per Cell ⁵
0.5% methylcellulose acetate solution	18	500	63.8	0	0	0.0	0	0	0	0	0.000 ± 0.000
TET-6720, 75 mg/kg	18	500	7.2	0	0	0.0	0	0	0	0	0.000 ± 0.000
150 mg/kg	18	500	7.1	0	0	0.0	0	0	0	0	0.000 ± 0.000
300 mg/kg	18	500	3.8	0	0	0.0	0	0	0	0	0.000 ± 0.000
450 mg/kg	18	500	2.6	NA							
CP, 30 mg/kg	18	500	4.2	0	**37	7.4	0	66	14	60	0.262 ± 0.112

¹Excluding gaps

²Including gaps; ** = p < 0.01 (Fisher's Exact Test).

³Includes chromosomal and chromosome breaks and fragments.

⁴Cell having at least 10 aberrations of any type, including pulverized chromosomes or cells.

NA = Numerical and structural aberrations not scored as per sponsor request

* The dose volume for all groups was 10 ml/kg

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TABLE 6
Chromosomal Damage in Bone Marrow of Female Rats Following Treatment with TEL-4720
Summary - 18 H

Treatment ^a	Time (Hr)	Total Number of Cells				Struct. Aberr. ² (%)	Number of Aberrations				Aberrations Per Cell ³
		Evaluated	Mitotic Index (%)	With Num. Aberr.	With Struct. Aberr.		Gaps	Breaks ¹	Exchs.	Severity Damaged Cells ⁴	
0.5% methylcellulose aqueous solution	18	500	6.8	0	0	0.0	0	0	0	0	0.000 ± 0.000
TEL-4720, 75 mg/kg	18	500	6.6	0	0	0.0	0	0	0	0	0.000 ± 0.000
150 mg/kg	18	500	5.7	0	0	0.0	0	0	0	0	0.000 ± 0.000
300 mg/kg	18	500	6.4	NA							
450 mg/kg	18	500	4.1	0	0	0.0	0	0	0	0	0.000 ± 0.000
CP, 30 mg/kg	18	500	2.4	0	**27	11.4	0	35	16	330	0.762 ± 0.532

¹Excluding gaps

²Excluding gaps; ** = p<0.01 (Fisher's Exact Test)

³Includes structural and chromosome breaks and fragments

⁴Cell having at least 10 aberrations of any type, including pulverized chromosomes or cells.

NA = Numerical and structural aberrations not scored as per Sponsor request.

^a The dose volume for all groups was 10 mL/kg.

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TABLE 9

Chromosomal Damage In Bone Marrow of Male Rats Following Treatment with TEI-6720
Summary - 43 H

Treatment ¹	Time (hr)	Evaluated	Total Number of Cells			Number of Aberrations					Aberrations Per Cell ²
			Mitotic Index (%)	With Num. Aberr.	With Struct. Aberr.	Struct. Aberr. ³ (%)	Gaps	Breaks ⁴	Exchs.	Severely Damaged Cells ⁵	
0.5% methylcellulose suspension solution	42	500	50	0	0	0.0	0	0	0	0	0.000 ± 0.000
TEI-6720, 500 mg/kg	42	500	56	0	0	0.0	0	0	0	0	0.000 ± 0.000
450 mg/kg	42	500	49	NA							

¹Excluding gaps

²Excluding gaps; ** = p<0.01 (Fisher's Exact Test)

³Includes chromatid and chromosome breaks and fragments.

⁴Cell having at least 10 aberrations of any type, including pulverized chromosomes or cells.

NA = Numerical and structural aberrations not scored as per Sponsor request.

* The dose volume for all groups was 10 mL/kg.

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TABLE 10
Chromosomal Damage in Bone Marrow of Female Rats Following Treatment with TEI-4720
Summary - 42 H

Treatment ¹	Time pHR	Total Number of Cells				Number of Aberrations					Aberrations Per Cell ²
		Evaluated	Mitotic Index (%)	With Num. Aberr.	With Struct. Aberr.	Struct. Aberr. ³ (%)	Gaps	Breaks ⁴	Exchs.	Severely Damaged Cells ⁵	
0.5% methylcellulose aqueous solution	42	500	5.0	0	0	0.0	0	0	0	0	0.000 ± 0.000
TEI-4720 100 mg/kg	42	500	3.7	NA							
450 mg/kg	42	500	4.3	0	0	0.0	0	0	0	0	0.000 ± 0.000

¹Excluding gaps
²Excluding gaps; ** = p<0.01 (Fisher's Exact Test).
³Includes chromatid and chromosome breaks and fragments.
⁴Cell having at least 10 aberrations of any type, including pulverized chromosomes or cells.
 NA = Numerical and structural aberrations not scored as per Sponsor request.
⁵The dose volume for all groups was 10 mL/kg.

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The toxicokinetics data are shown in the table below.

Gender	Dose (mg/kg)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC ₀₋₂₄ (ng·h/ml)	AUC ₀₋₄₈ (ng·h/ml)	$t_{1/2}$ (h)	C_{24} (ng/ml)	AUC ₀₋₄₈ (ng·h/ml)
Male	75	0.5	41.430	503.331	666.110	12.2	0.552	6.711
	150	1.0	86.831	882.364	901.377	3.9	0.578	5.882
	300	0.5	156.176	1433.698	1516.361	5.7	0.521	4.739
	450	0.5	200.187	1973.534	2653.694	21.7	0.449	4.386
Female	75	1.0	67.203	434.490	455.261	2.4	0.896	6.066
	150	1.0	108.272	911.743	925.452	3.7	0.688	6.078
	300	0.5	165.347	1182.915	1285.772	6.6	0.551	3.943
	450	0.5	156.715	1759.302	2678.834	18.0	0.548	3.910

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No chromosomal aberration was noted in male and female rats at the end of 42 hour treatment. The sponsor stated that the exposure was increased dose dependently in male and female rats.

2.6.6.5 Carcinogenicity

Rat study (Report # 4259-4260 in M4.2.3.4.1.2):

A two year carcinogenicity study was conducted at 3, 6, 12 and 24 mg/kg in F344 Fisher rats. The study was reviewed under IND 58,229 and results were presented to ECAC on March 16, 2004.

Male rats showed increased incidences of transitional cell papilloma and carcinoma in the urinary bladder at 24 mg/kg (16 times MRHD based on AUC). The tumorigenic effect on the urinary bladder was secondary to the calculus formation in the kidney and urinary bladder.

Increased size of thyroid was noted in the reproductive safety study in rats at 48 mg/kg. However, chronic treatment up to 24 mg/kg in the carcinogenicity study did not show any treatment related histopathological change in rats except follicular cell hyperplasia of thyroid in 3 out of 50 male rats at 24 mg/kg. One control female also showed thyroid follicular cell hyperplasia. The finding at 24 mg/kg in male rats was statistically not significant.

Toxicokinetics: The pharmacokinetic data in F344 rats were presented in the PK section. The average exposure in male and female F344 rats at 24 mg/kg is 198109 ng.hr/ml. The exposure at 120 mg/day human dose is 11960 ng.hr/ml. Therefore exposure to the F344 rats at 24 mg/kg is 16 times human exposure at maximum recommended human dose of 120 mg.

Mouse study (Report # 4257-011-025 in M4.2.3.4.1.1):

The mouse carcinogenicity study was reviewed under IND 58,229, and results were presented to ECAC on Sept 30, 2003.

A two-year carcinogenicity study was conducted in B6C3F1 mice at 3, 7.5 and 18.75 mg/kg. Female mice at 18.75 mg/kg (56.25 mg/m²) had increased incidences of transitional cell papilloma and carcinoma in the urinary bladder. However, it was statistically not significant. The ECAC recommended that these findings be incorporated in the package insert. The effect in the urinary bladder was secondary to calculus formation. Male mice did not show treatment related tumor.

Toxicokinetics: The exposure was 27.8 and 96.5 µg.hr/ml at 18.75 mg/kg for male and female mice, respectively. Since male and female mice showed different exposure, the exposure ratio between female mice and human was calculated using 96.5 µg.hr/ml for female mouse and 11.96 µg.hr/ml for humans at maximum recommended human dose of 120 mg. Therefore, the exposure ratio for mouse to human is 8.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Study on oral administration of TEI-6720 prior to and in the early stage of pregnancy in rats

Key study findings: TMX-67 did not show treatment related effect on the mating performance and fertility in male and female rats.

Study no.: S05410R100

Volume #M4, 4.2.3.5.1, and page #: 1

Conducting laboratory and location: Teijen Institute for Biomedical Research, 4-3-2 Asahigaoka, Hino-shi, Tokyo 191, Japan

Date of study initiation: July 5, 1994

GLP compliance: Yes

QA reports: yes (x) no ()

Drug lot # and % purity: 5072999 and 99.3-99.8%

Methods

Doses: 3, 12 and 48 mg/kg

Species/strain: Male and female Slc: SD strain rats were used in the experiment.

Male rats were 7 weeks old and weighed 197-243 g at the beginning of the dosing.

Female rats were 11 weeks old and weighed 203-248 g at the beginning of the dosing.

Number/sex/group: 24/group/sex

Route, formulation, volume, and infusion rate: Rats were treated at 5 ml/kg by oral gavage. The drug substance was suspended in 0.5% methylcellulose and the control animals were treated with the vehicle. The suspensions were within 100.2 – 109.2% of the nominal concentration. The high dose was selected on the basis of a five week toxicity study in which deaths, reduced food intake and body weight gain were observed at 75 and 150 mg/kg in male and female rats. Calculi in the kidney (yellow granules) were noted at 15 mg/kg and higher doses.

Satellite groups used for toxicokinetics: Nil

Study design: The dose groups are shown in the table below.

Group	Dose, mg/kg	Number of animals/sex	Animal number
1. Control	0	24	0101-0124 (male) 0201-0224 (female)
2. Low	3	24	1101-1124 (male) 1201-1224 (Female)
3. Mid	12	24	2101-2124 (male) 2201-2224 (female)
4. High	48	24	3101-3124 (male) 3201-3224(female)

One male and one female rats were cohabited in one cage for mating. Males were treated from 64 days before mating and throughout mating. The total duration of treatment for male rats was about 91 days. Female rats were treated from 15 days before mating, during mating and through gestation day 7. Non-copulated female rats were treated for 7 days after the end of scheduled mating period. The sponsor estimated that the maximum duration of treatment in female rats was 43 days. The total mating period was 21 days. During first 14 days, male and female rats were allowed to mate for each group. Mating was confirmed by the presence of sperms in the vaginal smears that was designated as day 0 of the gestation. If any female failed to mate during first two weeks, female rats were cohabited with a proven fertile male from the same group for another 7 days. Also, the unmated male was cohabited with an untreated female rat for 7 days. The sponsor stated that a total of 7 female rats were allotted for the purpose. Female rats were sacrificed on gestation day 20 for examining the uterus.

Male rats were sacrificed by exsanguination on day 7 or 8 after mating (total duration of treatment was 91-92 days). Gross changes and weights of the liver, kidneys, adrenals, spleen, thymus, thyroid, testes, epididymides, prostate and seminal vesicle were recorded. Samples of testes, epididymides, prostate, seminal vesicle, pituitary gland, and organs with macroscopical changes were fixed in 20% phosphate buffered formalin and stored. However, testes, epididymides and pituitary gland from non-copulated or infertile animals were histologically examined.

Gross examination was conducted for any change in copulated female rats. Ovary, pituitary and any organ with a gross change were fixed in 20% formalin and stored. Histological examinations were conducted for ovary and pituitary for female rats that failed to copulate or were non-pregnant.

Parameters and endpoints evaluated:

General signs and mortality were noted every day. Body weights of male rats were recorded twice a week. The body weight of female rats were recorded during pre-mating, mating and on gestation days 0, 4, 8, 12 and 20.

The food consumption of male rats was recorded twice a week up to day 63 of dosing and mean daily food intake was calculated. Food consumption of female rats was recorded twice a week before mating and on gestation days 4, 8, 12, 16 and 20.

Vaginal smears were examined at the start of dosing up to mating to determine estrus cycle. The presence of sperms was examined during the mating to confirm copulation.

Sperm samples from 10 animals per group were collected at necropsy from caudal epididymides for the examination of sperm motility and counts. Sperm motility was expressed as the percent of motile sperm out of total sperms. Also, sperm motile efficiency was calculated.

Pregnant females were sacrificed on gestation day 20. The ovary and uterus from mated animals were removed for examination.

Untreated females unsuccessful for mating with treated males were sacrificed on day 20 after cohabitation by sodium pentobarbital anesthesia. The uterus was removed to confirm the absence of implantations.

Untreated females mated with treated males were sacrificed on gestation day 14 or 15. The ovary and uterus were removed for confirmation of pregnancy. The sponsor stated that only fertility index was determined for control female # 0214.

The following parameters were calculated or determined for each group:

1. Male fertility index= number of impregnating male rats/ number of males with successful copulation x 100; Copulation index = number of animals with successful copulation/number of animals mated x100
2. Female fertility index = number of pregnant females/number of females with successful copulation x 100
3. Number of corpora lutea
4. Number of implantation sites, pre and post implantation losses
5. Number of resorptions, early resorptions, late resorptions, dead fetuses and live fetuses
6. External anomalies of fetuses, fetal body weight, sex ratio and placental weight

Results

Mortality: Male rats:

One control and one high dose rats were found dead on days 53 and 19, respectively. The cause of death was due to gavage error. One male rat was sacrificed on day 52 at 3 mg/kg due to loss of incisor teeth.

Female rats:

No mortality due to the treatment was reported in the study.

Clinical signs: Occasionally, salivation was noted at 48 mg/kg in male and female rats. Loss of hair was also noted in the control and treated rats. However, it was not treatment related.

Body weight: The body weight (g) is shown in the table below.

Male rats:

Group	Day 0	Day 63	Wt gain	Day 77	Wt gain	Day 96	Wt gain
1	221	419	198	437	216	455	235

2	221	420	199	439	218	462	241
3	220	416	196	433	213	456	236
4	219	407	188	427	208	447	228
% decrease in wt gain in gr 4			5%		4%		3%

The body weight of male rats at the beginning of mating, end of first mating and at the terminal sacrifice showed 3-5% reduction in the body weight gain at 48 mg/kg compared to the control. These changes are not toxicologically significant.

Body weights (g) of female rats are shown in the table below.

Group	Day 0, pre mating	Day 14, pre mating	G 0	G 8	G 16	G 20
1	223	238	244	268	310	360
BW gain		16	22	46	88	137
2	224	240	251	275	320	373
BW gain		16	27	51	96	149
3	223	240	247	271	317	371
BW gain		17	24	48	94	148
4	224	236	244	268	315	365
BW gain		12	20	44	91	141

G = Gestation day

Body weight gain was calculated by the difference of the weight from day 0 of the treatment. Based on the data, body weight gain in the treated animals was comparable to the control.

Food consumption: The food consumption (g/day) of male rats is shown in the table below.

Group	Day 3	Day 63	loss
1 (control)	22.8	21.3	1.5
2 (3 mg/kg)	22.9	21.0	1.9
3 (12 mg/kg)	22.0	20.8	1.2
4 (48 mg/kg)	20.8	20.6	0.2

Above data show that the food consumption in male rats was not affected by the treatment.

Food consumption (g/day) in the female rat is shown in the table below.

Group	Day 3, pre mating	Day 14, pre mating	G 4	G8	G16	G20
1	15.3	16.2	17.8	18.6	20.7	21.1
gain		0.9	2.5	3.3	5.4	5.8
2	15.8	16.3	18.4	19.4	21.4	22.6
gain		0.5	-0.4	3.6	5.6	6.8
3	16.1	16.4	19.0	19.0	21.5	22.4
gain		0.3	2.9	2.9	5.4	6.3
4	15.3	15.1	17.5	18.6	22.3	22.9
gain		-0.2	2.2	3.3	7	7.6

Above data suggest that there was no treatment related change in the food consumption in treated female rats before mating and during the gestation period.

Organ weight:

Male rats at 48 mg/kg showed a slight increase in the weight of spleen (g) and thyroid (mg) as shown in the table below.

Group	Spleen	% body weight	Thyroid	% body weight
1	0.78	0.17	20.7	0.0045
2	0.83	0.17	21.0	0.0046
3	0.80	0.17	20.2	0.0044
4	0.92*	0.20	24.7*	0.0055*

Toxicokinetics: No TK data were submitted in the report.

Necropsy:

Gross changes in the male rats at 48 mg/kg were small kidney, yellowish substance on kidney and yellowish color in the urinary bladder. No histological examination was conducted for these tissues. The sponsor stated that yellowish deposition was due to xanthine crystal formation.

Female rats sacrificed on gestation day 20 also showed yellowish white substance in kidney at 48 mg/kg. The number of non-copulated and non pregnant females was 2, 3, 3 and 1 at control, 3, 12 and 48 mg/kg, respectively. White and yellowish substances in the kidney were also noted in one non-copulated female rat at 48 mg/kg.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Mating performance of male rats showed a few non-copulated or infertile rats. The sponsor stated that there was no definite cause for the infertility. The comparison between

the control and treated groups does not suggest a treatment related effect. The following table presents the data.

Group	Number of male did not mate
1 (control)	2
2 (3 mg/kg)	3
3 (12 mg/kg)	2
4 (48 mg/kg)	3

Male and female mating performance data are shown in the table below. Data suggest that mating performance of male, female rats, fertility or pregnancy index were not affected by the treatment. Female rats also did not show any treatment related changes in the duration of estrus cycle.

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Table 19 Summary of fertility data

Control (kg)	7	12	40
No. of males examined			
23	23	24	23
No. of males with successful copulation			
1st mating	20	24	21
2nd mating	2	0	1
Total (%)	22(95.7)	24(100)	22(95.7)
No. of implanting sites (x10 ²)			
21195.71	20790.71	22791.71	20790.81
No. of females examined			
24	24	24	24
No. of females with successful copulation			
1st mating	13	24	22
2nd mating	0	0	2
Total (%)	13(54.2)	24(100)	24(100)
No. of pregnant females (%)			
22195.71	21191.71	22791.71	23795.81
Estrous cycle and ovulation period (days, Mean±S.E.)			
4.15±0.2	4.15±0.2	4.15±0.2	4.15±0.2

(a) : Males failing to show evidence of copulation after an initial 14-day period were retested for an additional 7 days, or until evidence of mating was found, with an untreated female.
 (b) : Copulation index = (No. of animals with successful copulation / No. of mated animals) x 100
 (c) : Implantation index = (No. of implanting sites / No. of males with successful copulation) x 100
 (d) : Female rats failing to show evidence of copulation after an initial 14-day period were retested until evidence of mating was found, with a pregnant male selected from the same group.
 (e) : Pregnant index = (No. of pregnant females / No. of females with successful copulation) x 100

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The treatment had no effect on sperm counts, motility or sperm motile efficiency of the male rats. Histology data for testes, epididymides, pituitary (male and female) and ovary

from non-copulated male and non-pregnant female rats did not show any treatment related change. Embryo and fetal data are shown in the table below.

Table 13 Summary of Maternal and Fetal Observations at Various Doses

Parameter	Control		1 mg/kg		5 mg/kg		15 mg/kg	
	No.	%	No.	%	No.	%	No.	%
No. of dams examined	22	100	22	100	22	100	22	100
No. of corpora lutea (CL)	10	45.5	10	45.5	10	45.5	10	45.5
No. of implantations (IM)	10	45.5	10	45.5	10	45.5	10	45.5
Pre-implantation loss (%)	0	0	0	0	0	0	0	0
No. of resorptions and dead fetuses	0	0	0	0	0	0	0	0
early resorptions	0	0	0	0	0	0	0	0
late resorptions	0	0	0	0	0	0	0	0
nonviable fetuses	0	0	0	0	0	0	0	0
dead fetuses	0	0	0	0	0	0	0	0
No. of live fetuses (LF)	10	45.5	10	45.5	10	45.5	10	45.5
Sex ratio (M/F)	5/5	100	5/5	100	5/5	100	5/5	100
Body weight (g, mean ± S.D.)	180 ± 10	100	180 ± 10	100	180 ± 10	100	180 ± 10	100
Male	180 ± 10	100	180 ± 10	100	180 ± 10	100	180 ± 10	100
Female	180 ± 10	100	180 ± 10	100	180 ± 10	100	180 ± 10	100

CL = corpora lutea; IM = implantations; LF = live fetuses; M/F = sex ratio; S.D. = standard deviation.

