

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
NDA 21-856

PHARMACOLOGY REVIEW(S)

1/7/09



FDA Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and Rheumatology Products
10903 New Hampshire Avenue, Silver Spring, MD 20993

**SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION**

NDA number: 21-856
Drug Substance: ULORIC® (febuxostat)
PDUFA Goal Date: 18-Jan-2009
Sponsor: Takeda Pharmaceuticals

Reviewer name: R. Daniel Mellon, Ph.D., Pharmacology Toxicology Supervisor
Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
Review completion date: 5-Jan-2009

Recommendation: Approval

NDA 21-856 was originally submitted on December 15, 2004. Drs. Asoke Mukherjee and Josie Yang (pharmacology toxicology supervisor at the time) recommended that the NDA may be approved from a nonclinical perspective with modifications to the proposed product labeling. The NDA was not approved at that time; however, there were no outstanding nonclinical deficiencies or requests for information in the October 14, 2005 action letter.

A complete response to the October 2005 action letter was received by the Agency on February 17, 2006. There were no new nonclinical data in the second cycle submission. At that time, a tertiary nonclinical pharmacology toxicology review was conducted by Dr. Kenneth Hastings, who concurred with the approval recommendation made by Drs. Mukherjee and Yang. However, the second cycle submission was deemed approvable by the Agency due to clinical concerns (see action letter dated 8/2/2006).

The current complete response to the August 2006 action letter contained several nonclinical studies, which were not requested by the Agency. I have read Dr. Asoke Mukherjee's review of the nonclinical pharmacology and toxicology sections of this NDA 21-856 complete response and agree with his conclusion that, from a nonclinical perspective, the NDA may be approved. I also concur with his recommendations for the nonclinical portions of the product labeling.

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/s/

R. Daniel Mellon
1/5/2009 02:55:47 PM
PHARMACOLOGIST

Paul Brown
1/7/2009 05:03:35 PM
PHARMACOLOGIST
Concur with approval recommendation and labeling as proposed by
reviewer.

12/29/08



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-856
SERIAL NUMBER: 000 / AZ; 000 / BZ
DATE RECEIVED BY CENTER: July 17, 2008
PRODUCT: Uloric® (febuxostat) tablets
INTENDED CLINICAL POPULATION: Proposed indication: Hyperurecemia in patients with gout
SPONSOR: Takeda Pharmaceuticals Inc.
DOCUMENTS REVIEWED: Updated nonclinical study reports related to cardiovascular safety
REVIEW DIVISION: Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)
PHARM/TOX REVIEWER: Asoke Mukherjee, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob A. Rappaport, M.D.
PROJECT MANAGER: Mathew Sullivan

Date of review submission to Division File System (DFS): December 29, 2008

Executive Summary

Background to third Cycle Submission:

A. Recommendation on Approvability

This application is approvable from a nonclinical perspective with some minor modifications of labeling as revised in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section "Pregnancy" section and non-clinical toxicity section.

B. Recommendation for Nonclinical Studies

No new non-clinical study is recommended.

C. Recommendations on Labeling as per PLR format

b(4)

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5 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

previous recommendations regarding the package insert of the product. A list of submitted studies is shown in the table below.

Study Title	Document #	Submission (Date)
An updated package insert for a review	M1	July 17, 2008
Sponsor's summary of neoplastic findings in the mouse carcinogenicity study of febuxostat	Study 4257-011-025	July 17, 2008
Prevention and treatment effects of xanthine oxidase inhibition on systemic overload-induced ventricular hypertrophy and congestive heart failure in mice	Study TAP—6-000875	July 17, 2008
Effect of acute xanthine oxidase inhibition with Febuxostat or allopurinol on myocardial energetics during basal and high cardiac workstates	Study TAP-06-000876	July 17, 2008
Effect of febuxostat on progression of renal disease in remnant kidney rats with and without coexisting hyperuricemia	Study TAP-07-900137	July 17, 2008
A 14-day repeated oral dose cardiovascular evaluation of TMX-67 in beagle dogs	Study n004049a	July 17, 2008

Summary of neoplastic findings, study # 4257-011-025:

The Applicant provided a summary stating that incidences of transitional cell papilloma and carcinoma were 3 and 1 respectively at 18.75 mg/kg in female B6C3F1 mice. Control, low and mid dose female mice did not show any tumor. Male mice also did not show any tumor. Female mice # 2303, 2316, and 2343 that showed transitional cell papilloma also showed calculi. Similarly female mouse # 2329 that showed transitional cell carcinoma also showed calculi in the urinary bladder. Considering the data, the Applicant stated that the transitional cell papilloma and carcinoma are due to calculi formation in the urinary bladder and a similar effect is not expected in clinical population because patients treated with Uloric did not show urinary calculus. However, the reviewer noted that urinary calculus formation was observed in some occasions in patients treated with other uric acid synthesis inhibitor. Therefore, the calculus formation in animals following chronic treatment needs to be indicated in the package insert.

The applicant concluded that, urinary bladder tumor in female mice at high dose had no significance to long term safety in humans.

The Applicant also provided AUC of febuxostat in mice as 62.2 and 123.1 ug.hr/mL at 12 and 24 mg/kg, respectively, for 13 weeks. However, the Applicant did not have real time exposure data in female mice at 18.75 mg/kg. The predicted data for exposure at 18.75 mg/kg was 96.6 ug.hr/mL. The NDA review dated Sept 1, 2005 also considered the extrapolated

exposure at 18.75 mg/kg as 96.6 ug.hr/mL. The summary also referred 120 mg as the maximum human dose. However, in view of a change in the proposed maximum human dose to 80 mg, the animal to human dose ration needs to be recalculated on the basis of 80 mg as the maximum human dose.

Since no new mouse carcinogenicity data were submitted with the complete response to the approvable letter, only the exposure ratio between animals and human needs to be adjusted to 80 mg human dose.

Updated Label:

An updated package insert was submitted for the NDA during this review cycle. The new package insert indicates that Uloric is recommended at 40 or 80 mg daily oral doses as tablets for the treatment of hyperuricemia in gout. The recommended maximum dose in the initial NDA submission was 120 mg. In the previous NDA review, the animal to human dose ration was compared using 120 mg daily human dose. The updated review would provide animal to human dose ratio at 80 mg human daily dose. It should be also noted that the new package insert added an animal toxicity section. Data from the chronic toxicity studies would be incorporated to the recommended label.

Following new non-clinical cardiovascular studies were submitted:

1. Prevention and treatment effects of xanthine oxidase inhibition on systolic overload-induced ventricular hypertrophy and congestive heart failure in mice (study # TAP-06-000875).

Left ventricular hypertrophy and congestive heart failure was induced in mice by transverse aortic constriction, a model of systemic overload progressing to congestive heart failure (CHF). Mice were treated with febuxostat for 8 days at 5 mg/kg/day via the oral route of administration after the surgery to assess the ability of febuxostat to prevent CHF. Another group of animals were treated at 0.05 mg/kg/day via the drinking water for three weeks beginning 7 days after the surgery to assess the ability of febuxostat to treat CHF. After end of treatment, ECG, plasma levels of uric acid, histopathology of the heart, collagen content and cardiac protein level was determined.

Febuxostat at 5 mg/kg shortly after surgery reduced ventricular hypertrophy, uric acid and induction of several proteins in the heart when compared to the untreated control without any effect on the post surgical survival of mice. However, febuxostat treatment for three weeks after the establishment of ventricular hypertrophy and dysfunction did not show efficacy in this model. The applicant concluded that inhibition of xanthine oxidase by febuxostat prevented the development of cardiac hypertrophy and congestive heart failure in mice but was not effective in the treatment of established cardiac hypertrophy and dysfunction.

2. Effect of acute xanthine oxidase inhibition with febuxostat or allopurinol on myocardial energetics during basal and high cardiac workstates (study # TAP-06-000876).

The applicant investigated the effect of febuxostat and allopurinol on catecholamine-induced myocardial workload by measuring myocardial blood flow, high energy phosphate level and oxygen consumption in open chest instrumented dogs. The dose of febuxostat was 4 mg/kg given as a slow IV infusion for 30 min showed a lowering of free ADP suggesting greater utilization of myocardial energy due to inhibition of oxidative states.

3. Effect of febuxostat on progression of renal disease in remnant kidney rats with and without coexisting hyperuricemia (study # TAP-07-900137).

The effect of febuxostat on microcirculation of the kidney after sub-total renal ablation was investigated in the Wistar rat model. Rats were also treated with oxonic acid, an Uricase inhibitor, to increase the uric acid levels. Febuxostat treatment was given in drinking water at 30 mg/L, about 3-4 mg/kg/day immediately after the surgery up to four weeks. Systemic blood pressure and the uric acid level in the plasma were recorded before surgery and at the end of treatment. Urinary protein excretion was measured at base line before the surgery, on weeks 2 and 4. Glomerular hemodynamics was determined by renal micropuncture under anesthesia at the end of week 4. Febuxostat reduced the renal glomerular pressure and proteinuria.

4. A 14-day repeated oral dose cardiovascular evaluation of TMX-67 in beagle dogs (study # N004049A).

This study was conducted at control, 5 or 50 mg/kg daily doses for 14 days to monitor cardiovascular effects in conscious dogs. Each group had 3 dogs/sex. The study was reviewed for the NDA on Sept 6, 2006 and concluded that the treatment had no effect on the ECG and respiration. However, the applicant reevaluated ECG of four dogs that showed hypotension, soon after dosing through almost 90 min. Dogs involved in hypotension are shown below.

Dog #	Dose	Day of dosing
201	5 mg/kg	7
252	5 mg/kg	6
301	50 mg/kg	1
351	50 mg/kg	1

Monitoring of ECG for dogs #301 and 351 for 3 hours showed transient small spikes and broad humps that were attributed to artifact. In absence of any such changes on subsequent days, the Applicant considered it as circumstantial findings. Since hypotension was not observed at high dose after the first day of treatment, it was concluded that transient hypotensive events in dogs were not related to arrhythmias.

Human PK at 80 mg dose:

Table 2.2.5.1.3. Mean Febuxostat Pharmacokinetic Parameters for Healthy Subjects in Phase 1 Studies^a Following Multiple 80 mg Oral Doses of Febuxostat.

	t _{max} (h)	C _{max} (µg/mL)	AUC ₂₄ (µg·h/mL)	t _{1/2z} ^b (h)	V _{ss} /F (L)	CL/F (L/h)	f _e (%)	Cl _r (L/h)
N	121	121	121	120	120	121	46	46
Mean	1.32	2.76	7.75	6.07 [5.35]	52.5	11.4	1.86	0.222
SD	0.775	1.34	2.63	2.28	19.9	3.67	2.06	0.274
Min	0.50	0.936	3.34	2.26	16.8	3.78	0.34	0.031
Median	1.00	2.40	7.46	5.60	49.9	10.7	1.12	0.126
Max	4.00	7.32	21.2	15.7	114	23.9	11.7	1.22
CV%	59	48	34	38	38	32	111	123

a Pooled pharmacokinetic parameters from Studies C02-013, C02-033, C02-034, C02-036, C03-059, C03-044, C03-054, TMX-99-001, TMX-01-008, TMX-01-010, TMX-01-012, TMX-01-014, TMX-01-016, TMX-02-017, and TMX-02-018 where applicable.

b Harmonic mean in brackets.

Update on information from publications and original NDA submission not include in the NDA review dated Sept 6, 2005. The informative does not impact the nonclinical approval recommendation for this product.

A comparative study for xanthine crystal formation in rats between TEI-6720 and allopurinol was reviewed from the literature (A comparative study on the hypouricemic activity and potency in renal xanthine calculus formation of two xanthine oxidase/xanthine dehydrogenase inhibitor: TEI-6720 and allopurinol in rats, Horiuchi H, Ota M, Kobayashi M, Kaneko H et al, Res. Comm. Mol. Path. and Pharmacol, vol 104, 307-319, 1999). Rats were fed high oxonate (uricase inhibitor) diet to induce hyperuricemia. Rats were treated at 1, 3, 10, 30 and 100 mg/kg of TEI-6720 and 3, 10, 30, 100 and 200 mg/kg of allopurinol. Calculi formation in the kidney was determined. Data show that TEI-6720 at 30 mg/kg and allopurinol at 100 mg/kg induced 100% and 96% calculi, respectively. These data suggest that TEI-6720 is 3 times more potent than allopurinol for urinary calculi formation in rats. It was also suggested that excretion of purine metabolites in rats was 25 times higher than that of humans.

The Applicant submitted an article, "Renal xanthine stone in Lesch-Nyhan syndrome treated with allopurinol" in Urology, July 1985, vol 26. The article stated that patients with HGPRT deficiency showed lymphoma. These patients were treated with chemotherapy and resulted in hyperuricemia due to rapid cell lysis. Patients were further treated with allopurinol for the condition. Autopsy of three patients showed xanthine stone or nephropathy in 3 cases.

The updated summary of mouse carcinogenicity information in the submission indicated "In comparison to rodents, this excessive pharmacological effect is unlikely to occur in humans, because of the species differences in nuclei acid metabolic turnover. The rate of purine

biosynthesis is much slower in human, compared to rodents and the relative volume of urine is much larger. Therefore, concentration of xanthine in human urine is unlikely to reach sufficiently high levels following febuxostat treatment to cause the formation of xanthine crystals and/or calculi.”

However, considering animal data for febuxostat and clinical data for allopurinol referred above, it is not completely assured that febuxostat would not have any chance to induce calculi in the urinary bladder. Therefore, it is recommended that the animal findings for calculi in the urinary bladder should be indicated in the label so as to alert the physicians for the long term use of the drug and its relationship to urinary stone formation.

Exposure ratio:

The animal and human exposure ratio would change for the comparison in the package insert because the maximum recommended human dose was modified to 80 mg/day as opposed to 120 mg/day proposed in the original NDA submission. Therefore, an updated exposure data is provided below. Animal data were taken from the NDA review dated Sept 6, 2005.

Species	Dose, mg/kg	Sex	Exposure, ng.hr/mL	Human exposure, 80 mg	Animal :Human
Mouse	18.75	Female	96.5 ug.hr/mL	7.75 ug.hr/mL	12.5
Rat	24	Male	194.5 ug.hr/mL		25.0
Rat	24	Female	201.8 ug.hr/mL		26.0
Rat	48	Male	238.5 ug.hr/mL		30.8
Rat	48	Female	305.2 ug.hr/mL		39.4
Pregnant Rabbit	3		9.3 ug.hr/mL		1.2
Pregnant Rabbit	12		58.5 ug.hr/mL		7.5
Pregnant Rabbit	48		394.6 ug.hr/mL		50.9
Dog	15	Male	31.9 ug.hr/mL		4.1
Dog	15	Female	38.6		4.9
Dog	45	Male	395.5		51.0
Dog	45	Female	572.7		73.9

Addendum to NDA 21-856 review dated Sept 6, 2005:

1. Study # S054C4D100, 52-week repeated dose toxicity study of TEI-6720 by oral administration in dogs.

The study was conducted at 5, 15 and 45 mg/kg. The original review indicated 3 mg/kg as the low dose. However, it should be 5 mg/kg. Therefore, the NOEL would be 5 mg/kg instead of 3 mg/kg.

2. The summary table of reproductive safety studies on page 137 showed exposure at 48 mg/kg in pregnant rabbits was 39406. It should be 394606 ng.hr/mL. However, overall conclusion and recommendations of the NDA would not be affected by the change.

Recommendation:

NDA 21-856 may be approved on the basis of non-clinical pharmacology/toxicology data. The non-clinical section of the package insert is indicated above. No new pharmacology/toxicology study is recommended before approval of the product.

C.C:

NDA 21-856 Div File
CDER/OND/DAARP/PM/Mathew Sullivan
CDER/OND/DAARP/Pharmacologist/Asoke Mukherjee
CDER/OND/DAARP/Supervisory Pharmacologist/Daniel Mellon
CDER/OND/DAARP/Medical Officer/Jane Gilbert

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/s/

Asoke Mukherjee
12/29/2008 02:11:10 PM
PHARMACOLOGIST

From Pharm/Tox point of view the NDA is approvable,
see labelling recommendations.

R. Daniel Mellon
12/29/2008 02:34:14 PM
PHARMACOLOGIST

I concur that from the nonclinical pharmacology toxicology perspective,
NDA 21-856 may be approved pending agreement on
the final labeling.

10/13/05

MEMORANDUM

Oct. 13, 2005

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-856

I concur with the recommendation by Dr. Asoke Mukherjee that Uloric (febuxostat) is approvable based on review of submitted pharmacology/toxicology data.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluations II & III

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/s/

Kenneth Hastings
10/13/2005 12:40:38 PM
PHARMACOLOGIST

9/6/05



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-856
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/15/2004
PRODUCT:	Febuxostat
INTENDED CLINICAL POPULATION:	Hyperuricemia
SPONSOR:	TAP Pharmaceuticals
DOCUMENTS REVIEWED:	eCTD
REVIEW DIVISION:	Division of Anesthesia, Analgesia and Rheumatology Products (DAARP)
PHARM/TOX REVIEWER:	Asoke Mukherjee, Ph.D.
PHARM/TOX SUPERVISOR:	Josie Yang, Ph.D.
DIVISION DIRECTOR:	Robert Rappaport, M.D.
PROJECT MANAGER:	Jane Dean

Date of review submission to Division File System (DFS): Sept 6, 2005

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability:

TMX-67 (TMX-67) is approvable on the basis of non-clinical safety and toxicity studies at 120 mg daily dose for the treatment of hyperuricemia.

B. Recommendation for nonclinical studies: No new non-clinical studies are recommended.

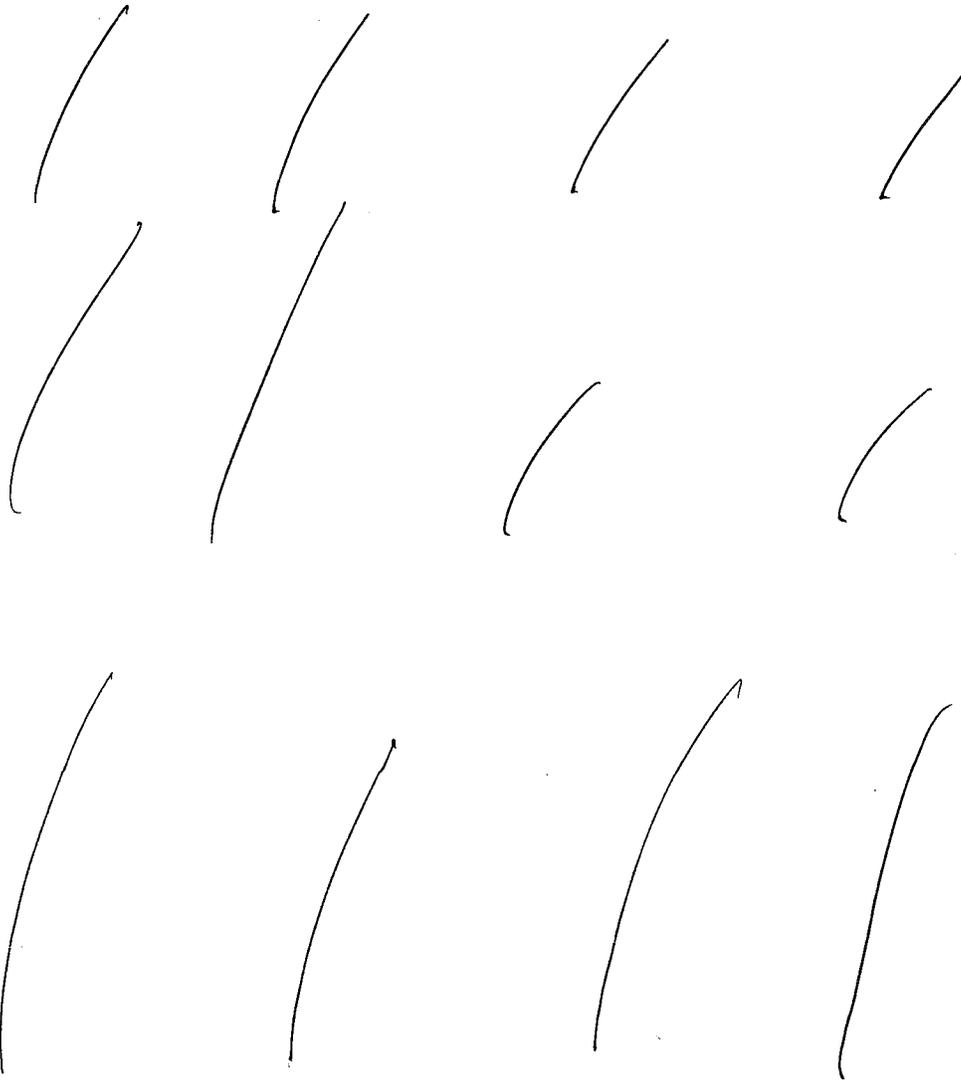
C. Recommendations on labeling:

Suggested labeling:

Label recommended by the reviewer:

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b(4)



b(4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

TMX-67 has no effect in the CNS, GI, cardiovascular and respiratory systems at pharmacodynamic doses. However, a transient hypotensive effect was observed in conscious beagle dogs at 5 and 50 mg/kg oral doses. TMX-67 showed a 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 humans. Glucuronidation

of TMX-67 by Phase II metabolism (TMX-67 glucuronide) is the principle metabolite in human urine. The metabolic profiles were qualitatively similar in rodents, dogs and humans. Female mice showed a gender difference in the exposure. One of the hydroxylated metabolites (M1) of TMX-67 was formed by stereospecific enzyme activity in humans and rodents. 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 cross placental transfer and was excreted in the milk in rats. This resulted in deposition of xanthine crystals in the kidney of pups nursed by rats treated with TMX-67 at 48 mg/kg dose. Hepatic induction of enzyme was not evident from the studies.

It showed chromosomal aberration in Chinese hamster lung fibroblast cells in the presence and absence of S-9 liver homogenates in vitro.

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 human exposure). The neoplastic effect of TMX-67 was secondary to xanthine crystal deposition in the kidney and urinary bladder.

Reproductive safety studies did not show any effect on the fertility and reproductive performance. No teratogenicity, variation or embryocidal effect of TMX-67 was observed. However, nursing performance (F0) and survival of F1 generation were affected by the treatment with TMX-67 at 48 mg/kg in rats (25 times human plasma exposure at MRHD at MRHD). The excretion of drug through milk was also evident. Xanthine crystals were present in the kidney of neonatal rats delivered 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 of calculus formation was noted in rats due to deposition of xanthine crystals at 48 mg/kg (23 times human exposure at MRHD) in the six-month study. A similar effect was also observed in the 2-year rodent carcinogenicity studies.

Therefore, it is concluded that TMX-67 is a xanthine oxidase inhibitor. It is devoid of inhibitory activities in several enzymes involved in the DNA synthesis. TMX-67 was genotoxic in the chromosomal aberration assay. TMX-67 showed papilloma and carcinoma of transitional cell in the urinary bladder in rodents. The neoplastic changes were secondary to calculus formation in the kidney and urinary tract. It is not teratogenic and did not affect fertility and reproductive performance in rats. Major toxicity profile based on the non-clinical studies is 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 3.0-16 folds human exposure based on AUC at MRHD.

B. Pharmacologic activity:

TMX-67 is a non-purinergic inhibitor of xanthine oxidase in vitro with a K_i value of 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 the inhibition of purine and pyrimidine synthesis at the doses showed xanthine oxidase inhibitory 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 could show anti-inflammatory activity in hyperuricemia and associated clinical conditions due to xanthine oxidase inhibition. TMX-67 inhibited LPS induced TNF- α release in rats at 10 mg/kg/oral. Clinical significance of this effect is unknown.

C. Non-clinical safety issues relevant to clinical use:

TMX-67 treatment would increase xanthine levels in the plasma and urine that may lead to form calculi in the kidney and urinary tract. Based on the non-clinical data, it is recommended that xanthine levels in the plasma and urine need to be monitored following chronic administration.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-856

Review number: One

Sequence number/date/type of submission: #000, Dec 15, 2004, 505 b(1)

Information to sponsor: Yes (x) No ()

Sponsor and/or agent: TAP Pharmaceuticals

Manufacturer for drug substance: Abbott Laboratories, 1401 N. Sheridan Road, Chicago, Il 60064. Febuxostat is licensed from Teijin Pharma Ltd. Japan.

Reviewer name: Asoke Mukherjee, Ph.D

Division name: Division of Anesthesia, Analgesic and Rheumatology Products

HFD #: 170

Review completion date: June 15, 2005

Drug:

Trade name: Pending

Generic name: Febuxostat

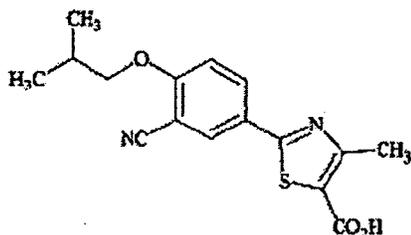
Code name: TEI-6720, TMX-67, TMX-6720, A-319198 and Abbott-319198

Chemical name: 2-[3-cyano-4-(2-methylpropoxy)phenyl]-4-methylthiazole-5-carboxylic acid

CAS registry number: 144060-53-7

Molecular formula/molecular weight: $C_{16}H_{16}N_2O_3S$ / 316.38

Structure:



Relevant INDs/NDAs/DMFs: IND 58,229

Drug class: Xanthine oxidase inhibitor

Intended clinical population: Management of hyperuricemia in patients with gout

Clinical formulation: The sponsor proposed 80 and 120 mg once a day dosing. Therefore, 80 and 120 mg tablets were developed for marketing. The composition of 80 mg tablet is shown in the table below.

Component	Compendial Reference	Role	Abbott Material Code	Unit Formula (mg/tablet)
Febuxostat (TEI-6720, A-319198.0)	In-house	Active		80.00
Lactose, Monohydrate.	NF			
Cellulose, Microcrystalline.	NF			
Hydroxypropyl Cellulose.	NF			
Croscarmellose, Sodium	NF			
Silicon Dioxide	NF			
Magnesium Stearate	NF			
Total Core Tablets				
Color Coating				
Opadry II, Green.	In-house	Color coating		
Total Coated Tablets				520.84

b(4)

The composition of the 120 mg tablet is shown below.

Component	Compendial Reference	Role	Abbott Material Code	Unit Formula (mg/tablet)		
Febuxostat (TEI-6720, A-319198.0)	In-house	Active		120.00		
Lactose, Monohydrate	NF					
Cellulose, Microcrystalline	NF					
Hydroxypropyl Cellulose	NF					
Croscarmellose, Sodium	NF					
Silicon Dioxide	NF					
Magnesium Stearate	NF					
Total Core Tablets						
Color Coating						
Opadry II, Green	In-house	Color coating		769.23		
Total Coated Tablets						

b(4)

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

In vitro mammalian chromosome aberration test.

In vitro mammalian cell gene mutation test in L5178Y/TK mouse lymphoma assay.

Reverse mutation test of TEI-6720 with bacteria.

Chromosomal aberration test of TEI-6720 in cultured Chinese Hamster cells.

Micronucleus test on TEI-6720 in BDF1 mice (intraperitoneal administration).

Study on in vivo unscheduled DNA synthesis (UDS) in rat hepatocytes treated with TEI-6720.

Mammalian bone marrow chromosome aberration test.

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

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

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

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

Pharmacokinetic study of TEI-6720 (2): Placental transfer and transfer into milk after single dose administration in rats.

Profiling study on the serum concentration of unchanged drug after repeated oral administration of TEI-6720 in pregnant rabbits.

Validation study of measurement method of TEI-6720 concentration in serum of rabbits.

Additional validation study for measurement of TEI-6720 in rabbit serum-concentration of stability in an autosampler, and effects of injection volume.

A multiple dose safety, pharmacokinetic, and pharmacodynamic study of oral TMX-67 in healthy volunteers.

Five-week repeated dose toxicity study of TEI-6720 by oral administration in rats.

Thirteen-week repeated dose toxicity study of TEI-6720 by oral administration in F344/Du — rats.

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Preliminary carcinogenicity study of TEI-6720 in mice.

Preliminary carcinogenicity study of TEI-6720 in rats.

Carcinogenicity study of TEI-6720 in rats.

Carcinogenicity study of TEI-6720 in mice.

52-week repeated dose toxicity study of TEI-6720 by oral administration in dogs.

26-week repeated dose toxicity study of TEI-6720 by oral administration in rats.

Single dose toxicity study of TEI-6720 by oral administration in rats.

Single dose toxicity study of TEI-6720 by oral administration in dogs.

Study on the measurement of urinary and fecal excretion following single oral administration of ^{14}C -TEI-6720 in rats.

Excretion study of ^{14}C -TEI-6720 in male chimpanzees following intravenous administration.

Comparative in vitro metabolism of ^{14}C -TMX-67 in male and female hepatocytes from mouse, rat and human.

In vitro metabolism of ^{14}C -TMX-67 by male liver microsomes from mouse, rat, dog and human.

Study on the metabolism of ^{14}C -TEI-6720 in male and female rat bile.

Analysis study of biliary metabolites after single oral administration of ^{14}C -TEI-6720 in rats.

Study of metabolite analysis in milk after single oral administration of ^{14}C -TEI-6720 in rats.

Study on the optical resolution of 67M-1 in human and rat urine.

Study on the analysis for rat urinary metabolites of TEI-6720.

Study on the determination of plasma and urinary concentration of metabolism following single oral administration of ^{14}C -TEI-6720 in rats.

In vivo metabolism of ^{14}C -TMX-67 in male and female mouse plasma, urine, bile and feces samples..

Pharmacokinetic study on TMX-67 in dogs.

Tissue distribution study of ^{14}C -TEI-6720 in rats after 2-week repeated oral administration.

Study of serum concentration of TEI-6720 during a 19-week repeated administration in mice.

Study of serum concentration of TEI-6720 during a 26-week repeated administration in rats.

Comparative pharmacokinetic study of TEI-6720 and allopurinol in rats with impaired renal function.

Pharmacokinetic study of ¹⁴C-allopurinol in rats with impaired renal function.

Comparative study on the hypouricemic effect of TMX-67 and allopurinol in rats.

Study on the effect of TMX-67 on the serum cytokine levels induced by LPS in rats.

Study on the effect of TMX-67 on the serum cytokine levels induced by LPS in rats.

Study on the effect of TMX-67 (TMX-6720) on human xanthine oxidase (XOD) and rat Aldehyde oxidase.

Study on the XOD inhibitors, TEI-6720, Allopurinol and oxipurinol on the activity of human purine nucleoside phosphorylase.

Study on the effect of TMX-67 on PNP.

Study on the effect of TMX-67 on hyperuricemic mouse model by oxonate feeding.

Study on the effect of albumin on the inhibitory efficacy of TMX-67 against bovine milk XOD.

Study on the effects of XOD inhibitors, TEI-6720, and allopurinol on the PRPP (5- α -phosphoribosylpyrophosphate) levels in mouse liver.

Study on the effect of TMX-67 on hyperuricemic mouse model by oxonate feeding.

Study on the superiority of TMX-67 with special reference to pyrimidine metabolism, changes in orotic acid and orotidine levels induced by allopurinol in normal mice.

Study on the superiority of TMX-67 with special reference to pyrimidine metabolism: Changes in orotic acid and orotidine levels induced by allopurinol in normal mice (comparison with TMX-67).

Study on the effect of TMX-67 on HGPRT.

Study on the effect of TMX-67 on OPRT.

Study on the effect of TMX-67 on OMPDC.

Comparative study on the effect of TMX-67 and allopurinol on hyperuricemic rat model by oxonate feeding.

Study on the efficacy of TMX-67 in rats with renal impairment.

Study on the inhibition of bovine milk XOD by human urinary metabolites (67-M-1-4) of TMX-67.

Study on the effect of TMX-67 on rabbit liver guanine deaminase activity.

The influence of allopurinol and TEI-compound on the level of uric acid in serum of chimpanzees/determination of xanthine and uric acid in urine and plasma.

Effect of Febuxostat and allopurinol on urate crystal-induced inflammation in a rat subcutaneous air pouch model.

Study on the effects of the XOD inhibitor TEI-6720 on general behavior in mice.

Study on the effects of the XOD inhibitor TEI-6720 on locomotor activity in mice.

Study on the effects of the XOD inhibitor TEI-6720 on hexobarbital-induced hypnosis in mice.

Study on the effects of the XOD inhibitor TEI-6720 on convulsion induction in mice.

Study on the effects of the XOD inhibitor TEI-6720 on pain perception in mice.

Study on the effects of TEI-6720 on body temperature in mice.

General pharmacological studies of TEI-6720-effects on contractile responses of isolated guinea-pig ileum (2).

General pharmacological studies of TEI-6720-effects on contractile responses of isolated guinea-pig ileum.

General pharmacological study of TEI-6720- effect on small intestine charcoal transport in rats.

Study on the effect of TEI-6720 on urine volume and urinary electrolyte excretion in rats.

Effect of the XOD inhibitor TEI-6720 on the respiratory and circulatory systems.

Effect of TMX-67 on action potential parameters in dog isolated cardiac purkinje fibers.

Effects of TMX-67 on the human cardiac sodium current expressed in mammalian cells.

Effect of TMX-67 on cloned hERG channels expressed in mammalian cells.

Effects of TMX-67 on cloned hERG channels expressed in Chinese hamster ovary cells (CHO).

A 14-day repeated oral dose cardiovascular evaluation of TMX-67 in beagle dogs.

Effects of TMX-67 on human platelets and blood coagulation.

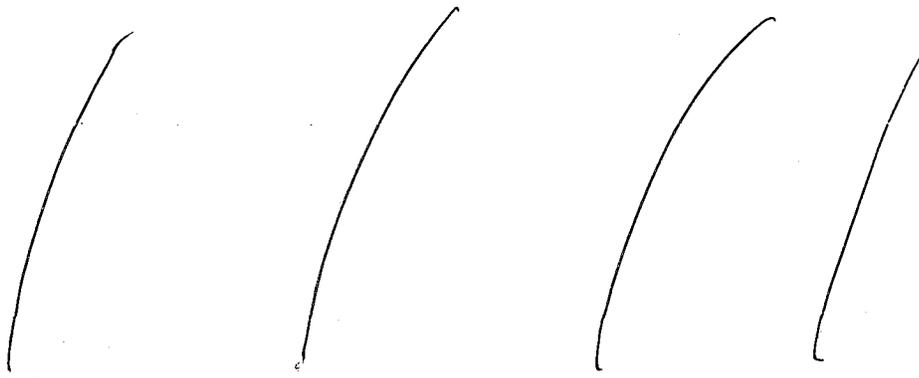
Effects of TMX-67 on action potentials in isolated cardiac purkinje fibers.

Effect of TMX-67 on the native cardiac L-type calcium current of guinea-pig cardiomyocytes.

Study on the effect of TMX-67 on the hypertensive activity of nifedipine in spontaneous hypertensive rats.

Study on the effect of TMX-67 on the hypoglycemic activity of glibenclamide.

Studies not reviewed within this submission:



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2 Page(s) Withheld

Trade Secret / Confidential (b4)

Draft Labeling (b4)

Draft Labeling (b5)

Deliberative Process (b5)

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## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary:

Studies were conducted to probe the effect of TMX-67 on xanthine oxidase, purine nucleotide phosphorylase (PNP), hypoxanthine-guanine phosphoribosyl transferase (HGPRT), guanine deaminase, orotate phosphoribosyl transferase (OPRT) and orotidine monophosphate decarboxylase (OMPDC) activities. Above enzymes are responsible for synthesis and metabolism of purine and pyrimidine bases. TMX-67 is a potent inhibitor of xanthine oxidase at 10 nM with negligible effect on other enzymes. These data suggest that TMX-67 is a potent inhibitor of uric acid formation. TMX-67 did not inhibit the salvage pathway of purine synthesis (HGPRT) and pyrimidine synthesis (OPRT and OMPDC) up to 100 mg/kg/oral in mice.

The sponsor indicated that allopurinol has a structural resemblance to purine base unlike TMX-67. Therefore, it was anticipated that allopurinol would have an effect on the purine and pyrimidine synthesis. However, experimental evidence provided in the report suggests neither allopurinol nor TMX-67 had substantial effect on the purine and pyrimidine biosynthesis.

In vivo uric acid inhibition was observed at 1 mg/kg/oral and above doses in rats and mice. A similar effect was noted in the chimpanzee model at 5 mg/kg/oral. TMX-67 showed inhibitory activity on the induction of TNF- $\alpha$  at 10 mg/kg in rats. The effect would have an impact on the efficacy and safety of the drug following chronic administration.

It is concluded that TMX-67 is a potent inhibitor of uric acid synthesis in vitro with a  $K_i$  value of 10 nM, in vivo at 1 mg/kg in rodents and 5 mg/kg in a chimpanzee model. TMX-67 did not affect the salvage pathway of purine synthesis and pyrimidine synthesis. Induction of cytokine TNF- $\alpha$  was inhibited at 10 mg/kg in rats that may have implication for the efficacy and safety of the drug.

### 2.6.2.2 Primary pharmacodynamics

#### Mechanism of action:

Several study reports were reviewed on Nov 26, 2002 under IND 58,229. The primary pharmacodynamic studies are summarized below.

Inhibition of xanthine oxidase in the human liver was noted at a  $k_i$  of 10 nM. Aldehyde oxidase activity in the rat liver was inhibited at 0.42-0.84 M of TMX-67 (study NP-P1). The sponsor also reported (18-P-96013) the inhibition of bovine milk xanthine oxidase activity with human metabolites e.g., 67M-1R, 67M-1S, 67M-2, 67M-3 and 67M-4. The activity was compared to TMX-67. The data for inhibition is shown in the table below.

<Table 3: Summary of the inhibition constant and the type of inhibition for TMX-67 and its metabolites>

| Compound evaluated | K <sub>i</sub> (nM) | Type of inhibition |
|--------------------|---------------------|--------------------|
| TMX-67             | 0.6                 | Mixed              |
| 67M-1R             | 0.6                 | Mixed              |
| 67M-1S             | 0.8                 | Mixed              |
| 67M-2              | 1.6                 | Mixed              |
| 67M-3              | 2.0                 | Mixed              |
| 67M-4              | 1.6                 | Mixed              |

Above data suggest that 67M1 isomers are equally potent as TMX-67. However, the sponsor mentioned in report # 18-K-96006 (reviewed in metabolism section) that the IC<sub>50</sub> for racemic 67M-1 is 1.67 nmol/L.

Allopurinol and its metabolite oxipurinol inhibited several enzymes responsible for purine and pyrimidine nucleoside biosynthesis (Report # 18-P-94002). These enzymes were purine nucleoside phosphorylase (PNP), hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and orotate phosphoribosyl transferase (OPRT). The inhibition of these enzymes was investigated.

Human blood derived PNP was purified. Inhibitory activities of TEI-6720, allopurinol and oxipurinol were compared. The sponsor stated that there were no remarkable differences in the inhibition of PNP by above compounds. TMX-67 showed 80%, 70% and 68% inhibition at 0.1, 1 and 10  $\mu$ M, respectively. Lineweaver-Burk and Dixon plots were used for the determination of K<sub>m</sub> and K<sub>i</sub> of allopurinol. The K<sub>m</sub> and K<sub>i</sub> of allopurinol were 16.4  $\mu$ M and 0.35 mM, respectively for the inhibition of PNP. It is concluded that both TMX-67 and allopurinol inhibited PNP.

The sponsor indicated that the substrate used in the above study # 18-P-94002 was 10  $\mu$ M hypoxanthine and formation of inosine was assayed. Actually this reaction is not catalyzed by PNP. Therefore, the effect of allopurinol, oxipurinol and TMX-67 in the above study could not provide information on the effects of the drugs on PNP.

In the study (18-P-98005) the effect of TMX-67 on guanosine to guanine formation was investigated as the effect on PNP enzyme. TMX-67 showed very weak activity for PNP inhibition up to 100  $\mu$ M when guanine production was measured. The source of PNP used in the study was from \_\_\_\_\_ and was derived from the human blood. Data are shown in the table below.

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| Conc of TMX-67, $\mu$ M/L | % Inhibition of PNP |
|---------------------------|---------------------|
| 1                         | -2.8                |
| 10                        | 7.3                 |
| 100                       | -8.7                |

Xanthine oxidase activity was measured in the presence and absence of albumin in vitro (study F4-97057). Data suggest that inhibitory activity of xanthine oxidase was reduced dose-dependently in the presence of increased plasma proteins.

The sponsor stated that the inhibition of nucleic acid synthesis is one of the causes of suppression of bone marrow following treatment with allopurinol. The effect of TMX-67 on PRPP levels would reflect the effect of the drug on salvage pathway of DNA synthesis (study # 18-G-95010). The sponsor stated that HGPRT activity in the presence of allopurinol and PRPP would generate allopurinol ribonucleotide and reduce the level of PRPP in the liver. A similar reduction would be anticipated if TMX-67 becomes a substrate of the reaction. However, allopurinol and TMX-67 did not reduce the PRPP levels in the mouse liver at 10 and 100 mg/kg oral doses, respectively. Based on the data, the role of allopurinol and TMX-67 for nucleic acid synthesis in the mouse liver is negligible in this experimental model.

The effect of TMX-67 on HGPRT (salvage pathway of purine synthesis) was investigated in vitro in study # 18-P-98001. Concentrations of TMX-67 were 1, 10 and 100  $\mu\text{M}$ . Results show that TMX-67 up to 10  $\mu\text{M}$  had no effect on HGPRT activity. About 14% inhibition was noted at 100  $\mu\text{M}$ . Data suggest that TMX-67 has a negligible effect on HGPRT activity in vitro.

The effect of TMX-67 on rabbit liver guanine deaminase activity is presented in report # 18-G-96007. Guanine deaminase is involved in the conversion of guanine to xanthine. The report indicated that TMX-67, allopurinol and oxipurinol were ineffective up to 100  $\mu\text{M}$ .

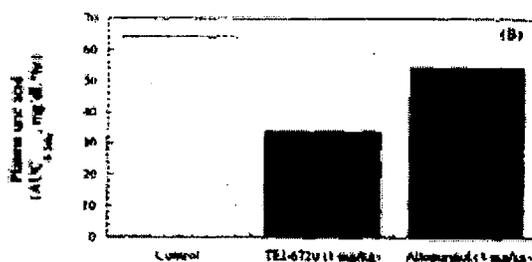
The in vitro effect of TMX-67 was investigated for the inhibition of orotate phosphoribosyltransferase (OPRT) and orotidine monophosphate decarboxylase (OMPDC) for pyrimidine synthesis (studies # 18-P-97002 and #18-P97003) at 1, 10 and 100  $\mu\text{M}$ . TMX-67 had no inhibitory activity against OPRT and OMPDC up to 100  $\mu\text{M}$ .

In order to substantiate above in vitro findings for pyrimidine synthesis, the sponsor determined the plasma and urinary levels of orotic acid (OA) and orotidine (OD) in allopurinol treated male Balb/c mice at 3, 30 and 300 mg/kg/oral doses (study F2-96049). An increase in the plasma and urinary OA and OD was observed at 300 mg/kg. The effect on OA and OD at 1 and 3 mg/kg dose of allopurinol was minimal. The report suggests that allopurinol had an effect on the pyrimidine synthesis by the inhibition of OPRT and OMPDC. It is concluded that pyrimidine synthesis was inhibited by allopurinol at 300 mg/kg. Although TMX-67 was not used in the study, TMX-67 did not increase OA and OD excretion in the urine up to 100 mg/kg in a separate study (F3-97019). It is concluded that TMX-67 is devoid of any effect on the pyrimidine synthesis up to 100 mg/kg in vivo, whereas, allopurinol inhibited pyrimidine synthesis at 300 mg/kg.

Drug activity related to proposed indication:

Rodent models:

1. Effect of TMX-67 on oxonate fed hyperuricemic mouse model was studied (study F3-98037). Male Balb/c mice were treated with 2.5% oxonate containing diet to induce hyperuricemia. Animals were treated with TMX-67 at 1 mg/kg/oral and allopurinol at 3 mg/kg/oral on day 7 after initiation of the special diet. The control group received methylcellulose vehicle. Blood samples were collected at various time points up to 24 hours. Plasma uric acid, BUN and creatinine levels were determined. The plasma level of BUN and creatinine was not affected by the treatment. The sponsor stated that TMX-67 had greater reduction of uric acid than allopurinol. The sponsor did not provide data tables for the report. However, reduction in the plasma AUC (mg.dL/hr) in the TMX-67 and allopurinol treated mice is shown in the figure below.



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It is concluded that TMX-67 showed hypouricemic effect at 1 mg/kg in vivo in mice.

2. A similar study was conducted in SD rats (18-P-98006). TMX-67 treatment was given at 1, 3 and 10 mg/kg/oral. Doses of allopurinol were 3, 10 and 30 mg/kg/oral. The ED<sub>50</sub> for reduction of plasma uric acid was 1.6 mg/kg for TMX-67 and 9.1 mg/kg for allopurinol. In vivo data suggest that TMX-67 was 5 times more potent than allopurinol in rats.

3. Renal impaired rats were treated at 1 mg/kg dose of TMX-67 and 10 mg/kg dose of allopurinol (study # 18-P-97001). Both inhibited uric acid levels in the plasma. However, allopurinol induced inhibition of uric acid was 4 times greater in the renal impaired rats compared to sham operated rats. TMX-67 showed 2.4 times higher inhibition of uric acid in renal impaired rats compared to sham operated rats. There was no change in the creatinine levels in any groups either with TMX-67 or allopurinol treatment. The sponsor submitted a summary of the study only.

4. Another study (18-P-96012) was conducted in renal impaired and sham-operated male SD rats at 0.3, 1 and 3 mg/kg/oral. Plasma uric acid, xanthine and BUN were measured. The data were compared with the sham-operated rats. TMX-67 showed inhibition of uric acid in the nephrectomized and sham operated rats dose dependently. However, the difference in the reduction of uric acid between sham-operated rats and nephrectomized rats was not significant. TMX-67 did not show any effect on the plasma BUN. The sponsor stated that the effect of TMX-67 treatment on the plasma xanthine levels was not always measurable. It is concluded that TMX-67 up to 3 mg/kg showed an inhibition of uric acid in the plasma.

#### Chimpanzee model:

Male chimpanzees were treated with TEI-6720, TEI-5620 and allopurinol at 5 mg/kg/oral for three days. Each group had three animals. Uric acid levels in the serum were decreased by 74% in TEI-6720 treated animals. Allopurinol and TEI-5620 showed a 45 and 16% reduction of serum uric acid, respectively. The sponsor stated that a transient elevation of liver transaminase activity was noted in animals. The average uric acid level in the serum was 2.8 mg/dL before the treatment. The sponsor did not identify if TEI-5620 is a metabolite of TEI-6720.

#### 2.6.2.3 Secondary pharmacodynamics:

1. TMX-67 (at oral doses  $\geq 10$  mg/kg) and allopurinol (100 mg/kg, the only dose tested) pretreatment inhibited TNF- $\alpha$  levels following iv LPS injection in Wistar rats (study #F3-97025).
2. Anti-inflammatory activity of TMX-67 and allopurinol was investigated in urate crystal-induced acute inflammation in rats. Urate crystals induced infiltration of inflammatory cells and mediators in the subcutaneous air-pouch. TMX-67 was investigated at 1, 3, 10 and 50 mg/kg. Allopurinol was investigated at 3, 10, 30 and 100 mg/kg. Colchicine was administered at 1 mg/kg. Treatments were given orally 30 min before urate crystal injections into the air pouch. Rats were sacrificed 24 hours after urate crystal injections. WBC counts, LTB4 and TNF- $\alpha$  levels were assayed. Pouch wall vascularity was examined histologically. Data for the WBC counts, LTB4 and TNF- $\alpha$  levels in the exudates are shown in the table below.

Table-2: Results

| GROUPS      | WBC Count        |                           | Percentage                 | Number of            | Air Pouch Fluid Concentration of |                       |                       |
|-------------|------------------|---------------------------|----------------------------|----------------------|----------------------------------|-----------------------|-----------------------|
|             | Blood*           | Air Pouch Fluid           | Phagocytosis               | Vessels              | LTB4                             | TNF $\alpha$          |                       |
|             | cells/mL         | cells/mm <sup>3</sup>     | %                          | cells/pouch          | ng/mL                            | ng/mL                 |                       |
| Controls    | Negative Control | 4387.1 $\pm$ 1729.5 (n=7) | 26 $\pm$ 27.4* (n=5)       | 0 (n=5)              | 6.7 $\pm$ 0.2* (n=4)             | 1.7 $\pm$ 0.66* (n=4) | 0.8 $\pm$ 0.06* (n=4) |
|             | Positive Control | 7362.5 $\pm$ 2309.5 (n=4) | 7272.2 $\pm$ 1550.9 (n=9)  | 6.4 $\pm$ 4.53 (n=9) | 13.0 $\pm$ 1.3 (n=8)             | 5.9 $\pm$ 1.16 (n=4)  | 1.2 $\pm$ 0.07 (n=4)  |
| Febuxostat  | Colchicine       | 3375 $\pm$ 45.7 (n=2)     | 3477.7 $\pm$ 645.7* (n=9)  | 6.2 $\pm$ 3.9 (n=9)  | 11.7 $\pm$ 1.7 (n=9)             | ND                    | 1.3 $\pm$ 0.1 (n=4)   |
|             | 1mg/kg           | ND                        | 5631.3 $\pm$ 3360.8 (n=8)  | 9.3 $\pm$ 6.6 (n=8)  | 12.0 $\pm$ 1.0 (n=4)             | ND                    | 0.98 $\pm$ 0.09 (n=4) |
|             | 3 mg/kg          | ND                        | 5043.7 $\pm$ 2480.4 (n=8)  | 11 $\pm$ 3.4 (n=8)   | ND                               | ND                    | 0.89 $\pm$ 0.09 (n=4) |
|             | 10 mg/kg         | ND                        | 7200.2 $\pm$ 2946.5 (n=8)  | 13 $\pm$ 6.7 (n=8)   | 8.9 $\pm$ 1.2* (n=8)             | ND                    | ND                    |
|             | 50 mg/kg         | 5915.7 $\pm$ 2101.3 (n=3) | 4973 $\pm$ 3604.7 (n=8)    | 7.9 $\pm$ 6.6 (n=8)  | 7.5 $\pm$ 1.4* (n=8)             | 8.5 $\pm$ 0.96 (n=4)  | 0.97 $\pm$ 0.15 (n=4) |
| Allopurinol | 3 mg/kg          | ND                        | 3850 $\pm$ 2583.9* (n=8)   | 2.7 $\pm$ 2.1 (n=8)  | 12.9 $\pm$ 2.5 (n=6)             | ND                    | 0.89 $\pm$ 0.03 (n=4) |
|             | 16 mg/kg         | ND                        | 3206.2 $\pm$ 2798.5* (n=8) | 2.9 $\pm$ 3.4 (n=8)  | 9.8 $\pm$ 2.9* (n=7)             | ND                    | ND                    |
|             | 30 mg/kg         | ND                        | 3400 $\pm$ 1758.4* (n=8)   | 3.9 $\pm$ 3.6 (n=8)  | ND                               | ND                    | 1.08 $\pm$ 0.08 (n=4) |
|             | 100 mg/kg        | 2400 $\pm$ 900* (n=3)     | 3551.2 $\pm$ 1494.7* (n=8) | 5.25 $\pm$ 3.6 (n=8) | 7.4 $\pm$ 0.4* (n=4)             | 9.2 $\pm$ 0.98 (n=4)  | 1.5 $\pm$ 0.4 (n=4)   |

\*Data is expressed as mean  $\pm$  SD. ND = not detected

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Above data show a minor reduction in WBC counts in inflammatory exudates at all doses of TMX-67 except 10 mg/kg. Contrarily, colchicine and allopurinol (at all dose levels) reduced WBC counts in the pouch fluid. However, LTB4 and TNF- $\alpha$  levels were not affected by the acute treatment. Overall role of TMX-67 as an anti-inflammatory agent up to 50 mg/kg in this model is not conclusive and its clinical significance is unknown.

Summary of the pharmacology studies:

Several pharmacology studies showed that TMX-67 had a potent inhibition of uric acid synthesis in animal models. TMX-67 inhibited xanthine oxidase activity at 10 nM in the human liver *in vitro*. TMX-67 did not interfere with the purine and pyrimidine synthesis. TMX-67 inhibited uric acid levels in the plasma at 1.6 and 5 mg/kg in rats and chimpanzee, respectively. Mice fed with 2.5% oxonate diet also showed hypouricemic effect at 1 mg/kg. TMX-67 inhibited LPS-induced TNF- $\alpha$  release in rats at 10 mg/kg. Its anti-inflammatory activity in the rat model is not conclusive.

2.6.2.4 Safety pharmacology

Neurological effects:

General behavior of male mice following treatment with TEI-6720 was investigated according to Irwin's method (study # 18-G-94007). Each group had 4 mice and animals were treated at 10, 30, 100 and 300 mg/kg/oral. Thirty minutes after the treatment, increased passivity and decreased locomotor activity were noted at 100 and 300 mg/kg. The locomotor activity of TEI-6720 was further investigated in the study # 18-G-94022 in male mice at 10, 30 and 100 mg/kg/oral. Each group had 4 mice. Sixty minutes after the treatment, locomotor activity at 30 mg/kg was decreased. However, in the absence of a similar effect at 100 mg/kg, the sponsor concluded that the treatment up to 100 mg/kg did not show significant effect on the locomotor activity in mice.

The effect of TEI-6720 on hexobarbital sleeping time was investigated (study # 18-G-94017) at 10, 30 and 100 mg/kg/oral in male mice. There was no indication of affecting sleeping time by TEI-6720 up to 100 mg/kg.

TEI-6720 was investigated for the effect on convulsions induced by pentamethylenetetrazole or by electroshock in male mice (study # 18-G-94012). Doses were 10, 30 and 100 mg/kg/oral. TEI-6720 had no effect on the chemically induced or electroshock-induced seizures. However, deaths were reported both in the control and treated animals.

Analgesic or hyperalgesic effect of TEI-6720 was studied in the acetic acid-induced writhing model and hot plate test in male mice at 10, 30 and 100 mg/kg/oral (study # 18-G-95001). However, TEI-6720 up to 100 mg/kg had no effect on the pain perception. TEI-6720 at 10, 30 and 100 mg/kg/oral had no effect on normal body temperature in male mice up to 4 hours after the treatment (study #18-G-94009).

#### Cardiovascular effects:

##### In vitro:

The effect of TMX-67 at 0.1, 1, 50 and 500  $\mu\text{M}$  on the rate of depolarization in the electrically stimulated isolated Purkinje fibers of dogs was evaluated (study #DTDE 1013). The tissue was stimulated at 0.5 and 1 Hz. TMX-67 did not show any effect on the resting membrane potential and rate of depolarization up to 1  $\mu\text{M}$ . The rate of depolarization was reduced by 12-17% and action potential was reduced by about 20-26% at 50  $\mu\text{M}$ . Most of the tissues were refractory to stimulation at 500  $\mu\text{M}$ . The data suggest that TMX-67 impairs cardiac conduction in the isolated tissues in vitro at 50  $\mu\text{M}$  and higher concentrations. The concentration was approximately 3 times higher than the plasma  $C_{\text{max}}$  of about 5  $\mu\text{g/ml}$  at the maximum therapeutic dose without factoring plasma protein binding of TMX-67. Considering 99.3% plasma protein binding of the drug, TMX-67 showed impairment of cardiac conduction at 42 times higher than the unbound drug at plasma  $C_{\text{max}}$  at maximum therapeutic dose. Sotalol was used as the positive control in the experiment. In a repeat experiment, TMX-67 did not show treatment related effect on resting potential and action potential up to 1000 nM in the electrically stimulated canine Purkinje fiber (report # 020722.UBQ).

The sponsor further investigated the effect of TMX-67 on the sodium ion channel from the human heart expressed in the human embryonic kidney cells. Depolarization was recorded by the single cell electrophysiological technique. Sodium channel dependent inward current was inhibited by 1, 15, 37, 57 and 89% at 1, 10, 50, 100 and 500  $\mu\text{M}$ . The calculated  $\text{IC}_{50}$  for the inhibition was 75  $\mu\text{M}$ . If the  $\text{C}_{\text{max}}$  at 120 mg daily dose is approximately 5  $\mu\text{g}/\text{ml}$ , the concentration at  $\text{IC}_{50}$  is about 5 times higher than that achieved at maximum clinical dose without considering the plasma protein binding of TMX-67. The concentration was about 64 times unbound drug at the maximum recommended human dose. Lidocaine was used as the positive control in the experiment (report #020501.UBQ).

The effect of the drug on potassium channel in the heart was investigated in the report # 020227.UBQ. hHERG (human ether-a-go-go-related gene products) channels in the heart are responsible for repolarization of the heart by potassium influx. Its blockade would induce prolongation of action potential and arrhythmia. The hERG channel was expressed in the embryonic kidney cells (HEK 293) and electrophysiology of the cell was recorded by the patch clamp techniques. TMX-67 up to 500  $\mu\text{M}$  did not show blockade of the potassium channel. On the other hand, slight agonist-like activity was observed. Terfenadine was used as the positive control. The study was repeated (study # 020503.UBQ) in CHO cell line transfected with hERG cDNA. It is stated that endogenous potassium channel in the HEK-293 cell line could have interfered the assay. Data in the CHO cell line also showed agonist type of response at positive voltage (voltage dependent) at 3-70 nM and did not block the hERG channel.