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Table 2: Summary of external observations of fetuses

No. of fetuses examined	Control		21	40
	1	21		
No. of fetuses examined	262	262	262	262
% of fetuses with malformations	0	0	0	0

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Above data suggest that the treatment had no effect on the corpora lutea and number of implantation. Early resorptions were slightly increased in treated groups. However, there was no dose related trend and it was statistically insignificant. The sponsor did not provide any historical control data. Published data for Sprague Dawley rats show 5.06 ± 2.46 % resorptions rate. It is concluded that the incidence is not due to the treatment.

The highest dose selected did not show any mortality. However, the presence of gross changes of yellow substance in the kidney at 48 mg/kg indicated deposition of xanthine calculi. Based on the finding, it is considered that the treatment was given up to a maximum tolerated dose. It is concluded that male and female rats treated with TMX-67 at 3, 12 and 48 mg/kg did not affect mating and fertility.

Embryo fetal development

Study title: Study for effects on embryo-fetal development in rats treated orally with TEI-6720

Key study findings: No teratogenicity was observed up to 48 mg/kg in rats.

Study no.: S05420R100

Volume #M4, 4.2.3.5.2.1, and page #: 1

Conducting laboratory and location: Teijin Institute for Biomedical Research, 4-3-2 Asahigaoka, Hino-shi, Tokyo 191-8512, Japan

Date of study initiation: June 24, 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug lot # and % purity: 7317 and 100.9%

Methods

Doses: the dose groups are shown in the table below.

Group	Dose, mg/kg	Number of copulated female	Animal number
1, control	0	24	0201-0224
2, Low	3	24	1201-1224
3. Mid	12	24	2201-2224
4. High	48	24	3201-3224

The dose level was selected on the basis of a preliminary study at 12, 48, 150 and 300 mg/kg in pregnant rats. Treatment was given during gestation days 7-17. Yellowish deposition was noted in the kidney at 48 mg/kg and higher doses. The sponsor stated that

the deposition was due to xanthine crystal formation in the kidney. The high dose in the study was selected at 48 mg/kg on the basis of the preliminary study.

Species/strain: Slc:SD strain pregnant rats were used in the experiment. Female rats were 12-13 weeks old and weighed 216-250 g.

Number/sex/group: Twenty four

Route, formulation, volume, and infusion rate: The drug substance was suspended in 0.5% methylcellulose and administered by oral gavage at 5 ml/kg volume during gestation days 7 to 17. The control rats were treated with methylcellulose suspensions. The suspensions were prepared once a week. Homogeneity and stability of the suspensions were determined. Average concentrations of the drug were between 98.9-103% of the nominal concentration.

Satellite groups used for toxicokinetics: The sponsor did not allot any satellite group in the study for TK analysis.

Study design: Female rats were mated with male rats from the same strain. Mating was confirmed by the presence of sperm cells in the vaginal smears. The day of confirmed mating was considered to be gestation day 0. Histopathological examinations were conducted for the kidney of animal # 1202 (3 mg/kg) and # 2215 (12 mg/kg) at autopsy due to abnormal gross finding.

Parameters and endpoints evaluated:

Animals were observed for survival and general signs twice a day during the treatment period. Animals were observed once a day for mortality and clinical signs once a day during non-treatment period. Body weights were recorded before administration, and on days 0, 4 and daily on gestation days 7 to 20. The food consumption was recorded on days 4, 7, 9, 11, 13, 15, 18 and 20.

Rats were sacrificed on gestation day 20 by cesarean section. Ovaries and uteri were removed for following observations:

early and late resorptions, macerated and dead fetuses, live fetuses and post implantation losses

Fetal weight, sex and external anomalies were examined. Placentas were weighed and fixed in 10% formalin. Half of live fetuses from the same litter were fixed in 70% ethanol and stained with Alizarin red for examining any skeletal variation and ossification according to Dawson's methods. Remaining fetuses were fixed in a mixture of formalin, n-propyl alcohol and acetic acid for the visceral examination of any malformation according to Wilson's methods. The sponsor stated that skeleton and visceral changes were examined for control and 48 mg/kg dose groups only. Statistical significance for abnormalities in the skeleton and viscera between the control and high dose treated rats was done by Wilcoxon rank test. Statistical significance for other parameters was

determined by analysis of variance and Dunnett test between the control and each treated group.

Results

Mortality (dams): No mortality was reported in the study.

Clinical signs (dams): No treatment related clinical sign was observed in the dams.

Body weight (dams):

The average body weight (g) of dams is shown in the table below.

Gestation day	Control	3 mg/kg	12 mg/kg	48 mg/kg
0	233.6	233.3	233.8	234.6
7	252.7	252	252.6	256.6
14	283.2	281.6	281.3	287.0
17	309.8	307.6	306.7	314.0
20	350.3	346.5	343.7	354.0

The body weight gain in the control and treated animals was comparable. The weight gain was 117, 113, 110 and 120 g in the control, 3, 12 and 48 mg/kg, respectively at the necropsy. It is concluded that the treatment had no effect on the body weight gain.

Food consumption (dams):

The food consumption (g/day) of the control and treated groups was comparable during the treatment period and at necropsy. Data are shown in the table below.

Gestation period (day)	Control	3 mg/kg	12 mg/kg	48 mg/kg
7-9	19.4	18.9	19.2	19.7
15-18	22.3	22.3	22.0	22.3
18-20	21.4	21.4	21.0	21.7

Toxicokinetics: No TK was measured.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

No treatment related change in the organ weight of dams was observed at necropsy. However, white or yellow substance was noted in the kidney of all dams at 48 mg/kg that

was treatment related. Two dams at 48 mg/kg dose also showed multiple dark red spot in the lung.

Animal # 1202 at 3 mg/kg and animal # 2215 at 12 mg/kg showed inflammation of the interstitial tissue of the kidney and pyelonephritis, respectively. These changes might be incidental.

Implantation and resorptions data did not show any treatment related changes between the control and treated groups. Data are shown in the table below.

Table 8 Summary of maternal and fetal observations at cesarean section

	Control	3mg/kg	12mg/kg	48mg/kg
No. of dams examined	24	24	24	24
No. of dams aborted	0	0	0	0
No. of dams with resorption of all embryos	0	0	0	0
No. of corpora lutea (mean: S.D.)	311(15.5 ± 1.7)	349(14.5 ± 1.3)	354(14.8 ± 1.5)	371(15.5 ± 1.3)
No. of implantation sites (mean: S.D.)	343(14.3 ± 2.3)	330(13.8 ± 1.3)	323(13.5 ± 3.6)	348(14.5 ± 2.2)
Pre-implantation loss (a)	7.27	5.19	10.46	6.20
No. of resorptions and dead fetuses (a) b)	32(11.58)	28(8.12)	42(12.13)	34(9.75)
early resorptions	25(10.68)	25(7.54)	38(11.02)	31(8.81)
late resorptions	3(0.91)	3(0.88)	4(1.09)	3(0.84)
macerated fetuses	0	1(0.30)	0	0
dead fetuses	0	0	0	0
No. of live fetuses (mean: S.D.)	311(13.0 ± 3.6)	301(12.5 ± 1.8)	281(11.7 ± 3.9)	314(13.1 ± 2.6)
male	165	151	142	177
female	146	150	139	137
Sex ratio (male, %)	54.32	49.82	50.86	55.69
Body weight of fetuses (g, mean: S.D.)				
male	3.667 ± 0.212	3.737 ± 0.289	3.735 ± 0.202	3.730 ± 0.289
female	3.515 ± 0.186	3.589 ± 0.283	3.500 ± 0.183	3.511 ± 0.258
Placental weight (g, mean: S.D.)				
male	0.477 ± 0.119	0.441 ± 0.058	0.461 ± 0.081	0.446 ± 0.052
female	0.443 ± 0.053	0.432 ± 0.042	0.419 ± 0.054	0.445 ± 0.057

a): [(No. of implantation sites / No. of corpora lutea) × 100]
 b): No. of resorptions and dead fetuses / No. of implantation sites × 100

Offspring (malformations, variations, etc.):

No macroscopic changes were observed in 311, 301, 281, 314 fetuses at 0, 3, 12 and 48 mg/kg, respectively. No teratogenicity was observed in the study. Data for the skeletal observation according to the Dawson's technique are shown in the table below.

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Table 11 Summary of skeletal observations of fetuses

Dose (mg/kg)	Control	48
No. of litters examined	23	24
No. of fetuses examined	159	163
No. of fetuses with abnormalities (N)	17(13.8)	24(14.8)
Dumbbell ossification of thoracic centrum	11(9.9)	13(8.2)
Bipartite ossification of thoracic centrum	2(1.2)	2(1.2)
Supernumerary rib	2(1.4)	10(6.1)
Cervical rib	1(0.6)	0
Missshapen sternebra	1(0.5)	0
No. of ossification centers (mean±S.D.)		
Sacral and caudal vertebrae	8.78±0.68	8.99±0.72
Forelimb : metacarpals	7.81±0.55	7.91±0.22
proximal phalanges	2.19±1.53	2.45±1.45
distal phalanges	9.95±0.26	10.00±0.00
Hindlimb : metatarsals	8.03±1.09	8.15±1.05
proximal phalanges	0.04±0.19	0.06±0.21
distal phalanges	9.79±0.98	9.90±0.51
No. of fetuses with ossified 5th sternebra (%)	85.56	90.24

Increased supernumerary ribs (extra ribs) were noted in 6.1% fetuses at 48 mg/kg compared to 1.4% in the control. The sponsor did not provide any historical control data. However, published data in Sprague Dawley rats showed 0.6-1.0% supernumerary ribs in the control animals. The incidences for each dam are shown in the table below.

Treatment	Dam#	#incidence
Control	0214	1
Control	0215	1
48 mg/kg	3207	1
48 mg/kg	3209	2
48 mg/kg	3210	4
48 mg/kg	3214	1
48 mg/kg	3216	1
48 mg/kg	3218	1

Only dam # 3210 showed higher than most of the dams. Therefore the incidence was not considered to be treatment related.

Conclusion:

Teratogenicity study was conducted in rats at 3, 12 and 48 mg/kg by treating animals during gestation days 7 to 17. No mortality and body weight changes due to the treatment were observed. The treatment at 48 mg/kg was considered to be at a maximum tolerated dose considering deposition of yellowish granules in the kidney. The sponsor indicated that the finding was due to deposition of xanthine crystals. Increased incidences of supernumerary ribs were noted at 48 mg/kg. However, it was considered not treatment related. TMX-67 is not teratogenic up to 48 mg/kg dose.

Study title: Study for effects on embryo-fetal development in rabbits treated orally with TEI-6720

Key study findings: TMX-67 was not teratogenic in rabbits.

Study no.: T-883

Volume #4.2.3.5.2.2 and page #: 1

Conducting laboratory and location: Higashi-Matsuyama Laboratory, Teijin Bio Laboratories Inc. 656-1, Oaza Higashi-taira, Higashi-matsuyama-shi, Saitama-ken, 355-0002, Japan

Date of study initiation: Nov 20, 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #7317, and % purity: 100.9%, the suspensions were within 98.4-103.8% of the nominal concentration.

Methods

Doses: 3, 12 and 48 mg/kg. The dose level was determined from a preliminary study in pregnant rabbits at 25, 50, 75 and 100 mg/kg. Mortality was observed at 100 mg/kg. The sponsor conducted a single dose PK study in non-pregnant rabbits at 25, 50 and 100 mg/kg. The exposure of TEI-6720 (AUC) at 100 mg/kg was similar to that of 50 mg/kg. The sponsor considered that a ceiling effect was present for the exposure above 50 mg/kg. Based on the preliminary study in pregnant animals and exposure data in non-pregnant animals, 48 mg/kg was selected as the high dose.

Species/strain: New Zealand white rabbits (Kbl: NZW, SPF) were used in the study. Male and female rabbits were mated. Pregnant females were used for the developmental toxicity testing. Confirmation of pregnancy from the vaginal smears was considered to be gestation day 0. The weight of rabbits was 3.29-3.91 kg and the age was 20 to 22 weeks on gestation day 0.

Number/sex/group: Twenty three copulated animals/group

Route, formulation, volume, and infusion rate: TEI-6720 was suspended in 0.5% methylcellulose and administered orally by gavage at 5 ml/kg from gestation days 6-18. The control animals were treated with the vehicle.

Satellite groups used for toxicokinetics: Nil

Study design: Clinical signs and mortality were observed twice daily during the treatment period and once daily after the treatment period. The body weight of rabbits was recorded on days 0, 2, 4, 6-19 (daily), 21, 23, 25, 27, and 28. The food intake was calculated daily. Dams were sacrificed on day 28 under pentobarbital anesthesia. Ovaries and uteri were removed for further examination of pregnancy and uterine contents. The following tissues were weighed and preserved in 10% formalin from the pregnant animals:

Brain, pituitary, heart, lung, liver, kidney, spleen, adrenal glands, ovaries and uterus. In addition, thyroid, thymus and urinary bladder were preserved in 10% formalin also.

Gross changes in the non-pregnant animals were recorded. Animals aborted during the study were sacrificed after gross examinations. Brain, pituitary, heart, lung, liver, kidney, spleen, adrenals, ovary, uterus, thymus, thyroid and urinary bladder from aborted rabbits were preserved in 10% formalin.

Parameters and endpoints evaluated:

Corpora lutea, number of implantations and pre-implantation losses, number of dead fetuses, resorptions, placental remnants, live fetuses, and post implantation losses were recorded. Fetal and placental weights were recorded also. Fetuses were examined for macroscopic changes, sex and external anomalies. Visceral changes were examined with a microscope. Fetuses were treated with acetone and stained with Alizarin red for skeletal anomalies and ossification. Statistical analysis was done by Dunnett's type rank test for comparisons between the control and treatment groups. Ossification of the fifth vertebra and skeletal anomalies were analyzed by Wilcoxon's rank test. Abortion rates were analyzed by the Fisher's direct probability test.

Results

Mortality (dams): Dam #2105 in the control group died on gestation day 10. Dam # 2301 at 12 mg/kg died on gestation day 17. Deaths were considered to be gavage accident.

Clinical signs (dams): Abortion was noted in dam # 2223 at 3 mg/kg on gestation day 19 and dam # 2406 at 48 mg/kg on gestation day 27. Both animals developed anorexia before abortion. No other clinical sign was observed in the treated rabbits.

Body weight (dams): The body weight (g) data are shown in the table below.

Gestation day	0 mg/kg	3 mg/kg	12 mg/kg	48 mg/kg
0	3.6	3.6	3.6	3.5
6	3.7	3.8	3.7	3.8

Gain	0.1	0.2	0.1	0.3
14	3.8	3.9	3.9	3.8
Gain	0.2	0.3	0.3	0.3
18	3.9	3.9	3.9	3.8
Gain	0.3	0.3	0.3	0.3
28	3.9	4.1	4.0	3.9
Gain	0.6	0.5	0.4	0.4

A slight reduction in the weight gain was observed at 12 and 48 mg/kg. The change could be incidental.

Food consumption (dams):

The food consumption (g/day) is shown in the table below.

Gestation day	0 mg/kg	3 mg/kg	12 mg/kg	48 mg/kg
1	166	177	166	187
6	175	172	168	179
Gain/loss	9	-5	2	-8
14	136	144	139	118
Gain/loss	-30	-33	-27	-69
18	144	162	136	128
Gain/loss	-22	-15	-30	-59
28	92	105	98	93
Gain/loss	-74	-72	-68	-94

Increased loss of food consumption (50%) was noted at 48 mg/kg on day 28. The control animal showed 45% reduction in the food consumption on day 28. Data suggest that a slight reduction in the food consumption was observed at 48 mg/kg before necropsy.

Toxicokinetics: Nil

Terminal and necropsic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

No treatment related gross change in the dam was observed in the scheduled necropsy. Also, dams that aborted did not show any abnormal gross changes.

The organ weight data showed a slight reduction in the weight of heart from 8.5 g in the control to 7.4 g at 48 mg/kg.

The uterine observation following cesarean section is presented in the table below. The preimplantation loss at 48 mg/kg was higher than the control. However, it is not statistically significant and was considered unrelated to the treatment.

Two dams (#2402 and 2409) at 48 mg/kg showed over 80% preimplantation loss that reflected in a reduction of live fetuses. Therefore, it was considered that TMX-67 was not embryocidal at 48 mg/kg.

Table 7 (continued) Implantation at cesarean section in dams treated orally with TMX-67

Dose		0 mg/kg	3 mg/kg	12 mg/kg	48 mg/kg
No. of dams		20	20	21	20
No. of corpora lutea	Total	198	181	187	197
	(Mean ± S.D.)	9.9 ± 1.7	9.0 ± 1.8	8.9 ± 1.3	9.8 ± 1.8
No. of implantation sites	Total	170	175	162	160
	(Mean ± S.D.)	8.5 ± 2.5	8.7 ± 2.3	7.7 ± 1.4	7.5 ± 2.0
Pre-implantation loss rate (%) ¹⁾	(Mean ± S.D.)	14.7 ± 17.0	12.2 ± 14.4	12.9 ± 13.1	23.2 ± 28.2
No. of lost implantations	Resorption	1	0	0	0
	Placental remnant	4	3	3	2
	Resorbed fetus	0	3	0	7
	Dead fetus	2	0	1	3
	Total	7	3	4	12
	(Mean ± S.D.)	0.4 ± 0.7	0.3 ± 0.5	0.3 ± 0.4	0.8 ± 0.8
Post-implantation loss rate (%) ²⁾	(Mean ± S.D.)	4.0 ± 7.5	3.3 ± 5.3	2.3 ± 4.9	11.8 ± 23.2
No. of live fetuses	Total	81	88	89	58
	Male	42	41	40	28
	Female	39	47	49	30
	Total	163	167	158	137
	(Mean ± S.D.)	8.2 ± 2.5	8.4 ± 2.3	7.5 ± 1.4	6.8 ± 2.0
Sex ratio	(Male %) ³⁾	50.7 ± 22.4	53.1 ± 21.8	42.7 ± 21.2	43.1 ± 20.8

- 1) : (No. of corpora lutea - No. of implantation sites) / No. of corpora lutea × 100.
 2) : (No. of resorptions and dead fetuses / No. of implantation sites) × 100.
 3) : (No. of males / No. of total live fetuses) × 100.

Table 7 (continued)

Dose		0 mg/kg	3 mg/kg	12 mg/kg	48 mg/kg	
No. of dams		20	20	21	20	
Fetal weights	Male	(g, Mean ± S.D.)	35.880 ± 4.814	37.009 ± 3.506	37.903 ± 3.553	35.504 ± 4.916
	Female	(g, Mean ± S.D.)	35.438 ± 4.091	36.898 ± 3.617	36.301 ± 3.221	33.488 ± 4.880
Placental weights	Male	(g, Mean ± S.D.)	5.058 ± 0.841	5.214 ± 0.732	5.153 ± 0.828	5.075 ± 0.773
	Female	(g, Mean ± S.D.)	4.878 ± 0.840	5.029 ± 0.848	4.755 ± 0.748	4.828 ± 0.919
No. of fetuses with external abnormalities	Total, (N)	1 (0.8)	0	0	0	
	Type of abnormalities					
	Omphalocele	1 (0.8)	0	0	0	
No. of placentas with abnormalities	Total, (N)	0	0	0	0	

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Offspring (malformations, variations, etc.):

Table 3 ^{CONFIDENTIAL} Visceral observation in fetuses from dams treated orally with TEI-8720

Dose	0 mg/kg	3 mg/kg	12 mg/kg	48 mg/kg
No. of dams	20	20	21	19
No. of fetuses examined	183	187	158	137
No. of live fetuses with abnormalities : Total, (%)	12 (7.4)	23 (12.0)	17 (10.8)	15 (10.9)
Thymic remnant in neck	7 (4.3)	10 (5.3)	14 (8.8)	10 (7.5)
Abnormal lung lobation	4 (2.5)	3 (1.6)	4 (2.5)	2 (1.5)
Malpositioned esophagus	1 (0.6)	0	0	0
Malpositioned of kidney	1 (0.6)	0	0	0
Hypoplasia of left auricle	1 (0.6)	0	0	0
Right-sided aortic arch	1 (0.6)	0	0	0
Absent left subclavian artery	1 (0.6)	0	0	0
Ventricular septal defect (VSD)	1 (0.6)	3 (1.6)	1 (0.6)	1 (0.7)
Persistent truncus arteriosus	0	1 (0.6)	1 (0.6)	1 (0.7)
Hypoplasia of atrioventricular ostium	0	1 (0.6)	0	0
Hypoplasia of left ventricle	0	1 (0.6)	0	0
Hypoplasia of right ventricle	0	0	1 (0.6)	0
Hypoplasia of pulmonary trunk	0	0	1 (0.6)	0

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Table 4 ^{CONFIDENTIAL} Skeletal observation on fetuses from dams treated orally with TEI-8720

Dose	0 mg/kg	48 mg/kg
No. of dams	20	19
No. of fetuses examined	183	137
No. of fetuses with abnormalities : Total, (%)	6 (3.7)	7 (5.1)
Cervical rib	3 (1.6)	0
Hirschman sternebra	2 (1.2)	1 (0.7)
Knobby rib	0	3 (2.2)
Bifid lumbar vertebral arches	0	1 (0.7)
Supernumerary lumbar vertebra	1 (0.6)	2 (1.5)
Degree of ossification		
% of ossified 5th sternebrae : Mean ± S.D.	79.7 ± 28.2	78.8 ± 29.8
No. of ossified sacro-caudal vertebrae : Mean ± S.D.	19.1 ± 0.3	19.2 ± 0.4

Increased thymic remnant in neck was observed at 48 mg/kg. Animal # 2106 in the control and #2410 at 48 mg/kg showed 3 and 5 incidences that contributed overall

increase of the incidence at 48 mg/kg. The incidence was statistically not significant and is not due to the treatment.