In vitro effect on ADP-induced platelet aggregation was studied in platelet rich plasma obtained from normal human volunteers (report # 18-G-96010) at 3, 10, 30 and 100  $\mu\text{M}$  concentrations of TMX-67. In addition, the effect of TMX-67 on coagulation parameters (APTT and PT) was evaluated. TMX-67 did not show treatment related changes in the platelet aggregation and coagulation parameters up to 100  $\mu\text{M}$ .

TMX-67 showed 4, 12, 14, and 37% blockade of calcium channel in the guinea-pig cardiac myocytes at 5, 50, 100 and 500  $\mu\text{M}$ . Nifedipine blocked the calcium channel related current at 0.1  $\mu\text{M}$ . Based on the data, it was concluded that the effect of TMX-67 on calcium channel was biologically insignificant ( study # 020502.UBQ).

Data signified that TMX-67 might not have adverse effect of Torsades-de-points based on the in vitro study. Further study in the in vivo system discussed below confirmed it.

#### In vivo:

1. Respiratory and hemodynamic effect of TEI-6720 was investigated in beagle dogs (18-G-94021). Dogs were anesthetized for monitoring respiration, arterial blood pressure, lead II ECG and left ventricular pressure (LVP) after appropriate instrumentation up to 4 hours post dose. TEI-6720 suspensions in 0.5% methyl cellulose was administered into

the duodenum at 10 and 100 mg/kg. The serum concentration of TEI-6720 was measured. Treatment up to 100 mg/kg did not show any effect on the respiratory and cardiovascular parameters. The sponsor stated that the serum concentration was 15 times higher than that of the therapeutic dose.

2. Report # N004049A:

Beagle dogs were treated at 5 and 50 mg/kg/day by oral gavage for 14 days. The control group was treated with 0.5% methylcellulose vehicle. Effects of the treatment on cardiovascular and respiratory parameters were examined in conscious dogs. Each group had three dogs per sex. Blood pressure, heart rates and ECG were monitored by implanted radiotelemetry (Holter arrhythmia analysis system) for recording RR, PR, QRS, QT and QTc intervals. Respiratory rates, clinical chemistry and pharmacokinetics of the drug were also determined. The sponsor stated that hypotension was noted by about 14-50% of the predose at 5 and 50 mg/kg that lasted up to 2 hours. However, these changes were transient and were not observed consistently when the average data for each day were compared between the control and treated groups. Heart rate did not show statistically significant change. There were no treatment related changes in the ECG. There were no treatment related changes in clinical pathology and respiratory rates noted. Serum uric acid level was about 0-0.1 mg/dL on day -4 of the treatment and did not show treatment related changes. Plasma levels of the drug are shown in the table below.

| Day | Gender | Dose mg/kg | t <sub>max</sub> (h) | C <sub>max</sub> (g/mL) | AUC <sub>0-24</sub> (g·h/mL) | AUC <sub>0-24</sub> (g·h/mL) | t <sub>1/2α</sub> (h) | C <sub>min</sub> /Dose | AUC <sub>0-24</sub> /Dose |
|-----|--------|------------|----------------------|-------------------------|------------------------------|------------------------------|-----------------------|------------------------|---------------------------|
| 2   | All    | 5          | 1.4                  | 3.0214                  | 5.4137                       | 5.5225                       | 0.5                   | 0.60                   | 1.10                      |
|     |        | 50         | 2.7                  | 28.9526                 | 319.3930                     | 321.5874                     | 1.4                   | 0.58                   | 2.43                      |
|     | Male   | 5          | 1.5                  | 4.8914                  | 8.7298                       | 8.8309                       | 0.4                   | 0.98                   | 1.77                      |
|     |        | 50         | 2.7                  | 17.5815                 | 78.0437                      | 82.4345                      | 0.8                   | 0.35                   | 1.65                      |
|     | Female | 5          | 1.3                  | 1.1514                  | 2.0977                       | 2.2142                       | 0.6                   | 0.23                   | 0.44                      |
|     |        | 50         | 2.7                  | 40.3236                 | 160.7403                     | 160.7403                     | 3.6                   | 0.81                   | 3.21                      |
| 14  | All    | 5          | 0.9                  | 1.7340                  | 2.2721                       | 2.3762                       | 0.7                   | 0.35                   | 0.47                      |
|     |        | 50         | 2.3                  | 38.1225                 | 149.5377                     | 149.7188                     | 2.0                   | 0.76                   | 2.99                      |
|     | Male   | 5          | 0.8                  | 1.7263                  | 2.0799                       | 2.1851                       | 0.9                   | 0.35                   | 0.44                      |
|     |        | 50         | 2.2                  | 29.5407                 | 118.3167                     | 118.6788                     | 1.4                   | 0.59                   | 2.37                      |
|     | Female | 5          | 1.0                  | 1.7417                  | 2.4643                       | 2.5554                       | 0.6                   | 0.35                   | 0.51                      |
|     |        | 50         | 2.3                  | 46.7042                 | 180.7588                     | 180.7588                     | 4.1                   | 0.93                   | 3.62                      |

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Above data suggest that the drug was absorbed rapidly and peak plasma levels were achieved within 3 hours following oral administration. There was a lack of dose proportionality in the plasma exposure at the high dose. The dose response relationship between the plasma exposure and hypotension was not evident.

It is concluded that treatment up to 50 mg/kg oral dose for 14 days did not show any treatment related changes in the ECG and respiration. Transient hypotension was observed at 5 and 50 mg/kg that was recovered within 2 hours.

Pulmonary effects:

The treatment had no effect on respiration. Data are reviewed under *in vivo* cardiovascular effect.

Renal effects:

Urine volume and electrolyte excretion in male Wistar rats was investigated (study # 18-G-94030). Rats were fasted over night and treated with TEI-6720 at 10, 30 and 100 mg/kg/oral. Rats were subjected to water load at 25 ml/kg of saline and placed in metabolism cage for the collection of urine up to 6 hour post dose. Each group had 6 animals. Urinary volume, sodium, potassium, chloride and xanthine levels were determined. Urinary xanthine levels were measured by HPLC methods. Data show that urine volume, potassium and chloride levels were increased at 100 mg/kg. Traces of xanthine were noted in the control rats. However, urinary xanthine levels ( $\mu\text{g/ml}$ ) were 390, 364 and 287 at 10, 30 and 100 mg/kg, respectively. These data show that potassium, chloride and xanthine excretion increased at 100 mg/kg in rats.

Gastrointestinal effects:

The effect of TEI-6720 on the contractility of isolated guinea-pig ileum was recorded in the presence of 3, 10 and 30  $\mu\text{M}$  concentrations *in vitro* (study # 18-G-95006). Data suggest that the spontaneous movement was slightly inhibited at 30  $\mu\text{M}$ . The spasmolytic effect of TEI-6720 was further studied in male mice *in vivo* at 10, 30 and 100 mg/kg (Study 18-G-94025). However, TEI-6720 did not show any effect on gastrointestinal transit time. The effect of TEI-6720 on mediator, neurotransmitter and ion channel-induced contractions by histamine, acetylcholine and barium chloride, respectively, was examined up to  $30 \times 10^{-3}$  M concentration in isolated guinea-pig ileum (study # 18-G-94028). TEI-6720 did not show any antihistaminergic, anticholinergic effect or blockade of calcium ion channel in the isolated guinea-pig ileum.

Abuse liability: Nil

Other: No other study reviewed for safety pharmacology

**Summary of safety pharmacology:**

Male mice treated up to 100 mg/kg/oral did not show any CNS and GI toxicity. TMX-67 showed diuretic effect at 100 mg/kg in rats. Increased excretion of potassium, chlorine and xanthine was noted. TMX-67 did not show any effect on heart, ECG and respiratory rates in beagle dogs at 50 mg/kg/oral for 14 days. However, transient hypotension was noted in some occasions after the treatment that lasted for 2 hours. TMX-67 had no effect on the platelet functions *in vitro*.

It is concluded that TMX-67 is devoid of effects on the heart, respiration and central nervous system. Transient hypotension may be expected after the oral doses.

### 2.6.2.5 Pharmacodynamic drug interactions

TMX-67 at 1 and 10 mg/kg showed no significant interactions with nifedipine in spontaneously hypertensive rat model (study # 18-G-98003).

Male Sprague Dawley rats were treated at 1 or 10 mg/kg oral doses of TMX-67. Animals were treated with glibenclamide orally at 3 mg/kg. The effect of TMX-67 treatment on the hypoglycemic activity of glibenclamide was determined. TMX-67 did not show any drug interaction for the hypoglycemic effect of glibenclamide up to 12 hours post dose (study # 18-G98002).

It is concluded that TMX-67 had no drug interactions for the pharmacodynamic effects of oral hypotensive and hypoglycemic agents.

### 2.6.3 PHARMACOLOGY TABULATED SUMMARY

Some of the studies for mechanism of action and in vivo pharmacodynamics are summarized in the table below.

| Parameter                                                         | Activity                                                                                                | Tissue/system                 |
|-------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-------------------------------|
| <u>In vitro</u> xanthine oxidase inhibition                       | Ki 10 nM                                                                                                | Human liver                   |
| <u>In vitro</u> xanthine oxidase inhibition                       | Ki 0.6 nM                                                                                               | Bovine milk                   |
| <u>In vitro</u> aldehyde oxidase inhibition                       | IC <sub>50</sub> 0.42 M                                                                                 | Rat liver                     |
| <u>In vitro</u> PNP inhibition                                    | 8% inhibition at 100 µM                                                                                 | Human blood                   |
| <u>In vitro</u> HGPRT inhibition                                  | 14% inhibition at 100 µM                                                                                | Commercially available enzyme |
| <u>In vitro</u> inhibition of guanine deaminase                   | No effect up to 100 µM                                                                                  | Rabbit liver                  |
| <u>In vitro</u> inhibition of OPRT/OMPDC                          | No effect up to 100 µM                                                                                  | Commercially available enzyme |
| <u>In vivo</u> activity of OPRT/OMPDC on OA/OD excretion in urine | TMX-67, no effect up to 100 mg/kg<br><br>Allopurinol, reduction of OA/OD excretion at 300 mg/kg         | Mice                          |
| <u>In vivo</u> inhibition of uric acid                            | ED <sub>50</sub> for plasma uric acid inhibition was 1.6 mg/kg for TMX-67 and 9.1 mg/kg for allopurinol | Rat                           |

| Parameter                                               | Activity         | Tissue/system |
|---------------------------------------------------------|------------------|---------------|
| In vivo inhibition of uric acid                         | 5 mg/kg          | Chimpanzee    |
| In vivo inhibition of uric acid                         | 1 mg/kg          | mouse         |
| In vivo inhibition of LPS-induced TNF- $\infty$ release | 10 and 100 mg/kg | Rat           |

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

TMX-67 is rapidly absorbed after oral doses. Traces of unchanged drug are excreted in urine, feces and milk. The drug is not accumulated in systemic organs. It crosses the placental barrier. Major human metabolite is glucuronidated TMX-67. It does not induce hepatic enzymes. Hydroxylation of TMX-67 generated isomers due to stereo-selective enzyme activity. Gender difference in the exposure was observed in mice only.

### 2.6.4.2 Methods of Analysis

TMX-67 and its metabolites were separated by HPLC and characterized by LC-Mass spectrometry as described in the study reports. The radioactivity was counted in the liquid scintillation spectrometer when  $^{14}\text{C}$ -TMX-67 was used.

### 2.6.4.3 Absorption

1. Study of serum concentration of TEI-6720 during a 19-week repeated administration in mice, study # 3881 (011-024), M. 4.2.2.2.9

The study was conducted in male mice at 3 and 24 mg/kg/oral. A summary of pharmacokinetic data is shown in the table below.

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**Table 7 Summary of pharmacokinetic parameters**

|        | 3mg/kg group                      |                           |                        | 24mg/kg group                     |                           |                        |
|--------|-----------------------------------|---------------------------|------------------------|-----------------------------------|---------------------------|------------------------|
|        | AUC <sub>0-24hr</sub><br>ng·hr/mL | C <sub>max</sub><br>ng/mL | T <sub>max</sub><br>hr | AUC <sub>0-24hr</sub><br>ng·hr/mL | C <sub>max</sub><br>ng/mL | T <sub>max</sub><br>hr |
| Day1   | 3999.5                            | 2025.0                    | 0.5                    | 31187.6                           | 19672.3                   | 0.5                    |
| Week5  | 2971.5                            | 1419.0                    | 0.5                    | 28859.7                           | 24056.3                   | 0.5                    |
| Week13 | 2348.9                            | 1399.9                    | 0.5                    | 24063.6                           | 22556.8                   | 0.5                    |
| Week19 | 4444.4                            | 2136.8                    | 0.5                    | 36519.2                           | 32350.5                   | 0.5                    |

Above data showed a dose proportionate increase in the exposure. Although differences in the exposure were noted between week 13 and 19, it is not clear whether this was due to variability of the exposure between animals. Microsomal protein content in the mouse liver did not show any increase due to the treatment. Data suggest that the treatment had no effect on the induction of microsomal enzymes in the liver.

2. Profiling study on the serum concentration of unchanged drug after repeated oral administration of TEI-6720 in pregnant rabbits, study # S05420K10P, in Module 4 # 4.2.2.2.12

This is a toxicokinetics study in pregnant rabbits, part of the segment 2 reproductive safety study of TEI-6720 (study # T883). The drug substance was suspended in 0.5% methylcellulose at 0.6, 2.4 and 9.6 mg/ml. The lot # of the drug substance was 980423A4. Doses were administered by oral gavage at 3, 12 and 48 mg/kg at 5 ml/kg volume. New Zealand white rabbits, kbl: NZW, were used in the study. The study design is shown in the table below.

| Group        | Treatment | Dose, mg/kg | # pregnant rabbits | Animal #  |
|--------------|-----------|-------------|--------------------|-----------|
| 1. Low dose  | TEI-6720  | 3           | 6                  | 1201-1206 |
| 2. Mid dose  | TEI-6720  | 12          | 6                  | 2201-2206 |
| 3. High dose | TEI-6720  | 48          | 6                  | 3201-3206 |

Pregnant animals were treated once a day from gestation days 6 to 18. Blood samples were collected on the first and last days of the treatment at 0.5, 1, 2, 4, 8 and 24 hours after the dosing. Serum levels of TEI-6720 were determined by the HPLC method. Blood samples were collected from the lateral ear vein. Animals were sacrificed on day 19 of the gestation by exsanguination under sodium pentobarbital anesthesia. One animal (#2205) at 12 mg/kg died due to dosing error on gestation day 8. Animal #2202 at 12 mg/kg was non-pregnant. Data for these animals were not included in the pharmacokinetic analysis. AUC (ng.hr/ml),  $C_{max}$  (ng/ml) and  $T_{max}$  (hr) were calculated.

The pharmacokinetic data are shown in the table below from page 47 of the report.

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Table 7 Summary of toxicokinetic parameters of TEI-6720 in pregnant rabbits

| Dose (mg/kg)                                    | 3                        | 12                        | 48                         |
|-------------------------------------------------|--------------------------|---------------------------|----------------------------|
| <b>First</b>                                    |                          |                           |                            |
| No. of dams examined                            | 6                        | 4                         | 6                          |
| C <sub>max</sub> (mean ± S.D., ng/mL)<br>(C.V.) | 2228.8 ± 2654.6<br>(119) | 8117.1 ± 4882.8<br>(60)   | 44749.0 ± 10001.5<br>(22)  |
| AUC(0-24hr) (mean ± S.D., ng·hr/mL)<br>(C.V.)   | 30111.2 ± 4494.9<br>(44) | 63899.9 ± 18513.4<br>(26) | 393084.9 ± 65789.0<br>(17) |
| T <sub>max</sub> (mean ± S.D., hr)<br>(C.V.)    | 2.3 ± 1.5<br>(66)        | 2.4 ± 1.9<br>(79)         | 2.4 ± 3.1<br>(126)         |
| <b>Final</b>                                    |                          |                           |                            |
| No. of dams examined                            | 6                        | 4                         | 6                          |
| C <sub>max</sub> (mean ± S.D., ng/mL)<br>(C.V.) | 1153.5 ± 397.4<br>(34)   | 6317.2 ± 1467.5<br>(28)   | 35292.5 ± 13252.1<br>(38)  |
| AUC(0-24hr) (mean ± S.D., ng·hr/mL)<br>(C.V.)   | 9315.2 ± 1772.9<br>(19)  | 58571.8 ± 12936.6<br>(22) | 394006.2 ± 99771.6<br>(25) |
| T <sub>max</sub> (mean ± S.D., hr)<br>(C.V.)    | 1.4 ± 1.4<br>(98)        | 3.3 ± 1.5<br>(46)         | 3.6 ± 3.7<br>(103)         |

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The exposure on GD 18 to 3, 12 and 48 mg/kg doses was 9315, 58571 and 394606 ng.hr/ml, respectively.

TEI-6720 was rapidly absorbed after the oral dose. The exposure data between the first and last dose did not show significant difference. The exposure increased with the dose. Validation of the assay was provided in the report # TBL-970606 located in M4.2.2.1.18. The calibration curve was prepared between 4 to 10000 ng/ml and TEI-6720 was measured within the concentration range in the serum. An additional validation experiment (study # TBL981203) in module 4.2.2.1.23 was conducted to validate increase in the injection volume from 40 to 75 µL in the HPLC assay.

3. Preliminary carcinogenicity study of TEI-6720 in mice, study #3500-011-022 and 3500-011-023), M.4.2.3.2.2.

A dose finding study was conducted in B6C3F1 mice at 3, 12, 24 and 48 mg/kg/oral for 13 weeks. The study was reviewed under IND 58229 (serial # 083) on Sept 10, 2003. Pharmacokinetic information for the three-month repeat dose study at 3, 12, 24 and 48 mg/kg was provided. However, the sponsor was asked to calculate the exposure at doses used for the carcinogenicity study (3, 7.5 and 18.75 mg/kg). Accordingly, the sponsor calculated the exposure at 7.5 and 18.75 mg/kg doses from the treatment duration vs. exposure data of the three-month toxicity study. The exposure data at 3 mg/kg was directly available from the result of the toxicokinetics study. The data are shown in the table below.

**Table 2.6g Exposure (AUC) and Blood Concentration (C<sub>30 min</sub>) Values at Week 13 for Dose Levels Used in the Mouse Carcinogenicity Study**

| Daily Dose (mg/kg/day)                    | 3    |        | 7.5  |        | 18.75 |        |
|-------------------------------------------|------|--------|------|--------|-------|--------|
|                                           | Male | Female | Male | Female | Male  | Female |
| AUC <sub>24</sub> (µg•hr/mL) <sup>a</sup> | 3.4  | 13.2   | 11.0 | 37.7   | 27.8  | 96.5   |
| C <sub>30 min</sub> (µg/mL) <sup>b</sup>  | 1.5  | 5.1    | NA   | NA     | 33.7  | 46.6   |

The exposure after 3-month repeated dosing was 27.8 and 96.5 µg.hr/ml at 18.75 mg/kg for male and female mice, respectively.

4. Preliminary carcinogenicity study of TEI-6720 in rats, study No# S054S2R10A, M.4.2.3.2.4.

The study was conducted for finding the doses for carcinogenicity study in rats. TMX-67 was orally administered at 3, 12, 24 and 36 mg/kg for 13 weeks by gavage to male and female F344/Du — rats. Blood samples were collected via jugular vein from animals for TK at 0.5, 1, 4, 8 and 24 hours post administration. The serum concentration of unchanged TEI-6720 was measured. AUC, C<sub>max</sub> and T<sub>max</sub> were calculated.

b(4)

Page 147 provided the pharmacokinetic data in male and female rats as shown in the table below.

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Table 12-3. Pharmacokinetic parameters of the unchanged TEI-6720 on day1 and on final day of the 13-week repeated oral administration of TEI-6720 to rats.

| sex/<br>dosing group | Day1                              |                           |                        | Final day                         |                           |                        |
|----------------------|-----------------------------------|---------------------------|------------------------|-----------------------------------|---------------------------|------------------------|
|                      | AUC <sub>0-24hr</sub><br>ng•hr/ml | C <sub>max</sub><br>ng/ml | T <sub>max</sub><br>hr | AUC <sub>0-24hr</sub><br>ng•hr/ml | C <sub>max</sub><br>ng/ml | T <sub>max</sub><br>hr |
| male/                |                                   |                           |                        |                                   |                           |                        |
| 3 mg/kg/day          | 6547.2                            | 1233.9                    | 1.0                    | 17314.7                           | 5512.3                    | 0.5                    |
| 12 mg/kg/day         | 36409.4                           | 8466.7                    | 0.5                    | 56677.6                           | 21340.7                   | 0.5                    |
| 24 mg/kg/day         | 103671.6                          | 25299.0                   | 0.5                    | 194449.8                          | 40337.0                   | 0.5                    |
| 36 mg/kg/day         | 152965.2                          | 38253.5                   | 0.5                    | 312966.7                          | 55588.0                   | 0.5                    |
| female/              |                                   |                           |                        |                                   |                           |                        |
| 3 mg/kg/day          | 8552.7                            | 2603.5                    | 0.5                    | 16934.2                           | 3815.0                    | 0.5                    |
| 12 mg/kg/day         | 47455.3                           | 11994.1                   | 0.5                    | 81110.8                           | 13526.3                   | 0.5                    |
| 24 mg/kg/day         | 128138.5                          | 35517.0                   | 0.5                    | 201770.6                          | 30831.4                   | 0.5                    |
| 36 mg/kg/day         | 185659.9                          | 41449.0                   | 1.0                    | 306814.4                          | 47709.3                   | 0.5                    |

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The drug was absorbed rapidly after oral dosing. The serum exposure was increased with dose. Male and female rats at 24 mg/kg showed almost similar exposure at the end of dosing. The exposure was increased at the end of dosing period compared to day 1. This could have resulted from a decrease elimination of the drug upon repeated dosing. The C<sub>max</sub> and AUC at 24 mg/kg in male F344 rats at the end of week 13 were 40337 ng/ml

and 194449 ng.hr/ml, respectively. The  $C_{max}$  and AUC at 24 mg/kg in female F344 rats were 30831 ng/ml and 201770 ng.hr/ml, respectively. Considering the similarity of exposure at 24 mg/kg in male and female rats, the average exposure in rats was 198109 ng.hr/ml in F344 rats. The exposure at 24 mg/kg was 16.56 times the maximum recommended human exposure at 120 mg/day.

5. A multiple dose safety, pharmacokinetic, and pharmacodynamic study of oral TMX-67 in healthy volunteers. Clinical PK study (TAP-02-001564-2.0), study # TMX-99-001.

At a maximum recommended clinical dose of 120 mg/day the PK parameters on day 1 is shown in the table below.

Table 29 Pharmacokinetic Parameters of TMX-67 on Day 1 Following Oral Administration of TMX-67 (120 mg QD) to Subject in Study TMX-99-001

| Subject                  | $T_{max}$<br>(h) | $C_{max}$<br>(ng/mL) | AUC <sub>0-24</sub><br>(ng·h/mL) | AUC <sub>0-∞</sub><br>(ng·h/mL) | AUC <sub>0-∞</sub><br>% Extrap | C <sub>tr</sub><br>(h) | $\lambda_z$<br>(h <sup>-1</sup> ) | Time for %   |              |                 | V <sub>d</sub> F<br>(L) | C <sub>tr</sub> F<br>(h) | C <sub>tr</sub> /D<br>(L <sup>-1</sup> ) | AUC <sub>0-24</sub><br>(h·L) |   |
|--------------------------|------------------|----------------------|----------------------------------|---------------------------------|--------------------------------|------------------------|-----------------------------------|--------------|--------------|-----------------|-------------------------|--------------------------|------------------------------------------|------------------------------|---|
|                          |                  |                      |                                  |                                 |                                |                        |                                   | Lower<br>(h) | Upper<br>(h) | No.<br>Patients |                         |                          |                                          |                              |   |
| 1203                     | 1.00             | 4.4427               | 12.6190                          | 12.4747                         | 3                              | 22.1                   | 0.081                             | 30.0         | 48.0         | 3               | 74.1                    | 9.67                     | 0.0370                                   | 0.1084                       |   |
| 1203                     | 0.50             | 4.5189               | 8.7795                           | 9.0487                          | 3                              | 13.1                   | 0.040                             | 16.0         | 36.0         | 4               | 64.5                    | 13.26                    | 0.0376                                   | 0.0734                       |   |
| 1204                     | 1.00             | 2.8638               | 7.3492                           | 7.4664                          | 2                              | 8.2                    | 0.085                             | 18.0         | 30.0         | 3               | 79.2                    | 16.07                    | 0.0230                                   | 0.0622                       |   |
| 1205                     | 0.50             | 5.9363               | 9.3382                           | 9.6361                          | 1                              | 4.7                    | 0.148                             | 6.0          | 24.0         | 6               | 36.7                    | 12.44                    | 0.0495                                   | 0.0804                       |   |
| 1205                     | 1.50             | 4.0038               | 9.5237                           | 9.8387                          | 3                              | 18.7                   | 0.041                             | 18.0         | 36.0         | 4               | 69.4                    | 12.17                    | 0.0335                                   | 0.0822                       |   |
| 1207                     | 0.50             | 3.2115               | 12.1656                          | 12.2732                         | 1                              | 6.0                    | 0.116                             | 16.0         | 36.0         | 4               | 38.1                    | 9.73                     | 0.0268                                   | 0.1029                       |   |
| 1208                     | 1.50             | 5.7068               | 14.7151                          | 14.9053                         | 1                              | 7.3                    | 0.095                             | 10.0         | 36.0         | 6               | 35.2                    | 8.03                     | 0.0416                                   | 0.1242                       |   |
| 1210                     | 2.00             | 3.9612               | 12.2119                          | 12.4106                         | 2                              | 11.9                   | 0.068                             | 30.0         | 48.0         | 3               | 67.5                    | 9.67                     | 0.0258                                   | 0.1084                       |   |
| 1211                     | 0.50             | 5.4475               | 13.8063                          | 13.8802                         | 1                              | 10.0                   | 0.069                             | 16.0         | 36.0         | 4               | 35.0                    | 8.79                     | 0.0537                                   | 0.1156                       |   |
| 1212                     | 0.50             | 7.3663               | 20.4499                          | 20.6803                         | 1                              | 9.7                    | 0.072                             | 16.0         | 36.0         | 4               | 24.4                    | 5.81                     | 0.0614                                   | 0.1720                       |   |
| AD Subject Summary Study |                  |                      |                                  |                                 |                                |                        |                                   |              |              |                 |                         |                          |                                          |                              |   |
| N <sup>a</sup>           | 9                | 9                    | 9                                | 9                               | 9                              | 9                      | 9                                 | 9            | 9            | 9               | 9                       | 9                        | 9                                        | 9                            | 9 |
| Mean                     | 1.00             | 4.4720               | 11.1204                          | 11.3131                         | 2                              | 11.4                   | 0.076                             | 17.3         | 48.0         | 4               | 57.8                    | 11.09                    | 0.0373                                   | 0.0949                       |   |
| SD                       | 0.38             | 1.3148               | 3.4228                           | 3.4203                          | 1                              | 5.8                    | 0.059                             | 8.0          | 0.0          | 1               | 17.5                    | 2.58                     | 0.0110                                   | 0.0202                       |   |
| Min                      | 0.50             | 2.8638               | 7.3492                           | 7.4664                          | 1                              | 4.7                    | 0.081                             | 6.0          | 48.0         | 3               | 35.0                    | 8.05                     | 0.0230                                   | 0.0622                       |   |
| Median                   | 1.00             | 4.4427               | 12.6190                          | 12.3731                         | 2                              | 10.0                   | 0.069                             | 16.0         | 48.0         | 4               | 64.6                    | 9.78                     | 0.0370                                   | 0.1022                       |   |
| Max                      | 2.00             | 5.4475               | 14.7151                          | 14.9053                         | 3                              | 22.1                   | 0.148                             | 30.0         | 48.0         | 6               | 79.2                    | 16.07                    | 0.0537                                   | 0.1242                       |   |
| C <sub>tr</sub> %        | 56               | 29                   | 22                               | 21                              | 40                             | 32                     | 30.370                            | 48           | 0.0          | 26              | 30                      | 23                       | 20                                       | 21                           |   |
| H <sub>1</sub> Mean      |                  |                      |                                  |                                 |                                |                        |                                   |              |              |                 |                         |                          |                                          |                              |   |

<sup>a</sup> Subject 1212 discontinued prior to receiving Day 14 dose and was therefore excluded from the descriptive statistics

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The PK data on day 14 following oral dosing at 120 mg/day to normal volunteers are shown in the table below.

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Table 30 Pharmacokinetic Parameters of TMX-67 on Day 14 Following Oral Administration of TMX-67 (120 mg QD) to Subject in Study TMX-99-001

| Subject                        | C <sub>max</sub> (ng/ml) | C <sub>min</sub> (ng/ml) | AUC <sub>0-24</sub> (ng·hr/ml) | AUC <sub>0-12</sub> (ng·hr/ml) | AUC <sub>12-24</sub> (ng·hr/ml) | t <sub>1/2</sub> (hr) | t <sub>1/2</sub> (hr) | Times for 1/2 A |       |               | V <sub>d</sub> (L) | Cl <sub>R</sub> (L/hr) | C <sub>min</sub> (ng/ml) | AUC <sub>0-24</sub> (ng·hr/ml) |
|--------------------------------|--------------------------|--------------------------|--------------------------------|--------------------------------|---------------------------------|-----------------------|-----------------------|-----------------|-------|---------------|--------------------|------------------------|--------------------------|--------------------------------|
|                                |                          |                          |                                |                                |                                 |                       |                       | Lower           | Upper | No. of Points |                    |                        |                          |                                |
| 1201                           | 0.50                     | 4.8802                   | 13.8469                        | 12.8837                        | 0.9632                          | 12.4                  | 0.028                 | 12.0            | 48.0  | 8             | 50.1               | 9.33                   | 0.0402                   | 0.1672                         |
| 1204                           | 0.50                     | 3.9265                   | 9.2155                         | 9.4638                         | 0.2483                          | 8.5                   | 0.084                 | 10.0            | 36.0  | 4             | 51.8               | 12.45                  | 0.0472                   | 0.1800                         |
| 1204                           | 1.50                     | 3.6525                   | 7.8833                         | 7.6834                         | 0.1999                          | 9.2                   | 0.035                 | 12.0            | 36.0  | 3             | 39.6               | 15.52                  | 0.0218                   | 0.0840                         |
| 1203                           | 0.50                     | 4.8291                   | 9.9632                         | 9.5632                         | 0.4000                          | 7.8                   | 0.059                 | 10.0            | 36.0  | 4             | 43.2               | 12.04                  | 0.0469                   | 0.1830                         |
| 1206                           | 0.50                     | 2.0814                   | 11.9170                        | 11.5093                        | 0.4077                          | 12.0                  | 0.028                 | 10.0            | 48.0  | 5             | 45.5               | 10.43                  | 0.0261                   | 0.0930                         |
| 1207                           | 1.50                     | 3.8828                   | 14.9285                        | 14.6454                        | 0.2831                          | 34.2                  | 0.025                 | 30.0            | 48.0  | 3             | 45.9               | 8.54                   | 0.0489                   | 0.1130                         |
| 1206                           | 1.50                     | 3.8306                   | 13.8460                        | 13.6055                        | 0.2405                          | 29.0                  | 0.024                 | 24.0            | 48.0  | 4             | 57.7               | 9.23                   | 0.0485                   | 0.1084                         |
| 1210                           | 3.00                     | 2.5013                   | 15.1817                        | 14.4711                        | 0.7106                          | 44.2                  | 0.015                 | 30.0            | 48.0  | 3             | 79.4               | 8.34                   | 0.0208                   | 0.1204                         |
| 1211                           | 1.50                     | 4.3521                   | 14.9699                        | 14.5522                        | 0.4177                          | 6.7                   | 0.109                 | 10.0            | 36.0  | 4             | 35.3               | 9.24                   | 0.0521                   | 0.1233                         |
| All Subjects: Cumulative Study |                          |                          |                                |                                |                                 |                       |                       |                 |       |               |                    |                        |                          |                                |
| N                              | 9                        | 9                        | 9                              | 9                              | 9                               | 9                     | 9                     | 9               | 9     | 9             | 9                  | 9                      | 9                        | 9                              |
| Mean                           | 1.11                     | 3.3358                   | 12.1618                        | 11.9499                        | 0.2444                          | 13.2                  | 0.038                 | 10.4            | 40.0  | 4             | 55.1               | 10.47                  | 0.0442                   | 0.0990                         |
| SD                             | 0.82                     | 1.4234                   | 2.5502                         | 2.4344                         | 0.2                             | 13.9                  | 0.032                 | 7.7             | 36.4  | 1             | 15.8               | 2.48                   | 0.0143                   | 0.0200                         |
| Min                            | 0.50                     | 2.0814                   | 7.8833                         | 7.6834                         | 0.1999                          | 6.7                   | 0.016                 | 10.0            | 24.0  | 3             | 33.3               | 8.34                   | 0.0208                   | 0.0540                         |
| Median                         | 1.00                     | 3.8828                   | 13.8460                        | 13.6055                        | 0.2405                          | 13.0                  | 0.038                 | 10.0            | 48.0  | 4             | 53.8               | 9.34                   | 0.0469                   | 0.1032                         |
| Max                            | 3.00                     | 3.6525                   | 15.1817                        | 14.5522                        | 0.7106                          | 44.2                  | 0.008                 | 30.0            | 48.0  | 6             | 79.6               | 15.52                  | 0.0261                   | 0.1233                         |
| 25%                            | 0.5                      | 3.2                      | 12.2                           | 12.0                           | 0.2                             | 10                    | 0.03                  | 10              | 36    | 3             | 45                 | 9                      | 0.04                     | 0.1                            |
| 75%                            | 1.5                      | 3.8                      | 14.0                           | 13.8                           | 0.3                             | 15                    | 0.04                  | 12              | 48    | 4             | 60                 | 11                     | 0.05                     | 0.13                           |

Above data suggest that C<sub>max</sub> (µg/ml) and AUC (µg·hr/ml) at the steady state at 120 mg/day oral dose was 5.31 and 11.96, respectively.

Study of serum concentration of TEI-6720 during a 26-week repeated administration in rats, Study # S054S2R10P, M.4.2.2.2.15

Serum concentrations of unchanged TEI-6720 were measured in Fisher F344/Du rats treated at 0.5 and 3 mg/kg. The exposure increased with the dose as shown in the table below.

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Table 1 Summary of pharmacokinetic parameters

|           | 0.5 mg/kg group     |                    |                  |                    | 3 mg/kg group    |                    |                     |                    |                  |                    |                  |  |
|-----------|---------------------|--------------------|------------------|--------------------|------------------|--------------------|---------------------|--------------------|------------------|--------------------|------------------|--|
|           | AUC <sub>0-24</sub> |                    | C <sub>max</sub> |                    | t <sub>max</sub> |                    | AUC <sub>0-24</sub> |                    | C <sub>max</sub> |                    | t <sub>max</sub> |  |
|           | µg·hr/ml            | ratio <sup>a</sup> | ng/ml            | ratio <sup>a</sup> | hr               | ratio <sup>b</sup> | ng·hr/ml            | ratio <sup>a</sup> | ng/ml            | ratio <sup>d</sup> | hr               |  |
| Day 1     | 912.4               | 1.0                | 126.3            | 1.0                | 1.0              | 1.0                | 6323.5              | 1.0                | 1514.7           | 1.0                | 0.5              |  |
| Week 4    | 1279.6              | 1.4                | 276.1            | 2.2                | 0.5              | 0.5                | 8593.9              | 1.4                | 2333.9           | 1.5                | 0.5              |  |
| Week 8    | 1516.0              | 1.7                | 310.0            | 2.4                | 0.5              | 0.5                | 11120.9             | 1.8                | 3682.2           | 2.4                | 0.5              |  |
| Week 13   | 1719.6              | 1.9                | 364.3            | 2.9                | 0.5              | 0.5                | 12618.6             | 2.0                | 4495.7           | 3.0                | 0.5              |  |
| Week 17   | 1419.7              | 1.6                | 199.3            | 1.6                | 0.5              | 0.5                | 11169.4             | 1.8                | 5640.5           | 3.7                | 0.5              |  |
| Week 21   | 1721.6              | 1.9                | 311.7            | 2.5                | 0.5              | 0.5                | 10873.4             | 1.7                | 3690.6           | 2.4                | 0.5              |  |
| Final day | 1711.9              | 1.9                | 310.9            | 2.5                | 0.5              | 0.5                | 13417.9             | 2.1                | 4823.6           | 3.2                | 0.5              |  |

<sup>a</sup> : ratio of values in Week 4, 8, 13, 17, 21 and on final day to value on day 1

Summary of the absorption studies:

Oral administration of TMX-67 absorbed rapidly in rodents and humans. The exposure increased dose dependently. Induction of microsomal protein was not observed in mouse liver.

2.6.4.4 Distribution

1. Tissue distribution study of <sup>14</sup>C-TEI-6720 in rats after 2-week repeated oral administration, study # AE-3133-G, M 4.2.2.3.1:

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Tissue distribution of TEI-6720 after a single dose and repeat doses for 14 days at 1 mg/kg in male Slc:SD SPF rats was determined. Rats were 7 weeks of age at the beginning of dosing. Most of the radioactivity was eliminated at 168 hours after the single dose. Most of the radioactivity was observed in the stomach and intestine, liver, kidney, adrenals, and urinary bladder up to 48 hours following a single dose. However, distribution of radioactivity in the brain and eyes was minimal. A similar distribution pattern of radioactivity was observed after 14 days of treatment at 1 mg/kg oral dose. TMX-67 was not accumulated in any tissues after the repeated administration.

2. Pharmacokinetic study of TEI-6720 (2): Placental transfer and transfer into milk after single dose administration in rats, study report #H-TB-9724, M4.2.2.3.4

<sup>14</sup>C labeled TEI-6720 was administered to pregnant Sprague Dawley rats at 19<sup>th</sup> day of pregnancy for examining the placental transfer of the drug. The drug substance was administered as 0.5% methylcellulose suspensions at 1 mg/kg (1.5 ml/kg). The distribution of radioactivity in mg/ml or mg in the placenta was determined at several hours post dose. The data for blood, plasma and placenta are shown in the table below.

| Observation | 1 hr | 4 hr | 8 hr | 24 hr |
|-------------|------|------|------|-------|
| Whole blood | 233  | 219  | 161  | 7.8   |
| Plasma      | 365  | 363  | 243  | 11.2  |
| Placenta    | 50   | 96   | 106  | 20    |
| Amnion      | 20   | 55   | 83   | 42    |

Above data suggest that the drug is distributed to the placenta after a oral dose and peak radioactivity was note in the placenta at 8 hour after dosing.

The milk to plasma ratio was also determined in the Sprague Dawley rats at 2 week postpartum. The treatment was given at 1 mg/kg orally in 1.93 ml/kg volume. The milk to plasma ratio data are shown in the table below.

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Radioactivity concentration in plasma and milk after a single oral administration of  
ID#-TEL-6720 to lactating rats

| Time (hr) | Concentration (ng eq./mL) |                  | Milk / Plasma ratio |
|-----------|---------------------------|------------------|---------------------|
|           | Maternal plasma           | Milk             |                     |
| 0.25      | 477.31 ± 291.58           | 152.93 ± 191.55  | 0.24 ± 0.19         |
| 0.5       | 688.74 ± 14.16            | 578.11 ± 45.54   | 0.84 ± 0.05         |
| 1         | 438.91 ± 92.74            | 761.23 ± 11.85   | 1.79 ± 0.41         |
| 2         | 331.53 ± 30.72            | 1505.47 ± 303.01 | 4.51 ± 0.48         |
| 4         | 225.17 ± 87.27            | 1557.26 ± 47.68  | 7.58 ± 2.65         |
| 8         | 156.92 ± 20.42            | 1125.01 ± 211.37 | 7.17 ± 0.88         |
| 24        | 26.70 ± 2.77              | 211.13 ± 31.26   | 7.89 ± 0.45         |
| 48        | 6.24 ± 0.53               | 40.40 ± 6.01     | 6.45 ± 0.44         |

Animals: Str: SD, female rats

Dose: 1.0 mg/kg, p.o.

Each value represents the mean ± S.D. of three animals.

Above data indicate that TMX-67 is distributed to the placenta and excreted through milk in the pregnant rats.

Summary of the distribution study:

TMX-67 is distributed mostly to organs of metabolism and excretion (kidney, urinary bladder and liver) and did not accumulate in the systemic organs at 1 mg/kg dose in rats. It is excreted in the milk and transported across the placental barrier in rats. Its distribution to central nervous system is minimal.

#### 2.6.4.5 Metabolism

1. Comparative in vitro metabolism of  $^{14}\text{C}$ -TMX-67 in male and female hepatocytes from mouse, rat and human, study #46473, M 4.2.2.4.1.

Hepatocytes from both genders of human, dog, rat and mouse were used to determine in vitro metabolism of  $25\ \mu\text{M}$   $^{14}\text{C}$ -TMX-67 in  $1 \times 10^6$  cells/ml incubation mixtures for 4 hours. Glucuronidation and hydroxylation of TMX-67 is the major metabolic pathway in vitro. About 95, 90, 81 and 76% of the parent compound was detected in human, dog, rat and mouse hepatocytes, respectively. Human, dog and mouse had negligible G1a and G1b (glucuronide conjugation of desbutyl TMX-67) metabolite. However, rat hepatocytes incubation showed the presence of G1a and G1b.

The following metabolites were identified:

**Table 1** Distribution of Total Radioactivity into Components: [<sup>14</sup>C]TMX-67 (25 μM) After 4 hr Incubations with and without Hepatocytes from Various Species

| Component ID <sup>1</sup>           | m/z <sup>2</sup> | Avg Control <sup>3</sup> | % of Total Radioactivity by HPLC |                  |             |             |             |             |             |             |
|-------------------------------------|------------------|--------------------------|----------------------------------|------------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                                     |                  |                          | Human (n=3)                      |                  | Dog (n=1)   |             | Rat (n=1)   |             | Mouse (n=1) |             |
|                                     |                  |                          | Female                           | Male             | Female      | Male        | Female      | Male        | Female      | Male        |
| U1                                  | ----             | 0.6                      | 0.6                              | 0.8              |             |             |             |             |             |             |
| G1a                                 | 437              | nd                       | nd                               | nd               |             |             |             |             |             |             |
| G1b                                 | -----            | -----                    | -----                            | -----            |             |             |             |             |             |             |
| M3                                  | 261              | 1.2                      | 1.4                              | 2.1              |             |             |             |             |             |             |
| M2a                                 | 333              | 0.7 <sup>4</sup>         | 1.6 <sup>5</sup>                 | 2.1 <sup>5</sup> |             |             |             |             |             |             |
| 67M-4                               | 347              | -----                    | -----                            | -----            |             |             |             |             |             |             |
| M2b                                 | 333              | 0.2                      | 0.9                              | 1.4              |             |             |             |             |             |             |
| G2a                                 | 493              | nd                       | nc                               | nd               |             |             |             |             |             |             |
| G2b-1                               | -----            | -----                    | -----                            | -----            |             |             |             |             |             |             |
| G2b-2                               | 493              | nd                       | 0.6 <sup>6</sup>                 | 1.2 <sup>6</sup> |             |             |             |             |             |             |
| G2b-3                               | -----            | -----                    | -----                            | -----            |             |             |             |             |             |             |
| U2                                  | ----             | 0.5                      | 0.4                              | nd               |             |             |             |             |             |             |
| U3                                  | ----             | 0.4                      | nd                               | nd               |             |             |             |             |             |             |
| U4                                  | ----             | 0.6                      | nd                               | nd               |             |             |             |             |             |             |
| TMX-67                              | 317              | 94.7                     | 94.3                             | 92.5             |             |             |             |             |             |             |
| U5                                  | ----             | 0.0                      | nd                               | nd               |             |             |             |             |             |             |
| U6                                  | ----             | 0.3                      | nd                               | nc               |             |             |             |             |             |             |
| U7                                  | ----             | 0.3                      | nd                               | nd               |             |             |             |             |             |             |
| <b>Total Identified<sup>7</sup></b> |                  |                          | <b>99.0</b>                      | <b>99.3</b>      | <b>98.0</b> | <b>97.3</b> | <b>97.6</b> | <b>98.5</b> | <b>98.5</b> | <b>97.7</b> |

<sup>1</sup> Components listed in order of retention time. See Table 3 for details.  
<sup>2</sup> Tentative identification based on HPLC retention time comparison and LC-MS/MS (Q1, PIS, MRM) techniques. m/z values listed are for (M+H)<sup>+</sup>  
<sup>3</sup> Average of peak percentages from five negative controls. See Appendix IV, Table IV-1 for details.  
<sup>4</sup> Quantitation is a combination of G1a and G1b.  
<sup>5</sup> Quantitation is a combination of M2a and 67M-4.  
<sup>6</sup> Quantitation is a combination of G2b-1, G2b-2 and G2b-3.  
<sup>7</sup> Total Identified = G1a, G1b, M3, M2a+67M-4, M2b, G2a, G2b-3 and TMX-67  
 nd Not detected.

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The components and their identities are shown in the table below.

| Component                     | Identity                |
|-------------------------------|-------------------------|
| G1a and G1b                   | Glucuronide of M3       |
| M3                            | Desbutyl TMX-67         |
| 67M-4                         | Carboxylated TMX-67     |
| M2a (TMX-M2) and M2b (TMX-M1) | Monohydroxylated TMX-67 |
| G2a/G2b                       | Glucuronide of TMX-67   |
| TMX-67                        | TMX-67                  |

It is concluded that human, dog, rat and mouse hepatocytes showed mostly unmetabolized TMX-67 after 4 hours of incubation. Desbutyl TMX-67 (M3) and monohydroxylated TMX-67 (M1 and M2) were major Phase I metabolites. Glucuronide

of TMX-67 (G2a.G2b) was the major Phase II metabolite. Traces of Glucuronide of M3 were detected mostly in rats. Gender difference in the metabolism was not evident in the study.

2. In vitro metabolism of <sup>14</sup>C-TMX-67 by male liver microsomes from mouse, rat, dog and human. Study # 46475, M 4.2.2.4.2.

Incubation was conducted with microsomes (1 mg/ml) and cofactors at TMX-67 concentration 25 μM for 30 min and 1 hour at 37°C. The concentration of TMX-67 was determined from the preliminary experiment. The rate of metabolism was in the order of rodents>dog>human as indicated in page 14 of the report. Like the hepatocytes experiment, very little metabolism was observed in the microsomal mixtures. Unmetabolized TMX-67 was 81-94% of the radioactivity.

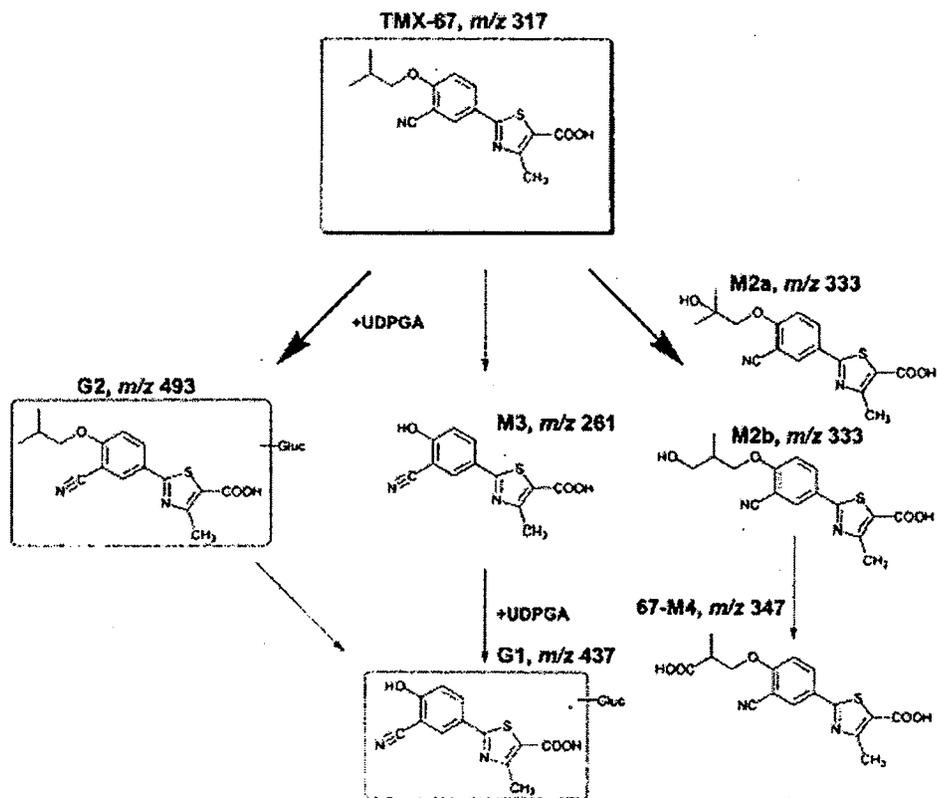
The metabolic profile in humans, dogs and rodents was qualitatively similar. Major Phase I metabolites in all species were M1 and M2 (hydroxylated TMX-67). About 53-78% M2 was detected among animal species tested and in human liver microsomes. Other metabolites were M3 (desbutyl) and M4 (carboxylated) metabolites of TMX-67. Major Phase II metabolite was G2 (70-88%).

Therefore, in vitro study in the human, dog, rat and mouse microsomal system showed hydroxylated (M1 and M2) and glucuronide (G2) as major Phase I and Phase II metabolites.

The proposed metabolic pathway is shown below.

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**Figure 1 Proposed In Vitro Biotransformation Pathway of TMX-67 in Various Species**



M2a = TMX-67 M2 and M2b = TMX-67 M1

3. Study on the metabolism of  $^{14}\text{C}$ -TEI-6720 in male and female rat bile, study # 18-A-95003, M. 4.2.2.4.4.

The excretion of radioactivity in the bile was monitored. Glucuronide of TEI-6720 was the principle metabolite excreted in the bile in male and female Slc:SD rats.

4. Analysis study of biliary metabolites after single oral administration of  $^{14}\text{C}$ -TEI-6720 in rats, Study # 18-K-02006, M.4.2.2.4.9.

Single oral dose of  $^{14}\text{C}$ -TEI-6720 was administered to male Sprague Dawley rats at 1 mg/kg. Bile duct was cannulated for the collection of the drug and its metabolites from 0-8 and 8-24 hours. Unchanged drug and metabolites were analyzed by HPLC.

Results show that about 74% of the total dose was excreted in the bile. Unchanged TMX-67 was 2.1%, TMX-67 glucuronide was 58% and M2 metabolite was about 5% of the total dose.

Data suggest that most of the parent compound was excreted in the bile as TEI-glucuronide conjugated metabolites with a single peak at 16 min retention time in the HPLC assay after 1 mg/kg oral dose in rats within 24 hours of administration.

5. Study of metabolite analysis in milk after single oral administration of  $^{14}\text{C}$ -TEI-6720 in rats. Study # 18-K-03001, M 4.2.2.4.10.

$^{14}\text{C}$ -TEI-6720 was administered to lactating SD rats on the 12<sup>th</sup> or 13<sup>th</sup> day after delivery at 1 mg/kg oral dose. Nursing mothers were separated from pups at 2 hour post dose. Oxytocin at 1 IU/ml was injected intraperitoneally ten minutes before collection of milk in a glass capillary tube. Excretion of the drug in milk at 4 hour post dose was 79%. Based on the method validation study, about 20% of the radioactivity was retained in the HPLC analyzing process. Considering the efficiency of the HPLC system, no metabolites were excreted in the milk after the oral dose. It was concluded that unchanged drug was excreted in the milk in lactating rats.

6. Study on the optical resolution of 67M-1 in human and rat urine, Study # 18-K-96006, M 4.2.2.4.12

Urine samples from human treated at 6.25 mg dose and rats treated at 1mg/kg with  $^{14}\text{C}$ -TMX-67 were analyzed for the chiral composition of the urinary metabolite 67M-1. Rat urine samples were collected from the study # 18-K-96005. Results suggest that 67-M1 metabolite is present as 67M-1R and 67M-1S isomers with R/S ratio of 1.3 in human urine samples. The R/S isomeric ratio of 67M-1 in rat urine was 2.6. The proportion of chirality in rat and human was different for 67M-1. Therefore, drug

metabolizing enzyme for 67M-1 formation in human and rats had different specificity. The racemic 67-M1 had lower IC<sub>50</sub> (1.67 nmol/l) for the inhibition of xanthine oxidase compared to the parent.

7. Study on the analysis for rat urinary metabolites of TEI-6720, Report # 18-A-95009, M.4.2.2.4.5

TEI-6720 metabolites were examined in rat urine. Urinary bladder was cannulated for the collection of urine under ice-cold conditions. Urinary metabolites were analyzed by HPLC. <sup>14</sup>C-TEI-6720 was administered orally at 1 mg/kg to male rats. Urine was collected up to 8 hours. In a separate experiment, urine samples were collected from non-cannulated rats also.

Results suggest that unchanged drug accounted for 1% of the radioactivity. A total of 5 polar peaks were present in addition to unchanged drug. Glucuronide conjugate of the parent drug (peak 4) represented about 10% of the radioactivity in the urine. Percent of radioactivity for peaks 1, 2, 3 and 5 was 56, 8.7, 21.9 and 0.3%, respectively. The sponsor indicated that CYP1A, CYP2B and CYP2E1 isozymes catalyzed the metabolism of TEI-6720.

It is concluded that 5 metabolites were excreted in the rat urine after a single dose. Only traces of unchanged drug were excreted in the urine.

8. Study on the determination of plasma and urinary concentration of metabolism following single oral administration of <sup>14</sup>C-TEI-6720 in rats. Study # 18-K-01001, M.4.2.2.4.16.

Single dose of <sup>14</sup>C-TEI-6720 at 3 and 36 mg/kg/oral in male Sprague Dawley rats showed the following plasma metabolites in addition to TEI-6720:

67M-1, 67M-2, 67M-3, 67M-4, 67-M1 sulfate, 67M-3 sulfate and 67M-3 glucuronide (G1).

In addition to above metabolites, TMX-67 glucuronide (G2) and CM-3 were identified in the rat urine.

67M-3 was the major plasma metabolite. 67M-3 and 67M-3-glucuronide (G1) were major urinary metabolites in rats.

Comparison of human and rat urinary excretion data showed that TMX-67 glucuronide (G2) was the major metabolite in human. However, desbutyl TMX-67 (M3) and its

glucuronide (G1 or glucuronide of M3) was the major metabolite in the rat urine. Data of urinary excretion as % of total radioactivity in the rat urine are shown in the table below.

Table 7. Urinary excretion (% of total radioactivity in urine)

[3 mg/kg dose group]

| Name               | Urinary excretion (% of total RA in urine) |       |      |       |      |      |
|--------------------|--------------------------------------------|-------|------|-------|------|------|
|                    | #1                                         | #2    | #3   | Mean  | S.D. | C.V. |
| TEI-8720           |                                            |       |      | 4.9   | 0.6  | 12.2 |
| 87M-1              |                                            |       |      | 29.2  | 2.9  | 9.9  |
| 87M-1-sul          |                                            |       |      | 8.5   | 1.8  | 27.7 |
| 87M-2              |                                            |       |      | 9.7   | 0.9  | 9.3  |
| 87M-3              |                                            |       |      | 25.5  | 7.8  | 30.6 |
| 87M-3-glu          |                                            |       |      | 10.9  | 3.9  | 35.8 |
| 87M-3-sul          |                                            |       |      | 7.1   | 1.1  | 15.5 |
| 87M-4              |                                            |       |      | 3.4   | 0.6  | 17.6 |
| CM-3 <sup>§)</sup> |                                            |       |      | 0.8   | 0.1  | 18.7 |
| TEI-8720-glu       |                                            |       |      | 2.7   | 2.6  | 96.3 |
| Sum total          | 101.3                                      | 100.4 | 99.7 | 100.5 | 0.8  | 0.8  |

[36 mg/kg dose group]

| Name               | Urinary excretion (% of total RA in urine) |       |       |       |      |       |
|--------------------|--------------------------------------------|-------|-------|-------|------|-------|
|                    | #1                                         | #2    | #3    | Mean  | S.D. | C.V.  |
| TEI-8720           |                                            |       |       | 1.3   | 0.3  | 23.1  |
| 87M-1              |                                            |       |       | 15.2  | 3.7  | 24.3  |
| 87M-1-sul          |                                            |       |       | 5.1   | 2.7  | 52.9  |
| 87M-2              |                                            |       |       | 15.2  | 1.7  | 11.2  |
| 87M-3              |                                            |       |       | 32.8  | 8.4  | 25.8  |
| 87M-3-glu          |                                            |       |       | 18.5  | 3.3  | 17.8  |
| 87M-3-sul          |                                            |       |       | 7.4   | 0.5  | 6.8   |
| 87M-4              |                                            |       |       | 4.6   | 0.4  | 8.7   |
| CM-3 <sup>§)</sup> |                                            |       |       | 0.5   | 0.1  | 20.0  |
| TEI-8720-glu       |                                            |       |       | 0.9   | 0.9  | 100.0 |
| Sum total          | 102.8                                      | 100.8 | 101.4 | 101.8 | 1.1  | 1.1   |

Abbreviations) -glu: -glucuronide, -sul: -sulfate  
 §) CM-3: unidentified metabolite

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respectively. Dogs excreted TMX-67M1, TMX-67M2, TMX-67M3 and TMX-67M4 in urine.