The sponsor presented data for skeletal changes for the control and 48 mg/kg groups only although the protocol did not specify that the skeletal changes would be examined in two groups only. Knobby rib was observed at 48 mg/kg in three fetuses (Two in #2403 and one in # 2407). The percent of knobby ribs was 2.2% among 137 fetuses examined. The sponsor provided historical control data on March 31, 2005 for Kbl:NZW rabbits between 1999-2002. Data showed minimum and maximum range of knobby ribs was between 0-2.62%. Control rabbits did not show knobby ribs in the present study. The incidence of knobby rib was statistically not significant and within the historical control. Based on the data, it was considered that the presence of knobby ribs was not treatment related.

Based on the data, TMX-67 is not teratogenic up to 48 mg/kg. Based on the mortality and body weight data, the high dose did not reach maximum tolerated dose. However, the sponsor indicated that further increase in the oral dose would not increase the exposure to the drug. Based on the information, the study is acceptable.

Prenatal and postnatal development

Study title: Study for effects on pre and postnatal development, including maternal function in rats treated orally with TEI-6720

Key study findings: TMX-67 did not show any treatment related effect on the gestation, labor and delivery of pregnant rats up to 48 mg/kg. The treatment had no effect on the behavior and reproductive performance of the second generation of rats. However, dams and pups at 48 mg/kg showed increased deposition of xanthine crystals in the kidney. Survival of pups nursed by dams treated at 48 mg/kg was reduced. TMX-67 treatment also showed deposition of xanthine crystals in the kidney and urinary bladder in dams treated at 12 mg/kg.

Study no.: S05430R100

Volume # M4, 4-2-3-5-3, **and page #:** 1

Conducting laboratory and location: Safety Research Center, Teijin Institute for Biomedical Research, Teijin Ltd., 4-3-2 Asahigaoka Hino, Tokyo 191-8512.

Date of study initiation: June 26, 1998

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot # 7317, and % purity: 100.9%, stability and homogeneity of the dosing solution were confirmed. The suspensions were within 101.6 to 103.1 % of the nominal concentration.

Methods

Doses: Dose groups are shown in the table below.

Group	Treatment	Dose, mg/kg	Concentration, mg/ml	# pregnant rats	Animal #
1, control	vehicle	0	0	24	0201-0224
2, low	TEI-6720	3	0.6	24	1201-1224
3, mid	TEI-6720	12	2.4	24	2201-2224
4, high	TEI-6720	48	9.6	24	3201-3224

The doses were selected on the basis of the study results of the embryo-fetal development in rats. The high dose 48 mg/kg was chosen on the basis of the yellowish white substance found in the kidney at 48 mg/kg in rats in the previous study.

Species/strain: Slc: SD rats, female rats weighed 226-258 g and 13 to 14 weeks old at the beginning of the mating

Number/sex/group: Twenty four

Route, formulation, volume, and infusion rate: The drug substance was suspended in 0.5% methylcellulose and administered by oral gavage at 5 ml/kg volume.

Satellite groups used for toxicokinetics: Nil

Study design: Male and female animals were mated. Pregnancy was confirmed by the presence of sperms in the vaginal smears that was designated as day 0 of pregnancy. Pregnant rats were treated once a day from day 7 of pregnancy to lactation day 20.

Parameters and endpoints evaluated:

Mortality and clinical signs were observed twice a day during the treatment period and once a day before the treatment. Body weight was recorded on days 0, 4 and daily throughout gestation and lactation period. Food consumption was recorded on days 7, 10, 14, 17, and 21 of gestation. The food consumption was recorded on lactation days 4, 7, 10, 14, 17 and 21.

F₀ dams were sacrificed by exsanguination on lactation day 21 and birth index was calculated.

Birth index = # live offspring at birth/number of implantations x 100

The weight of following organs was recorded:

Pituitary, adrenals, thyroid, ovaries, heart, lungs, thymus, liver, kidneys and spleen.

Above organs, organs with any gross changes, stomach, mammary gland, urinary bladder were fixed in 10% neutral buffered formalin.

Dead animals were necropsied. Tissues with gross changes were fixed in 10% neutral buffered formalin. Dams that had no litters or total resorptions were sacrificed. Macroscopic changes were recorded. The following tissues and tissues with gross changes were fixed in 10% buffered formalin.

Pituitary, thyroid, trachea, pharynx, esophagus, thymus, heart, lungs, liver, spleen, kidneys, adrenals, urinary bladder, ovaries, uterus, uterine contents, vagina, skin and mammary glands.

Tissues from non-pregnant rats were not preserved.

The sponsor stated that histopathology of the preserved organs would be conducted if necessary.

F₀ dams allowed to litter:

The number of live and still births was recorded. Sex and external abnormalities of F₁ pups were recorded. Pups were nursed until day 21 and nursing performance was observed once a day. Mortality of F₁ pups was recorded daily. On day 4, total surviving pups per litter were reduced to 4 males and 4 females only by sacrificing any extra pups. The body weight of F₁ pups was recorded on postnatal days 0, 4, 7, 14 and 21. The body weight was recorded once a week after weaning. F₁ pups were observed for the eruption of lower incisors, separation of ear auricula, opening of eye lids, vaginal opening and cleavage of balanopreputial gland during the weaning period and sexual maturity.

The following behavioral and functional tests were conducted for F₁ pups at three weeks of age:

Pinna reflex, righting reflex, negative geotaxis, pain response, Preyer's reflex (hearing reflex) and pupillary reflex.

The following tests were conducted for one male and one female per F₁ litter at 4-5 weeks of age:

Open field test for 3 minutes once a day for 2 days.

The following test was conducted in 10 males and 10 female of F₁ rats per group at 6-7 weeks of age:

Water maze test at four times a day for 3 consecutive days.

Following parameters were calculated for F₀ dams:

Gestation index = # dams with live offspring/ # dams allowed to deliver x 100.

Viability index = # of live offspring on day 4 / # of live offspring at birth x 100

Weaning index = # of live offspring on day 21 / # of live offspring on day 4 x 100

Still born or dead F₁ pups were fixed in 10% neutral buffered formalin and preserved. Tissues and organs of F₁ rats sacrificed after birth for reduction of the size of litter to a maximum of 8 were fixed and preserved in 10% neutral buffered formalin.

Necropsy of F₁ animals:

Except those F₁ animals allowed to mate or used for water maze test and open field test, F₁ rats were necropsied by exsanguination at 3 weeks of age. Gross changes of tissues or organs were made. The kidney, urinary bladder and any tissue with gross change were fixed in 10% neutral buffered formalin and preserved. Skeleton was fixed in 70% ethanol and stained with Alizarin red for examination of any skeletal abnormalities according to the Dawson's technique.

F₁ rats used for open field and water maze tests were necropsied by exsanguination at 10 weeks of age. Weights of the adrenal, thyroid, heart, thymus, lungs, liver, kidneys, spleen, ovaries, epididymides and pituitary gland were recorded. These tissues and urinary bladder, eyes and organs with any abnormalities were fixed in 10% neutral buffered saline and preserved. The eye tissues were fixed in 2.5% glutaraldehyde.

Reproductive performance of F₁ rats:

One male and one female from each litter were allowed to mate at 12-13 weeks of age. Estrus cycle was monitored 14 days before mating. Litter mates were avoided for the mating. Animals were cohabited for 10 days. The presence of sperms in the vaginal smear was considered to be day 0 of the pregnancy.

In the absence of copulation, male rats were cohabited with untreated females (females not from treated F₀ animals) for another 10 days. Unmated female rats were cohabited with a male of proven fertility from the same group for 10 days. Copulation index of male and female rats was determined. Copulation index = number of animals with successful copulation / number of mated animals x 100.

The body weight of pregnant rats (excluding non F₁ females used for second mating) was recorded on gestation days 0, 7, and 14. Animals were sacrificed by exsanguination on gestation day 14. Organs were examined for gross changes and fixed in 10% neutral buffered saline.

Ovaries and uteri were removed for checking pregnancy, corpora lutea, implantations and fetuses. Number of corpora lutea, implantations, resorptions, dead and live fetuses were counted. Pre and post-implantation losses were calculated. Pituitary and ovaries from

non-pregnant animals were preserved in 10% neutral buffered formalin. The uteri were preserved in 50% glycerin.

Fertility index for male and female animals were calculated.

Fertility index of female rats: # pregnant females/# females with successful copulation x 100

Fertility index of male rats: # of impregnating males/ # of male with successful copulation x 100.

No uncopulated F₁ female rat was observed in the study. Therefore all female rats were sacrificed on pregnancy day 14.

Non-F₁ females allotted for F₁ males for mating (those did not mate during the first mating period) were sacrificed on gestation day 14 if copulation was confirmed by the presence of sperm in the vaginal smears. Ovaries and uteri were removed for confirmation of pregnancy. In the absence of copulation, females were sacrificed between days 14-16 after mating period.

F₁ males used for mating were sacrificed at 17 weeks of age by exsanguination. Tissues with gross changes, testes, epididymides and pituitary gland were preserved in 10% neutral buffered formalin. Histopathology examinations were conducted for the kidney of animal #2206-101 and 3221-101 at 12 and 48 mg/kg, respectively.

Statistical analysis between the control and the dosing groups was done by the Fisher's exact test for mortality, gestation index, copulation index and fertility index. The body weight, food consumption, organ weight data were analyzed by variance. Statistical significance for behavioral, functional and other pregnancy data were analyzed by the Dunnett's method between the control and treated groups.

Results

F₀ in-life:

No death was reported for the control, 3 and 12 mg/kg during gestation. Moribund condition was noted at 48 mg/kg for dam # 3203 on gestation day 22 with incomplete delivery and the animal was sacrificed on day 22. One dam # 3209 at 48 mg/kg did not deliver and sacrificed on gestation day 23. Dam at 48 mg/kg (#3202) died on lactation day 4.

Control dam #0206 was sacrificed on lactation day 8 due to total litter death. Dam # 3206, 3217, 3219 and 3222 at 48 mg/kg were sacrificed on lactation days 9, 7, 13, and 7, respectively due to total litter death.

There were incidental findings of loss of hair (control and treated animals), swelling of forelimbs (#3208 at 48 mg/kg), tissue mass around perioral region (#3219 at 48 mg/kg) and vaginal hemorrhage (#1202 at 3 and #3201 at 48 mg/kg) after delivery. However, some of the above mentioned incidences were noted in the control also. These incidences were sporadic and not treatment related.

The mean body weight during the gestation period is shown in the table below.

The body weight (g) of F₀ dams during the gestation period is shown in the table below.

Gestation day	Control	3 mg/kg	12 mg/kg	48 mg/kg
0	241.6	241.4	240.7	241.6
7	267.1	264.8	264.7	265.1
Gain	25.5	23.4	24	23.5
14	296.7	298.1	294.7	292.2
Gain	55.1	56.7	54	50.6
21	375.7	375.7	376.6	364.0
Gain	134.1	134.3	135.9	122.4

The body weight gain at 48 mg/kg was reduced by 8.8% compared to the control at the end of gestation day 21.

The body weight of F₀ dams (g) during the lactation period is shown in the table below.

Lactation Day	Control	3	12	48 mg/kg
0	291.7	291.2	286.7	278.5
4	282.8	277.6	275.5	253.4
Gain	-8.9	-13.6	-11.2	-25.1
7	291.8	287.4	281.5	246.0
Gain	0.1	-3.8	-5.2	-32.5
14	309.2	306.0	304.3	281.0
Gain	17.5	14.8	17.6	2.5
21	301.1	299.1	299.1	283.9
Gain	9.4	7.9	12.4	5.4

Above data show that there was a loss of body weight gain on days 4 and 7. The loss of weight gain was most at 48 mg/kg. Rats at control, 3 and 12 mg/kg recovered from the loss of weight gain on days 14 and 21. However, the weight gain was reduced by 43% at 48 mg/kg at the end of lactation period. Therefore, dams at 48 mg/kg showed loss of body weight gain during lactation due to the treatment.

Therefore, treatment at 48 mg/kg showed a reduction in the weight gain. The effect of TMX-67 on the body weight gain during gestation and lactation at 48 mg/kg was due to a reduction of food consumption.

F₀ necropsy (table 10 of the report):

Gross changes of F₀ dams at necropsy on lactation day 21 were small thymus, large kidney, yellowish white substance in the kidney and urinary bladder, large thyroid at 48 mg/kg. Yellowish white substance was also observed at 12 mg/kg in the kidney and urinary bladder.

The dead dam#3202 at 48 mg/kg showed white substance in the heart, kidney, small thymus and large adrenal.

Dams # 3203 and #3209 (dams did not deliver) showed white substance in the kidney. One of the animals showed white substance in the heart also.

Dams # 3206, 3217, 3219 and 3222 at 48 mg/kg (dams with total litter death) showed white substance in the kidney and large thyroid. Dam # 0206 in the control did not show any change in the kidney and thyroid.

The sponsor stated that increased thyroid weight was observed in several toxicity studies including a 26-week toxicity study in rats at 48 mg/kg. However, no histological change in the thyroid was observed in the 26-week toxicity study in rats.

The effect on the kidney and urinary bladder was due to xanthine crystal deposition as seen in several toxicity studies in rats. Oral toxicity studies in rats did not show any histological changes in the thymus despite a reduction in the weight of thymus.

These gross changes are shown in the table below.

Animal #	Dose, mg/kg	Gross change
0206	Control	No change in the kidney and thyroid
3202	48	White substance in the kidney, heart, small thymus, large adrenals
3203	48	White substance in kidney
3209	48	White substance in the kidney
3206	48	White substance in the kidney and large thyroid
3217	48	White substance in the kidney and large thyroid
3219	48	White substance in the kidney and large thyroid
3222	48	White substance in the kidney and large thyroid

Organ weight data for selected organs are shown in the table below (table 11).

Organ	Control, (%BW)	3 mg/kg	12 mg/kg	48 mg/kg, (%BW)
Thyroid , mg	16, (0.053)	16	18	27*, (0.094*)
Ovary, mg	92, (0.30)	93	92	80*, (0.28)
Thymus, g	0.23, (0.079)	0.21	0.23	0.14*, (0.05*)
Kidney, g	2.0, (0.68)	2.0	2.1	3.0*, (1.06*)
Spleen, g	0.61, (0.20)	0.62	0.61	0.69*, (0.24*)

*statistically significant

An increase in the absolute and relative weight of thyroid, kidney and spleen was noted at 48 mg/kg. The weight of ovary and thymus was decreased compared to the control.

Delivery (F₀) and offspring (F₁) information are shown in the table below (tables 12 and 13).

Observation	Control	3 mg/kg	12 mg/kg	48 mg/kg
# dams examined	24	24	24	24
# dams undelivered and sacrificed	0	0	0	2
# dam died during lactation period	0	0	0	1
# of implantations (%)	325 (13.6%)	332 (13.8%)	333 (13.9%)	310 (14%)
#dams with total litter death	1	0	0	4
Abnormal nursing behavior	1	0	0	3
# live offspring at birth	306	301	312	286
Birth index (%)	94	90	93	92
Sex ratio (% male)	53	48	45	53
#live offspring on postnatal day 4	298	293	297	264
Viability index (%)	97.3	97.2	95	92.3

Observation	Control	3 mg/kg	12 mg/kg	48 mg/kg
#live offspring on postnatal day 4, after reduction	192	192	192	168
#live offspring postnatal day 7	189	191	192	149
#live offspring postnatal day 14	183	189	192	131
#live offspring postnatal day 21	183	188	192	129
Weaning index (%)	95.3	97.9	100	76.8
#dams with abnormal nursing posture	1	0	0	3
#dams with live offspring at delivery	24	24	24	22
Gestation index (%)	100	100	100	91.7
#dams with live offspring on lactation day 4	24	24	24	21
#dams with live offspring on lactation day 21	23	24	24	17
Gestation period (day)	21.4	21.3	21.3	21.3
# of still births	4	6	2	0
BW of offspring at birth, male (g)	5.8	5.8	5.8	5.5
BW on day 4, male, (g)	8.1	8.3	7.9	7.0
BW on day 7, male, (g)	12.3	12.5	12.0	9.7
BW on day 14, male, (g)	26.6	25.3	24.8	20.0
BW on day 21, male, (g)	41.2	39.2	38.6	31.5
BW at birth,	5.5	5.5	5.47	5.27

Observation	Control	3 mg/kg	12 mg/kg	48 mg/kg
female, (g)				
BW on day 4, female, (g)	7.7	7.9	7.5	6.8
BW on day 7, female, (g)	11.8	11.9	11.5	9.4
BW on day 14, female, (g)	25.6	24.4	23.9	19.3
BW on day 21, female, (g)	39.6	37.9	37.4	30.9

Gestation index = #dams with live offspring/#dams allowed to deliver x 100

Birth index = # of live offspring at birth/# of implantations x100

Viability index = # of live offspring on day 4/ # of live offspring on day 1 x 100

Weaning index = # of live offspring on day 21/ # of live offspring on day 4 after reduction

Above data show that the treatment had no adverse effect on the duration of gestation. Number of dams with live offspring was slightly reduced (8%) at 48 mg/kg that resulted a reduction of gestation index. The sponsor did not provide any historical control data. However, published historical control data during 1992-1994 for Sprague Dawley rats showed 97.3 ± 10.26 % of females gave live births. Considering the data, the reduction in the gestation index was not considered to be treatment related. Based on the data, TMX-67 did not show any treatment related change for the labor and delivery.

The viability index was slightly reduced at 48 mg/kg. However, it was not statistically significant. The published historical control data show $2.7\% \pm 2.7$ reduction of viability index in pups. The reviewer suggested that the reduction in the viability of F₁ pups at 48 mg/kg was due to maternal toxicity to the treatment. However, the secondary reviewer concluded that total litter death in four dams at 48 mg/kg resulted from the treatment and TMX-67 should be considered to have embryo-fetal toxicity. Accordingly, pregnancy category C was recommended.

Number of live F₁ rats (weaning index) during the weaning period and the body weight of F₁ rats were significantly reduced at 48 mg/kg during the nursing period. The published historical control data from 1992-1994 showed 0.71 ± 1.28 % reduction in the survival of pups during the weaning period. Therefore, the reduction in the number of live litter at 48 mg/kg was due to nursing behavior of dams as shown in table 12 as well as exposure to TMX-67 during nursing. Dams at 48 mg/kg also showed a loss of body weight gain and food consumption during the lactation.

A reduction of the body weight of male and female F₁ rats was observed on postnatal day 21.

F₁ physical development:

The following observations were made for F₁ rats during nursing period (day 1-21):

1. Small body size at 12 and 48 mg/kg
2. Hypothermia at 48 mg/kg

There was no postnatal change in the separation of ear auricular and eruption of lower incisor. The time for separation of eye lids was slightly increased from 16.5 to 17.5 days at 48 mg/kg. However, biological significance of the change is unknown. Only one of 286 F₁ rats at 48 mg/kg was dwarf when examined for external abnormality after weaning. However, it was considered to be an isolated event.

The body weight (g) of F₁ rats after weaning is shown in the table below. The body weight from weaning day 21 to 17 weeks of age for male is presented in table 21 of the study report. The body weight of female F₁ rats from weaning day 21 to day 105 is presented in table 21 of the study report.

Day of age	Sex	Control (n)	3 (n)	12 (n)	48 mg/kg (n)
21	male	41.7 (48)	39.8 (48)	38.9 (48)	31.5 (33)
63	male	325 (48)	336 (48)	324 (47)	306 (29)
gain		283.3	296.2	285.1	274.5
119	male	514 (23)	529 (24)	502 (24)	472 (16)
gain		472.3	489.2	463.1	440.5
21	female	39.7	38.4	37.5	31.6
63	female	211.3	213.7	212	199
gain		171.6	175.3	174.5	167.4
77	female	245 (23)	244 (24)	245 (34)	232 (17)
gain		205.3	205.6	207.5	200.4
91	female	268 1(10)	278 (10)	274 (13)	258 (11)
gain		228.3	239.6	236.5	226.4
105	female	318 (1)	315 (2)	317 (3)	-
gain		278.3	276.6	279.5	

The body weight gain in male F₁ rats after weaning period was decreased at 48 mg/kg. Female F₁ rats at 48 mg/kg showed a decrease in the body weight gain up to day 77. However, the body weight gain was comparable to the control at the end of day 91.

F₁ behavioral and functional evaluation:

On day 21, all F₁ rats showed no treatment related changes in sensory function and neuromuscular activity, pinna reflex, righting reflex, negative geotaxis, pain response, Preyer's reflex and papillary reflex. The total number of F₁ rats examined was 91, 96, 96 and 68 at control, 3, 12 and 48 mg/kg, respectively. Data for behavior and functional tests are shown in table 26,

Open field behavior including latency, ambulation, rearing, preening, defecation and urination did not show any treatment related change. Data for the open-field test is presented in table 27.

Data for the learning ability of F₁ rats in water maze swimming test suggest that the learning ability to swim on successive days improved in the control and drug treated rats and there was no treatment related effect in F₁ rats. Data for the learning ability test using a water maze is presented in table 28.

F₁ reproduction:

The duration for cleavage of balanopreputial gland and vaginal opening among F₁ male and female rats, respectively was not affected by the treatment.

The fertility data for F₁ rats are shown in the table below.

Observation	Control	3	12	48 mg/kg
# males examined	23	24	24	16
#males with successful copulation, 1 st mating	21	22	19	13
2 nd mating	2	2	5	1
Copulation index (%)	100%	100%	100%	87%
Fertility index (%)	100%	87.5%	79.2%	100%
# female examined	23	24	24	17
# females with successful copulation, 1 st mating	21	22	19	14
2 nd mating	2	2	5	3
Copulation index (%)	100%	100%	100%	100%
Fertility index	100%	87.5%	79.2%	100%

Observation (%)	Control	3	12	48 mg/kg
Estrous cycle before mating, days	4.6	4.5	4.4	4.4

Copulation index= # animals with successful copulation/#mated animals x100

Fertility index= #pregnant females/ #females mated x100

The fertility index in male and female F₁ rats was not affected. The copulation index of male rats was reduced at 48 mg/kg compared to the control. However, the difference was not statistically significant. The estrous cycle was not affected in the F₁ females. No clinical sign in the pregnant dams was observed before cesarean section. Also, the body weight of F₁ dams in the control and treated animals was comparable. No treatment related abnormal gross changes were observed in F₁ dams at necropsy.

It is concluded that reproductive performance of F₁ male and female rats was not affected due to the treatment.

Dams were sacrificed on gestation day 14 and uteri and ovaries were examined. The following table shows the fetal information as shown in the table 33 of the study report.

Observation	Control	3	12	48 mg/kg
# dams	22	21	19	17
# corpus lutea	303	304	271	232
Corpus lutea/dam	13.8	14.4	14.2	13.6
#implantations	280	239	248	205
Implantation/dam	12.7	11.4	13.0	12.0
#resorptions	17	20	29	18
#resorption/dam	0.77	0.95	1.5	1.0
#resorption/#implantation (%)	5.9	7.3	15.2	7.1
#live fetuses	263	218	217	189
#live fetus/dam	11.9	10.4	11.4	11.1

Resorptions/dam was slightly increased in the treated groups compared to the control in the F₁ female. The sponsor did not provide any historical control data. However, resorption/dam in Sprague Dawley rats is 0.82 ± 0.34. Therefore, the resorption in groups 2, 3 and 4 dams was considered unrelated to the treatment of F₀ dams.

It is concluded that cesarean section data of F₁ dams on day 14 of the pregnancy did not show any effect of the drug on reproduction due to the treatment of F₀ dams. The effect of the treatment of F₀ dams on the variation and malformation of F₂ generation was not investigated.

The non pregnant F₁ rats did not show any gross change.

Overall it is concluded that the reproductive function of F₁ rats was not affected by the treatment.

The sponsor submitted a study report (see under PK section) to indicate that TMX-67 is distributed to the placenta and excreted through the milk in rats.

F₁ gross changes at week 3 of necropsy (table 17):

At necropsy white and yellowish substances in the kidney and urinary bladder were observed at 48 mg/kg. These changes were also observed in the F₀ dams and were considered to be due to deposition of xanthine crystals. Therefore, it is possible that TMX-67 was ingested by the F₁ pups through milk and inhibited uric acid, resulting the deposition of white substance (xanthine crystals) in the kidney and urinary bladder in 3-week old animals. The deposition of white substance in the kidney and urinary tract could also contribute to the high mortality of F₁ rats at 48 mg/kg during the nursing stage as shown from the necropsy findings of F₁ rats died during postnatal days 5-21 (data shown below). The effect on the F₁ rats also suggests that TMX-67 is excreted in the milk. The pharmacokinetic data reviewed in the PK section also support the finding.

The data from F₁ rats necropsied after day 21 are shown in the table below.

Observation	Control	3 mg/kg	12 mg/kg	48 mg/kg
# litter examined	23	24	24	17
# offspring examined	92	92	96	62
Kidney, white substance	0	0	2	37
Kidney, yellowish white substance	0	0	0	16
Urinary bladder, yellowish white substance	0	0	9	44

No treatment related skeletal abnormality was noted in F₁ rats sacrificed at 3 weeks of age.

Necropsy findings for F₁ rats that died between days 5 and 21 are shown in the table below (table 20).

Observation	Control	3 mg/kg	12 mg/kg	48 mg/kg
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Observation	Control	3 mg/kg	12 mg/kg	48 mg/kg
Dead	9	4	0	34
Necropsied	2	1	-	18
Intracranial hemorrhage	1	0		0
Thyroid enlargement	0	0		3
Yellowish white substance in kidney	0	0		11
Yellow substance in kidney	0	0		17
Yellowish substance in urinary bladder	0	0		14
Yellowish substance in ureter	0	0		4

F₁ gross changes at week 10 of necropsy (table 24):

Observation	Sex	Control	3 mg/kg	12 mg/kg	48 mg/kg
# animals	Male	23	24	23	12
Kidney, dilated renal pelvis	Male	1	1	2	3
#animals	Female	22	24	23	17
Liver, diaphragmatic hernia	Female	1	1	0	2

Absolute organ weight data for animals necropsied on week 10 did not show remarkable treatment related change in male and female rats.

Mortality, necropsy findings and clinical observations of F₁ rats after weaning:

The following F₁ rats were found dead after weaning (table 20).

Observation	Control	3 mg/kg	12 mg/kg	48 mg/kg
Death	0	0	2	5
			#2224-102 on day 24 # 2224-202 on	#3204-101 on day 24 #3205-102 on

			day 25	day 26 #3211-101 on day 28 #3211-102 on day 66 #3221-102 on day 24
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Dilated renal pelvis, yellowish white substance in the kidney, calculi in the urinary bladder and ureter were noted in one of 5 dead F₁ rats at 48 mg/kg.

Among surviving F₁ rats, exophthalmos and reddish discharge from the eye were noted at 48 mg/kg after weaning (table 19). No other treatment related developmental change in F₁ rats was observed after weaning.

Those F₁ males allowed to mate were sacrificed on week 17 and gross changes were recorded (table 35). Nephroblastoma (table 36), a rare tumor was noted in one animal in each of 12 and 48 mg/kg. Number of male rats sacrificed on week 17 was 23, 24, 24 and 16 at control, 3, 12 and 48 mg/kg, respectively. The relationship of nephroblastoma to the treatment is unknown.

F₂ findings:

Pregnant F₁ rats were sacrificed on gestation day 14 and fetal data were presented above under pregnancy findings of F₁ dams. There was no other data for F₂ animals were presented.

Summary:

Sprague Dawley rats were treated at 3, 12 and 48 mg/kg oral doses daily from gestation day 7 to lactation day 21. Rats were allowed to deliver and the effect of the treatment on gestation and viability of F₁ pups at birth on days 4 and 21 were observed. The behavior, function and reproduction of F₁ rats were examined. Some of the F₁ rats were mated and the pregnant rats were sacrificed on the gestation day 14 for examination of the uterus and ovary. The pregnancy status, corpora lutea, implantation, resorption, dead and live fetuses were examined.

A slight reduction in the body weight (8%) was noted at 48 mg/kg during gestation. However, F₀ dams at 48 mg/kg showed about 43% reduction in the body weight gain at the end of lactation period.

F₀ dams were sacrificed on the lactation day 21. Deposition of white substance in the kidney and urinary bladder was noted at necropsy at 12 and 48 mg/kg. The sponsor stated that the white substance was xanthine crystals and a similar effect was noted in other toxicity studies in rats. Larger thyroid was also observed at 48 mg/kg.

The treatment had no effect on the gestation period of dams. However, nursing performance of some rats was not adequate at 48 mg/kg. Increased mortality of F₁ rats was noted at the end of weaning period at 48 mg/kg. Deposition of xanthine crystals in the kidney, urinary bladder in the dams and F₁ rats at 48 mg/kg and the nursing behavior of F₀ dams were contributed to high mortality of F₁ rats. The sponsor conducted a pharmacokinetic study to support that TMX-67 is distributed to the placenta and also excreted through milk. The deposition of xanthine crystals was not observed as the F₁ animals matured to 10 weeks of age. Learning behavior, reflexes and physical changes of control and treated dams were comparable except a reduction of the body weight gain of F₁ rats during and after the weaning period.

A second generation reproductive study among F₁ male and female rats did not show any effect. However, F₁ dams were sacrificed on gestation day 14.

Conclusion of the prenatal and postnatal study:

TMX-67 showed no treatment related effect on gestation, labor and delivery of F₀ dams at a maternally non-toxic dose of 3 mg/kg. Deposition of xanthine crystals in the kidney and urinary bladder was observed in dams treated at 12 and 48 mg/kg. Behavioral, functional, developmental and reproductive performance of the second generation rats was also not affected due to the treatment. However, at 48 mg/kg dose, survival of weaning rats was reduced due to the toxicity of TMX-67 in the kidney and urinary bladder characterized by the deposition of xanthine crystals. TMX-67 crosses the placenta, and readily available to fetuses and excreted in the milk.

Conclusion of the reproductive safety study:

Fertility, teratogenicity, prenatal and postnatal studies were conducted. TMX-67 has no adverse effect on the fertility of male and female rats at 3 (18 mg/m²), 12 (72 mg/m²) and 48 mg/kg (288 mg/m²) oral doses. TMX-67 is not teratogenic in rats at 3 (18 mg/m²), 12 (72 mg/m²) and 48 mg/kg (288 mg/m²). Teratogenicity study in New Zealand rabbits at 3 (36 mg/m²), 12 (144 mg/m²) and 48 mg/kg (576 mg/m²) did not show any teratogenicity also.

TMX-67 had no adverse effect on gestation, labor and delivery of pregnant rats at 3 (18 mg/m²), 12 (72 mg/m²) and 48 mg/kg (288 mg/m²). However, survival and body weight gain of F₁ neonatal rats was reduced at 48 mg/kg (288 mg/m²). No adverse effect on the behavior, functional and reproductive capacity was observed in the multi-generation study.

It should be noted that deposition of xanthine crystals in the kidney and urinary tract was noted in dams treated at 12 and 48 mg/kg. New born pups nursed to TMX-67 treated dams at 48 mg/kg also showed xanthine crystals in the kidney. Kinetics and histopathology data suggest that the drug is excreted in the milk that contributed to the xanthine deposition in the kidney. Care should be taken to avoid nursing a baby in TMX-67 treated patients. Rabbits did not show deposition of xanthine crystals in the kidney up

to 48 mg/kg (576 mg/m²) dose in the segment 2 reproductive safety study. The maximum recommended human dose is 120 mg/day. Considering 60 kg body weight of the patient, the human dose will be 2 mg/kg (74 mg/m²)

The exposure ratio in rats at 3, 12 and 48 mg/kg was reviewed under the 6-month toxicity study in rats. The human exposure at 120 mg daily dose is 11960 ng.hr/ml. Animal to human exposure ratios are shown in the table below.

Species	Dose, mg/kg	Sex	Exposure, ng.hr/ml	Human exposure, ng.hr/ml	Exposure ratio
Rat	48	M and F	271838.5	11960	22.7
Rat	48	F	305194	11960	25
Rat	12	F	86190	11960	7
Rat	3	F	16363	11960	1.3
Rabbit	48	F	39406	11960	33

2.6.6.7 Local tolerance: No local tolerance study was reviewed

2.6.6.8 Special toxicology studies: No special toxicology studies were reviewed

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

The following tables were provided by the sponsor.

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2.6.7.3 Toxicokinetics Overview of Toxicokinetics Data Test Article: Febuxostat

Dose (mg/kg)	Mouse		Steady State AUC (ng·hr/mL)				Rabbit	Human
	M	F	M	F	M	F		
0.1								M&F 930.3 ^h
0.3								2112.5 ^h
0.4								3588.1 ^h
0.5			1713.9 ^g					4299.3 ^h
0.6								4378.5 ^h
0.9								6948.9 ^h
1.3								9648.7 ^h
1.5								11959.9 ^h
2.1								12282.3 ^h
3	3266.0 ^g , 4443.4 ^g	13227.8 ^g	12828.2 ^g 17314.7 ^g 13417.9 ^g	16763.9 ^g 18934.2 ^g			9515.2 ^h	14976.3 ^h
5					1315.0 ^g 4932.4 ^g	3609.4 ^g 5056.5 ^g		
12	18755.5 ^g	62247.1 ^g	52895.3 ^g 86477.6 ^g	86190.7 ^g 81110.8 ^g			58571.6 ^g	
15					31875.8 ^g	38659.5 ^g		
20					10544.3 ^g	42950.3 ^g		
24	34647.4 ^g , 36519.2 ^g	123134.2 ^g	194440.8 ^g	201770.8 ^g				
30			312966.7 ^g	306814.4 ^g				

b S054S27100, Week 13 c S054C3R100, Week 26 d 3500 (011-023), 3501 (011-023), Week 13
 e 3831 (011-024), Week 19 f S054S2R10A, Week 13 g S054S2R1G, Week 26
 h TMX-99-001, Week 2, 10 mg QD i TMX-99-001, Week 2, 30 mg QD
 j TMX-99-001, Week 2, 40 mg QD k TMX-99-001, Week 2, 20 mg QD l TMX-99-001, Week 2, 70 mg QD
 m TMX-99-001, Week 2, 50 mg QD n TMX-99-001, Week 2, 30 mg QD o TMX-99-001, Week 2, 70 mg QD
 p TMX-99-001, Week 2, 50 mg QD q TMX-99-001, Week 2, 120 mg QD r TMX-99-001, Week 2, 160 mg QD
 s TMX-99-001, Week 2, 180 mg QD t TMX-99-001, Week 2, 240 mg QD u S054C4D100, Week 32
 v S054C4D100, Day 13

APPEARS THIS WAY
 ON ORIGINAL

2.6.7.7F Repeat-Dose Toxicity Report Title: 26 Week Repeated -Dose Toxicity Study of TEL-6720 by Oral Administration in Rats Test Article: Febuxostat

Species/Strain: Rat/SLC:SD Duration of Dosing: 26 Weeks Study No: S054C3R100
 Initial Age: 6 Weeks Duration of Post-Dose: 6 Weeks Location in CTD: 4.2.3.2.6
 Study Start Date: 11 Apr. 94 Method of Administration: Gavage GLP Compliance: Yes
 Special Features: Toxicokinetics Vehicle/Formulation: 0.5% aqueous methylcellulose
 NOAEL: 3 mg/kg/day

Daily Dose (mg/kg)	0 (Control)		3		12		48	
Number of Animals Main	M:30 ^a	F:30 ^a	M:15	F:15	M:15	F:15	M:30 ^a	F:30 ^a
Toxicokinetics								
Toxicokinetics AUC _{0-24h} (ng·hr/mL)								
Week 26	NA	NA	12828	16364	52896	66191	238494	305194
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	1 ^b	0
Body Weight (%)	49.2 g	27.8 g	0	0	0	+3.0	-3.3	-3.3
Food Consumption (%)	18.2 g	12.7 g	-5.5	-5	-5.0	-2.4	0	0
Water Intake (%) Day 182	37.9 mL	38.4 mL	-18.2	+8.6	-26.6	-7.6	+91.0 ^{**}	+49.0 ^{**}
Recovery (Day 40)	34.8 mL	29.3 mL	NA	NA	NA	NA	-79.3 ^a	-41.9
Clinical Observations ^{**}								
Salivation	-	-	1	1	10	1	29	22

- No noteworthy findings
 Dunnett's (equal no. of animals) or Scheffé's (unequal no. animals) test *p<0.05, **p<0.01
 a Includes 15/sex in recovery group.
 b Death occurred post administration on Day 78 and was attributed to a gavage error.
 c At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
 d Number of animals with observations.
 e Following changes were seen in some animals in every group except control females (only loss of fur): loss of fur, wounds or hemorrhage, incisor loss, ear swelling, red nasal discharge and loose stool.
 NA = Not Applicable

2.6.7.FE Repeat-Dose Toxicity Study No: S054C3R100 (Cont) Test Article: Febuxostat

Daily Dose (mg/kg)	0 (Control)		3		12		48	
	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Hematology								
End Dose								
Erythrocyte Count (x 10 ¹² /dL)	1019	928	1015	896	1013	906	936**	884*
Hemoglobin (g/dL)	16.3	14.4	15.7	15.2	16.8	16.3	15.7	15.6
Hematocrit (%)	48.1	49.8	50.2	48.6	50.5	48.9	47.0*	47.1*
MCV (fL)	48.1	53.8	49.3*	54.5	49.9**	53.9	50.5**	55.3
MCH (pg)	16.0	17.7	16.6	18.2	16.6*	17.9	16.9**	17.8
MCHC (g/dL)	33.1	32.9	33.2	33.3	33.3	33.3	33.4	33.5*
Lymphocyte (%)	61.8	58.9	58.3	68.5	68.9*	68.3	58.7	58.8**
White cell count (x 10 ⁹ /dL)	53	53	57	31	58	39	72**	48**
Neutrophil-segmented	29.8	23.1	24.9	14.0	22.0**	25.3	31.9	32.3*
Recovery								
Erythrocyte count (x 10 ¹² /dL)	1022	919	1015	896	1013	906	936**	884*
Hemoglobin (g/dL)	16.8	14.9	NA	NA	NA	NA	15.3*	16.1*
Hematocrit (%)	50.3	50.4	NA	NA	NA	NA	46.8**	48.6
MCV (fL)	49.3	54.9	NA	NA	NA	NA	51.1**	54.1
MCH (pg)	16.3	18.3	NA	NA	NA	NA	16.8*	17.9*
MCHC (g/dL)	33.0	33.6	NA	NA	NA	NA	32.9	33.2
White cell count (x 10 ⁹ /dL)	52	30	NA	NA	NA	NA	61*	39*
Neutrophil-segmented	25.2	21.3	NA	NA	NA	NA	24.3	29.1
Lymphocyte (%)	65.2	68.5	NA	NA	NA	NA	67.9	61.4*
Bone Marrow (myelogram)								
End Dose								
Total myeloid (%)	39.1	24.3	37.9	30.9	36.8	29.0	34.2	35.8*
Neutrophilic metamyelocytes (%)	10.6	5.2	11.5	7.5	10.4	7.4	9.7	9.4**
Recovery								
Total myeloid (%)	10.6	5.2	11.5	7.5	10.4	7.4	9.7	9.4**
Neutrophilic metamyelocytes (%)	11.7	6.7	NA	NA	NA	NA	10.9	8.1