Hydroxylation of TMX-67 at the butyl chain was an important Phase I metabolite (M1). However, hydroxylation involved two stereo isomers of hydroxylated TMX-67 (M1). Therefore, hydroxylation involves stereo-specific enzymes in the animals and humans. The metabolite (M1) showed lower pharmacodynamic activity than the parent drug. It should be noted that TMX-67M2 is also another hydroxylated metabolite of TMX-67.

TMX-67 is excreted unchanged in the milk of lactating rats.

#### **2.6.4.6 Excretion**

1. Urinary and fecal excretion of <sup>14</sup>C-TEI-6720 after single oral dose in male SD rats was investigated in Study # 18-K-02002 (M4.2.2.5.2). The dose administered was 1 mg/kg. About 97% of the radioactivity was excreted at 168 hours in the urine and feces. However, about 95% of the radioactivity was excreted within 24 hours. The percent of radioactivity in the urine and feces was 37 and 59%, respectively. Oral bioavailability was about 79% based on the ratio of urinary radioactivity after oral and IV doses.

It was concluded that most of the radioactivity was excreted in the urine and feces as the major routes of excretion in rats.

2. Excretion study of <sup>14</sup>C-TEI-6720 in male chimpanzees following intravenous administration, study #WSRC941009 (M 4.2.2.5.1)

<sup>14</sup>C-TEI-6720 was injected at 0.3 mg/kg to three animals weighed 24-45 kg. Urine and feces were collected up to 240 hours for the detection of radioactivity. About 49% of the radioactivity was excreted in the urine and 24% was excreted in the feces. Total recovery of radioactivity was 85%. Most of the urinary and fecal radioactivity was excreted within 6 and 48 hours, respectively. Cage washing represented rest of the radioactivity.

It is concluded that urine was the major route of excretion of radioactivity in the chimpanzee.

#### **2.6.4.7 Pharmacokinetic drug interactions : Nil**

#### **2.6.4.8 Other Pharmacokinetic Studies**

Comparative pharmacokinetic study of TEI-6720 and allopurinol in rats with impaired renal functions (5/6 nephrectomized rats), study # 18-P-94003, M.4.2.2.7.1.

Accumulation of TEI-6720 in renal impaired Sprague Dawley rats was investigated to probe if renal insufficiency would cause greater toxicity. Right kidney and two-third of the left kidney were removed in rats. <sup>14</sup>C-TEI-6720 or <sup>3</sup>H-allopurinol was injected by iv

route. Radioactivity in the blood and urine was determined. Sham control animals were also used in the experiment. All rats were treated at 0.5 mg/kg. Results of the study show that elimination half-life of TEI-67 increased from 6.2 hours in the sham operated animals to 10.4 hours in the nephrectomized animals. Data indicate that impaired renal function would reduce elimination TEI-6720 and metabolites that could contribute to its toxicity. A similar study was conducted using <sup>14</sup>C-allopurinol in rats (study # 18-A-94018 in M 4.2.2..7.2. Data suggest that allopurinol excretion was also delayed in nephrectomized rats.

#### **2.6.4.9 Discussion and Conclusions**

Oral administration of TMX-67 in rats, mice, dogs and humans showed rapid absorption. The exposure increased dose proportionately. Gender difference in the exposure was not evident in rats and dogs. However, female mice showed about 3 times higher exposure than male mice. TMX-67 did not induce hepatic enzymes based on the microsomal protein content in mice treated with TMX-67. The drug did not accumulate in the systemic organs after repeated dosing. TMX-67 showed minimal distribution in the brain. TMX-67 and its metabolites were excreted in the urine and feces. Pregnant and lactating animals showed transplacental distribution and excretion of TMX-67 in the milk.

Terminal plasma half life ( $T_{1/2}$ ) in humans was about 18 hours. HPLC assays showed plasma half life of about 4-6 hours in rodents and about 18 hours in dogs.

Metabolic pattern of TMX-67 was qualitatively similar in animals and humans. TMX-67 glucuronide (G2) is the major human metabolite. TMX-67M1, TMX-67M3 and glucuronide of Desbutyl TMX-67 (G1) were major metabolites in rats. TMX-67 glucuronide (G2) and hydroxylated TMX-67 (M2) were major metabolites for mice. Dogs excreted hydroxylated TMX-67 (M1 and M2), Desbutyl TMX-67 (M3) and carboxylated TMX-67 (M4) as major metabolites. Phase I metabolism of TMX-67 involved CYP1A1, CYP1A2 and CYP2C9-1. It is important to note that in human and rat liver, hydroxylation of TMX-67 (M1) involved stereoselective enzymes to form hydroxylated TMX-67 isomers of TMX-67M1.

It is concluded that TMX-67 is metabolized by Phase I and Phase II enzymes and excreted in the urine and feces. Hepatic induction of drug metabolizing enzymes was not evident. Glucuronidation of TMX-67 is the major human metabolite.

#### **2.6.4.10 Tables and figures to include comparative TK summary**

##### **Comparative TK summary**

Average combined data for male and female animals are shown in the table below. See individual study review for details.

| Study #      | Species         | Dose, mg/kg/Duration | AUC, ng.hr/ml | C <sub>max</sub> , ng/ml | T <sub>max</sub> , hr | T <sub>1/2</sub> , hr |
|--------------|-----------------|----------------------|---------------|--------------------------|-----------------------|-----------------------|
| 3881-011-024 | Mouse           | 3/19 wk              | 4444          | 2136                     | 0.5                   |                       |
|              |                 | 24/19 wk             | 36520         | 32350                    | 0.5                   |                       |
| S05420K10P   | Pregnant rabbit | 3/day 18             | 9315          | 1153                     | 1.4                   |                       |
|              |                 | 12/day 18            | 58571         | 5317                     | 3.3                   |                       |
|              |                 | 48/day 18            | 394606        | 35292                    | 3.6                   |                       |
| 3500-011-022 | Mouse           | 3/13 WK              | 8300          |                          |                       |                       |
|              |                 | 7.5/13 WK            | 24350         |                          |                       |                       |
|              |                 | 18.75/13 WK          | 62150         |                          |                       |                       |
| S054s2r10a   | Rat             | 3/13 WK              | 17124         | 4663                     | 0.5                   |                       |
|              |                 | 12/13 wk             | 73893         | 17433                    | 0.5                   |                       |
|              |                 | 24/13 wk             | 198109        | 35584                    | 0.5                   |                       |
|              |                 | 36/13 wk             | 309890        | 51648                    | 0.5                   |                       |
| TMX-99-001   | Human           | 120 mg QD/Day 14     | 11960         | 5310                     | 1.11                  | 18.2                  |

**2.6.5 PHARMACOKINETICS TABULATED SUMMARY**

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

**APPEARS THIS WAY  
 ON ORIGINAL**

2.6.5.3.4 Pharmacokinetics: Absorption After a Single Dose - Study Number: 18-K-96007

Test Article: Febuxostat  
 Location in CTD: 4.2.2.2  
 Study Number: 18-K-96007

| Species                        | Rat                  | Rat                  | Rat                  | Rat                  |
|--------------------------------|----------------------|----------------------|----------------------|----------------------|
| Gender (M/F)                   | M/F per time point   |
| Number of Animals              |                      |                      |                      |                      |
| Feeding Conditions             | Fasted               | Fed                  | Fed + High Protein   | Fed + High Fat       |
| Vehicle/Formulation            | 0.5% Methylcellulose | 0.5% Methylcellulose | 0.5% Methylcellulose | 0.5% Methylcellulose |
| Method of Administration       | Oral gavage          | Oral gavage          | Oral gavage          | Oral gavage          |
| Dose (mg/kg)                   | 1                    | 1                    | 1                    | 1                    |
| Sample                         | Plasma               | Plasma               | Plasma               | Plasma               |
| Analyte                        | Total Radioactivity  | Total Radioactivity  | Total Radioactivity  | Total Radioactivity  |
| Assay                          | LSC                  | LSC                  | LSC                  | LSC                  |
| <b>PK Parameters</b>           |                      |                      |                      |                      |
| $t_{max}$ (hr)                 | 0.25                 | 0.25                 | 0.5                  | 0.25                 |
| $C_{max}$ (ng eq/mL)           | 2133.83              | 1624.16              | 1607.54              | 1628.23              |
| $AUC_{0-\infty}$ (ng eq.hr/mL) | 6961.97              | 4487.62              | 7547.62              | 5974.30              |
| $AUC_{0-t}$ (ng eq.hr/mL)      | -                    | -                    | -                    | -                    |
| $t_{1/2}$ (hr)                 | -                    | -                    | -                    | -                    |
| CL (mL/hr/kg)                  | -                    | -                    | -                    | -                    |
| $V_d$ (mL/kg)                  | -                    | -                    | -                    | -                    |

Additional Information: Febuxostat bioavailability is reduced when administered with food. Neither high protein nor high fat in the diet appeared to have a substantial effect on febuxostat absorption in rat.  
 Lot number = 50729

2.6.5.3.5 Pharmacokinetics: Absorption After a Single Dose - Study Number: 18-K-96001

Test Article: Febuxostat  
 Location in CTD: 4.2.2.2  
 Study Number: 18-K-96001

| Species                        | Rat                          | Rat                          | Rat                            | Rat                            |
|--------------------------------|------------------------------|------------------------------|--------------------------------|--------------------------------|
| Gender (M/F)                   | M/F per time point           | M/F per time point           | M/F per time point             | M/F per time point             |
| Number of Animals              |                              |                              |                                |                                |
| Feeding Conditions             | Fed                          | Fasted                       | Fasted                         | Fasted                         |
| Vehicle/Formulation            | 0.5% Methylcellulose, pH = 4 | 0.5% Methylcellulose, pH = 4 | 0.5% Methylcellulose, pH = 2.9 | 0.5% Methylcellulose, pH = 7.1 |
| Method of Administration       | Oral gavage                  | Oral gavage                  | Oral gavage                    | Oral gavage                    |
| Dose (mg/kg)                   | 1                            | 1                            | 1                              | 1                              |
| Sample                         | Plasma                       | Plasma                       | Plasma                         | Plasma                         |
| Analyte                        | Total Radioactivity          | Total Radioactivity          | Total Radioactivity            | Total Radioactivity            |
| Assay                          | LSC                          | LSC                          | LSC                            | LSC                            |
| <b>PK Parameters</b>           |                              |                              |                                |                                |
| $t_{max}$ (hr)                 | 0.25                         | 0.25                         | 0.5                            | 0.25                           |
| $C_{max}$ (ng eq/mL)           | 396.92                       | 1618.55                      | 1175.41                        | 2236.72                        |
| $AUC_{0-\infty}$ (ng eq.hr/mL) | 2480.74                      | 4724.79                      | 5212.61                        | 5190.45                        |
| $AUC_{0-t}$ (ng eq.hr/mL)      | -                            | -                            | -                              | -                              |
| $t_{1/2}$ (hr)                 | -                            | -                            | -                              | -                              |
| CL (mL/hr/kg)                  | -                            | -                            | -                              | -                              |
| $V_d$ (mL/kg)                  | -                            | -                            | -                              | -                              |

Additional Information: Febuxostat bioavailability is reduced when administered with food to rats. Overall, the pH of the dose solution did not appear to have a large effect on absorption of total radioactivity.  
 Lot number = 50729

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 ON ORIGINAL

2.6.5.3.7 Pharmacokinetics: Absorption After a Single Dose - Study Number: 18-A-95004

Test Article: Febuxostat  
 Location in CTD: 4.2.2.2  
 Study Number: 18-A-95004

| Species                            | Rat                       | Rat                       | Rat                      |
|------------------------------------|---------------------------|---------------------------|--------------------------|
| Gender (M/F)/<br>Number of Animals | M/F per timepoint         | M/F per timepoint         | M/F per timepoint        |
| Feeding Conditions                 | Fasted                    | Fasted                    | Fasted                   |
| Vehicle/Formulation                | 0.5% Methylcellulose/A    | 0.5% Methylcellulose/B    | 0.5% Methylcellulose/C   |
| Method of Administration           | Oral                      | Oral                      | Oral                     |
| Dose (mg/kg)                       | 3                         | 3                         | 3                        |
| Sample                             | Serum                     | Serum                     | Serum                    |
| Analyte                            | Febuxostat                | Febuxostat                | Febuxostat               |
| Assay                              | HPLC                      | HPLC                      | HPLC                     |
| PK Parameters                      | Formulation A (Lot 50729) | Formulation (Lot 324-152) | Formulation (Lot 335-91) |
| $t_{max}$ (hr)                     | 1.00                      | 0.25                      | 0.25                     |
| $C_{max}$ (ng/mL)                  | 1391.68                   | 2262.30                   | 2265.68                  |
| $AUC_{0-24}$ (ng·h/mL)             | 9669.91                   | 10446.52                  | 10314.91                 |
| $AUC_{inf}$ (ng·h/mL)              | -                         | -                         | -                        |
| $t_{1/2}$ (hr)                     | -                         | -                         | -                        |
| AUC Ratio vs A                     | 1.00                      | 1.08                      | 1.07                     |

Additional Information: Febuxostat was used to make the febuxostat clinical drug supply lots.

has little effect on febuxostat bioavailability in rats.

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 ON ORIGINAL

2.6.5.3.9 Pharmacokinetics: Absorption After a Single Dose - Study Number: 18-K-01001

Test Article: Febuxostat  
 Location in CTD: 4.2.2.4  
 Study Number: 18-K-01001

| Species                            | Rat                  | Rat                  | Rat                  | Rat                  | Rat                  | Rat                  |
|------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Gender (M/F)/<br>Number of Animals | M/3 per time point   |
| Feeding Conditions                 | Fasted               | Fasted               | Fasted               | Fasted               | Fasted               | Fasted               |
| Vehicle/Formulation                | 0.5% Methylcellulose |
| Method of Administration           | Oral gavage          |
| Dose (mg/kg)                       | 3                    | 3                    | 3                    | 3                    | 3                    | 3                    |
| Sample                             | Plasma               | Plasma               | Plasma               | Plasma               | Plasma               | Plasma               |
| Analyte                            | Total radioactivity  | Febuxostat           | 67M-1                | 67M-2                | 67M-3                | 67M-4                |
| Assay                              | LSC                  | LSC                  | LSC                  | LSC                  | LSC                  | LSC                  |
| PK Parameters                      |                      |                      |                      |                      |                      |                      |
| $t_{max}$ (hr)                     | 0.5                  | 0.5                  | 0.5                  | 0.5                  | 0.5                  | 0.5                  |
| $C_{max}$ (ng eq/mL)               | 4356.3               | 3464.6               | 69.4                 | 60.3                 | 376.4                | 10.9                 |
| $AUC_{0-24}$ (ng eq·hr/mL)         | 15730.3              | 8953.3               | 186.0                | 135.5                | 1578.5               | 36.3                 |
| $AUC_{0-12}$ (ng eq·hr/mL)         | 15730.3              | 10391.8              | 197.4                | 143.9                | 2009.4               | 39.3                 |
| $AUC_{inf}$ (ng eq·hr/mL)          | 15775.7              | 10417.2              | 197.5                | 144.0                | 2020.3               | 39.3                 |
| $t_{1/2}$ (hr)                     | 3.4                  | 2.7                  | 1.9                  | 1.9                  | 3.5                  | 3.1                  |
| $Cl_{ex}$ F (mL/hr/kg)             | 196.1                | 288.0                | -                    | -                    | -                    | -                    |
| $Cl_r$ (mL/h)                      | 15.3                 | 1.2                  | 368.6                | 167.5                | 31.6                 | 208.7                |

2.6.5.3.9 (Continued)

Pharmacokinetics: Absorption After a Single Dose

Test Article: Febuxostat  
 Location in CTD: 4.2.2.4  
 Study Number: 18-K-01001

| Species                        | Rat                 | Rat                 | Rat                 | Rat                 | Rat                 | Rat                 |
|--------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Gender (M/F)                   | M/3 per time point  |
| Number of Animals              |                     |                     |                     |                     |                     |                     |
| Feeding Conditions             | Fasted              | Fasted              | Fasted              | Fasted              | Fasted              | Fasted              |
| Vehicle/Formulation            | 0.5% Methycellulose |
| Method of Administration       | Oral gavage         |
| Dose (mg/kg)                   | 3                   | 3                   | 3                   | 36                  | 36                  | 36                  |
| Sample                         | Plasma              | Plasma              | Plasma              | Plasma              | Plasma              | Plasma              |
| Analyte                        | 67M-1-sulfate       | 67M-3-glucuronide   | 67M-3-sulfate       | 67M-1-sulfate       | 67M-3-glucuronide   | 67M-3-sulfate       |
| Assay                          | LSC                 | LSC                 | LSC                 | LSC                 | LSC                 | LSC                 |
| <b>PK Parameters</b>           |                     |                     |                     |                     |                     |                     |
| $t_{max}$ (hr)                 | 0.5                 | 0.5                 | 0.5                 | 1                   | 1                   | 1                   |
| $C_{max}$ (ng eq/mL)           | 38.0                | 11.9                | 16.4                | 728.9               | 303.7               | 97.9                |
| $AUC_{0-16}$ (ng eq.hr/mL)     | 128.9               | 39.1                | 46.5                | 1501.3              | 981.5               | 437.4               |
| $AUC_{0-24}$ (ng eq.hr/mL)     | 130.4               | 41.6                | 50.3                | 1022.3              | 1478.0              | 665.5               |
| $AUC_{0-\infty}$ (ng eq.hr/mL) | 130.4               | 41.6                | 50.3                | 1018.5              | 1568.9              | 709.5               |
| $t_{1/2}$ (hr)                 | 1.1                 | 1.9                 | 2.2                 | 3.2                 | 5.9                 | 6.1                 |
| $Cl_{app}/F$ (mL.hr/kg)        | -                   | -                   | -                   | -                   | -                   | -                   |
| $Cl_r$ (mL/h)                  | 134.2               | 651.4               | 347.9               | 36.5                | 257.5               | 234.0               |

Additional Information: Febuxostat  $AUC_{0-\infty}$  appeared to increase in a slightly greater than dose-proportional manner between 3 and 36 mg/kg doses, suggesting a possible reduction in non-renal clearance or enhancement in oral absorption with the higher dose.

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ON ORIGINAL

2.6.5.3.10 Pharmacokinetics: Absorption After a Single Dose - Study Number: C-TB-9400-01

Test Article: Febuxostat  
 Location in CTD: 4.2.2.3  
 Study Number: C-TB-9400-01

| Species                          | Rat                   | Rat                | Rat                | Rat                          |
|----------------------------------|-----------------------|--------------------|--------------------|------------------------------|
| Gender (M/F)/Number of Animals   | M/5                   | M/5                | M/5                | M/5                          |
| Feeding Conditions               | Fasted                | Fasted             | Fasted             | Fasted                       |
| Vehicle/Formulation              | 5%<br>Methylcellulose | 5% Methylcellulose | 5% Methylcellulose | Phosphate buffered<br>saline |
| Method of Administration         | Oral gavage           | Oral gavage        | Oral gavage        | IV                           |
| Dose (mg/kg)                     | 1                     | 3                  | 10                 | 1                            |
| Sample                           | Plasma                | Plasma             | Plasma             | Plasma                       |
| Analyte                          | Febuxostat            | Febuxostat         | Febuxostat         | Febuxostat                   |
| Assay                            | HPLC                  | HPLC               | HPLC               | HPLC                         |
| <b>PK Parameters</b>             |                       |                    |                    |                              |
| $t_{max}$ (hr)                   | 0.25                  | 0.25               | 0.25               | -                            |
| $C_{max}$ (ng eq/mL)             | 1669.0                | 7012.9             | 21719.0            | -                            |
| AUC <sub>0-∞</sub> (ng eq hr/mL) | 3093.6                | 11524.9            | 38376.2            | 4253.1                       |
| AUC <sub>0-t</sub> (ng eq hr/mL) | 3707.4                | 16490.0            | 45920.3            | 4730.6                       |
| $t_{1/2}$ (hr)                   | 3.4                   | 4.8                | 3.0                | 4.2                          |
| CL (mL/hr/kg)                    | 269.7                 | 181.9              | 217.3              | 211.4                        |
| $V_d$ (mL/kg)                    | 1313.4                | 1286.4             | 947.4              | -                            |

2.6.5.3.10 (Continued) Pharmacokinetics: Absorption After a Single Dose

Test Article: Febuxostat  
 Location in CTD: 4.2.2.3  
 Study Number: C-TB-9400-01

| Species                          | Rat                 | Rat                 | Rat                 | Rat                       |
|----------------------------------|---------------------|---------------------|---------------------|---------------------------|
| Gender (M/F)/Number of Animals   | M/5                 | M/5                 | M/5                 | M/5                       |
| Feeding Conditions               | Fasted              | Fasted              | Fasted              | Fasted                    |
| Vehicle/Formulation              | 5% Methylcellulose  | 5% Methylcellulose  | 5% Methylcellulose  | Phosphate buffered saline |
| Method of Administration         | Oral gavage         | Oral gavage         | Oral gavage         | IV (injected? Pg. 06030)  |
| Dose (mg/kg)                     | 1                   | 3                   | 10                  | 1                         |
| Sample                           | Plasma              | Plasma              | Plasma              | Plasma                    |
| Analyte                          | Total Radioactivity | Total Radioactivity | Total Radioactivity | Total Radioactivity       |
| Assay                            | LSC                 | LSC                 | LSC                 | LSC                       |
| <b>PK Parameters</b>             |                     |                     |                     |                           |
| $t_{max}$ (hr)                   | 0.25                | 0.25                | 0.25                | -                         |
| $C_{max}$ (ng eq/mL)             | 1971.30             | 7306.50             | 23654.03            | -                         |
| AUC <sub>0-∞</sub> (ng eq hr/mL) | 5894.0              | 21666.3             | 67465.4             | 6916.3                    |
| AUC <sub>0-t</sub> (ng eq hr/mL) | 5945.1              | 21791.5             | 67416.0             | 6967.1                    |
| $t_{1/2}$ (hr)                   | 42.1                | 55.7                | 18.0                | 43.5                      |
| CL (mL/hr/kg)                    | 168.2               | 137.7               | 147.9               | 143.5                     |
| $V_d$ (mL/kg)                    | 10230.5             | 11053.7             | 3643.8              | -                         |

Additional Information: The AUC values based on total radioactivity and parent drug appeared to increase in a dose-proportional manner between 1 and 10 mg/kg, suggesting that the absorption and bioavailability of febuxostat in rats were independent of dose within that range.

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 ON ORIGINAL

2.6.5.3.13 Pharmacokinetics: Absorption After a Single Dose - Study Number: S054A0R10P

Test Article: Febuxostat  
 Location in CTD: 4.2.2.2  
 Study Number: S054A0R10P

| Species                        | Rat                  | Rat                  | Rat                  | Rat                  |
|--------------------------------|----------------------|----------------------|----------------------|----------------------|
| Gender (M/F)/Number of Animals | M/5                  | M/5                  | M/5                  | M/5                  |
| Feeding Conditions:            | Fed                  | Fed                  | Fed                  | Fed                  |
| Vehicle/Formulation            | 0.5% Methylcellulose | 0.5% Methylcellulose | 0.5% Methylcellulose | 0.5% Methylcellulose |
| Method of Administration       | Oral gavage          | Oral gavage          | Oral gavage          | Oral gavage          |
| Dose (mg/kg)                   | 3                    | 15                   | 75                   | 150                  |
| Sample                         | Serum                | Serum                | Serum                | Serum                |
| Analyte                        | Febuxostat           | Febuxostat           | Febuxostat           | Febuxostat           |
| Assay                          | HPLC                 | HPLC                 | HPLC                 | HPLC                 |
| <b>PK Parameters</b>           |                      |                      |                      |                      |
| $t_{max}$ (hr)                 | 0.4                  | 0.4                  | 0.8                  | 0.8                  |
| $C_{max}$ (ng/mL)              | 4142                 | 30820                | 77964                | 86556                |
| $AUC_{0-4}$ (ng hr/mL)         | 12773.0              | 91619.1              | 489269.3             | 1033865.5            |
| $AUC_{inf}$ (ng hr/mL)         | -                    | -                    | -                    | -                    |
| $t_{1/2}$ (hr)                 | -                    | -                    | -                    | -                    |
| CL (mL/hr/kg)                  | -                    | -                    | -                    | -                    |
| $V_d$ (mL/kg)                  | -                    | -                    | -                    | -                    |

2.6.5.4.1 Pharmacokinetics: Absorption After Repeated Doses - Study Number: C-TB-9400-01

Test Article: Febuxostat  
 Location in CTD: 4.2.2.3  
 Study Number: C-TB-9400-01

| Species                        | Rat           | Rat                 |
|--------------------------------|---------------|---------------------|
| Gender (M/F)/Number of Animals | M/5           | M/5                 |
| Feeding Conditions:            | Fed           | Fed                 |
| Vehicle/Formulation            | 0.5% methanol | 0.5% methanol       |
| Method of Administration       | Oral gavage   | Oral gavage         |
| Dose (mg/kg/day)               | 1             | 1                   |
| Sample                         | Plasma        | Plasma              |
| Analyte                        | Febuxostat    | Total Radioactivity |
| Assay                          | HPLC          | LSC                 |
| <b>PK Parameters</b>           |               |                     |
|                                | Day 14        | Day 14              |
| $t_{max}$ (hr)                 | 0.25          | 0.25                |
| $C_{max}$ (ng eq/mL)           | 637.67        | 1016.22             |
| $AUC_{0-4}$ (ng eq hr/mL)      | 2345.3        | 5445.0              |
| $AUC_{inf}$ (ng eq hr/mL)      | 3483.1        | 5653.0              |
| $t_{1/2}$ (hr)                 | 5.7           | 58.4                |
| CL (mL/hr/kg)                  | 287.1         | 176.9               |
| $V_d$ (mL/kg)                  | 2351.0        | 14900.8             |

Additional Information: Febuxostat accumulation in rats is minimal after once daily dosing for 14 days.

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2.6.5.3.11 (Continued) Pharmacokinetics: Absorption After a Single Dose Test Article: Febuxostat  
 Location in CTD: 4.2.2.4  
 Study Number: 18-K-93007

| Species                            | Dog                     | Dog                     | Dog                     | Dog                     | Dog                     | Dog                     |
|------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Gender (M/F)/<br>Number of Animals | M/3                     | M/3                     | M/3                     | M/3                     | M/3                     | M/3                     |
| Feeding Conditions                 | Fasted                  | Fasted                  | Fasted                  | Fasted                  | Fasted                  | Fasted                  |
| Vehicle/Formulation                | 0.5%<br>Methylcellulose | 0.5%<br>Methylcellulose | 0.5%<br>Methylcellulose | 0.5%<br>Methylcellulose | 0.5%<br>Methylcellulose | 0.5%<br>Methylcellulose |
| Method of<br>Administration        | Oral                    | Oral                    | Oral                    | Oral                    | Oral                    | Oral                    |
| Dose (mg/kg)                       | 1                       | 1                       | 1                       | 1                       | 1                       | 1                       |
| Sample                             | Plasma                  | Plasma                  | Plasma                  | Plasma                  | Plasma                  | Plasma                  |
| Analyte                            | Total<br>Radioactivity  | Febuxostat              | 67M-1                   | 67M-2                   | 67M-3                   | 67M-4                   |
| Assay                              | HPLC                    | HPLC                    | HPLC                    | HPLC                    | HPLC                    | HPLC                    |
| <b>PK Parameters</b>               |                         |                         |                         |                         |                         |                         |
| $t_{1/2}$ (hr)                     | 0.4                     | 0.4                     | 0.7                     | nd                      | 1.8                     | 0.5                     |
| $C_{max}$ (ng/mL)                  | 537.0                   | 294.2                   | 15.1                    | nd                      | 19.3                    | 5.1                     |
| AUC <sub>0-∞</sub> (ng hr/mL)      | 797.2                   | 370.1                   | 34.3                    | -                       | 119.1                   | 5.1                     |
| AUC <sub>0-t</sub> (ng hr/mL)      | 838.8                   | 419.5                   | 41.1                    | -                       | 161.3                   | 45.9                    |
| $t_{1/2}$ (hr)                     | 7.1                     | 18.1 <sup>a</sup>       | 3.6                     | -                       | 6.4                     | 7.3                     |
| CL (mL/hr/kg)                      | -                       | -                       | -                       | -                       | -                       | -                       |
| $V_d$ (mL/kg)                      | -                       | -                       | -                       | -                       | -                       | -                       |

Additional Information: Comparison of the mean AUC<sub>0-∞</sub> value after oral and intravenous administration indicated that approximately 45% of the total radioactivity administered was absorbed and that the bioavailability of febuxostat was 48%.

nd = Not determined.

a Individual half-life values for the 3 dogs indicated that the half-life obtained for one dog (51.3 h) appeared to be an outlier since it was much longer compared to the remaining two dogs (1.70 and 1.31 h). Excluding the dog with the long half-life value, the mean half-life from the remaining two dogs was approximately 1.5 hours.