NA = Not Applicable
 Dunnett's (equal no. of animals) or Scheffe's (unequal no. animals) test *p<0.05, **p<0.01

APPEARS THIS WAY
 ON ORIGINAL

2.6.7.FE Repeat-Dose Toxicity Study No: S054C3R100 (Cont) Test Article: Febuxostat

Daily Dose (mg/kg)	0 (Control)		3		12		48	
	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Serum Chemistry								
End Dose								
BUN (mg/dL)	17.8	18.9	17.2	19.3	17.8	19.9	37**	25.7
Creatine (mg/dL)	0.57	0.65	0.48	0.55*	0.48	0.55*	0.97**	0.75
Total protein (g/dL)	6.0	6.7	5.9	6.2	5.9	6.8	5.6**	6.6
Ca (mg/dL)	10.12	10.58	10.28	10.86	10.09	10.66	10.63**	10.94
Phospholipid (mg/dL)	103	130	109	139	107	133	123*	138
Cholinesterase (IU/L)	523	3544	572	3939	575	3606	310**	5500
GGT (IU/L)	1.1	2.6	1.3	2.3	1.5	2.4	2.4**	3.0
IP (mg/dL)	4.7	4.1	5.3	4.5	5.1	4.5	6.2*	5.1**
A/G ratio	0.65	0.80	0.67	0.79	0.70	0.80	0.66	0.73**
Recovery								
BUN (mg/dL)	17.2	18.5	NA	NA	NA	NA	42.3**	30.4*
Creatine (mg/dL)	0.55	0.36	NA	NA	NA	NA	0.90**	0.81*
Total protein (g/dL)	6.1	5.8	NA	NA	NA	NA	5.7**	6.7
Ca (mg/dL)	10.41	10.97	NA	NA	NA	NA	10.71	11.42*
Phospholipid (mg/dL)	103	179	NA	NA	NA	NA	158**	238**
Cholinesterase (IU/L)	568	4777	NA	NA	NA	NA	654	4203
GGT (IU/L)	0.8	2.2	NA	NA	NA	NA	1.1	2.3
IP (mg/dL)	4.7	4.0	NA	NA	NA	NA	5.7*	4.3
A/G ratio	0.66	0.81	NA	NA	NA	NA	0.61	0.73**

NA = Not Applicable
 Dunnett's (equal no. of animals) or Scheffe's (unequal no. animals) test *p<0.05, **p<0.01
 = Trace -Mild --Moderate ---Marked

2.6.7.7F Repeat-Dose Toxicity Study No: S054C3R100 (Cont.) Test Article: Febuxostat

Daily Dose (mg/kg)	0 (Control)		3		12		48	
	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Urinalysis								
End Dose								
Volume (mL/24hr)	10.0	9.4	11.0	9.9	11.8	11.1	24.3**	16.7**
Specific gravity	1.037	1.027	1.033	1.024	1.031	1.022	1.017**	1.015*
Na (mg/24hr)	2.20	3.56	1.63	3.14	1.76	2.66	5.75**	4.72
K (mg/24hr)	47.08	30.99	45.59	29.51	44.23	29.51	60.89**	36.46
Cl (mg/24hr)	3.31	3.32	7.00	3.26	7.80	7.59	17.52**	11.56**
pH	6.8	7.0	7.0	6.7	7.2	6.9	7.5**	7.0
Creatinine (mg/24hr)	12.38	6.14	11.92	6.48	12.48	6.68	13.08	7.18**
Protein (mg/dL)	9+, 6+++	2=, 3+	3=, 6+++, 1+++	3=, 5+	11+, 3+++, 1+++	3=, 1+	9=, 2+++, 3+++	1=, 4+, 5=, 5---
Recovery								
Volume (mL/24hr)	9.0	6.5	NA	NA	NA	NA	18.8**	11.7*
Specific gravity	1.043	1.040	NA	NA	NA	NA	1.027**	1.030*
Na (mg/24hr)	3.29	4.86	NA	NA	NA	NA	6.98**	5.57
K (mg/24hr)	52.73	34.12	NA	NA	NA	NA	73.93**	43.78*
Cl (mg/24hr)	11.33	9.90	NA	NA	NA	NA	15.87*	11.61
pH	6.5	6.5	NA	NA	NA	NA	6.6	6.6
Creatinine (mg/24hr)	11.87	6.56	NA	NA	NA	NA	13.39	7.57**
Protein (mg/dL)	7+, 2=+, 6+++	3=, 8+, 1+++	NA	NA	NA	NA	1=, 4+, 9+++	1=, 4+, 3=, 5---

NA = Not applicable
 Dunnett's (equal no. of animals) or Scheffe's (unequal no. animals) test *p<0.05, **p<0.01
 = Trace + Mild ++ Moderate +++ Marked

APPEARS THIS WAY
 ON ORIGINAL

2.6.7.7F Repeat-Dose Toxicity Study No: S054C3R100 (Cont.) Test Article: Febuxostat

Daily Dose (mg/kg)	0 (Control)		3		12		48	
	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Histopathology								
End Dose								
Number examined	15	15	15	15	15	15	15	15
Kidney								
Palvic calculi	0	0	0	0	2=	0	2=, 10=, 1=	3=, 3+
Palvic cellular infiltration	0	1=	0	1=	3=	2=	5=, 8+	1=, 7=, 6=+
Palvic mucosal hyperplasia	0	0	0	0	0	0	8=	7=
Tubular dilatation	0	0	0	1=	1=	1=	2=, 1=, 12=+	2=, 4=, 9=+
Tubular epithelial basophilic change	0=	1=	6=, 1=	1=	7=, 1+	3=	1+, 14=+	3=, 6=, 6=+
Tubular basement membrane thickening	5=	1=	2=	0	3=	0	1=, 1=, 13=+	7=, 3=, 4=+
Cellular infiltration	6=	1=	3=	1=	4=	2=	2+, 13=+	1=, 7=, 6=+
Hyaline casts	3=	1=	2=, 1=	2=	3=	1=	1=, 6+, 8=+	4=, 0+
Leukocyte casts	0	0	0	0	0	0	1=, 8=, 3=+	3=, 2=, 5=
Tubular basophilic deposition	0	0	0	0	0	0	3=, 11+	2=, 4=
Foreign body giant cells	0	0	0	0	0	0	6=, 5=	5+
Bowman's capsule basement membrane thickening	0	0	0	0	0	0	11=, 4=+	4=, 0+
Tubular epithelial cell necrosis	0	0	0	0	0	0	1=, 12=, 1=+	3=, 6=
Pigment deposition	3=	0	2=	1=	1=	0	15=	6=, 6=
Glomerular sclerosis	0	0	0	0	0	0	3=	0
Interstitial fibrosis	0	0	0	0	0	0	1=, 2=, 12=+	3=, 6=, 4=+
Urinary Bladder								
Calculi	0	0	0	0	0	0	1=	0
Dilatation	1=	0	0	0	0	0	1=	0
Hyperplasia	0	0	0	0	0	0	3=, 2=	0
Lung								
Cellular infiltration	0	0	0	1=	0	0	1=	3=

= Slight + Mild ++ Moderate +++ Marked

2.6.7.F Repeat-Dose Toxicity Study No: S054C3R100 (Cont.) Test Article: Febuxostat

Daily Dose (mg/kg)	0 (Control)		3 ^a		12 ^a		49	
Number of Animals	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
Histopathology (Cont.)								
Recovery								
Number Examined	15	15	NA	NA	NA	NA	14	13
Kidney								
Pelvic calculi	0	0	NA	NA	NA	NA	1=, 1+	1=
Pelvic cellular infiltration	0	0	NA	NA	NA	NA	0=, 2+	4=, 3-, 1+
Pelvic mucosal hyperplasia	0	3	NA	NA	NA	NA	2=	3=, 1+
Tubular dilatation	0	0	NA	NA	NA	NA	2=, 4+, 7+	4=, 1-, 4+
Leukocyte casts	0	0	NA	NA	NA	NA	5=, 1+	2=
Tubular epithelial basophilic change	10=	1=	NA	NA	NA	NA	5-, 9+	5+, 6+
Tubular basement membrane thickening	4=	1=	NA	NA	NA	NA	1=, 3-, 10+	1=, 4-, 6+
Cellular infiltration	4=	0	NA	NA	NA	NA	4-, 10+	4-, 6+
Hyalin casts	3=, 2+	2=	NA	NA	NA	NA	4=, 2+, 6+	7=, 2-, 4+
Leukocyte casts	0	0	NA	NA	NA	NA	5=, 1-	2=
Tubular basophilic deposition	0	0	NA	NA	NA	NA	4=	0
Foreign body giant cells	0	0	NA	NA	NA	NA	0	1=
Bowman's capsule basement membrane thickening	0	0	NA	NA	NA	NA	2=, 12+	2=, 3+
Tubular epithelial cell necrosis	0	0	NA	NA	NA	NA	3=, 9-	5-
Pigment deposition	2=	0	NA	NA	NA	NA	3=, 10+	5=, 6+
Glomerular sclerosis	0	0	NA	NA	NA	NA	6=	3=
Interstitial fibrosis	0	0	NA	NA	NA	NA	1=, 4-, 9+	3+, 6+

= Slight + Mild → Moderate ↔ Marked
 d Recovery data was not collected for the mid and low doses.
 NA = Not Applicable

APPEARS THIS WAY
 ON ORIGINAL

2.6.7.H Repeat-Dose Toxicity Report Title: 52 Week Repeated-Dose Toxicity Study of TEL-6720 by Oral Administration in Dog Test Article: Febuxostat

Species/Strain: CSK Beagle Dog Duration of Dosing: 52 Weeks Study No: S054C4D100
 Initial Age: 7 months Duration Post-Dose: 13 Weeks
 Date of First Dose: 2 Sep 97 Method of Administration: Oral Location of CTD: 4.2.3.2.3
 Special Features: Toxicokinetics Vehicle/Formulation: Wafer in Galatin Capsule
 NOAEL: 5 mg/kg/day No Observed Toxic Effect Level: 15 mg/kg/day GLP Compliance: Yes

Daily Dose (mg/kg/day)	0 (Control)		5		15		45	
Number of Animals	M:6 ^a	F:6 ^a	M:3	F:3	M:3	F:3	M:6 ^b	F:6 ^b
Toxicokinetics								
AUC_{0-12h} (ng·hr/mL)								
Day 1	ND	ND	5544	1067	17952	25691	169053 ^c	155745 ^c
Day 183	ND	ND	2267	6906	27751	31784	335067 ^d	511542 ^d
Day 365	ND	ND	4032	5067	31876	33060	393441 ^e	572723 ^e
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	1 ^f	1 ^f
Body Weight (%^g)								
Day 364	11.30 kg	10.87 kg	+3.3	-5.2	-2.9	-7.5	-5.8	-4.1
Recovery Day 91	12.69 kg	11.70 kg	ND	ND	ND	ND	-5.4	-9.3
Food Consumption (%^g)								
-								
Chemical Obs.^h								
Vomiting	4	5	2	2	3	3 ⁱ frequency	5	6 ⁱ frequency
Salivation	-	-	-	-	-	-	2	3

a Includes 3 dogs/sex in recovery group.
 b N=3 (Values for 3 animals that vomited excluded).
 c N=5 (One animal sacrificed in moribundity of Day 190).
 d N=3 (Value for animal that vomited excluded).
 e N=5 (One animal sacrificed in moribundity on Day 176).
 f Moribund sacrifice (recovery group animals).
 g Mean weights shown for control. Percent differences from control shown for treated animals.
 h Reported incidences for main study animals only, number of animals with observation.
 ND = Not Done
 ↑ increased
 - No noteworthy findings

2.6.7.7H Repeat-Dose Toxicity

Study No: S054C4D100 (Cont.)

Test Article: Febuxostat

Daily Dose (mg/kg/day)	0 (Control)		5		15		45	
	M:6 ^a	F:6 ^a	M:3	F:3	M:3	F:3	M:6 ^a	F:6 ^a
Number of Animals								
Noteworthy Findings (Cont.)								
Urinalysis (Week 52)								
Volume (mL/24hr)	475	298	367	252	350	340	365	679
Specific gravity	1.027	1.055	1.032	1.043	1.027	1.035	1.031	1.017
Occult blood	5-, 1+	5-, 1+	2-, 1+	3-	5-, 1+	3-	2-, 3+	2-, 3+
Round granule in sediment	6-	6-	3-	3-	3-	3-	4-, 1+	3-, 1+, 1+
Erythrocytes in sediment	5-, 1+	5-, 1+	3-	3-	3-	3-	4-, 1+	3-, 2+, 2+
Leucocytes in sediment	4-, 1=, 1+	3-, 1=, 2+	1-, 3+, 1+	2-, 1-	1-, 1=, 1+	2-, 1+	1-, 1+	1-, 1+
Recovery (Week 13)								
Occult blood	3-	3-	ND	ND	ND	ND	2-	2-
Erythrocytes in sediment	3-	3-	ND	ND	ND	ND	2-	1-, 1+

a Includes 3 dogs/sex in recovery group. Week 51 values for survivors.
 - negative = Trace + Slight ++ Moderate
 ND = Not Determined

2.6.7.7H Repeat-Dose Toxicity

Study No: S054C4D100 (Cont.)

Test Article: Febuxostat

Daily Dose (mg/kg/day)	0 (Control)		5		15		45	
	M:6 ^a	F:6 ^a	M:3	F:3	M:3	F:3	M:6 ^a	F:6 ^a
Number of Animals								
Noteworthy Findings (Cont.)								
Bone Marrow	-	-	-	-	-	-	-	-
Ophthalmology	-	-	-	-	-	-	-	-
Gross Pathology ^b								
Kidney								
Yellow granules	-	-	-	-	-	1	1	-
Yellow calculi pelvis	-	-	-	-	-	-	2	3
Dilatation pelvis	-	-	-	-	-	-	-	3
Hard surface	-	-	-	-	-	-	2	-
Uneven surface	-	-	-	-	-	-	1	3
Whitened surface	-	-	-	-	-	-	1	2
Urinary Bladder	-	-	-	-	-	-	-	-
Yellow granules	-	-	-	-	-	-	1	-
Lower mucosa red portion	-	-	-	-	-	-	1	-
Mucosa red masses	-	-	-	-	-	-	-	1
Lumbar lymph nodes (periaxillary swellings)	-	-	-	-	-	-	-	1
Organ Weights								
Kidney								
Absolute (g) ^b	-	43.59±g	-	+9.5	-	+2.7	-	+28.8*
Relative (%)	-	0.413	-	-15.6	-	-9.2	-	+37.5*
Pancreas								
Absolute (g) ^b	-	22.643g	-	0	-	+1.5	-	+20.3
Relative (%)	-	0.213	-	+3.8	-	-8.0	-	-30.0**

a Includes 3 dogs/sex in recovery group. Week 51 values for survivors.
 - No noteworthy findings.
 Dunnett's multicomparison test: *p<0.05, **p<0.01
 g Mean weights shown for control. Percent differences from control shown for treated animals.
 h Reported incidences for main study only, number of animals with observation.

APPEARS THIS WAY
 ON ORIGINAL

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ON ORIGINAL

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

TMX-67 is a non-purinergeric inhibitor of xanthine oxidase activity in vitro and in vivo. The in vitro K_i for inhibition of xanthine oxidase is about 10 nM. TMX-67 inhibited uric acid levels in the plasma at 1.6 and 5 mg/kg in rats and chimpanzee, respectively. It has negligible activity for purine and pyrimidine synthesis at the doses showed xanthine oxidase activity. Therefore, it is not expected to have any anti-metabolite-like effect. The sponsor did not investigate its anti-inflammatory activity in appropriate models of experimental inflammation except urate crystal induced exudates formation in rats. However, the anti-inflammatory activity of TMX-67 could not be predicted from the results of the experiment. TMX-67 inhibited LPS induced TNF- α release in rats at 10 mg/kg/oral. Clinical significance of this effect is unknown. TMX-67 has no effect in the CNS, GI, cardiovascular and respiratory systems at pharmacodynamic doses. However, transient hypotensive effect was observed in conscious beagle dogs at 5 and 50 mg/kg oral doses. TMX-67 showed diuretic effect at 100 mg/kg single dose in rats associated with increased excretion of potassium, chloride and xanthine in the urine.

TMX-67 is rapidly absorbed after oral dosing and excreted as hydroxylated, carboxylated and desbutyl derivatives as Phase I metabolites in animals and human. However, TMX-67 glucuronide is the major urinary metabolite in humans. Hydroxylation of TMX-67 formed R and S isomers of M1 in rat and human urine. The metabolic profiles were qualitatively similar in rodents, dogs and humans. Female mice showed a gender difference in the exposure. Pharmacodynamic activity of the metabolites was not greater than TMX-67 for xanthine oxidase inhibition. Therefore, it is not considered to be a prodrug. P450 CYP1A1, CYP1A2 and CYP2C9 isozymes were involved in the Phase I metabolism. TMX-67 showed across placental transfer and was excreted in the milk in rats. Therefore, rats treated with TMX-67 at 48 mg/kg during lactation resulted in higher pup mortality and deposition of xanthine crystals in the kidney. Hepatic induction of enzyme was not evident from the studies.

TMX-67 is not mutagenic in the Ames assay in Salmonella typhimurium and E. coli. It also showed increased chromosomal aberration in Chinese hamster lung fibroblast cells in the presence and absence of S-9 liver homogenates in vitro. The sponsor conducted the recommended battery of mutagenicity studies.

TMX-67 showed papilloma and carcinoma of transitional cells in the urinary bladder in male F 344 rats at 24 mg/kg (16 times plasma exposure at maximum recommended human dose, MRHD). A similar effect was also noted in female B6C3F1 mice at 18.75 mg/kg (8 times plasma exposure at MRHD). The effect of TMX-67 was secondary to xanthine crystal deposition in the kidney and urinary bladder.

Reproductive safety study did not show any effect for the fertility and reproductive performance. No teratogenicity, variation or embryocidal effect of TMX-67 was observed. However, nursing performance, survival and weight gain of second generation of rats were affected by the treatment with TMX-67 at 48 mg/kg in rats (25 times human plasma exposure). The excretion of drug through milk was also evident from the deposition of xanthine crystals in the kidney in new born rats nursed by dams treated with TMX-67 at 48 mg/kg.

Long term studies were conducted in rats and dogs to determine organ system toxicity and clinical signs. Salivation, diarrhea and vomiting were noted in beagle dogs. Salivation and decreased activity were noted in rats as clinical signs. A 12-month toxicity study in beagle dogs showed deposition of xanthine crystals and calculi in kidneys at 15 mg/kg (3.0 times human exposure at MRHD). A similar effect on the calculus formation was noted in rats due to deposition of xanthine crystals at 48 mg/kg (23 times human exposure) in the six-month study. A similar effect was also observed in the 2-year study in rats.

Therefore, it is concluded that TMX-67 is a xanthine oxidase inhibitor. It is devoid of antimetabolite-like activity based on its pharmacological effect reviewed in the submission. TMX-67 was genotoxic in the chromosomal aberration assay in Chinese lung fibroblasts, and showed papilloma and carcinoma of transitional cell in the urinary bladder in rodents secondary to calculus formation in the kidney and urinary tract. It is not teratogenic and did not affect fertility. However, total litter death was observed in four dams at 48 mg/kg when treated from gestation day 7 to lactation day 20 in a rat study. Therefore, pregnancy category C is recommended. Major toxicity profile based on the non-clinical studies was increased xanthine deposition and formation of crystals in the kidney and urinary tract due to low solubility of xanthine (1 mg/15 ml of water). Therefore, the proposed clinical dose of 120 mg daily (2 mg/kg) is safe from the organ system toxicity based on the non-clinical studies. However, the possibility of calculus formation in the kidney and urinary tract could not be ruled out as it was observed in rats, mice and dogs following chronic treatment at doses that had ranged 3.0-16 folds human exposure based on AUC at MRHD.

Unresolved toxicology issues (if any): Nil

Recommendations:

Based on the non-clinical data, TMX-67 (TMX-67) is approvable at 120 mg daily dose for the treatment of hyperuricemia. It is recommended that kidney functions and levels of xanthine in the urine need to be monitored for chronic treatment due to the formation of calculi in the kidney and urinary tract observed in animals. The proposed changes in the package insert are shown below.

Suggested labeling:

3 Page(s) Withheld

 Trade Secret / Confidential (b4)

 / Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

/ / / /

b(4)

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

C.C:

NDA 21-856 Div File
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NDA21856Jan182005.doc

APPENDIX/ATTACHMENTS

1. Histopathology inventory (optional)

Study	12-mo	6-mo
Species	Beagle dog	SD rats
Adrenals	x	x
Aorta	x	x
Bone Marrow smear	x	x
Bone (femur)	x	x
Brain		
Cecum	x	x
Cervix		
Cerebrum	x	x
Cerebellum	x	x
Colon	x	x
Duodenum	x	x
Epididymis	x	x
Esophagus	x	x
Eye	x	x
Fallopian tube		
Gall bladder	x	
Gross lesions		
Harderian gland		x
Heart	x	x
Ileum	x	x
Injection site		
Jejunum	x	x
Kidneys	x	x
Lachrymal gland		
Larynx		x
Liver	x	x
Lungs	x	x
Lymph nodes, cervical		
Lymph nodes mandibular		
Lymph nodes, mesenteric	x	x
Lymph nodes, submaxillary		x
Mammary Gland	x	x
Medulla oblangata	x	
Nasal cavity		
Optic nerves	x	x

Study	12-mo	6-mo
Species	Beagle dog	SD rats
Ovaries	x	
Pancreas	x	x
Parotid glands	x	x
Parathyroid	x	x
Peripheral nerve		
Pharynx	x	
Pituitary	x	x
Preputial gland		x
Prostate	x	x
Penis	x	
Rectum	x	x
Salivary gland		
Sciatic nerve	x	x
Seminal vesicles		x
Skeletal muscle (thigh)	x	x
Skin	x	x
Spinal cord	x	x
Spleen	x	x
Sternum	x	x
Stomach	x	x
Submandibular gland	x	
Sublingual gland	x	
Submaxillary gland		x
Testes	x	x
Thymus	x	x
Thyroid	x	x
Tongue	x	x
Trachea	x	x
Urinary bladder	x	x
ureter	x	
Uterus	x	
Vagina	x	
Zymbal gland		

X, histopathology performed

2. Review of rat carcinogenicity data and ECAC minutes are attached below.

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 58,229

Review number: Four

Sequence number/date/type of submission: Serial # 070, May 2, 2002 and serial # 123, Sept 2, 2003 and Serial # 141, Jan 30, 2004.

Information to sponsor: Yes (x) No ()

Sponsor and/or agent: TAP Pharmaceuticals, Chicago, IL

Manufacturer for drug substance: Abbott Laboratories, Chicago, IL

Reviewer name: Asoke Mukherjee, Ph.D.

Division name: Division of Anti-inflammatory, Analgesic and Ophthalmic Drug Products.

HFD #: 550

Review completion date: Feb 23, 2004

Drug:

Trade name: Uloric

Generic name (list alphabetically): Febuxostat

Code name: TMX-67, TEI-6720, A-319198, A-319108.0, Abbott-319198

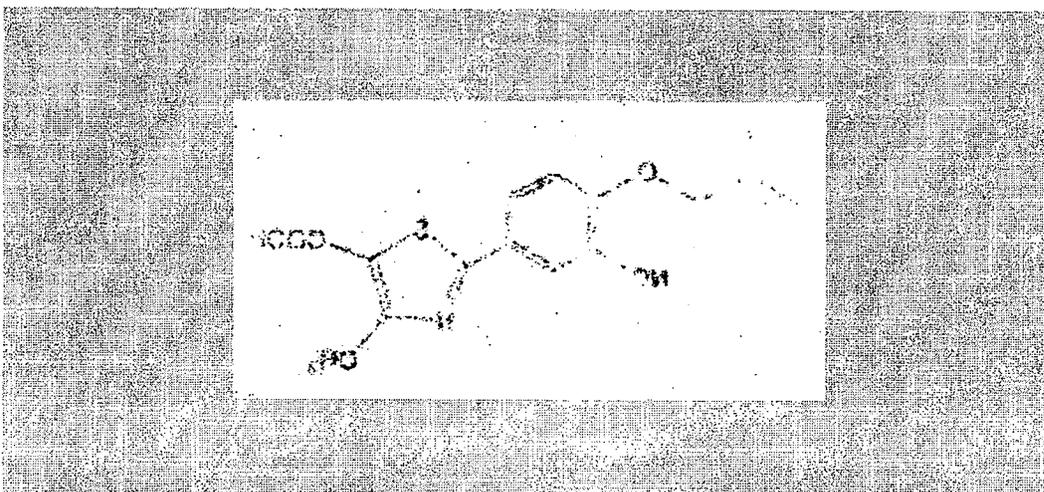
Chemical name: 2-(3-cyano-4-isobutoxyphenyl-4-methyl-5-thiazole carboxylic acid

CAS registry number: 144060-53-7

Mole file number:

Molecular formula/molecular weight: C₁₆H₁₆N₂O₃S, MW 316.38

Structure:



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Relevant INDs/NDAs/DMFs:

Drug class: Xanthine Oxidase inhibitor

Indication: Gout

Clinical formulation: See review dated Nov 26, 2002.

Route of administration: Oral

Proposed clinical protocol: Nil in this amendment

Previous clinical experience: See review dated Nov 26, 2002.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: See review dated Nov 22, 2002.

Studies reviewed within this submission:

1. Carcinogenicity study of TEI-6720 in rats. (Serial #70, vol 2, page 202, and Serial # 141).

Studies not reviewed within this submission: Nil

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ON ORIGINAL**

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY: 1

II. SAFETY PHARMACOLOGY:..... 1

III. PHARMACOKINETICS/TOXICOKINETICS:..... 1

IV. GENERAL TOXICOLOGY: 1

V. GENETIC TOXICOLOGY:..... 1

VI. CARCINOGENICITY:..... 1

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY: 10

VIII. SPECIAL TOXICOLOGY STUDIES:..... 10

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:..... 10

X. APPENDIX/ATTACHMENTS: 12

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ON ORIGINAL**

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

No pharmacology data were submitted in the amendment.

II. SAFETY PHARMACOLOGY:

No safety pharmacology data were submitted in the IND.

III. PHARMACOKINETICS/TOXICOKINETICS:

Toxicokinetic data were presented in the toxicity reviews dated Nov 26, 2002.

IV. GENERAL TOXICOLOGY:

No general toxicity study report was submitted in the amendment.

V. GENETIC TOXICOLOGY:

No genotoxicity data were submitted in the amendment.

VI. CARCINOGENICITY:

Study title: Carcinogenicity study of TEI-6720 in rats

Key study findings: Increased incidence of urinary bladder transitional cell papilloma and carcinoma in male rats was observed at 24 mg/kg doses.

Study number: 4259 (011-027) and 4260 (011-028)

Volume # 2, and page #: 201

Conducting laboratory and location: _____

Date of study initiation: July 12, 1999

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot # and % purity: TMX-RP-120 (2000.3-2001.8), 100.1% purity

CAC concurrence: CAC discussed the protocol and did not agree to the dose selection (see attached CAC meeting minutes dated Sept 21, 1999). However, based on data from the repeat dose toxicity study in Fisher rats, the ECAC concluded that the selection of 24 mg/kg as the high dose was acceptable (see attached ECAC meeting minutes dated May 15, 2001).

Study Type (2 yr bioassay, alternative model etc.): Two year bioassay

Species/strain: F344 Fisher rats.

b(4)

Number/sex/group; age at start of study:

The study design is shown below.

Group/Dose	Gr 1, 0 mg/kg		Gr 2, 3 mg/kg		Gr 3, 6 mg/kg		Gr 4, 12 mg/kg		Gr 5, 24 mg/kg	
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. animals	50	50	50	50	50	50	50	50	50	50
	1001-1050	2001-2050	1101-1150	2101-2150	1201-1250	2201-2250	1301-1350	2301-2350	1401-1450	2401-2450

Animal housing: Animals were housed individually. The room temperature was 23°C with 55% relative humidity. Rats were fed — rat formula. The diet was analyzed every 3 months. The composition of the diet is: _____

b(4)

Formulation/vehicle: The drug substance was suspended in 0.5% methyl cellulose in distilled water. The suspensions were prepared once a week and stored in dark at room temperature.

Drug stability/homogeneity: The 0.6 and 9.6 mg/ml suspensions were stable for 2 weeks at room temperature. Samples from 0.6, 1.2, 2.4 and 4.8 mg/ml were taken while stirring the suspensions at the end of dosing at weeks 78, 91 and 104. The concentrations were within 103.8-105.5% of the nominal concentration. The uniformity of the suspensions was determined by taking samples from upper, middle and lower layers. The percent of the label claim was within 96-104.7%.

Methods:

Doses: Animals were treated at 3, 6, 12 and 24 mg/kg/day.

Basis of dose selection:

A 13-week dose selection study was conducted in F344 rats at 3, 12, 24 and 36 mg/kg oral doses. Basophilic pigmentation in the renal tubules and collecting ducts, inflammation of kidney were noted at 24 and 36 mg/kg. Xanthine crystals in the urine were also noted at 24 and 36 mg/kg.

The serum PK parameters of TMX-67 in F344 rats treated at 3, 12, 24 and 36 mg/kg on day 1 and at week 13 were provided by the sponsor and are shown in the table below. Data are provided in page 2322, vol. 7 of serial #070.

Dose, mg/kg	T _{max} (h)		C _{max} (µg/ml)		AUC ₀₋₂₄ (µg.h/ml)	
	Day 1	Week 13	Day 1	Week 13	Day 1	Week 13
3, male	1.0	0.5	1.23	5.51	6.55	17.31
3, female	0.5	0.5	2.60	3.82	8.55	16.93
12, male	0.5	0.5	8.47	21.34	36.41	66.68
12, female	0.5	0.5	11.99	13.53	47.46	81.11
24, male	0.5	0.5	25.30	40.34	103.67	194.45
24, female	0.5	0.5	35.52	30.83	128.14	201.77
36, male	0.5	0.5	38.25	55.59	152.96	312.97
36, female	1.0	0.5	41.45	47.71	185.66	306.81

The sponsor submitted the human pharmacokinetic data at 240 mg/day dose (maximum dose used in the Phase 3 study) as shown below.

IND 58,229

The mean (\pm SD) peak and total plasma exposures to febuxostat following multiple dosing with 120 mg QD^a, 240 mg QD^b, and 300 mg QD^b of febuxostat

Dosage	N	C _{max} ^c (μ g/mL)	AUC ₀₋₂₄ ^c (μ g.h/mL)
120 mg QD	9	5.31 \pm 1.68	11.96 \pm 2.42
240 mg QD	8	11.26 \pm 4.04	34.98 \pm 9.91
300 mg QD	10	14.25 \pm 5.44	48.36 \pm 15.70

a TMX-99-001 study

b C02-02-023 study

c at steady state

The exposure ratio is about 4.8 based on AUC. The limitation of the exposure comparison was that data were obtained from plasma samples for humans and from serum samples for rats. The sponsor stated that AUC values of the 12 mg/kg group represented about 25-fold human exposure. However, available data do not support the conclusion. It appears that the 24 mg/kg would be qualified as a MTD if basophilic changes and inflammation in the kidney were considered treatment-related toxicity following 3-month treatment in SD rats. The ECAC committee suggested that the 3-month toxicity study be repeated for the selection of appropriate MTD although kidney lesions e.g., tubular basement membrane thickening and basophilic deposits were observed at 36 mg/kg. The CAC committee concluded that 24 mg/kg could not be considered as the MTD because the sponsor did not show that a dose higher than 36 mg/kg was not tolerated. The sponsor conducted another 90-day toxicity study in Fisher rats at 48, 75 and 150 mg/kg doses. The ECAC accepted the carcinogenicity study design based on the data of this 90-day repeat dose study in Fishers rats.

Restriction paradigm for dietary restriction studies: Nil

Route of administration: Oral gavage for 104 weeks

Frequency of drug administration: Once a day

Dual controls employed: No

Interim sacrifices: Nil

Satellite PK or special study group(s): Five rats/sex from groups 2, 4 and 5 were allotted for the toxicokinetic study. These animals were sacrificed after blood collection. Samples were collected after first dose at week 13 and week 26. However, the

summary on page 211 stated that 15 males and 15 females were used each in the 3, 12 and 24 mg/kg groups for the determination of serum concentration of TEI-6720.

Deviations from original study protocol:

A minor modification was made for the facilities of TK sample analysis.

Statistical methods: Homogeneity of the data was analyzed by Bartlett's variance test. Homogenous data were further analyzed by Dunnett's multiple comparison test. Heterogeneous data were analyzed by Kruskal-Wallis rank analysis. The survival and neoplastic or non neoplastic lesions were analyzed by Fisher's Exact test. Peto's trend test was applied to the tumor data.

Observations and times:

Clinical signs: All animals were observed at least twice a day. Palpation was performed once a week for examining any palpable tumor.

Body weights: Body weights were recorded once a week.

Food consumption: The leftover food was measured once a week and food consumption was recorded. The weekly food consumption was calculated as g/week. The percent food efficiency was calculated until week 52.

Hematology: Blood samples were collected from all surviving animals at the end of dosing. Animals were fasted for 16 hours prior to the blood collection. Blood samples were collected from the abdominal aorta under ether anesthesia.

Clinical chemistry: Blood samples were collected from first 10 animals for clinical chemistry analysis at the end of the dosing period. Plasma samples were separated for the assay of standard parameters.

Organ weights: Animals were sacrificed by exsanguination under ether anesthesia. Weights of following organs were recorded.

Brain, heart, liver, kidney, spleen, adrenal glands, testes, ovaries and thymus. Organ weight was normalized to the body weight.

Gross pathology: The body surface, body cavities and organs were examined for gross changes at necropsy. Tissues were fixed in 10% formalin. Eyes were fixed in Davidson's fixative.

Histopathology: Initially histopathology was conducted on tissues from the control, high dose, moribund and dead animals. In response to the review division's request, the sponsor submitted histopathology data for all animals in a separate amendment, Amendment #141 dated Jan 30, 2004.

Toxicokinetics: Blood samples were collected from 5 male and 5 female rats each in the 3, 12 and 24 mg/kg groups on the first day, and at weeks 13 and 26 after dosing. Blood samples were collected from the jugular vein at 30 min and 24 hours after the dose. Serum levels of TEI-6720 were determined by the HPLC method.

Results:

Mortality: Percent mortality is shown in the table below. The actual number of deaths is shown in the parentheses.

Week	Control		3 mg/kg		6 mg/kg		12 mg/kg		24 mg/kg	
	M	F	M	F	M	F	M	F	M	F
53-65	0	0	0	0	2% (1)	0	2% (1)	0	0	0
66-78	2% (1)	2% (1)	0	0	4% (1)	4% (2)	4% (1)	6% (3)	2% (1)	6% (3)
79-91	8% (3)	8% (3)	2% (1)	8% (4)	12% (4)	20% (8)	10% (3)	16% (5)	16% (7)	16% (5)
92-104	24% (8)	22% (7)	20% (9)	30% (11)	18% (3)	30% (5)	20% (5)	40% (12)	36% (10)	34% (9)

Above data show that male and female animals showed a similar trend in the mortality. The high dose showed higher mortality than the control. However, the increased mortality in the high dose group was not statistically significant.

Clinical signs: There were no treatment related clinical signs observed in the study. Some of the changes are shown in the table below.

Sign, Week, Sex	Control	3	6	12	24 mg/kg
Wasting, 53-78, male	1	0	1	1	2
Wasting, 79-104, male	6	9	7	6	13
Wasting, 53-78, female	1	1	1	4	3
Wasting, 79-104, female	8	10	12	15	12
Piloerection, male, 79-104	5	10	6	7	12
Decrease motor activity, male, 79-104	5	9	5	4	11
Pallor/auricles, 53-78, female	0	0	1	4	3
Pallor/auricles, 79-104, female	8	14	12	16	10

Body weights: The body weight gain (g) is shown in the table below. There was no treatment-related change in the body weight and body weight gain.

Dose, mg/kg	Week 1, male	Week 104, male	Wt Gain, male	Week 1, female	Week 104, female	Wt Gain, female
0	114	349	259	91	253	178
3	115	364	273*	91	249	173
6	113	354	263	91	250	174
12	114	356	265	91	254	177
24	115	344	254	91	256	179

Food consumption:

The food consumption in g/day is shown in the table below.

Dose, mg/kg	Week 1, M	Week 2, F	Week 104, M	Week 104, F	Total Food Consumption, M	Total Food Consumption, F
Control	12	10	14	13	10232	8146
3	12	10	15	13	10371	8195
6	12	10	15	13	10577*	8334
12	13	10	15	13	10569*	8352
24	13	10	15	14	10610*	8597*

No treatment-related changes in weekly food consumption were observed although there was a slight increase in total food consumption by 3.7 and 5.5% in the high dose males and females, respectively.

Hematology:

Hematology data at the end of 104 weeks of the treatment did not show any treatment-related changes. Statistically significant changes in the mean corpuscular hemoglobin levels were observed in the male rats at 12 and 24 mg/kg doses. However, the change was minimal and not considered to be biologically significant. The MCH (pg) levels were 16.9, 17.1, 16.9, 16.7 and 16.3 at 0, 3, 6, 12 and 24 mg/kg doses, respectively.

Clinical chemistry:

Some of the changes at the end of 104 weeks of treatment are shown in the table below. However, these changes were minimal and do not have toxicological significance.

	Control		3		6		12		24 mg/kg	
	M	F	M	F	M	F	M	F	M	F
BUN, mg/dl	15.1	12.6	14.9	13.5	15.5	14.1*	15.7	12.9	22.5*	14.5*

Animal #1414 (Male) at 24 mg/kg showed BUN levels of 49.2 mg/dL. Otherwise, all values were close to the normal range.

Organ weights:

	0		3 mg/kg		6 mg/kg		12 mg/kg		24 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Heart (g)	1.05	0.83	1.10	0.83	1.07	0.84	1.09	0.88	1.08	0.88*
Kidney (R)	1.25	0.91	1.28	0.96	1.25	0.90	1.28	0.96	1.21	0.94
Kidney (L)	1.24	0.90	1.26	0.93	1.25	0.91	1.29	0.96*	1.31	0.96*
Adrenals (mg)	64	65	60	67	61	67	67	69	60	72*
Testes	3.35		3.60		4.50*		4.20*		4.21*	
Spleen	1.38	0.88	1.31	0.68	1.50*	0.72	1.38*	1.21	1.19	0.83

Female rats showed a slight increase in the weight of left kidney that could be incidental. Male rats showed an increase in the testicular weight with a corresponding gross change of hypertrophy. No other changes were biologically significant.

Gross pathology:

Gross changes in 50 animals/sex/group are shown in the table below.

Observations	0		3 mg/kg		6 mg/kg		12 mg/kg		24 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Stomach, black patch	1	1	0	0	0	0	1	1	2	4
Liver, granular	7	4	9	13*	5	12*	6	15*	6	11*
Kidney, granular	1	0	4	1	5	0	1	1	15*	2
Kidney, scarred	1	1	5	2	2	0	5	4	17*	8*
Urinary bladder, calculus	0	0	2	0	0	0	2	0	21*	7*
Urinary bladder, nodule	0	0	1	0	0	0	1	0	17*	0
Urinary bladder, thick	0	0	0	0	0	0	0	0	6*	1
Testes, hypertrophic	2		3		11*		5		8*	

* statistically significant

Calculi in the urinary bladder were observed at 24 mg/kg dose in male and female rats. Also hypertrophy of testes were noted in several animals in the treated groups.

Histopathology:

Non-neoplastic:

Some of the changes are shown in the table below.