2.6.5.4.2 Pharmacokinetics: Absorption After Repeated Doses - Study Number: N004049A

Test Article: Febuxostat  
 Location in CTD: 4.2.2.2  
 Study Number: N004049A

| Species                               | Dog                          | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gender (M/F)/<br>Number of<br>Animals | M/3                          | F/3                          | M/3                          | F/3                          | M/3                          | F/3                          | M/3                          | F/3                          |
| Feeding<br>Conditions                 | Dosed prior<br>to feeding    |
| Vehicle/<br>Formulation               | 0.5%<br>methyl-<br>cellulose |
| Method of<br>Administration           | Oral                         |
| Dose (mg/kg/day)                      | 5                            | 5                            | 50                           | 50                           | 5                            | 5                            | 50                           | 50                           |
| Sample                                | Plasma                       |
| Analyte                               | Febuxostat                   |
| Assay                                 | HPLC                         |
| <b>PK Parameters</b>                  |                              |                              |                              |                              |                              |                              |                              |                              |
| $t_{1/2}$ (hr)                        | Day 2<br>1.5                 | Day 2<br>1.3                 | Day 2<br>2.7                 | Day 2<br>2.7                 | Day 14<br>0.8                | Day 14<br>1.0                | Day 14<br>2.2                | Day 14<br>2.3                |
| $C_{max}$ (ng/mL)                     | 4891.4                       | 1151.4                       | 17581.5                      | 40323.6                      | 1726.3                       | 1741.7                       | 39540.7                      | 46704.2                      |
| AUC <sub>0-∞</sub> (ng hr/mL)         | 8729.8                       | 2097.7                       | 78045.7                      | 160740.3                     | 2079.9                       | 2464.3                       | 118316.7                     | 180758.8                     |
| AUC <sub>0-t</sub> (ng hr/mL)         | 8830.9                       | 2214.2                       | 82434.5                      | 160740.3                     | 2185.1                       | 2555.4                       | 118678.8                     | 180758.8                     |
| $t_{1/2}$ (hr)                        | 0.4                          | 0.6                          | 0.8                          | 3.6                          | 0.9                          | 0.6                          | 1.4                          | 4.1                          |
| $C_{max}/Dose$                        | 978                          | 230                          | 352                          | 808                          | 345                          | 348                          | 591                          | 934                          |
| AUC <sub>0-t}/Dose</sub>              | 1766                         | 443                          | 1649                         | 3215                         | 437                          | 511                          | 2374                         | 3615                         |

Additional Information: Dose-normalized  $C_{max}$  and AUC<sub>0-t</sub> values increased with increasing dose, indicating a possible saturation of febuxostat elimination at these high dose levels, and a lack of dose-proportionality in the pharmacokinetics of febuxostat within the dosage range studied.

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2.6.5.3.8 Pharmacokinetics: Absorption After a Single Dose - Study Number: PK-914

Test Article: Febuxostat  
 Location in CTD: 4.2.2.4  
 Study Number: PK-914

| Species                         | Mouse                                         | Mouse                                         | Mouse                                         | Mouse                                         |
|---------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Gender (M/F)                    | M3/time point                                 | F3/time point                                 | M3/time point                                 | F3/time point                                 |
| Number of Animals               |                                               |                                               |                                               |                                               |
| Feeding Conditions              | Fasted                                        | Fasted                                        | Fasted                                        | Fasted                                        |
| Vehicle/Formulation             | Phosphate buffered saline (50 mmol/L, pH 8.5) |
| Method of Administration        | IV                                            | IV                                            | Oral gavage                                   | Oral gavage                                   |
| Dose (mg/kg)                    | 1                                             | 1                                             | 3                                             | 3                                             |
| Sample                          | Plasma                                        | Plasma                                        | Plasma                                        | Plasma                                        |
| Analyte                         | Total radioactivity                           | Total radioactivity                           | Total radioactivity                           | Total radioactivity                           |
| Assay                           | LSC                                           | LSC                                           | LSC                                           | LSC                                           |
| <b>PK Parameters</b>            |                                               |                                               |                                               |                                               |
| $t_{1/2}$ (hr)                  | 0.1                                           | 0.1                                           | 0.5                                           | 2.0                                           |
| $C_{max}$ (ng eq/g)             | 3850                                          | 5097                                          | 1670                                          | 2028                                          |
| AUC <sub>0-∞</sub> (ng eq.hr/g) | 3355                                          | 6039                                          | 5037                                          | 11420                                         |
| AUC <sub>0-t</sub> (ng eq.hr/g) | 3372                                          | 6063                                          | 5051                                          | 11465                                         |
| $t_{1/2}$ (hr)                  | 3.2                                           | 3.2                                           | 2.9                                           | 3.0                                           |

Additional Information: Absorption of total radioactivity in male and female mice was approximately 50% and 63%, respectively, of the [<sup>14</sup>C]Febuxostat dose administered.

2.6.5.3.12 Pharmacokinetics: Absorption After a Single Dose - Study Number: RJ-960122-1

Test Article: Febuxostat  
 Location in CTD: 4.2.2.2  
 Study Number: RJ-960122-1

| Species                        | Mouse                | Mouse                | Mouse                | Mouse                |
|--------------------------------|----------------------|----------------------|----------------------|----------------------|
| Gender (M/F)/Number of Animals | M/5                  | M/5                  | F/5                  | F/5                  |
| Feeding Conditions             | Fed                  | Fed                  | Fed                  | Fed                  |
| Vehicle/Formulation            | 0.5% Methylcellulose | 0.5% Methylcellulose | 0.5% Methylcellulose | 0.5% Methylcellulose |
| Method of Administration       | Oral gavage          | Oral gavage          | Oral gavage          | Oral gavage          |
| Dose (mg/kg)                   | 12                   | 96                   | 12                   | 96                   |
| Sample                         | Serum                | Serum                | Serum                | Serum                |
| Analyte                        | Febuxostat           | Febuxostat           | Febuxostat           | Febuxostat           |
| Assay                          | HPLC                 | HPLC                 | HPLC                 | HPLC                 |
| <b>PK Parameters</b>           |                      |                      |                      |                      |
| $t_{1/2}$ (hr)                 | 0.4                  | 1                    | 0.5                  | 0.4                  |
| $C_{max}$ (ng/mL)              | 16492                | 92942                | 13395                | 170396               |
| AUC <sub>0-∞</sub> (ng.hr/mL)  | 34173                | 515964               | 64814                | 1005937              |

Additional Information: Mean AUC values increased in a greater than dose-proportional manner with increasing dose. Febuxostat exposure (based on AUC) was approximately 2-fold higher in females as compared to males.

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2.6.5.4.3 Pharmacokinetics: Absorption After Repeated Doses - Study Number: 3500(011-022), 3501(011-023)  
 Test Article: Febuxostat  
 Location in CTD: 4.2.2.2  
 Study Number: 3500(011-022), 3501(011-023)

| Species                        | Mouse                | Mouse                | Mouse                | Mouse                |
|--------------------------------|----------------------|----------------------|----------------------|----------------------|
| Gender (M/F)/Number of Animals | M/30                 | M/30                 | M/30                 | M/30                 |
| Feeding Conditions             | Fed                  | Fed                  | Fed                  | Fed                  |
| Vehicle/Formulation            | 0.5% Methylcellulose | 0.5% Methylcellulose | 0.5% Methylcellulose | 0.5% Methylcellulose |
| Method of Administration       | Oral gavage          | Oral gavage          | Oral gavage          | Oral gavage          |
| Dose (mg/kg/day)               | 3                    | 12                   | 24                   | 48                   |
| Sample                         | Serum                | Serum                | Serum                | Serum                |
| Analyte                        | Febuxostat           | Febuxostat           | Febuxostat           | Febuxostat           |
| Assay                          | HPLC                 | HPLC                 | HPLC                 | HPLC                 |
| <b>PK Parameters</b>           | <b>Day 1</b>         | <b>Day 1</b>         | <b>Day 1</b>         | <b>Day 1</b>         |
| $t_{1/2}$ (hr)                 | 0.5                  | 0.5                  | 0.5                  | 0.5                  |
| $C_{max}$ (ng/mL)              | 5294.9               | 13456.9              | 43550.0              | 55268.7              |
| AUC <sub>0-12</sub> (ng.hr/mL) | 7561.0               | 29377.9              | 76602.9              | 129259.9             |
| AUC <sub>0-24</sub> (ng.hr/mL) | -                    | -                    | -                    | -                    |
| $t_{1/2}$ (hr)                 | -                    | -                    | -                    | -                    |
| CL (mL/hr/kg)                  | -                    | -                    | -                    | -                    |
| $V_d$ (mL/kg)                  | -                    | -                    | -                    | -                    |

2.6.5.4.5 Pharmacokinetics: Absorption After Repeated Doses - Study Number: TAP-TD00-801  
 Test Article: Febuxostat  
 Location in CTD: 4.2.2.2  
 Study Number: TAP-TD00-801

| Species                             | Mouse                | Mouse                | Mouse                | Mouse                | Mouse                | Mouse                |
|-------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Gender (M/F):<br>Number of Animals: | M/4 per time point   | M/4 per time point   | M/4 per time point   | F/4 per time point   | F/4 per time point   | F/4 per time point   |
| Feeding Conditions                  | Fed                  | Fed                  | Fed                  | Fed                  | Fed                  | Fed                  |
| Vehicle/Formulation                 | 0.5% Methylcellulose |
| Method of Administration            | Oral gavage          |
| Dose (mg/kg/day)                    | 62.5                 | 125                  | 250                  | 62.5                 | 125                  | 250                  |
| Sample                              | Plasma               | Plasma               | Plasma               | Plasma               | Plasma               | Plasma               |
| Analyte                             | Febuxostat           | Febuxostat           | Febuxostat           | Febuxostat           | Febuxostat           | Febuxostat           |
| Assay                               | HPLC                 | HPLC                 | HPLC                 | HPLC                 | HPLC                 | HPLC                 |
| <b>PK Parameters</b>                | <b>Day 1</b>         |
| $t_{1/2}$ (hr)                      | 0.5                  | 0.5                  | 0.5                  | 0.5                  | 0.5                  | 0.5                  |
| $C_{max}$ (ng/mL)                   | 43928.1              | 54856.6              | 190219.0             | 69038.8              | 73762.4              | 164205.9             |
| AUC (ng.hr/mL)                      | 84761.7              | 217897.3             | 561480.4             | 347565.9             | 726501.8             | 1230815.2            |
| AUC <sub>0-12</sub> (ng.hr/mL)      | 121864.7             | 307901.5             | 562057.0             | 348040.3             | 727690.2             | 1239147.3            |
| $t_{1/2}$ (hr)                      | 4.8                  | 4.2                  | 2.4                  | 2.3                  | 2.4                  | 3.0                  |
| $C_{max}/Dose$                      | 700                  | 440                  | 760                  | 1100                 | 590                  | 660                  |
| AUC/Dose                            | 1950                 | 2460                 | 2250                 | 5570                 | 5820                 | 4960                 |

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## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

#### General toxicology:

Single oral dose at 600 mg/kg showed mortality in rats. Clinical signs were salivation and decreased activity. Beagle dogs tolerated doses up to 2000 mg/kg/oral as a single dose. Salivation, vomiting and diarrhea were observed. A repeat dose toxicity study was conducted in Sprague Dawley rats at 3, 12 and 48 mg/kg/oral for 26 weeks. Calculi formation was noted in the kidney at 48 mg/kg (23 times human exposure). The no effect dose was 12 mg/kg. Beagle dogs treated at 3, 15 and 45 mg/kg/oral for 52 weeks also showed calculi in the kidney at 45 mg/kg (40 times human exposure). The no effect dose was 3 mg/kg. The effect of the drug on calculi formation in the kidney was not reversible in rats and dogs.

#### Genetic toxicology:

Mutagenicity studies were conducted in human peripheral blood lymphocytes, mouse lymphoma L5178 TK<sup>+/−</sup> cell line, salmonella and *E. coli* bacteria (Ames test), Chinese hamster lung fibroblast cells and unscheduled DNA synthesis in rat hepatocytes *in vitro*. TMX-67 also showed chromatid break and exchange in Chinese hamster lung fibroblast (V79) cell line *in vitro* in the absence and presence of S-9 liver homogenates. TMX-67 is negative in micronucleus assay in mice and chromosomal aberration in rat bone marrow cells *in vivo*. Data suggest that TMX-67 is mutagenic.

#### Carcinogenicity:

Two year carcinogenicity studies were conducted in F344 Fisher rats at 3, 6, 12 and 24 mg/kg and in B6C3F1 mice at 3, 7.5 and 18.75 mg/kg. Male rats showed increased incidences of papilloma and carcinoma of the transitional cell in the urinary bladder at 24 mg/kg (144 mg/m<sup>2</sup>). The exposure in rats at 24 mg/kg was about 16 times human exposure at MRHD. Female mice at 18.75 mg/kg (56.25 mg/m<sup>2</sup>) showed increased incidences of transitional cell papilloma and carcinoma in the urinary bladder. The exposure in female mice at 18.75 mg/kg was 8 times the human exposure at MRHD.

#### Reproductive toxicology:

TMX-67 did not show any untoward effect on the fertility and mating performance in rats up to 48 mg/kg. Teratogenicity study in rats and rabbits also did not show any effect on the embryo up to 48 mg/kg. TMX-67 had no effect on the gestation, labor and delivery of rats up to 48 mg/kg. The behavior and mating performance of second generation rats

were also not affected by the treatment. However, pups (F1 generation) nursed by dams treated at 48 mg/kg showed deposition of xanthine crystals in the kidney that reduced the survival of weaning rats. The effect was due to excretion of TMX-67 in the milk and its pharmacodynamic activity. The F0 dams at 12 and 48 mg/kg also showed xanthine crystal deposition in the kidney and urinary bladder. Therefore, TMX-67 treated females should not breast feed to avoid xanthine crystal formation in the kidney.

Special toxicology: No special toxicity study was reviewed.

#### **2.6.6.2 Single-dose toxicity**

##### **1. Single dose toxicity study of TEI-6720 by oral administration in rats, 4.2.3.1.1**

Single dose toxicity in rats was conducted in eleven week old male and female Slc: SD rats. The drug substance was suspended in 0.5% methylcellulose and administered orally at 150, 300 and 600 mg/kg. The control animals received the vehicle only. Each group had 5 rats/sex.

A total of five male rats died at 600 mg/kg. Four male rats died within 24 hours and one male rat died on day 4. Three female rats died at 600 mg/kg within 24 hours. Clinical signs and autopsy at the end of 14 days showed salivation and distension of stomach. One male rat showed yellowish granules in the kidney at 600 mg/kg.

Salivation and decrease locomotor activity were observed in male rats treated at 300 mg/kg. However, no major clinical signs were noted in female rats at 300 mg/kg.

It is concluded that the highest single dose tolerated was 300 mg/kg in male and female rats. Mortality was observed at 600 mg/kg in male and female rats. Clinical signs were salivation and decreased activity.

##### **2. Single dose toxicity study of TEI-6720 by oral administration in dogs. 4.2.3.1.2**

Male beagle dogs weighing 7.9 -9.5 kg was used in the study. Animals were approximately 8 months old at the time of dosing. Each group had 2 animals. Dogs were treated at 500, 1000 and 2000 mg/kg orally in gelatin capsules. The control dogs received empty gelatin capsules.

All animals survived through 14 days of observation period. Vomiting, salivation and lack of activity were noted in the treated animals. Kinetic data show average AUC of 239143, 1243464 and 387423 ng.hr/ml at 500, 1000 and 2000 mg/kg, respectively. The variation in the exposure data could be due to vomiting following the treatment.

It is concluded that male beagle dogs tolerated 2000 mg/kg single dose. Vomiting, diarrhea, salivation were observed as clinical signs dose dependently.

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The sponsor provided metabolic profile in human urine at 25 mg per day dose for 7 days as shown below.

Table 8. Pharmacokinetic parameters in human

| [Day 1]                       |                |             |             |             |                      |
|-------------------------------|----------------|-------------|-------------|-------------|----------------------|
| Parameters                    | TEI-6720       | 67M-1       | 67M-2       | 67M-4       | TEI-6720-glucuronide |
| AUC <sub>0-6</sub> (ng*hr/mL) | 2480.3 ± 317.0 | 53.0 ± 12.6 | 64.9 ± 19.5 | 65.1 ± 22.6 | -                    |
| % of TEI-6720                 | -              | 2.0 ± 0.3   | 2.5 ± 0.6   | 2.0 ± 0.7   | -                    |
| C <sub>max</sub> (ng/mL)      | 712.5 ± 246.9  | 19.1 ± 4.1  | 19.5 ± 4.8  | 19.3 ± 5.2  | -                    |
| % of TEI-6720                 | -              | 2.7 ± 0.6   | 2.7 ± 0.6   | 2.6 ± 0.7   | -                    |
| F <sub>e</sub> (% of dose)    | 1.62 ± 0.59    | 4.90 ± 0.83 | 5.20 ± 1.22 | 2.73 ± 0.89 | 39.78 ± 5.88         |

| [Day 7]                       |                |             |             |             |                      |
|-------------------------------|----------------|-------------|-------------|-------------|----------------------|
| Parameters                    | TEI-6720       | 67M-1       | 67M-2       | 67M-4       | TEI-6720-glucuronide |
| AUC <sub>0-6</sub> (ng*hr/mL) | 3096.6 ± 293.2 | 37.4 ± 10.0 | 43.9 ± 13.2 | 52.9 ± 17.6 | -                    |
| % of TEI-6720                 | -              | 1.1 ± 0.2   | 1.3 ± 0.3   | 1.6 ± 0.5   | -                    |
| C <sub>max</sub> (ng/mL)      | 812.1 ± 200.6  | 15.1 ± 3.4  | 15.1 ± 3.6  | 18.8 ± 8.3  | -                    |
| % of TEI-6720                 | -              | 1.8 ± 0.1   | 1.8 ± 0.4   | 2.1 ± 0.3   | -                    |
| F <sub>e</sub> (% of dose)    | 2.22 ± 1.01    | 5.97 ± 1.20 | 5.31 ± 1.16 | 3.45 ± 1.13 | 41.60 ± 6.01         |

AUC<sub>0-6</sub> and C<sub>max</sub> are quoted from AUClast and C<sub>max</sub> estimated at the previous study (Study No. 18-K-99002)  
 F<sub>e</sub> is quoted from the cumulative urinary excretion between 0-24 hr estimated at the previous study (Study No. 18-K-98008)  
 Healthy male subjects were orally given 25 mg of TEI-6720, QD, for 7 days.  
 - : not determined or not calculated

It is concluded that most of the TEI-6720 was excreted as metabolites in the rat and human urine. TMX-67M1, TMX-67M3 and TMX-67M3 glucuronide were major metabolites in rat urine. Major metabolite in human urine is TEI-6720 glucuronide (G2).

9. In vivo metabolism of <sup>14</sup>C-TMX-67 in male and female mouse plasma, urine, bile and feces samples, study # QKan-2002-0900-BIO, M. 2.2.4.17.

Single dose of <sup>14</sup>C-TMX-67 was administered at 1 mg/kg/iv and 3 mg/kg/oral to C57BL-6 male and female mice. Excretion of the parent and its metabolites in the urine, bile, feces and its levels in the plasma were analyzed. The thiazole ring of TMX-67 had the <sup>14</sup>C label with specific activity of 185.4 μCi/mg. Parent and the metabolites were detected by HPLC and liquid scintillation counter. Structures of the peaks were determined by Radio-LC-MS equipments.

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The following table represents the structure of the parent metabolites identified in the study.

| Identity                                  | Chromatographic Peak | samples with major metabolite |
|-------------------------------------------|----------------------|-------------------------------|
| TMX-67                                    | P12                  | Urine, Feces, Plasma          |
| Hydroxylated TMX-67 (67M-1)               | P8                   |                               |
| Hydroxylated TMX-67 (67M2)                | P7                   | Feces                         |
| Dicarboxylic acid (67M-4)                 | P5                   |                               |
| Glucuronide of TMX-67                     | P9-P11,              | Urine, Bile                   |
| Sulfated conjugate of hydroxylated TMX-67 | P6                   |                               |

It is concluded that M2 (hydroxylated) and glucuronide of TMX-67 were major metabolites in the mouse. Urine and feces were routes of excretion of the parent and metabolites.

#### 10. Pharmacokinetic study on TMX-67 in dogs, study # 18-K-98007, M.4.2.2.4.15.

<sup>14</sup>C-TMX-67 was administered by IV or oral route at 1 mg/kg to male beagle dogs 7-9 months of age. Three dogs were allotted for the cross-over study with a 10-day washout period. Absorption, metabolism and excretion patterns were examined by separating metabolites, the parent drug by HPLC and counting the radioactivity by the liquid scintillation counter. Retention time of the parent and metabolites was compared to the standard solutions. Oral absorption was 68%. However, bioavailability after oral dose was 48%. Elimination through feces was the major route of excretion of the drug and its metabolites (85% of the dose) after the iv dose. Urinary excretion of radioactivity was 9% of the total iv dose. A similar data was obtained after the oral dose.

Some of the urinary metabolites were TMX-67M1, TMX-67M2, TMX-67M3 and TMX-67M4. The sponsor did not provide fecal metabolites in the report.

#### Summary of metabolism studies:

In vitro and in vivo metabolism of TMX-67 was studied in rats, mice and dogs. The metabolic profile in human and experimental animals used in the study was qualitatively similar. TMX-67 is extensively metabolized by CYP2C9-1, CYP1A1 and CYP1A2 isozymes based on the data reviewed under IND 58,229 dated Nov 26, 2002. TMX-67 and its metabolites were excreted in the urine and feces. TMX-67 glucuronide is the major Phase II metabolite in human. However, hydroxylated TMX-67 (M1), desbutyl TMX-67 (M3) and its glucuronide (G1) were major urinary metabolites in rats. Major metabolites in mice were TMX-67 glucuronide and hydroxylated TMX-67 (M2) in urine and feces,

### 2.6.6.3 Repeat-dose toxicity

**Study title:** 52-week repeated dose toxicity study of TEI-6720 by oral administration in dogs

**Key study findings:** Kidney calculi were observed at 45 mg/kg in male and female dogs. Calculi formation was not completely reversed during the three month recovery.

**Study no.:** S054C4D100

**Volume #M4.2.3.2.8, and page #:** 1

**Conducting laboratory and location:** Safety Research Center, Teijin Institute for Bio-Medical Research, Tokyo 191-8512, Japan

**Date of study initiation:** July 8, 1997

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug and % purity:** lot # 7317 and purity 100.9% by titration method

#### Methods

Doses: Study design is shown in the table below.

| Group        | Dose, mg/kg | Sex    | # for toxicity | # for recovery |
|--------------|-------------|--------|----------------|----------------|
| 1, Control   | 0           | Male   | 3 (0104-0106)  | 3 (0101-0103)  |
| 1. Control   | 0           | Female | 3 (0204-0206)  | 3 (0201-0203)  |
| 2. Low dose  | 3           | Male   | 3 (1101-1103)  |                |
| 2. Low dose  | 3           | Female | 3 (1201-1203)  |                |
| 3. Mid dose  | 15          | Male   | 3 (2101-2103)  |                |
| 3. Mid dose  | 15          | Female | 3 (2201-2203)  |                |
| 4. High dose | 45          | Male   | 3 (3104-3106)  | 3 (3101-3103)  |
| 4. High dose | 45          | Female | 3 (3204-3206)  | 3 (3201-3203)  |

Species/strain: Beagle dogs

Number/sex/group or time point (main study): See the study design above.

Route, formulation, volume, and infusion rate: The test article was administered orally in gelatin capsules before food once a day for 52 weeks.

Satellite groups used for toxicokinetics or recovery: Satellite groups for the control and high dose animals were allotted for recovery for three months.

Age: 7 months old at the beginning of the dose

Weight: 6.77 to 8.29 kg at the beginning of the one month quarantine

Sampling times: -Shown under the individual parameter

Unique study design or methodology (if any): Nil

**Observations and times:** (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: Twice a day

Clinical signs: Twice a day

Body weights: Once a week

Food consumption: Once a day

Ophthalmoscopy: Ophthalmological examinations were conducted at predose, on weeks 13, 24, 38 and 50. Ophthalmological examinations were conducted on weeks 5 and 12 during the recovery. The eye examination was conducted by a slit lamp and ophthalmoscope after dilatation of the pupil by Mydrin-P.

EKG: EKG was recorded at predose, weeks 12, 24, 38 and 50 during the treatment and on recovery weeks 5 and 11. PR time, QRS interval and QTc time were recorded by a six channel recorder. All recordings were made before the dosing and at 24 hours after dosing during the treatment period.

Rectal temperature:

The temperature was recorded before dosing and at 24 hours after the dose on weeks 11, 23, 37, and 49.

Hematology: Hematological tests were conducted at predose, weeks 13, 26, 39 and 51 of the treatment, and weeks 6 and 12 during the recovery period. Blood samples were collected from the cephalic vein. Standard hematological and coagulation parameters were tested.

Clinical chemistry: Serum chemistry was performed at pretreatment, weeks 13, 25, 39 and 51 during the treatment period, and during recovery weeks 6 and 12. LDH and CPK were determined from the plasma samples. Blood samples were collected from the cephalic vein. Standard parameters were evaluated.

Urinalysis: Urine samples were collected at predose, and on weeks 14, 26, 40 and 52 during the treatment period, and recovery weeks 7 and 13. Five-hour and 24-hour urine samples were collected from the metabolism cage for examining standard parameters.

Gross pathology: Surviving animals were sacrificed by exsanguination under sodium pentobarbital anesthesia at the end of dosing and recovery.

Organ weights (specify organs weighed if not in histopath table):

Organ weights of the following organs were recorded:

Brain, pituitary, heart, lungs, thymus, liver, kidney, adrenal, spleen, pancreas, thyroid, submandibular glands, testes, epididymides, prostate, ovaries and uterus

Histopathology: Adequate Battery: yes (x ), no ( )—explain

Peer review: yes ( ), no ( x )

Histopathology of protocol specified tissues was conducted on animals sacrificed at the end of the dosing and recovery periods. Histopathology for animal # 3102 and #3202 sacrificed in moribund conditions was also conducted.

Toxicokinetics:

Blood samples were collected from the cephalic vein after the first, 183<sup>rd</sup> and 365<sup>th</sup> dose at 1, 2, 4, 6, 8 and 24 hours post dose. Serum levels of unchanged TEI-6720 were determined by HPLC.

Other: Parts of the liver and kidneys were taken at necropsy and after sacrifice in moribund conditions. These tissues were fixed according to electron microscopic procedures. However, kidneys from the control and high dose treated animals were examined by transmission electron microscope when any abnormality was noted in the light microscopic examinations.

Bone marrow:

Bone marrow nucleated cells were counted following collection of the bone marrow from the 4<sup>th</sup> rib. Bone marrow samples were collected at terminal necropsy and at the time of sacrifice of moribund animals. Bone marrow smears were prepared and stained by Giemsa stain.

Hepatic function:

Hepatic function was tested on animals during pretest, weeks 12, 24, 38 and 50 and during recovery weeks 5 and 11. Hepatosulphalein dye was injected intravenously and blood samples were taken after 30 min to determine serum levels of the dye.

Renal function:

Renal function was examined by determining the plasma levels of phenolsulfonphthalein after iv injection at 1 mg/kg at the predose, treatment weeks 11, 23, 37 and 49 and recovery weeks 4 and 10. The assay of the dye in plasma was conducted after 60 minutes.

**Results**

Mortality: No mortality was reported. However, male dog # 3102 and female dog # 3202 at 45 mg/kg was sacrificed in moribund conditions on days 190 and 176, respectively.

Clinical signs: Clinical signs for animal # 3102 were vomiting, diarrhea and tachypnea. Body temperature was reduced to 37.6° on day 190. Vomiting, salivation, loss of appetite and hypothermia (36.5° C) were observed for animal # 3202. Although vomiting and diarrhea were noted in the control and 5 mg/kg, incidences of vomiting and diarrhea were

increased at 45 mg/kg. Salivation was also observed in surviving male and female dogs at 45 mg/kg.

Vomiting, diarrhea and salivation were clinical signs at 45 mg/kg in male and female dogs and recovered during the treatment free period. However, presence of vomiting was reported in one out of 3 male dogs in the control group during the recovery period.

Body weights (table 3 of the report):

The average body weight (kg) on day 91, 182 and 365 is shown in the table below.

| Day          | Control |      | 5 mg/kg |      | 15 mg/kg |      | 45 mg/kg |      |
|--------------|---------|------|---------|------|----------|------|----------|------|
|              | M       | F    | M       | F    | M        | F    | M        | F    |
| 0            | 9.5     | 8.8  | 9.8     | 8.7  | 9.8      | 8.5  | 9.7      | 9.0  |
| 91           | 10.7    | 9.9  | 11.3    | 9.8  | 11.1     | 9.6  | 10.7     | 9.8  |
| Gain         | 1.2     | 1.1  | 1.5     | 1.1  | 1.3      | 1.1  | 1.0      | 0.8  |
| 182          | 10.9    | 10.3 | 11.6    | 10.0 | 11.4     | 9.7  | 10.9     | 10.2 |
| Gain         | 1.4     | 1.5  | 1.8     | 1.3  | 1.6      | 1.2  | 1.2      | 1.2  |
| 365          | 11.3    | 10.9 | 11.8    | 10.3 | 11.8     | 10.6 | 10.8     | 10.4 |
| Gain         | 1.8     | 2.1  | 2       | 1.6  | 2        | 2.1  | 1.1      | 1.4  |
| % of control |         |      |         |      |          |      | 61%      | 66%  |

Both male and female dogs at 45 mg/kg showed a reduction in the weight gain during the treatment period. However, there were no differences in mean body weight. Therefore, the reduction of weight gain might not be of toxicological significance.

Although one dog died in the recovery group, average weight in the control and at 45 mg/kg was comparable at the end of recovery period in male and female dogs (table 4 of the report).

Food consumption (tables 5 and 6 of the report):

The average food consumption (g) in the control and at 45 mg/kg in male and female dogs was 250 g per day and the food consumption was not affected by the treatment in male and female dogs.

Ophthalmoscopy:

No ophthalmological changes related to the treatment were observed.

EKG:

Male and female dogs did not show any treatment related change in the heart rate, PR, QRS, QT and QTc intervals.

Hematology:

Male:

Male dogs showed a slight increase in platelet counts and % of reticulocyte at 45 mg/kg. Platelet counts were within the normal range. Only dog #3105 at 45 mg/kg showed 80% reticulocyte. However, the average % reticulocyte did not show any statistical significance compared to the control. Average data for some of the hematology parameters are shown in the table below.

| Parameter                      | Sex  | Control | 5 mg/kg | 15 mg/kg | 45 mg/kg |
|--------------------------------|------|---------|---------|----------|----------|
| Platelet (10 <sup>4</sup> /μL) | Male | 37.5    | 36.8    | 33.0     | 40.2*    |
| Reticulocyte (%)               | Male | 39.4    | 28      | 37.3     | 47.2     |

It should be noted that average reticulocyte counts (% RBC) is 0.0.7% in beagle dogs. Therefore, reticulocyte counts reported in the report are abnormally high.

Male dog # 3102 at 45 mg/kg showed increased erythrocyte counts, hemoglobin, hematocrit and APTT on day 189 before sacrifice in moribund the condition. Data are shown in the table below.

| Parameter                         | Predose | Day 189 |
|-----------------------------------|---------|---------|
| Erythrocyte (10 <sup>4</sup> /μL) | 637     | 900     |
| Hemoglobin (g/dL)                 | 14.2    | 21.1    |
| Hematocrit (%)                    | 46.5    | 50      |
| APTT (sec)                        | 13.4    | 19.8    |

Bone marrow of dog # 3102 at 45 mg/kg showed an increase in the myeloid to erythroid ratio (M:E). Male dog #3102 showed an increase in the erythrocyte parameters and APTT. However, average data for bone marrow did not show statistically significant changes in the M:E ratio and normochromatic erythroblast (%), although % normochromatic erythroblast in all treated groups was lower than the control at the end of 52 weeks treatment as shown in the table below.

| Parameter                       | Control | 5    | 15   | 45 mg/kg |
|---------------------------------|---------|------|------|----------|
|                                 | N=3     | N=3  | N=3  | N=3      |
| Normochromatic erythroblast (%) | 2.9     | 1.4  | 1.0  | 1.9      |
| M:E ratio                       | 0.79    | 0.90 | 0.79 | 0.71     |

Female:

The erythrocyte counts ( $10^4/\mu\text{L}$ ) in animal # 3206 at 45 mg/kg were slightly reduced from 606 before the treatment to 564 at the end of week 51. Hemoglobin level (g/dL) in animal # 3206 was 13.0 at the end of 51 week from 14.5 before the dosing period. These changes were not of toxicological significance and within the normal range. However, average erythrocyte counts and hemoglobin levels at 45 mg/kg did not show significant change from the pretreatment period.

The platelet counts were slightly increased at 45 mg/kg at the end of week 51. However, it was within the normal range of 160000-525000/ $\mu\text{L}$ . Increased reticulocyte (%) was noted at 45 mg/kg that was statistically significant at the end of week 51.

| Parameter        | Sex    | Control | 5 mg/kg | 15 mg/kg | 45 mg/kg |
|------------------|--------|---------|---------|----------|----------|
| Reticulocyte (%) | Female | 30.7    | 60      | 52       | 65.2*    |

Female dog #3202 at 45 mg/kg showed an increase in the myeloid to erythroid ratio. However, the average normochromatic erythroblasts (%) and M: E ratio was not affected by the treatment at the end of week 52 as shown in the table below.

| Parameter                       | Control | 5    | 15   | 45 mg/kg |
|---------------------------------|---------|------|------|----------|
|                                 | N=3     | N=3  | N=3  | N=3      |
| Normochromatic erythroblast (%) | 2.5     | 1.5  | 2.0  | 2.5      |
| M:E ratio                       | 0.73    | 0.95 | 0.68 | 0.78     |

Above data suggest that there was no hematological effect of the treatment except a slight increase in reticulocytes at 45 mg/kg at the end of the treatment period in female dogs.

The treatment at 45 mg/kg showed a decrease in the erythroid cells in the bone marrow in one male and one female dogs at 45 mg/kg at the time of moribund sacrifice. Therefore, chronic treatment of the drug had an effect on the bone marrow at 45 mg/kg that resulted a reduction in erythroid cells in two of six dogs.

#### Clinical chemistry:

##### Male dogs:

Average clinical chemistry data showed a reduction in the alkaline phosphatase and CPK activities both in the control and at 45 mg/kg at the end of week 51. However, these changes were incidental and control dogs also showed a reduction over time.

| Parameter                   | Week         | Control | 5   | 15  | 45 mg/kg |
|-----------------------------|--------------|---------|-----|-----|----------|
| Alkaline phosphatase (IU/L) | Pretreatment | 253     | 280 | 224 | 246      |

|                             |              |     |     |     |     |
|-----------------------------|--------------|-----|-----|-----|-----|
| Alkaline phosphatase (IU/L) | 51           | 113 | 116 | 104 | 95  |
| CPK (IU/L)                  | Pretreatment | 326 | 326 | 194 | 244 |
| CPK (IU/L)                  | 51           | 124 | 136 | 122 | 121 |

Average total bilirubin (mg/dL) was not changed statistically during the treatment at 5, 15 and 45 mg/kg. The control animals showed 0.06 to 0.07 mg/dL during the pretest and treatment period. Few dogs at 45 mg/kg listed below showed slightly higher levels during the treatment. However, these levels were within the normal range for beagle dogs (0.1-0.3 mg/dL).

| Animal # | Sex  | Week | Total bilirubin (mg/dL) |
|----------|------|------|-------------------------|
| 1102     | Male | 39   | 0.11                    |
| 2102     | Male | 51   | 0.1                     |
| 3104     | Male | 51   | 0.1                     |

BUN (mg/dl) and creatinine were within the normal range of 7-24 mg/dL and 0.7-1.4 mg/dL, respectively for most of dogs. However, dog # 3102 at 45 mg/kg showed creatinine level of 4.36 and BUN level of 96.3 on day 189 before the sacrifice.

No treatment related changes in the serum chemistry was observed in the male dogs. However, moribund animal showed an elevated level of creatinine and BUN.

Female dogs:

Female dogs did not show any treatment related change in total bilirubin (mg/dL). Alkaline phosphatase activity was significantly reduced at 45 mg/kg. However, a similar trend was noted in the control dogs. The biological significance of the change is unknown. Normal range of alkaline phosphatase is 35-100 IU/L.

BUN (mg/dL) was increased statistically at 45 mg/kg on week 51 and became normal during the recovery period. Most of the values for BUN were within the normal range for beagle dogs (10-20 mg/dL) with the exception of animal # 3204 and 3205. Animal # 3204 and 3205 showed BUN levels of 26.9 and 27.2 at the end of week 52 from 9.5 and 13.6 at pretreatment, respectively.

The average data for alkaline phosphatase and BUN (mg/dL) are shown in the table below.

| Parameter                   | Week         | Control | 5   | 15  | 45 mg/kg |
|-----------------------------|--------------|---------|-----|-----|----------|
| Alkaline Phosphatase (IU/L) | Pretreatment | 253     | 204 | 237 | 248      |
|                             | Week 51      | 148     | 111 | 111 | 98*      |

|                    |              |      |      |      |       |
|--------------------|--------------|------|------|------|-------|
| Phosphatase (IU/L) |              |      |      |      |       |
| BUN (mg/dL)        | Pretreatment | 11.3 | 9.9  | 11.8 | 11.0  |
| BUN (mg/dL)        | Week 51      | 12.7 | 13.0 | 14.2 | 20.2* |

A statistically significant increase in the average creatinine (mg/dL) was noted at 45 mg/kg in female dogs on weeks 13, 25, 39 and 51 as shown in the table below. Data were within the normal range for dogs (0.7-1.4 mg/dL) for most of animals except the average value for group 4 animals and dog #3202. Dog # 3202 sacrificed in moribund condition on day 176 showed an elevated level of creatinine that was 15.19 mg/dl on week 25.

| Parameter  | Week         | Control | 5   | 15  | 45 mg/kg |
|------------|--------------|---------|-----|-----|----------|
| Creatinine | Pretreatment | 0.7     | 0.6 | 0.6 | 0.7      |
| Creatinine | 13           | 0.7     | 0.7 | 0.7 | 0.9*     |
| Creatinine | 25           | 0.8     | 0.8 | 0.8 | 3.4*     |
| Creatinine | 39           | 0.7     | 0.7 | 0.7 | 1.1*     |
| Creatinine | 51           | 0.7     | 0.7 | 0.7 | 1.1*     |

Chloride levels of #3202 was 89 mmol/L on week 25 compared to 111.7 at predose.

Serum chemistry data showed elevated BUN and creatinine in male and female moribund animals at 45 mg/kg. Higher levels of BUN were also noted in female dogs at 45 mg/kg at the end of treatment that was reversible.

Urinalysis:

Male and female dogs did not show treatment related changes in the urine chemistry, presence of urinary calculi (precipitates), pH, volume, specific gravity and occult blood. An increase in volume and a decrease in the specific gravity were noted in female dogs treated at 45 mg/kg in week 40. However, it was considered to be unrelated to the treatment.

The plasma concentration of phenolsulfonphthalein ( $\mu\text{g/dL}$ ) was not changed due to the treatment. Phenolsulfonphthalein is an organic anion that is excreted by the kidney. Drugs that induce kidney tubular toxicity can be monitored by intravenous injection of phenolsulfonphthalein and measurement of the plasma levels over time. Since the plasma levels of phenolsulfonphthalein were not changed, it appeared that the renal tubular activity was not affected by TMX-67 treatment in dogs.

Gross pathology:

Gross pathology of male and female dogs showed yellowish granules and calculi at 45 mg/kg in the kidney. Animals examined were #3104, 3105, 3106 for male dogs and #3204, 3205 and 3206 for female dogs.

One female dog # 2202 at 15 mg/kg also showed yellow granules in the kidney. Uterus in # 1203 at 5 mg/kg and #2203 at 15 mg/kg showed masses.

Male dogs in recovery groups previously treated at 45 mg/kg also showed yellowish calculi in the kidney. One male dog #3103 showed calculi in the ureter. Female dogs in the recovery group previously treated at 45 mg/kg also showed yellowish calculi in the kidney.

Moribund animals:

Male # 3102, at 45 mg/kg was sacrificed in moribund conditions on day 190. Yellow calculi in the kidney pelvis, urethra and large peripancreatic lymph nodes were observed.

Female # 3202 at 45 mg/kg also showed yellow granules in the kidney, yellow calculi in the ureter and large peripancreatic lymph nodes.

Data suggest that yellow calculi were present at 45 mg/kg in male and female dogs and were not reversible during two months of recovery. Yellowish granules were present in one female dog only at 15 mg/kg. Mass in the uterus was not present in all doses except 45 mg/kg.

Organ weights (specify organs weighed if not in histopathology table):

Male:

Absolute and relative organ weight data in male dogs did not show any treatment related change.

Female:

The absolute and relative weight of the liver was increased significantly at 45 mg/kg. The liver weight for female # 3206 was 68.5 g compared to the average weight of 43.6 g in the control animals. The average weight of pancreas was increased from 22.6 g in the control to 27.2 g at 45 mg/kg. The average weight of ovary was 0.97 and 0.61 in the control and 45 mg/kg groups, respectively. The average weight of uterus was decreased at 45 mg/kg compared to the control. However, dog #0206 in the control group showed a higher weight of uterus (18.7 g) that contributed to the difference.

Moribund animals:

Dog #3102 showed an increase in the weight of kidney (103 g) compared to the average weight of 53 g in the control dogs. The weight of kidney in #3202 was comparable to the control.

Histopathology:

Some of the histopathology data are shown in the table below. Histopathology data for 3 dogs/sex/group are presented.

The sponsor provided following notation to histopathology findings:

- = no change, ± = slight, + = mild, ++ = moderate, +++ = severe

| Lesion                        | Sex             | Control     | 5     | 15   | 45 mg/kg   |
|-------------------------------|-----------------|-------------|-------|------|------------|
| Thymus atrophy                | Male            | 2 (1 ±, 1+) | 1, ++ | 2, ± | 2, +       |
| Thymus atrophy                | Recovery male   | 3, ±        |       |      | 3, +       |
| Thymus atrophy                | Female          | 0           | 2, ±  | 3, ± | 2, ±       |
| Thymus atrophy                | Recovery female | 1,+         |       |      | 1, ±       |
| Pelvis dilation, kidney       | Male            | 0           | 0     | 0    | 2, +       |
| Pelvis dilation, kidney       | Recovery male   | 0           |       |      | 0          |
| Pelvis dilation, kidney       | Female          | 0           | 0     | 0    | 3, ++      |
| Pelvis dilation, kidney       | Recovery female | 0           |       |      | 1,++       |
| Interstitial fibrosis, kidney | Male            | 0           | 0     | 0    | 2, ±       |
| Interstitial fibrosis, kidney | Recovery male   | 0           |       |      | 1, ±       |
| Interstitial fibrosis, kidney | Female          | 0           | 0     | 0    | 3(2+, 1++) |
| Interstitial                  | Recovery        | 0           |       |      | 1,+        |

| Lesion                | Sex             | Control | 5 | 15 | 45 mg/kg      |
|-----------------------|-----------------|---------|---|----|---------------|
| fibrosis, kidney      | female          |         |   |    |               |
| Atrophy, renal tubule | Male            | 0       | 0 | 0  | 2,+           |
| Atrophy, renal tubule | Recovery male   | 0       |   |    | 0             |
| Atrophy, renal tubule | Female          | 0       | 0 | 0  | 3 (1±,1+,1++) |
| Atrophy, renal tubule | Recovery female | 0       |   |    | 1,+           |
| Hyperplasia, kidney   | Male            | 0       | 0 | 0  | 2,±           |
| Hyperplasia, kidney   | Female          | 0       | 0 | 0  | 3(2±,1+)      |
| Calculus, Kidney      | Male            |         |   |    | 2 (1++,1+++)  |
| Calculus, Kidney      | Recovery male   | 0       |   |    | 2,+           |
| Calculus, Kidney      | Female          | 0       | 0 | 1± | 3 +++         |
| Calculus, Kidney      | Recovery female | 0       |   |    | 1, ±          |

Above data suggest that male and female dogs at 45 mg/kg showed calculi in the kidney and other pathological changes in the kidney due to calculus formation. Kidney calculus formation was not completely reversible during three months of recovery. One female at 15 mg/kg also showed kidney calculi. The differences observed were due to the differences in exposure. Females had higher exposure levels than males.

Animal # 3102 (male) and 3202 (female) at 45 mg/kg were sacrificed on day 190 and 176 of the treatment, respectively. Both animals showed kidney calculi and secondary changes related to calculus formation in the kidney.

It is concluded that chronic treatment between 15-45 mg/kg induced calculi in the kidney.

Toxicokinetics:

Male TK data are shown in the table below.

<Male>

|          | AUC(0-24hr) (ng•hr/ml) |          |                        | C <sub>max</sub> (ng/ml) |         |                       | T <sub>max</sub> (hr) |       |                   |
|----------|------------------------|----------|------------------------|--------------------------|---------|-----------------------|-----------------------|-------|-------------------|
|          | First                  | 183rd    | 365th                  | First                    | 183rd   | 365th                 | First                 | 183rd | 365th             |
| 5 mg/kg  | 5543.7                 | 3366.5   | 4032.4                 | 3007.4                   | 1717.3  | 2271.9                | 1.7                   | 1.7   | 2.7               |
| 15 mg/kg | 17951.5                | 27751.1  | 31875.8                | 7706.6                   | 12272.6 | 11342.9               | 2.7                   | 2.0   | 2.7               |
| 45 mg/kg | 149055.2 <sup>a)</sup> | 335067.2 | 395440.5 <sup>a)</sup> | 22206.7 <sup>a)</sup>    | 65018.2 | 74646.4 <sup>b)</sup> | 4.0 <sup>a)</sup>     | 3.3   | 4.0 <sup>b)</sup> |

a) N=3 [Values for animals (3103, 3104 and 3106) that showed vomiting were excluded]

b) N=5 [Animal No. 3102 was sacrificed in moribundity on Day 190 of administration]

TK data for female animals are shown below.

<Female>

|          | AUC(0-24hr) (ng•hr/ml) |                        |                        | C <sub>max</sub> (ng/ml) |                       |                       | T <sub>max</sub> (hr) |                   |                   |
|----------|------------------------|------------------------|------------------------|--------------------------|-----------------------|-----------------------|-----------------------|-------------------|-------------------|
|          | First                  | 183rd                  | 365th                  | First                    | 183rd                 | 365th                 | First                 | 183rd             | 365th             |
| 5 mg/kg  | 1966.8                 | 6906.0                 | 5056.5                 | 600.3                    | 3589.3                | 2093.4                | 4.0                   | 1.7               | 1.7               |
| 15 mg/kg | 23691.1                | 31783.7                | 38659.5                | 12663.4                  | 14770.6               | 18617.5               | 4.0                   | 1.7               | 1.7               |
| 45 mg/kg | 155744.7 <sup>c)</sup> | 511541.7 <sup>d)</sup> | 572724.1 <sup>d)</sup> | 15720.8 <sup>c)</sup>    | 70706.8 <sup>d)</sup> | 90561.6 <sup>d)</sup> | 4.2 <sup>c)</sup>     | 4.4 <sup>d)</sup> | 3.6 <sup>d)</sup> |

c) N=5 [Values for the animal (3206) that showed vomiting were excluded]

b) N=5 [Animal No. 3202 was sacrificed in moribundity on Day 176 of administration]

The exposure in male dogs at the end of treatment was 31875 ng.hr/ml (31.9 µg.hr/ml) and 395440 ng.hr/ml (395.5 µg.hr/ml) at 15 and 45 mg/kg, respectively. The male dog to human exposure ratio was 2.7 and 33 at 15 and 45 mg/kg, respectively, considering human exposure at 120 mg/day dose was of 11.96 µg.hr/ml.

The exposure in female dogs at the end of treatment was 38659 ng.hr/ml (38.6 µg.hr/ml) and 572724 ng.hr/ml (572.7 µg.hr/ml) at 15 and 45 mg/kg, respectively. The female dog to human exposure ratio was 3.2 and 48 folds at 15 and 45 mg/kg, respectively, considering human exposure at 120 mg/day was 11.96 µg.hr/ml.

Data show that female dogs had higher exposure than male dogs particularly at 45 mg/kg.

Therefore, kidney calculi were noted in female dogs at 3.2 times human exposure. Male and female dogs showed kidney calculi at 33 and 48 times human exposure, respectively.

Other:

Hepatic function test was conducted by measuring serum levels of hepatosulphalein (BSP). BSP is excreted by bile and have higher bile to plasma ratio. An increase in the plasma level would indicate inability of the liver to excrete through bile. Male and female dogs did not show treatment related change. Isolated incidences of elevation of BSP

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(6.4%) in #2101 male at 15 mg/kg on week 12 and a decrease in BSP (0.35%) in female dog # 2201 at 15 mg/kg on week 50 were observed.

**Study summary:**

Male and female beagle dogs were treated with TMX-67 at 3, 15 and 45 mg/kg once a day for one year. One male and one female dog at 45 mg/kg were sacrificed in moribund conditions before the scheduled necropsy. Vomiting, diarrhea and salivation were noted in male and female dogs at 45 mg/kg. The weight gain in male and female dogs at 45 mg/kg was reduced by 34-39%. However, there were no differences in mean body weight. Therefore, the reduction in weight gain might not be of toxicological significance. The reduction in the body weight gain was not due to a reduction in the food consumption.

Hematology and clinical chemistry data of moribund animals at 45 mg/kg showed an increase in the M:E ratio and higher creatinine levels. A slight elevation of average BUN was also noted in female dogs at 45 mg/kg.

Yellowish granules were noted in the kidney of male and female dogs at 45 mg/kg. One female dog at 15 mg/kg also showed yellowish granules in the kidney. The differences in the calculus formation in the kidney between male and female dogs at 15 mg/kg were as a consequence of higher exposure. Histopathology data confirmed the presence of calculi in the kidney at 45 mg/kg in male and female dogs including animals sacrificed in moribund conditions. Calculus formation was not completely reversed during the three-month recovery period. Secondary changes in the kidney due to calculus formation were dilatation of kidney pelvis, interstitial fibrosis, reduced renal tubular atrophy and hyperplasia of kidney.

It is concluded that the treatment at 15 mg/kg or higher (3.0 and higher exposure to human maximum dose) induced calculi in kidney. The no effect dose was 3 mg/kg. The high dose reached MTD.

**Study title:** 26-week repeated dose toxicity study of TEI-6720 by oral administration in rats

**Key study findings:** Calculi in the kidney were observed at 48 mg/kg in male and female rats. It was not completely recovered after 5-week post treatment.

**Study no.:** S054C3R100

**Volume # 4.2.3.2.6 and page #:** 1

**Conducting laboratory and location:** Safety Research Department, Teijin Institute for Biomedical Research, Tokyo, Japan

This GLP study was conducted in Slc: SD (SPF) rats at 3, 12 and 48 mg/kg once a day by oral gavage for 26 weeks. Since the long term effect of the drug was reviewed for carcinogenicity testing, data for the study are summarized. Animals were six weeks old at the beginning of dosing. Satellite groups (8/sex) were assigned for the determination of serum levels of the drug at 3, 12 and 48 mg/kg. Each group had 15 animals/sex. Additional 15 rats/sex were assigned to the control and 48 mg/kg dose group for 5 weeks of recovery. The high dose was selected on the basis of findings from a 5-week study in which animals were treated at 3, 15, 75 and 150 mg/kg. The drug substance was suspended in 0.5% methylcellulose. The control rats received the vehicle only.

The blood samples for TK study was collected at 1 and 24 hour post dose on days 1 and day 90, and at 1, 3, 6 and 24 hours post dose on day 175.

No treatment related change in the body weight was observed. Hematology data showed an increase in the WBC counts from  $52 \times 10^3/\mu\text{L}$  in the control to  $72 \times 10^3/\mu\text{L}$  at 48 mg/kg in male rats. WBC counts for the female rats was increased from  $33 \times 10^3/\mu\text{L}$  in the control to  $48 \times 10^3/\mu\text{L}$  at 48 mg/kg. However, above data were within the normal range and of no toxicological significance. Biologically significant difference in the M:E ratio in male and female rats was not observed.

Serum creatinine levels were increased from 0.57 mg/dL in the control to 0.97 mg/dL at 48 mg/kg in male rats which was within the normal range. Male rats in the recovery group also showed a similar increase. Female rats did not show treatment related increase in the creatinine levels.

Yellow granules in the kidneys were observed in the high dose (48 mg/kg) male and female rats at terminal and after a 5-week recovery sacrifices.

Histopathology data did not show any treatment related lesions in the thyroid, parathyroid, heart and liver in male and female rats.

Male rats showed basophilic deposition in renal tubules, hyaline droplet degeneration and necrosis of the renal tubular epithelium, interstitial fibrosis, calculi in the kidney pelvis, and epithelial hyperplasia of urinary bladder at 48 mg/kg. No treatment related cardiovascular lesion was observed in male rats.

Female rats showed basophilic deposition in the renal tubules, tubular dilatation, pelvic calculi and interstitial fibrosis at 45 mg/kg. No treatment related lesion was observed in the urinary bladder at 45 mg/kg.

Male and female rats treated at 45 mg/kg did not show complete recovery from kidney calculus formation at the end of 5 weeks of recovery period.

The average serum exposure (AUC, ng.hr/ml) to the drug in male and female rats at the end of week 26 is shown in the table below.

|        |       |       |          |
|--------|-------|-------|----------|
| Sex    | 3     | 12    | 48 mg/kg |
| Male   | 12828 | 52895 | 238483   |
| Female | 16363 | 86190 | 305194   |

The exposure was increased dose dependently. The average exposure in male and female rats at 48 mg/kg is 271838.5 ng.hr/ml. The exposure in rats at 48 mg/kg is 23 times human exposure at maximum recommended human dose 120 mg. Male and female rats did not show a difference in the exposure.

It is concluded that male and female rats showed calculi in the kidney and secondary changes due to calculus formation in the kidney at 48 mg/kg following treatment for six months. Recovery animals also showed similar changes at 48 mg/kg. The no effect dose was 12 mg/kg.

#### 2.6.6.4 Genetic toxicology

**Study title:** In vitro mammalian chromosome aberration test

**Key findings:** TMX-67 is not mutagenic in chromosomal aberration assay in human peripheral lymphocytes.

**Study no.:** TAP-T99-82

**Volume #4.2.2.2.1.4 and page #: 1**

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Sept 23, 1999

**GLP compliance:** Yes

**QA reports:** yes ( x ) no ( )

**Drug lot # and % purity:** TMX-03-RP-01 and 90-110% of theoretical values

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#### Methods

**Strains/species/cell line:** Peripheral blood lymphocytes obtained from a healthy human volunteer were used in the assay. Heparinized blood was incubated with 1% PHA for 48 hours at 37° C. Cells were resuspended in the medium with or without S-9 liver homogenates, the test article solutions or solvent.

The incubation mixture containing the drug substance, positive or negative control was incubated for 4 or 20 hours in the absence of S-9 rat liver homogenates. At the end of 4 hour incubation, the medium was replaced by the fresh medium containing 1% PHA and incubated for another 16 hours. Two hours before harvesting, cells were treated with colcemid at 0.1 µg/ml.

In the S-9 activated system, cells were exposed to the drug substance, solvent or the positive control for 4 hours. The treatment medium was replaced with a fresh medium containing 1% PHA and incubated for an additional 16 hours. Two hours before harvesting, cells were treated with colcemid at 0.1 µg/ml. All incubations were made at 37 °C in the presence of 5% CO<sub>2</sub> balanced air.

Metaphase cells were harvested and incubated in 0.075 M KCl for 20 min. Cells were fixed in methanol:glacial acetic acid at 3:1 v/v. Cells were air dried on a glass slide and stained with 5% Giemsa stain. A total of 200 metaphase cells per treatment concentration were examined for breaks, exchange and rearrangements. The mitotic index was determined as the percent of cells in the mitosis per 500 cells counted. The percent polyploidy and endoreduplicated cells was determined per 100 metaphase cells.