Lesion	1, M	2, M	3, M	4, M	5, M	1, F	2, F	3, F	4, F	5, F
Liver, atrophy	6	2	0	3	4	5	9	4	13	12
Liver, focal necrosis	5	2	0	4	2	4	4	4	6	9
Liver, single cell necrosis	3	1	2	2	0	4	7	6	10	9
Kidney, calculi	0	2	1	11	47	0	0	1	19	44
Kidney, interstitial nephritis	0	0	0	2	15	0	0	0	3	9
Kidney, fibrosis	0	0	0	0	34	0	1	0	0	11
Kidney, regeneration	1	2	3	8	25	1	3	2	14	21
Kidney, transitional cell hyperplasia	1	2	3	7	41	6	4	0	14	37
Urinary bladder, calculi	0	0	0	2	12	0	0	0	1	2
Urinary bladder fibrosis	0	0	0	4	13	2	0	2	0	1
Urinary bladder, transitional cell hyperplasia	0	1	0	9	38	1	3	1	0	13
Urethra, calculi	0	0	0	7	15	0	0	0	0	2
Urethra, transitional cell hyperplasia	1	1	1	4	13	1	1	0	0	3
Parathyroid, focal hyperplasia	17	18	20	19	30	20	21	21	21	22

Severity of above lesions in the high dose group is shown in the table below.

Target organ	Toxicity	Male	Female
Liver	Atrophy		Slight=12

Target organ	Toxicity	Male	Female
Kidney	Focal necrosis		Slight=9
	Single cell necrosis		Slight=9
	Calculi	Slight=45 Moderate=2	Slight=44
	Interstitial nephritis	Slight=8 Moderate=4 Marked=3	Slight=7 Moderate=1 Marked=1
	Fibrosis	Slight=30 Moderate=2 Marked=2	Slight=10 Moderate=1
Urinary bladder	Regeneration	Slight=11 Moderate=12 Marked=2	Slight=9 Moderate=8 Mark=4
	Transitional cell hyperplasia	Slight=41	Slight=37
	Calculi	Slight=12	
Urethra	Fibrosis	Slight=13	
	Transitional cell hyperplasia	Slight=34 Moderate=4	Slight=12 Moderate=1
Parathyroid	Calculi	Slight=15	
	Transitional cell hyperplasia	Slight=12 Moderate=1	Slight=3
	Focal hyperplasia	Slight=30	Slight=22

Major organs of toxicity were kidney, urinary bladder and urethra. Formation of calculi in the urinary system was responsible for pathological changes in these organs.

Neoplastic:

Some of the histological findings for neoplastic lesions are shown in the table below.

Lesion	1, M	2, M	3, M	4, M	5, M	1, F	2, F	3, F	4, F	5, F
Lymph node, malignant lymphoma	0	0	0	0	1	0	0	0	0	1
Urinary bladder, Transitional cell papilloma	0	0	0	0	10	1	0	0	1	1
Urinary bladder, Transitional cell carcinoma	0	0	0	0	7	0	0	0	0	0
Uterus, Deciduoma						0	0	1	1	1

Animals that showed neoplastic lesions and calculi in the urinary bladder (at high dose) are shown in the table below. Although, table 1, page 116 of the submission # 141 showed 10 male rats had transitional cell papilloma in the urinary bladder, individual animal data showed 9 male rats had such lesions. The individual data also showed 9 male rats had urinary bladder calculi. However, the summary table showed 12 male rats had calculi in the urinary bladder.

It appears that calculus in the urinary bladder was responsible for the lesions. However, not all animals with urinary transitional cell papilloma or carcinoma have calculi in the urinary bladder.

Lesion	# Male	# Female
Lymph node, malignant lymphoma	1415	
Urinary bladder, Transitional cell papilloma	1404, 1405, 1407, 1414, 1420, 1421, 1429, 1436, 1447	2436
Urinary bladder, transitional cell carcinoma	1406, 1411, 1412, 1416, 1426, 1431, 1446	
Urinary bladder calculi	1405, 1406, 1411, 1414, 1420, 1421, 1431, 1446, 1447	2417, 2436

Toxicokinetics:

Serum concentration of TMX-67 (ng/ml) in male and female F344 rats after first dose, and at weeks 13 and 26 are shown in the following table (p. 547, vol 2, serial #70).

Day or week	Time after dose	3 mg/kg		12 mg/kg		24 mg/kg	
		Male	Female	Male	Female	Male	Female
Day 1	30 min	858	541	7957	5757	13722	10256
Day 1	24 hr	10.6	18.7	31.7	26.9	55.7	47.6
Week 13	30 min	1956	2492	9714	15891	21131	23864
Week 13	24 hr	35.4	42.1	90.6	169.9	252.1	388.5
Week 26	30 min	2112	2562	12993	17584	23702	38510
Week 26	24 hr	34.2	40.4	94.3	197.7	255.7	341.4

Above data show that the serum TMX-67 levels were increased dose proportionately.

Summary of individual study findings:

Above data show that treatment at doses up to 24 mg/kg, increased incidences of urinary bladder papilloma and carcinoma with increased lesions of urinary bladder fibrosis and transitional cell hyperplasia in male rats were observed. Increased incidences of calculi and toxicity in the kidney (renal fibrosis, regeneration, and interstitial nephritis) were also noted in male and female rats. It appears that the neoplastic lesions were secondary to calculus formation.

The macroscopic change (hypertrophy) in the testes was noted without any histopathological correlates.

Adequacy of the carcinogenicity study and appropriateness of the test model:

The study is adequate to characterize the carcinogenic potential of TMX-67. However, increased calculus formation and pathological changes secondary to the accumulation of calculi in the kidney and urinary bladder were the dose limiting factors in this bioassay. Calculus formation in the renal-urinary tract is rodent specific and its relevance to human is questionable.

Evaluation of tumor findings:

Carcinogenicity summary: Urinary transitional cell papilloma and carcinoma was noted in the high dose in male rats. The difference between male and female rats with respect to neoplastic findings was not due to the differences in the pharmacokinetics of the drug.

Carcinogenicity conclusions: Treatment of TMX-67 at doses up to 24 mg/kg/day for 2 years caused increased incidences of the urinary bladder transitional cell carcinoma in male rats. These neoplastic findings are considered secondary to the calculus formation

Recommendations for further analysis: Nil

Labeling Recommendations:

Male rats treated with TMX-67 up to 24 mg/kg oral dose showed increase incidences of papilloma and carcinoma in the transitional cell of urinary bladder. b(4)

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

No reproductive safety data were submitted in the amendment.

VIII. SPECIAL TOXICOLOGY STUDIES:

No studies were submitted.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: A 104 week carcinogenicity study in Fisher rats showed increased incidences of papilloma and carcinoma of transitional cell of the urinary bladder in male rats. Increased incidence of urinary bladder calculi was also observed. It is concluded that the neoplastic changes were secondary to the calculus formation. Based on the toxicity and tumor findings in the urinary bladder, the study is adequate.

Recommendations to the Sponsor:

1. Please update Investigator's Brochure with inclusion of neoplastic and non-neoplastic findings from the rat 2-year carcinogenicity study.
2. Please include urinary bladder neoplastic findings of this study in the labeling.

Labeling with basis for findings:

Reviewer signature: _____ b(4)

Supervisor signature: Concurrence - _____

Non-Concurrence - _____

cc: list:

IND 58,229

HFD-550/PM/Jane Dean

HFD-550/Reviewer/A. Mukherjee

HFD-550/Team Leader/J. Yang

HFD-550/Chemist/Puttagunta Rao

HFD-550/MO/J. Sciffenbauer

C:\DATA\IND\IND58229 RAT CA STUDY.DOC

REVISED ON FEB 23, 2004, FEB 26, 2004 AND FEB 27, 2004.

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X. APPENDIX/ATTACHMENTS:

**1. List of organs and tissues examined
 Histopathology Inventory for IND 58,229**

Study	104 week			
Species	F344 rats			
Adrenals	x			
Aorta				
Bone Marrow smear	x			
Bone (femur)	x			
Brain	x			
Cecum	x			
Cervix				
Colon				
Duodenum	x			
Epididymis	x			
Esophagus				
Eye	x			
Fallopian tube				
Gall bladder				
Gross lesions				
Hindlimb	x			
Harderian gland	x			
Heart	x			
Ileum	x			
Injection site				
Jejunum	x			
Kidneys	x			
Lachrymal gland				
Larynx				
Liver	x			
Lungs	x			
Lymph nodes, cervical	x			
Lymph nodes mandibular				
Lymph nodes, mesenteric				
Mandibular gland	x			
Mammary Gland	x			
Nasal cavity				
Optic nerves				
Oviduct	x			
Ovaries	x			
Pancreas	x			
Parathyroid	x			
Peripheral nerve				
Pharynx				
Pituitary	x			

Prostate	x			
Rectum	x			
Salivary gland				
Sciatic nerve	x			
Seminal vesicles	x			
Skeletal muscle	x			
Skin	x			
Spinal cord	x			
Spleen	x			
Sternum	x			
Stomach	x			
Tail	x			
Testes	x			
Thymus	x			
Thyroid	x			
Tongue	x			
Trachea				
Ureter	x			
Urethra	x			
Urinary bladder	x			
Uterus	x			
Vagina	x			
Zymbal's gland				
Standard List				

X, histopathology performed

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/s/

Asoke Mukherjee
3/31/04 09:55:57 AM
PHARMACOLOGIST

Josie Yang
3/31/04 10:09:53 AM
PHARMACOLOGIST

Executive CAC

Date of Meeting: March 16, 2004

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Josie Yang, Ph.D., HFD-550, Supervisory Pharmacologist
Asoke Mukherjee, Ph.D., HFD-550, Presenting Reviewer

Author of Draft: Asoke Mukherjee, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND # 58,229

Drug Name: Uloric

Sponsor: TAP Pharmaceuticals

Background:

Uloric (TMX-67, TEI-6720 or Febuxostat), a thiazolecarboxylic acid derivative xanthine oxidase/xanthine dehydrogenase (XOD) inhibitor, is under development for the treatment of gout. Phase 3 studies are underway at 240 mg daily maximum doses. The mouse carcinogenicity data were already presented to the committee on Sept 30, 2003.

Rat Carcinogenicity Study: The study was conducted at 3, 6, 12 and 24 mg/kg doses given orally by gavage to F344 rats (50 rats/sex/dose) for 104 weeks. The treatment did not show statistically significant changes in the mortality and body weight. Several non-neoplastic changes in the kidney and urinary bladder were noted at 24 mg/kg doses in male and female rats. These changes were calculi in the kidney and urinary bladder, kidney fibrosis, interstitial nephritis in the kidney, transitional cell hyperplasia in the kidney and urinary bladder, fibrosis in the urinary bladder, and calculi in the urethra.

Treatment-related neoplastic changes, urinary bladder transitional cell papilloma (10/50) and carcinoma (7/50), were in male rats at 24 mg/kg only. The difference in the prevalence of these observed urinary bladder neoplastic lesions, secondary to the calculus formation, in male and female rats was not due to the difference in the drug exposure. Based on the histopathology data, the maximum tolerated dose was reached and the study is acceptable.

Executive CAC Recommendations and Conclusions:

The committee concluded that there was a treatment-related increase in the incidence of urinary bladder transitional cell papilloma and carcinoma in male rats at 24 mg/kg. Observed urinary bladder neoplasms in male rats were secondary to the excess calculus formation. The study was acceptable.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\

/Division File, HFD-550

/Team leader/ HFD-550/J. Yang

/Reviewer/ HFD-550/A. Mukherjee

/CSO/PM/ HFD-550/J. Dean

/A Seifried, HFD-024

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/s/

David Jacobson-Kram
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3. **Review of mouse carcinogenicity data and ECAC minutes dated Sept 21, 1999 and Sept 30, 2003 are attached below.**

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 58,229

Review number: Two

Sequence number/date/type of submission: Serial # 083, Sept 6, 2002 and serial # 0112 dated May 22, 2003.

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: TAP Pharmaceuticals, Chicago, IL

Manufacturer for drug substance : Abbott Laboratories, Chicago, IL.

Reviewer name: Asoke Mukherjee, Ph.D.

Division name: Division of Anti-inflammatory, Analgesic and Ophthalmic Drug Products.

HFD #:550

Review completion date: Sept 10 , 2003

Drug:

Trade name: Uloric

Generic name (list alphabetically): Febuxostat

Code name: TMX-67, TEI-6720, A-319198, A-319108.0, Abbott-319198

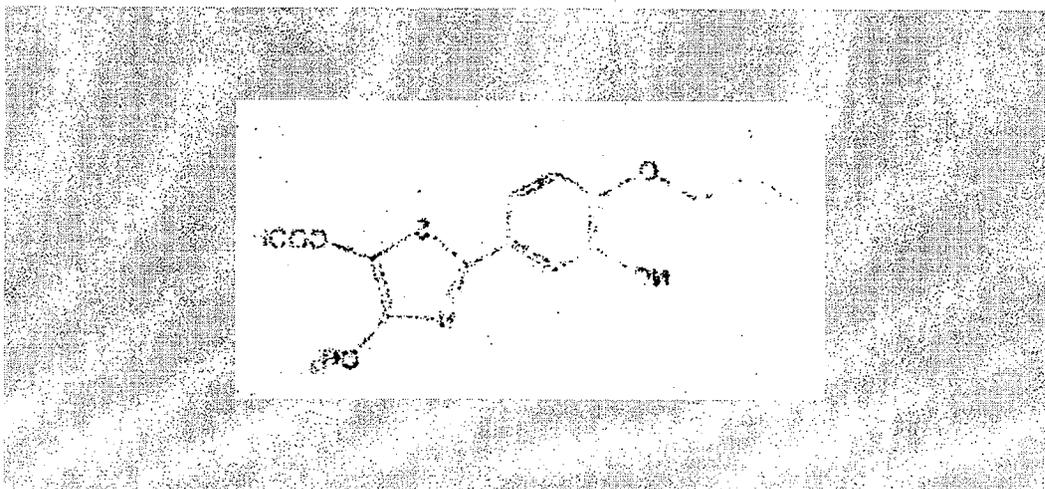
Chemical name: 2-(3-cyano-4-isobutoxyphenyl-4-methyl-5-thiazole carboxylic acid

CAS registry number: 144060-53-7

Mole file number: Not available

Molecular formula/molecular weight: C₁₆H₁₆N₂O₃S, MW 316.38

Structure:



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Relevant INDs/NDAs/DMFs: _____

b(4)

Drug class: Xanthine Oxidase Inhibitor.

Indication: Gout

Clinical formulation: See review dated Nov 26, 2002.

Route of administration: Oral

Proposed clinical protocol: Nil in this amendment.

Previous clinical experience: See review dated Nov 26, 2002.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: See review dated Nov 22, 2002.

Studies reviewed within this submission:

1. A carcinogenicity study of TEI-6720 in mice, page 010, vol 1.

Studies not reviewed within this submission: Nil

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TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY: 1

II. SAFETY PHARMACOLOGY:..... 1

III. PHARMACOKINETICS/TOXICOKINETICS:..... 1

IV. GENERAL TOXICOLOGY: 1

V. GENETIC TOXICOLOGY:..... 3

VI. CARCINOGENICITY:..... 3

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY: 12

VIII. SPECIAL TOXICOLOGY STUDIES:..... 12

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:..... 12

X. APPENDIX/ATTACHMENTS: 14

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ON ORIGINAL**

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

No pharmacology data submitted in the amendment.

II. SAFETY PHARMACOLOGY:

No safety pharmacology data submitted in the IND.

III. PHARMACOKINETICS/TOXICOKINETICS:

Toxicokinetic data presented in the toxicity reviews.

IV. GENERAL TOXICOLOGY:

No toxicity study report was submitted in the amendment.

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Histopathology Inventory for IND # 58,229

Study	24 mos			
Species	Mice, B6C3 F1			
Adrenals	X			
Aorta	X			
Bone Marrow smear	X			
Bone (femur)	X			
Brain	X			
Cecum	X			
Cervix				
Colon	X			
Duodenum	X			
Epididymis	X			
Esophagus	X			
Eye	x			
Fallopian tube				
Gall bladder	X			
Gross lesions				
Harderian gland	x			
Heart	X			
Ileum	X			
Injection site				
Jejunum	X			
Kidneys	X			
Lachrymal gland				
Larynx				
Liver	X			
Lungs	X			
Lymph nodes, cervical				
Lymph nodes mandibular	X			
Lymph nodes, mesenteric	X			
Mammary Gland	X			
Nasal cavity				
Optic nerves				
Ovaries	X			
Pancreas	X			

Parathyroid	X			
Peripheral nerve				
Pharynx				
Pituitary	x			
Prostate	X			
Rectum	X			
Salivary gland				
Sciatic nerve	X			
Seminal vesicles	X			
Skeletal muscle	X			
Skin	X			
Spinal cord	X			
Spleen	X			
Sternum	X			
Stomach	X			
Testes	X			
Thymus	X			
Thyroid	X			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	X			
Urethra	X			
Vagina	X			
Zymbal gland				
Standard List				

X, histopathology performed

V. GENETIC TOXICOLOGY:

No genotoxicity data submitted in the amendment.

VI. CARCINOGENICITY:

Study title: A carcinogenicity study of TEI-6720 in mice (24-month study)

Key study findings: Treatment of mice at 18.75 mg/kg showed increased calculi in the urinary bladder and urinary transitional cell papilloma (3/50) and carcinoma (1/50) in female mice.

Study number: 4257 (carcinogenicity), 4258 Toxicokinetics

Volume #, and page #: Page 010 and vol 1

Conducting laboratory and location: _____

b(4)

Date of study initiation: July 12, 1999

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: TMX-RP-0120 (2000.3-2001.8), purity 100.1%, certificate of analysis is provided on page R-054, vol 1.

CAC concurrence: No, the protocol was discussed on Sept 21, 1999. The CAC minutes stated that transitional cell hyperplasia in the urinary bladder should not be considered for MTD determination. The committee also suggested considering conducting carcinogenicity study in the p53^{+/-} mouse model.

Study Type (2 yr bioassay, alternative model etc.): 2-year bioassay

Species/strain: B6C3F1 mice

Number/sex/group; age at start of study: Age of animals at the beginning of dosing was 5 weeks. Study design is shown in the table below.

Group	Dose, mg/kg	Animals, M/F	Animal # , Male	Animal #, Female
1	0, vehicle	50/ 50	1001-1050	2001-2050
2	3	50/ 50	1101-1150	2101-2150
3	7.5	50/ 50	1201-1250	2201-220
4	18.75	50/ 50	1301-1350	2301-2350

Animal housing: Animals were individually kept in aluminum cages at 23° C and 55% relative humidity.

Formulation/vehicle: The drug substance was suspended in 0.5% methylcellulose and used within one week.

Drug stability/homogeneity: The sponsor stated that the suspensions were stable for two weeks under the dark condition. The concentrations and homogeneity of the drug substance in the suspensions were confirmed.

Methods:

Doses: See the study design above.

Basis of dose selection:

The sponsor conducted a dose finding study in the mouse for 13 weeks duration at 3, 12, 24 and 48 mg/kg/day. Urinary calculi was present at 24 and 48 mg/kg in male and 12, 24 and 48 mg/kg in female mice. Transitional cell hyperplasia was noted in the urinary bladder in male and female mice at 24 and 48 mg/kg. The sponsor stated that the MTD was selected between 12 and 24 mg/kg. The sponsor also stated that AUC value of 12 mg/kg treated animals was 25-fold higher in the mice compared to that for human at a maximum clinical dose. Considering the outcome of the toxicity findings and exposure, 18.75 mg/kg was considered by the sponsor to be the MTD for the carcinogenicity study.

The Exec. CAC committee recommended that male mouse should be treated at 6, 16 and 48 mg/kg and a second dose ranging study should be conducted in female mice for

selecting the MTD. The Exec. CAC committee also commented that serum chemistry data would provide dose-limiting toxicity in the kidney. Collection of the histology data for all treated and control groups were recommended. The sponsor stated that the carcinogenicity studies were already undertaken by the time Exec. CAC recommendations were made available to the sponsor.

Restriction paradigm for dietary restriction studies: Nil

Animals were fed with commercially available _____ rat and mouse diet. The feeders were changed once a week. Results of the analysis of the diet are shown below.

b(4)

Route of administration: orally by gavage at a dose volume of 0.5 ml/100 g body weight.

Frequency of drug administration: Once daily

Dual controls employed: No

Interim sacrifices:

Satellite PK or special study group(s): 45 mice/sex were allotted for each of group 2 and 4 for the toxicokinetic study. 15/sex were sacrificed on each of day 1, week 13 and week 26 of the study.

Deviations from original study protocol:

Statistical methods: Survival rate and tumor data were analyzed by Fisher's Exact Test and Peto's trend test.

Observations and times:

Clinical signs: All animals were observed for clinical signs two or three times a day. Animals were examined for the presence of tumor growth by palpitation once a week.

Body weights: Body weights were recorded once a week up to 26 weeks and biweekly thereafter.

Food consumption: Food consumption was recorded once a week up to week 26 and biweekly thereafter. Food consumption g/day and food efficiency were calculated.

Hematology: Hematological tests were performed on all surviving animals at the end of dosing. Blood samples were collected from the abdominal aorta.

Clinical chemistry: Blood samples were taken at the end of dosing period from 10 surviving animals for plasma chemistry.

Organ weights: Mice were sacrificed by exsanguination under ether anesthesia. Animals found dead were necropsied immediately. Weights of following organs were recorded.

Brain, heart, liver, kidney, spleen, adrenal, testes, ovaries and thymus.

Gross pathology: Gross examination of the body surface, body cavities and organs were conducted. Protocol specified tissues were fixed in 10% formalin. Eye tissues were fixed in Davidson's fixative.

Histopathology: Histological examinations were conducted on tissues from the control, high dose treated animals, and moribund and dead animals. However, at the request of the reviewer, histology of the low and mid dose treated animals was conducted. Tissues were stained with H&E.

Toxicokinetics: Blood samples were collected from 5 mice/sex at 30 min, 8 and 24 hours post dose on day 1, and weeks 13 and 26. About 0.5 ml of the blood was collected from the abdominal aorta and serum levels were determined. Toxicokinetics were determined at 3 and 18.75 mg/kg.

Results:

Mortality: Percent mortality data during the treatment period are shown in the table below (taken from page 061, vol 2).

Group	Week	Male, %	Female, %
1	66-78	0	0
2	66-78	0	0
3	66-78	0	0
4	66-78	6	2
1	79-91	0	10
2	79-91	2	4
3	79-91	6	10
4	79-91	8	6
1	92-104	8	18
2	92-104	8	16
3	92-104	14	24
4	92-104	20	24

As shown in the table, mortality in the male mice at 7.5 and 18.75 mg/kg was higher than the control. The female mice also showed higher mortality at 7.5 and 18.75 mg/kg compared to the control. However, the magnitude of increase was smaller than that observed for the male mice.

Clinical signs: Several clinical signs were noted in the control and treated animals. However, some of the high dose treated male animals showed ulcer in the urogenital area and dirty hair in the whole body that were not present in the other groups. Female animals did not show any treatment-related clinical signs.

Clinical signs, number of observations and time of signs first observed are shown in the table below.

Observation	Gr 1 (week)	Gr 2 (week)	Gr 3 (week)	Gr 4 (week)
-------------	-------------	-------------	-------------	-------------

1. Loss of teeth	1 (96)	3 (70)	4 (78)	1 (52)
2. Wasting	2(98)			2 (63)
3. Piloerection	2 (98)	2 (97)	3 (93)	3 (63)
4. Abdominal mass	10 (68)	8 (87)	9 (78)	11 (60)
Tachypnea	2 (98)	1 (94)	3 (91)	2 (70)
Dirty Hair, whole body				2 (70)
Nodule at scrotal site			1 (77)	
Tissue mass	1 (67)		5 (78)	
Swelling of orbit	6 (60)	6 (73)	2 (73)	2 (70)
Ulcer, urogetital region				3 (67)
Corneal opacity	1 (93)			

Body weights:

Male mice showed above 10% reduction of the body weight gain in all drug treated animals during the 104-week treatment period. There were no differences in mean body weight. However, difference in weight gain was minimal in the female mice. Data for the body weight (g) are shown in the table below. The sponsor stated that there was no statistically significant difference compared to body weight gain in the control group.

Week	1M	1F	2M	2F	3M	3F	4M	4F
0	19.7	16.6	19.7	16.6	19.7	16.6	19.7	16.6
1	21.6	18.3	21.6	18.3	22.1	18.3	22.1	18.3
104	42.2	31.2	39.5	31.3	39.7	30.6	39.7	31.2
Wt gain, 0-104 wk	22.5	14.5	19.8	14.7	20	14	20.1	14.6
%Change BW gain			-12%	+1.3%	-11%	-3.5%	-10.7%	+0.7%

Food consumption:

The food consumption (g/day) during the treatment period was not affected by the treatment. Data are shown below.

Week	1M	1F	2M	2F	3M	3F	4M	4F
1	4.2	4.4	4.1	4.3	4.4	4.6	4.2	4.5
104	5.0	4.9	4.8	5.0	5.0	5.2	5.0	5.2

Hematology:

No treatment related changes in the hematology were noted.

Clinical chemistry:

Plasma chemistry data did not show any treatment-related change except there was a slight increase in BUN to 27.8 mg/dl at 18.75 mg/kg dose in female mice. The control female mice had BUN level of 17.0 mg/dl. The observed range of values for BUN (mg/dl) in the control and high dose groups are shown in the table below.

Group	Male	Female
Control	19.2-34.3	12.9-20.7
18.75 mg/kg	18.1-36	17.7-39.6

Organ weights:

No treatment related change in the organ weight was noted. Weight of kidney as percent of the body weight show a statistically significant increased at 18.75 mg/kg in female mice. The biological significance of this change is not known. Data as percent of body weight are shown in the table below.

Organ	1F	2F	3F	4F
% wt of kidney, Right	0.76±0.08	0.90±0.89	0.80±0.13	0.85*±0.15
% wt of kidney, Left	0.73±0.07	0.86±0.89	0.78±0.18	0.81*±0.17

*statistically significant

Gross pathology:

Gross pathology findings in the terminal sacrificed, dead and moribund animals are shown in the table below. Total number of animals examined are shown in the parenthesis.

	1M	1F	2M	2F	3M	3F	4M	4F
Enlarged lymph node	11 (50)	6 (50)	6 (50)	9 (50)	14 (50)	13 (50)	8 (50)	14 (50)
Enlarged spleen	3 (50)	5 (50)	6 (50)	9 (50)	7 (50)	14 (50)	6 (50)	14 (50)
Urinary bladder calculi	0 (50)	0 (50)	0 (50)	0 (50)	1 (50)	0 (50)	48 (50)	44 (50)
Urinary bladder dilated lumen	1 (50)	0	0	0	1	0	6 (50)	4 (50)
Atrophy, seminal vesicle	2 (50)		0 (50)		0 (50)		4 (50)	

Data suggested that urogenital and hemapoietic systems were involved in the gross changes.

Histopathology:

Non-neoplastic:

The sponsor provided data for the control and high dose groups on page 186, vol 1 under non-neoplastic findings. Data for non-neoplastic findings of urinary bladder for low and mid dosed females are also provided. In another submission, page 051, serial # 112, dated May 22, 2003, non-neoplastic findings in all animals were presented. Some of the findings are presented in the table below.

Lesion	1M	2M	3M	4M	1F	2F	3F	4F
Kidney, calculi	0/50	0/50	0/50	0/50	0/50	0/50	0/50	13/50
Urinary bladder, edema	0/50	0/50	0/50	0/50	0/50	0/50	0/50	42/50
Urinary bladder, Hyalin droplet	1/50	0/50	0/50	36/50	0/50	0/50	0/50	24/50
Urinary bladder, fibrosis	1/50	0/50	0/50	1/50	0/50	0/50	0/50	34/50
Urinary bladder, transitional cell hyperplasia (slight)	0/50	0/50	3/50	3/50	0/50	1/50	0/50	40/50
Urethra, calculus	0/50	0/50	0/50	12/50	2/50	0/50	0/12	1/50
Uterus, atrophy					0/50	0/50	0/50	3/50
Kidney, fibrosis	0	0	2/50	1/50	1/50	0	3/50	7/50

Female mice showed a greater number of non-neoplastic lesions mostly in the urinary system.

Neoplastic:

Some of the neoplastic lesions are listed in the table below. A complete histology data has been provided on page 036, vol 1, serial # 112 dated May 22, 2003.

Lesion	1, M	2, M	3, M	4, M	1, F	2, F	3, F	4, F
Spleen hemangiosarcoma	3/50	0/50	0/50	0/50	0/50	1/50	1/50	2/50
Ileum, malignant lymphoma	0/50	1/50	0/50	1/50	0/50	0/50	0/50	2/50
Urinary bladder, transitional cell papilloma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	3/50
Urinary bladder, transitional cell carcinoma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
Subcutaneous tissue, Rhabdomyosarcoma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
Zymbal's gland	0	0	0	0	0	0	0	1/50

carcinoma								
Multiple organ metastasis	47/50	10/50	40/50	30/50	67/50	40/50	108/50	74/50

Female # 2329 showed transitional cell hyperplasia and carcinoma in the urinary bladder at 18.75 mg/kg. In addition to the finding, another 3 female animals in the high dose group also showed transitional cell papilloma in the urinary bladder.

Toxicokinetics:

The sponsor provided exposure data from the 13-week dose finding study in male and female B6C3F1 mice (page 007, vol 1 dated May 22, 2003, serial # 112). Data are shown in the table below.

Dose, mg/kg	3	12	24	48
Animals, M,F	12, 12	12, 12	12, 12	12, 12
Male AUC µg.hr/ml	3.4	18.8	34.8	91.4
Female AUC, µg.hr/ml	13.2	62.2	123.1	316.8

Serum levels (ng/ml) of the drug at 30 min, and 8 and 24 hours after treatment are shown in the table below.

Day/Week	3 mg/kg			18.75 mg/kg		
	30 min	8 hr	24 hr	30 min	8 hr	24 hr
Day 1, male	2199	578	ND	23973	334	9.8
Day 1, female	7443	392	7.2	47279	1329	31.3
Week 13, male	1456	48.3	2.3	33732	189	41.5
Week 13, female	5114	164	11.4	46558	723	81
Week 26, male	1784	67.4	2.2	27887	359.4	17.2
Week 26, female	6110	303	16.7	50703	1830.8	70.1

Serum level data obtained at the end of 26 weeks show that female mice were exposed to higher levels of the drug. In the absence of data at the mid dose, it is not certain that the serum level at 18.75 mg/kg was the maximum that could be achieved. Kinetic data from the 3-month dosing also suggest differences in the kinetics between male and female animals. The sponsor needs to provide PK parameters including AUC values for the toxicokinetic study.

The sponsor provided human plasma exposure data on Sept 11, 2003 at the request of the reviewer as shown below.

The mean (\pm SD) peak and total plasma exposures to febuxostat following multiple dosing with 120 mg QD^a, 240 mg QD^a, and 300 mg QD^b of febuxostat

Dosage	N	C _{max} ^c (μ g/mL)	AUC ₂₄ ^c (μ g.h/mL)
120 mg QD	9	5.31 \pm 1.68	11.96 \pm 2.42
240 mg QD	8	11.26 \pm 4.04	34.98 \pm 9.91
300 mg QD	10	14.25 \pm 5.44	48.36 \pm 15.70

a TMX-99-001 study

b C02-02-023 study

c at steady state

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:

The treatment of mice for 104 weeks with TMX-67 up to 18.75 mg/kg/day oral dose showed increased incidences of urinary bladder calculi in both males and females, and fibrosis in females. The female mice also showed calculi and fibrosis in the kidney, and edema in the urinary bladder. Male and female mice showed an increase in the hyalin droplet in the urinary bladder. These data suggested that deposition of calculi in the urinary system is the dose limiting effect of the drug that resulted other lesions. Female animals were affected greater than male mice partly due to increased exposure of the drug. It is stated in the literature that administration of purine analogs and adenine to rodents resulted in a deposition of calculi. Inhibition of xanthine oxidase by TMX-67 causes accumulation of xanthine and hypoxanthine that formed calculi in the urinary tract of mice. Similar findings have not been reported yet in humans or other large animal species. Based on the findings, it appears that the treatment showed an optimal effect and male mice had significantly higher mortality compared to the control considering P value of 0.0352 in the dose mortality trend. Any increase in the dose could have affected the survival of the mice. Neoplastic and non-neoplastic lesions in the animals were due to the consequence of calculi deposition in the urinary tract. Female animals also showed a slight increase in the BUN that is indicative of the kidney toxicity. However, the toxicological significance of this finding is minimal. Based on the fact that male mice showed higher mortality, female animals showed renal fibrosis, carcinoma and papilloma of transitional cells in the urinary bladder and increase incidences of calculi in both genders at the high dose, the reviewer concluded that the experiment was conducted at adequate doses considering the limitation of the mouse model for the drug.

Evaluation of tumor findings:

There is no dose-related mortality trend in female mice. Statistically significant lesions are urinary bladder transitional cell papilloma with a P value of 0.026 in the trend test. Male mice showed statistically significant mortality trend (P =0.03). No statistically significant trend was observed for the occurrence of tumor lesions in male mice. There were some incidences of histiocytic sarcoma in several organs. However, the trend test did not show statistically significant findings. The reviewer requested the statistical reviewer to further analyze the data considering grouping

the data for hemangiosarcoma, histiocytic sarcoma and lymphoma for the whole body, respectively. No statistically significant trend was noted.

Carcinogenicity summary:

The mouse carcinogenicity study was conducted at 3, 7.5 and 18.75 mg/kg/oral doses by gavage for 104 weeks. Male mice showed an increase in the mortality. Female mice showed a slight increase in the plasma BUN. Male and female mice showed increased incidence in the calculi and hyalin droplets in the urinary bladder. Increased incidence of renal fibrosis was seen in female mice. Data suggested that the study was conducted at optimal doses to reach an MTD or near a MTD. Male mice did not show a treatment-related increase in neoplastic lesions. However, female mice showed increased incidence of transitional cell papilloma (3/50 vs 0/50 in the control) in the urinary bladder at 18.75 mg/kg. The maximum human dose in the Phase III trial is 240 mg QD. The dose is about 4 mg/kg for a 60 kg patient or 148 mg/m². The mouse to human dose ratio for equal surface area is 1.5.

Carcinogenicity conclusions:

Treatment up to 18.75 mg/kg/day by oral gavage to mice for 104 weeks showed increased incidence of urinary bladder transitional cell papilloma and carcinoma in female mice.

Recommendations for further analysis: Nil

Labeling Recommendations:

The label should indicate that treatment at 18.75 mg/kg _____ for 104 weeks showed increased incidence of transitional cell papilloma and carcinoma in female mice secondary to the calculus formation in the urinary bladder.

b(4)

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

No reproductive study report was submitted in the amendment.

VIII. SPECIAL TOXICOLOGY STUDIES:

No special toxicity report was submitted in the amendment.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Chronic treatment with TMX-67 to mice showed increased calculi in the urinary tract of mice. The effect could be due to deposition of xanthine and hypoxanthine. A similar effect for purine base analogs in the rodent models is published in the literature. Formation of calculi in the urinary tract contributed to other changes mostly in the female mice e.g., urinary bladder edema, transitional cell papilloma in the urinary bladder and fibrosis of the urinary bladder and kidney. The systemic exposure levels in female mice were higher and that contributed to higher toxicity in female mice. However, high and mid-dose male mice also

showed higher mortality than the control. Considering the toxicity, it is concluded that the treatment reached or nearly approached the maximum tolerated dose. Transitional cell papilloma which is an extremely rare tumor in the urinary bladder of female mice was the only neoplastic lesion in the 104-week carcinogenicity study in mice. Although the incident of urinary transitional cell papilloma is not statistically significant, it might have a biological significance.

General Toxicology Issues: Nil

Recommendations to the sponsor:

1. The finding of transitional cell papilloma and carcinoma in the urinary bladder in female mice should be included in the label, patients' informed consent and the investigator's brochure (see Exec. CAC minutes of September 30, 2003 for additional comments).
2. Please provide plasma PK parameters including AUC for mouse carcinogenicity study.

Labeling with basis for findings:

((((

(4)

Reviewer signature: _____

Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

**APPEARS THIS WAY
ON ORIGINAL**

cc: list:

IND 58,229
HFD-550/PM/J/ Dean
HFD-550/Reviewer/ A. Mukherjee
HFD-550/Team Leader/ J. Yang
HFD-550/ Chemist/ Puttagunta Rao
HFD-550/MO/J. Sciffenbauer
HFD-550/Medical Team Leader/J. Witter

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Revised on Sept 22, 2003, Sept 23, 2003, and Oct 9, 2003.

X. APPENDIX/ATTACHMENTS:

Addendum to review:

Exec. CAC executive committee report dated September 30, 2003.

Other relevant materials (appended consults, etc.): N/A

Any compliance issues: Nil

**APPEARS THIS WAY
ON ORIGINAL**

Executive CAC

Date of Meeting: September 30, 2003

Committee: Abigail Jacobs, Ph.D., HFD-540, Acting Chair
Joseph Contrera, Ph.D., HFD-901, Member
David Morse, Ph.D., HFD-150, Alternate Member
Josie Yang Ph.D., HFD-550, Team Leader
Asoke Mukherjee Ph.D., HFD-550, Presenting Reviewer

Author of Draft: Asoke Mukherjee

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND # 58,229

Drug Name: TMX-67; TEI-6720; Febuxostat

Sponsor: TAP Pharmaceuticals, Lake Forest, IL 60045

Background:

TMX-67 also referred to as TEI-6720 or Febuxostat, a thiazolecarboxylic acid derivative xanthine oxidase/xanthine dehydrogenase (XOD) inhibitor, is manufactured by Teijin Ltd., Yamaguchi, Japan. Its proposed indication is for the treatment of gouty b(4) the sponsor had submitted the protocols for both mouse and rat carcinogenicity studies for review in September, 1999. The proposed doses for both studies were not concurred with by the Exec. CAC (see Exec. CAC meeting minutes of September 21, 1999). The original Exec CAC recommendations for the mouse carcinogenicity study were:

- (1) to use doses of 48, 16, and 6 mg/kg/day for male mice,
- (2) to conduct a second dose-range finding study utilizing higher doses to clearly demonstrate an MTD in female mice, and
- (3) to consider conducting the mouse carcinogenicity study in an alternative model, specifically the p53^{+/-} mouse model, as TMX-67 was positive in the chromosomal aberration assay in CHO cells. A dose range-finding study should be conducted to clearly identify both an MTD and the NOAEL. A positive control group should be included and perform histopathological evaluations on all dose groups if an alternative carcinogenicity study is conducted.

Although the study was not conducted in accordance with the original dose recommendations of the Exec. CAC, the sponsor has requested (Sept 6, 2002 and May 22, 2003) that Exec. CAC provide feedback on the acceptability of the completed mouse carcinogenicity study.

Mouse Carcinogenicity Study: The mouse carcinogenicity study was conducted in B6C3F1 mice

at 3, 7.5 and 18.75 mg/kg oral doses given by gavage for 104 weeks. The control animals received vehicle (0.5% methylcellulose) only. Each group had 50 mice/sex. The mortality in the high-dose male mice was higher than the control group (20% vs 8%) although there was no statistically significant difference in the intercurrent mortality test. All treated male mice also showed about 10% reduction in the body weight gain at the end of the study. Female mice showed higher plasma drug levels (approximately 2-4x) than male mice. Male and female mice at 18.75 mg/kg had a higher incidence of urinary bladder calculi that resulted in increased non-neoplastic lesions of urinary bladder e.g., fibrosis in both male and female mice, and kidney fibrosis in female mice. The high-dose female mice also showed a higher but not statistically significant incidence of transitional cell papilloma (3/50 vs 0/50 in the control) and transitional cell carcinoma (1/50 vs 0/50 in the control) in the urinary bladder. However, this observation is treatment-related and biologically significant as the urinary transitional cell papilloma and carcinoma are extremely rare.

Executive CAC Recommendations and Conclusions:

The Committee determined that the study is acceptable based on the observation of calculi in the urinary bladder and calculus associated-lesions in the urinary bladder and kidneys. Observed increased incidences of urinary bladder transitional cell papilloma and carcinoma in female mice are considered secondary to the calculus formation. The committee recommended that the findings be included in the product label, despite incidences not being statistically significant at the maximum dose tested.

An additional carcinogenicity study in p53 mouse model is not necessary.

The committee recommended using mouse to human exposure (AUC) ratios to express the multiples of human equivalent dose in the label.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\n
/Division File, HFD-550
/Team leader, HFD-550/JYang
/Reviewer, HFD-550/AMukherjee
/CSO/PM, HFD-550/JDean
/ASeifried, HFD-024

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/s/

Abby Jacobs

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/s/

Asoke Mukherjee
10/10/03 09:08:46 AM
PHARMACOLOGIST

Josie Yang
10/10/03 09:14:20 AM
PHARMACOLOGIST

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/s/

Asoke Mukherjee
9/6/2005 04:43:05 PM
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Josie Yang
9/6/2005 04:50:56 PM
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