The test article was considered to be positive when the percent of aberrant cells was increased in a dose dependent manner and one or more concentrations showed a statistically significant increase from the solvent control.

Doses used in definitive study:

Absence of S-9 mixtures:

Doses chosen for 4-hour incubation with the test substance were 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 and 750 µg/ml.

Doses chosen for the 20-hour incubation period were 12.5, 25, 50, 75, 100, 125, 150, 175 and 200 µg/ml.

Presence of S-9 mixtures:

Doses chosen for the assay were 2.5, 5, 12.5, 25, 50, 100, 150, 200, 300 and 400 µg/ml.

Basis of dose selection: Cytotoxicity was determined on the basis of the effect of the test substance on mitotic index. The drug substance was precipitated at 1670 µg/ml in the treatment medium. Concentrations of 500 µg/ml or less were soluble in the medium. Also, about 50% reduction in the mitotic index was observed at doses higher than 500 µg/ml in the non-activated system at 4 hour incubation period with the drug substance. Cytotoxicity was noted at 167 µg/ml in the non-activated system for 20 hour exposure in the absence of S-9 mixtures. Cytotoxicity was noted at 5, 50, 500 and 1670 µg/ml following 4 hour incubation in the presence of S-9 mixtures. These data were considered for choosing the concentrations in the definitive study.

Negative controls: DMSO was used as the solvent control for the test article.

Positive controls: Mitomycin C (MMC) was used as a positive control in the non-activated system at 0.25 and 0.5 µg/ml dissolved in distilled water. Cyclophosphamide

was used as a positive control in S-9 activated system at 25 and 50 µg/ml dissolved in distilled water.

Incubation and sampling times: See the study description above.

Historical control for % aberrant cells in the solvent:

|                    | Non-activated system | Activated system |
|--------------------|----------------------|------------------|
| Mean               | 1.4                  | 1.6              |
| Standard deviation | 1.3                  | 1.4              |
| Range              | 0-6.0                | 0-6.5            |

### Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): As stated in the protocol, the test was considered to be valid if the structural aberrations of cells in the solvent control were within the historical control and the positive control showed a statistically significant increase in the chromosomal aberration.

Study outcome:

The mitotic index at various treatments is shown in the table below.

| Treatment, µg/ml | S-9 | Treatment (hr) | Mitotic Index | % Change |
|------------------|-----|----------------|---------------|----------|
| DMSO             | -   | 4              | 3.6           |          |
| 0.167            | -   | 4              | 4.2           | 17       |
| 0.5              | -   | 4              | 3.8           | 6        |
| 1.67             | -   | 4              | 4.0           | 11       |
| 5                | -   | 4              | 3.4           | -6       |
| 16.7             | -   | 4              | 3.0           | -17      |
| 50               | -   | 4              | 3.6           | 0        |
| 167              | -   | 4              | 3.8           | 6        |
| 500              | -   | 4              | 1.4           | -61      |
| 1670             | -   | 4              | 0             | -100     |
| DMSO             | +   | 4              | 4.0           |          |
| 0.167            | +   | 4              | 3.8           | -5       |
| 0.5              | +   | 4              | 2.8           | -30      |
| 1.67             | +   | 4              | 2.8           | -30      |
| 5                | +   | 4              | 2.0           | -50      |
| 16.7             | +   | 4              | 2.4           | -40      |
| 50               | +   | 4              | 2.0           | -50      |
| 167              | +   | 4              | 2.8           | -30      |
| 500              | +   | 4              | 0             | -100     |

| Treatment, $\mu\text{g/ml}$ | S-9 | Treatment (hr) | Mitotic Index | % Change |
|-----------------------------|-----|----------------|---------------|----------|
| 1670                        | +   | 4              | 0             | -100     |
| DMSO                        | -   | 20             | 3.6           |          |
| 0.167                       | -   | 20             | 4.0           | 11       |
| 0.5                         | -   | 20             | 4.0           | 11       |
| 1.67                        | -   | 20             | 3.6           | 0        |
| 5                           | -   | 20             | 3.4           | -6       |
| 16.7                        | -   | 20             | 2.6           | -28      |
| 50                          | -   | 20             | 2.8           | -22      |
| 167                         | -   | 20             | 0.6           | -83      |
| 500                         | -   | 20             | 0             | -100     |
| 1670                        | -   | 20             | 0             | -100     |

No aberration due to TMX-67 treatment was noted at 4 hour incubation in the absence of S-9 mixtures. The highest concentration was 300  $\mu\text{g/ml}$  at which 50% reduction of mitotic index was noted and aberrations at higher doses were not recorded. The positive control MMC showed a significant increase in the aberration. TMX-67 did not show any aberration up to a cytotoxic dose of 100  $\mu\text{g/ml}$  after 20 hours of incubation in the absence of S-9 mixtures.

A similar data was obtained in the presence of S-9 mixtures at 4 hour incubation. Chromosome aberrations were examined up to 100  $\mu\text{g/ml}$  due to a reduction of mitotic index by 50% at 100  $\mu\text{g/ml}$ . The % aberrant cells were increased slightly compared to the solvent control. However, it was not statistically significant and the data were within historical control provided by the sponsor.

Based on the data TMX-67 was considered to be non-clastogenic in peripheral human lymphocytes. The data for the chromosomal aberration study are shown below.

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| Treatment <sup>1</sup> | S9 Activation | Treatment Time | Mean Mitotic Index | Cells Scored | Aberrations Per Cell <sup>2</sup><br>(Mean +/- SD) | Cells With Aberrations <sup>3</sup> |                |
|------------------------|---------------|----------------|--------------------|--------------|----------------------------------------------------|-------------------------------------|----------------|
|                        |               |                |                    |              |                                                    | Numerical (%)                       | Structural (%) |
| DMSO                   | -             | 4              | 4.0                | 200          | 0.000 ±0.000                                       | 0.0                                 | 0.0            |
| TEI-6720               |               |                |                    |              |                                                    |                                     |                |
| 150 ug/mL              | -             | 4              | 2.9                | 200          | 0.015 ±0.122                                       | 0.0                                 | 1.5            |
| 200 ug/mL              | -             | 4              | 3.0                | 200          | 0.005 ±0.071                                       | 0.5                                 | 0.5            |
| 300 ug/mL              | -             | 4              | 2.0                | 200          | 0.020 ±0.140                                       | 0.5                                 | 2.0            |
| MMC,<br>0.5 ug/mL      | -             | 4              | 1.1                | 200          | 0.265 ±0.683                                       | 0.5                                 | 16.5**         |
| DMSO                   | +             | 4              | 4.2                | 200          | 0.000 ±0.000                                       | 0.0                                 | 0.0            |
| TEI-6720               |               |                |                    |              |                                                    |                                     |                |
| 25 ug/mL               | +             | 4              | 3.0                | 200          | 0.005 ±0.071                                       | 0.0                                 | 0.5            |
| 50 ug/mL               | +             | 4              | 2.6                | 200          | 0.010 ±0.100                                       | 0.0                                 | 1.0            |
| 100 ug/mL              | +             | 4              | 2.1                | 200          | 0.015 ±0.122                                       | 0.0                                 | 1.5            |
| CP,<br>25 ug/mL        | +             | 4              | 0.6                | 200          | 0.220 ±0.745                                       | 0.0                                 | 12.5**         |
| DMSO                   | -             | 20             | 3.9                | 200          | 0.000 ±0.000                                       | 0.0                                 | 0.0            |
| TEI-6720               |               |                |                    |              |                                                    |                                     |                |
| 25 ug/mL               | -             | 20             | 3.2                | 200          | 0.000 ±0.000                                       | 0.0                                 | 0.5            |
| 50 ug/mL               | -             | 20             | 2.5                | 200          | 0.000 ±0.000                                       | 0.0                                 | 0.0            |
| 100 ug/mL              | -             | 20             | 2.0                | 200          | 0.005 ±0.071                                       | 0.0                                 | 0.5            |
| MMC,<br>0.25 ug/mL     | -             | 20             | 1.5                | 200          | 0.280 ±0.635                                       | 0.0                                 | 18.5**         |

**Study title:** In vitro mammalian cell gene mutation test in L5178Y/TK<sup>±</sup> mouse lymphoma assay.

**Key findings:** TMX-67 is not mutagenic in the mouse lymphoma assay.

**Study no.:** T99-827

**Volume #4.2.2.1.2 and page #: 1**

**Conducting laboratory and location:** \_\_\_\_\_

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**Date of study initiation:** Sept 23, 1999

**GLP compliance:** Yes

**QA reports:** yes (X ) no ( )

**Drug lot # and % purity:** TMX-03-RP-01 and 100%

## Methods

### Strains/species/cell line:

The L5178Y cell line obtained from \_\_\_\_\_ was used in the assay. Liver homogenates from Arochlor-1254 treated male Sprague Dawley rats were used along with appropriate cofactors.

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### Doses used in definitive study:

In the absence of S-9 mixtures:

Concentrations used in the initial assay were 50, 60, 80, 100, 125 and 150 µg/ml.  
Concentrations used in the repeat experiment were 5.1, 7.6, 13, 18, 25 and 38 µg/ml.

In the presence of S-9 mixtures:

Concentrations used were 50, 100, 125, 150, 175, 200 and 250 µg/ml.  
Assay was not repeated in the presence of S-9 mixtures.

Basis of dose selection: A preliminary toxicity study was conducted at several concentrations up to 1667 µg/ml. The exposure periods in the absence of S-9 mixtures were 4 and 24 hours. The exposure period in the presence of S-9 mixtures was 4 hours. Cell density was determined at 24 and 48 hours after the incubation with the test substance. The toxicity was determined on the basis of the growth of the cell suspensions compared to the solvent control.

Negative controls: DMSO was used in duplicate cultures as solvent controls.

Positive controls: The positive control in absence of S-9 mixtures was methyl methanesulfonate (MMS) and the positive control in the presence of S-9 mixtures was 7, 12-dimethyl-benzo (a)-anthracene (DMBA).

### Incubation and sampling times:

An initial and a repeat mutagenicity test were conducted in duplicate cultures in the absence and presence (not repeated) of S-9 mixtures. The treatment was carried out using

$6 \times 10^6$  cells, 4 ml of the medium or S-9 activation systems, 100  $\mu$ L of the test solution or the solvent. The total volume of the incubation mixtures was 10 ml. The tubes were incubated in the dark for 4 or 24 hours at 37 °C. Cells were washed with the fresh medium and resuspended. Cells were further incubated for 48 hours for the expression of the mutant cells. During this period the cell counts were adjusted to  $3 \times 10^5$  cells/ml at the end of 24 and 48 hours. The cytotoxicity of the test substance was determined from the cell density and growth rate relative to the solvent control.

Mutant phenotype was expressed by placing the cells in a cloning medium containing 0.23% granulated agar and trifluorothymidine (TFT) as the toxic base. TK<sup>-/-</sup> cells would grow in the presence of TFT if mutation occurred. Cell counts were adjusted to  $3 \times 10^6$  cells per 100 ml of cloning medium containing TFT. Cells counts were also adjusted to 600 cells/100 ml of cloning medium in the absence of TFT (called viable count) for the determination of viability of cells. After shaking the flask for 15 minutes at 125 rpm, 33 ml of the cell suspension was distributed to each of 3 Petri dishes and further incubated for 10-14 days at 37° C.

The total number of colonies per plate was counted for TFT and non-TFT containing medium after the incubation period. The mutant frequency was determined by average # TFT colonies/average # non-TFT (viable count) colonies x200.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): An increase in the mutant frequency at a highly toxic dose was not considered biologically relevant. A dose that showed less than 20% of total growth was considered to be toxic dose. A positive response would show a concentration related increase in mutant frequency. The sponsor also indicated that  $\geq 100$  mutants/ $10^6$  cells at one or more non-toxic doses was considered for a positive response. The result was considered as equivocal if the mutant frequency in the drug treated cultures was between 55-99 mutants/ $10^6$  clonable cells over the background levels. Number of mutant colonies smaller than 55 per  $10^6$  cells over the background level were considered to be a negative response.

Following criteria were selected for the validity of the test:

1. Negative controls: Spontaneous mutant frequency of the solvent control must be within 20-100 TFT resistant mutants/ $10^6$  cells. The cloning efficiency of the solvent control must be greater than 50%. Cloning efficiency (CE %) = mean colonies per plate/Total cells per plate x 100.
2. Positive controls: At least one concentration of each positive control should show mutant frequency of  $\geq 100$  mutants per  $10^6$  cells over the solvent control. The colony size distribution for MMS positive control must show an increase in both small and large colonies.

3. Test article treated cultures: A minimum of 4 analyzable concentrations with mutant frequency data would be required.

Historical control data for mean mutation frequency are shown in the table below.

|                    | Solvent (non-activated, 4 hr) | Solvent (non-activated 24 hr) | Solvent (activated) |
|--------------------|-------------------------------|-------------------------------|---------------------|
| Mean               | 42.8                          | 47                            | 60.4                |
| Standard deviation | 12.4                          | 119.4                         | 18.4                |
| Maximum            | 74                            | 85                            | 100                 |
| Minimum            | 22                            | 24                            | 27                  |

Study outcome:

Results of the preliminary cytotoxicity assay showed no cell growth at 150 µg/ml and higher concentrations with a 4-hour incubation period in the absence of S-9 mixtures. Also, at concentrations 150 µg/ml and higher, no cell growth was noted at 24- hour incubation in the absence of S-9 mixtures. The test substance precipitated at 1000 µg/ml in the medium.

In the presence of S-9 mixtures, concentrations higher than 500 µg/ml showed no growth compared to the control. In all cases, cultures containing less than  $0.3 \times 10^6$  cells/ml on day 1 and day 2 were considered as having 0 suspension growth.

The initial mutagenesis assay in the activated and non-activated systems was negative. No dose response for the increase in the mutant frequency was noted. Whereas, the positive control showed a higher mutant frequency than the solvent control. An independent repeat assay was conducted with the 24-hour incubation in the absence of S-9 mixtures. Cultures treated with 5.1, 7.6, 13, 18 and 25 µg/ml were cloned. Cloning data did not show any dose-related increase in the mutant colonies over the control. The test article did not show 55 mutant cells/ $10^6$  clones over the control. The positive control showed an increase in the mutant frequency. Based on the data, TMX-67 was considered to be negative in the mouse lymphoma assay. Data for the initial experiment with S-9 mixtures and repeat experiment without S-9 mixtures (24-hr incubation) are shown in the table below.

| Concentration µg/ml | S-9 mixtures | Mutant frequency | Induced mutant frequency* | % Total growth |
|---------------------|--------------|------------------|---------------------------|----------------|
| Solvent             | -            | 21               | -                         | -              |
| Solvent             | -            | 26               | -                         | -              |
| 5.1                 | -            | 35               | 11                        | 62             |
| 5.1                 | -            | 35               | 11                        | 50             |
| 7.6                 | -            | 36               | 13                        | 45             |

| Concentration<br>µg/ml | S-9 mixtures | Mutant<br>frequency | Induced mutant<br>frequency* | % Total growth |
|------------------------|--------------|---------------------|------------------------------|----------------|
| 7.6                    | -            | 46                  | 23                           | 37             |
| 13                     | -            | 32                  | 9                            | 24             |
| 13                     | -            | 35                  | 12                           | 22             |
| 18                     | -            | 29                  | 6                            | 28             |
| 18                     | -            | 44                  | 20                           | 25             |
| 25                     | -            | 53                  | 29                           | 21             |
| 25                     | -            | 28                  | 5                            | 26             |
| 2.5 (MMS)              | -            | 79                  | 56                           | 82             |
| 5 (MMS)                | -            | 179                 | 155                          | 47             |
| Solvent                | +            | 28                  |                              |                |
| Solvent                | +            | 40                  |                              |                |
| 50                     | +            | 16                  | -18                          | 100            |
| 50                     | +            | 25                  | -9                           | 98             |
| 100                    | +            | 15                  | -19                          | 107            |
| 100                    | +            | 23                  | -11                          | 90             |
| 125                    | +            | 21                  | -13                          | 87             |
| 125                    | +            | 14                  | -20                          | 82             |
| 150                    | +            | 27                  | -7                           | 77             |
| 150                    | +            | 20                  | -14                          | 79             |
| 175                    | +            | 34                  | 0                            | 46             |
| 175                    | +            | 30                  | -4                           | 34             |
| 200                    | +            | 31                  | -3                           | 18             |
| 2.5 (DMBA)             | +            | 146                 | 112                          | 71             |
| 4                      | +            | 251                 | 217                          | 36             |

\*Induced mutant frequency per  $10^6$  surviving cells = mutant frequency – average mutant frequency of solvent controls.

No clonable cells were observed without S-9 mixtures at 38 µg/ml and with S-9 mixtures at 250 µg/ml.

It is concluded that TMX-67 is not mutagenic in the mouse lymphoma assay.

**Study title:** Reverse mutation test of TEI-6720 with bacteria

**Key findings:** TMX-67 increased the number of revertant colonies over the solvent control only at 2500 µg/plate in the absence of S-9 mixtures in TA 1535. It is considered to be non-mutagenic in the Ames assay.

**Study no.:** S054M0B900

**Volume #4.2.3.3.1.1 and page #: 1**

**Conducting laboratory and location:** Safety Research Department, Pharmaceutical Development Research laboratories Teijin Ltd., 4-3-2 Asahigaoka, Hino-shi, Tokyo, Japan

**Date of study initiation:** Jan 20, 1994

**GLP compliance:** Yes

**QA reports:** yes ( x ) no ( )

**Drug lot # and % purity:** 50729 and 99.4%

## Methods

**Strains/species/cell line:** TA 1535, TA 1537, TA 98 and TA 100 strains of Salmonella typhimurium were used. In addition, E. coli strain WP2uvrA was used in the assay. The strains selected in the assay meet requirements of the ICH guidelines. The S-9 liver homogenates were prepared from male Sprague Dawley rats. Rats were treated with phenobarbitone or 5,6-benzoflavone for induction of hepatic enzymes. Appropriate cofactors were added to the liver homogenate for metabolic activation.

**Doses used in definitive study:** Doses used in the definitive study were 78, 156, 313, 625, 1250, 2500, 5000 µg/plate. Duplicate cultures were used in each concentration. Triplicate cultures were used for the solvent control.

**Basis of dose selection:** A dose finding assay was conducted up to 5000 µg/plate. Doses used in the dose range finding study were 39, 78, 156, 313, 625, 1250, 2500, 5000 µg/plate. A total inhibition of colony growth was noted at 5000 µg/plate in TA 1537. Inhibition of the colony was noted at 2500 and 5000 µg/plate in TA 98 in the absence of S-9 mixtures. Cytotoxicity was also noted at 2500 µg/plate but not at 5000 µg/plate in TA 1535 in the absence of S-9 mixtures.

Cytotoxicity was noted at 2500 and 5000 µg/plate in TA 1537 in the presence of S-9 mixtures. Cytotoxicity was noted at 5000 µg/plate in TA 1535 in the presence of S-9. Cytotoxicity was also noted at 1250 µg/plate and higher concentration in TA 100 in the presence of S-9 mixtures.

**Negative controls:** DMSO was used as the solvent control or negative control in triplicates.

### Positive controls:

| Strain  | Without S-9                                              | With S-9                                |
|---------|----------------------------------------------------------|-----------------------------------------|
| TA 1535 | N-ethyl-N-nitro-N-nitrosoguanidine (ENNG),<br>5 µg/plate | 2-Aminoanthracene (2-AA),<br>2 µg/plate |
| TA 1537 | Acridine (ICR-191),<br>1 µg/plate                        | 2-AA, 2 µg/plate                        |
| TA 98   | Aminofluorene (AF-2), 0.1                                | 2-AA, 0.5 µg/plate                      |

| Strain  | Without S-9                           | With S-9                             |
|---------|---------------------------------------|--------------------------------------|
|         | $\mu\text{g}/\text{plate}$            |                                      |
| TA100   | AF-2, 0.01 $\mu\text{g}/\text{plate}$ | 2-AA, 1.0 $\mu\text{g}/\text{plate}$ |
| WP2uvrA | AF-2, 0.01 $\mu\text{g}/\text{plate}$ | 2-AA, 20 $\mu\text{g}/\text{plate}$  |

Incubation and sampling times: Bacterial suspensions were incubated with the test substance or solvent control with or without S-9 mixtures for 20 min at 37° C. Soft agar was prepared and sterilized in the autoclave. The soft agar was maintained at 45° C to avoid solidifying. The medium was supplemented with tryptophan for E. coli and histidine for S. typhimurium strains. Bacterial suspensions were poured on soft agar plate and incubated for 48 hours at 37° C for the growth of bacterial colonies. After 48 hours samples were cooled to 4° C for avoiding further growth of the colony. Colonies visible to the naked eye were counted using a — Colony Counter.

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## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): A valid study would have appropriate numbers of revertant colonies in the positive and negative controls. The test system should not be contaminated with other strains of bacteria. Four or more dose levels tested would not provide any antibacterial effect. The result was considered to be positive if the number of revertant colonies was twice or greater than the negative control. A dose dependent increase in the revertant colonies was necessary for a positive response. The sponsor did not use any statistical analysis for the evaluation.

### Study outcome:

The number of revertant colonies in the positive control treated plates was substantially higher than the solvent control. As shown in the table below, the number of revertant colonies at 2500  $\mu\text{g}/\text{plate}$  increased to 354 and 399 in replicate cultures in the absence of S-9 mixtures in TA 1535 strain. The next higher dose did not show a similar effect. No other doses showed any increase in the revertant colonies in the presence of S-9 mixtures.

A dose response was not observed for the increase in revertant colonies in TA 1535 strain. Preliminary data showed cytotoxicity at 2500  $\mu\text{g}/\text{plate}$  in TA 1535. However, cytotoxicity was not observed at 5000  $\mu\text{g}/\text{plate}$  in the main study. The reviewer concluded that TMX-67 is mutagenic in TA 1535 without S-9 mixtures at 2500  $\mu\text{g}/\text{plate}$ . However, the Pharmacology/Toxicology Team Leader concluded that the observation is not considered as positive.

The result of dose finding study is shown below.

| Test substance concentration (µg/plate) | number of plates | Reversion Colonies (Colonies/plate) |       |        |       |         |       |                 |  |
|-----------------------------------------|------------------|-------------------------------------|-------|--------|-------|---------|-------|-----------------|--|
|                                         |                  | Base-pair substitution type         |       |        |       |         |       | frameshift type |  |
|                                         |                  | TA 1537                             |       | TA 150 |       | WP2uvrA |       | TA 98           |  |
| mean                                    | stdev            | mean                                | stdev | mean   | stdev | mean    | stdev |                 |  |
| Solvent control (DMSO)                  | -                | 9                                   | 127   | 27     | 8     | 19      |       |                 |  |
| 59                                      | -                | 10                                  | 114   | 29     | 5     | 11      |       |                 |  |
| 76                                      | -                | 6                                   | 115   | 119    | 32    | 29      | 4     | 13              |  |
| 156                                     | -                | 12                                  | 178   | 32     | 5     | 19      |       |                 |  |
| 313                                     | -                | 10                                  | 131   | 129    | 24    | 28      | 2     | 14              |  |
| 625                                     | -                | 6                                   | 100   | 26     | 3     | 15      |       |                 |  |
| 1250                                    | -                | 6                                   | 130   | 115    | 28    | 27      | 3     | 12              |  |
| 2500                                    | -                | 10                                  | 173   | 30     | 5     | 15      |       |                 |  |
| 5000                                    | -                | 7                                   | 120   | 118    | 34    | 32      | 11    | 16              |  |
| Solvent control (DMSO)                  | +                | 8                                   | 111   | 111    | 24    | 10      | 7     | 11              |  |
| 59                                      | +                | 6                                   | 7     | 84     | 32    | 8       | 8     | 12              |  |
| 76                                      | +                | 8                                   | 7     | 107    | 96    | 20      | 26    | 7               |  |
| 156                                     | +                | 5                                   | 67    | 27     | 7     | 14      |       |                 |  |
| 313                                     | +                | 6                                   | 6     | 75     | 71    | 33      | 25    | 6               |  |
| 625                                     | +                | 3                                   | 0     | 43     | 0     | 29      | 0     | 5               |  |
| 1250                                    | +                | 0                                   | 0     | 46     | 45    | 30      | 30    | 4               |  |
| 2500                                    | +                | 22                                  | 0     | 130    | 0     | 25      | 0     | 0               |  |
| 5000                                    | +                | 8                                   | 20    | 54     | 92    | 28      | 28    | 0               |  |
| Solvent control (DMSO)                  | -                | 11                                  | 120   | 36     | 10    | 27      |       |                 |  |
| 59                                      | -                | 6                                   | 113   | 34     | 13    | 18      |       |                 |  |
| 76                                      | -                | 8                                   | 6     | 125    | 119   | 34      | 12    | 28              |  |
| 156                                     | -                | 6                                   | 116   | 30     | 13    | 26      |       |                 |  |
| 313                                     | -                | 14                                  | 10    | 130    | 126   | 34      | 17    | 15              |  |
| 625                                     | -                | 7                                   | 137   | 28     | 11    | 38      |       |                 |  |
| 1250                                    | -                | 11                                  | 9     | 132    | 115   | 37      | 10    | 30              |  |
| 2500                                    | -                | 6                                   | 7     | 137    | 130   | 27      | 24    | 16              |  |
| 5000                                    | -                | 8                                   | 7     | 137    | 130   | 27      | 24    | 16              |  |
| Solvent control (DMSO)                  | +                | 9                                   | 119   | 26     | 17    | 27      |       |                 |  |
| 59                                      | +                | 8                                   | 0     | 125    | 123   | 32      | 34    | 11              |  |
| 76                                      | +                | 5                                   | 7     | 103    | 108   | 43      | 12    | 34              |  |
| 156                                     | +                | 3                                   | 7     | 112    | 108   | 27      | 35    | 10              |  |
| 313                                     | +                | 0                                   | 4     | 76     | 72    | 29      | 31    | 31              |  |
| 625                                     | +                | 6                                   | 4     | 86     | 72    | 26      | 25    | 10              |  |
| 1250                                    | +                | 2                                   | 0     | 52     | 0     | 34      | 0     | 10              |  |
| 2500                                    | +                | 9                                   | 6     | 10     | 56    | 23      | 29    | 5               |  |
| 5000                                    | +                | 2                                   | 0     | 23     | 0     | 27      | 3     | 5               |  |
|                                         |                  | 2                                   | 2     | 21     | 22    | 28      | 28    | 4               |  |

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| Positive control | Name       | TA1537    | TA150    | WP2uvrA   | TA98     |
|------------------|------------|-----------|----------|-----------|----------|
| -59              | 3.0        | 3.01      | 0.01     | 0.01      | 0.1      |
|                  | 3.1 mean   | 4.72 mean | 84 mean  | 3728 mean | 299 mean |
|                  | 3.0 stdev  | 454       | 463      | 73        | 78       |
|                  |            | 4342      | 4015     | 281       | 250      |
| Positive control | Name       | ZAA       | ZAA      | ZAA       | ZAA      |
| +59              | F.D        | 1.0       | 20.0     | 2.0       | 0.5      |
|                  | 1.23 mean  | 325 mean  | 840 mean | 61 mean   | 177 mean |
|                  | 1.58 stdev | 141       | 310      | 518       | 403      |
|                  |            | 627       | 65       | 63        | 107      |
|                  |            |           |          |           | 172      |

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Results of the main study are shown below.

CV:

| Test substance concentration (µg/plate) | with or without 59mic | Revertant colonies (Colonies/plate) |        |         |         |                 |      |      |      |      |  |
|-----------------------------------------|-----------------------|-------------------------------------|--------|---------|---------|-----------------|------|------|------|------|--|
|                                         |                       | Base-pair substitution type         |        |         |         | Frameshift type |      |      |      |      |  |
|                                         |                       | TA 1535                             | TA 100 | WPZ101A | TA 1537 | TA 98           | mean | mean | mean | mean |  |
| Solvent control (DMSO)                  | -                     | 8                                   | 81     | 14      | 2       | 13              |      |      |      |      |  |
|                                         |                       | 4                                   | 84     | 28      | 9       | 15              |      |      |      |      |  |
|                                         |                       | 4                                   | 76     | 81      | 15      | 19              | 4    | 5    | 14   | 14   |  |
| 78                                      | -                     | 4                                   | 93     | 16      | 4       | 14              |      |      |      |      |  |
|                                         |                       | 5                                   | 101    | 97      | 24      | 20              | 5    | 5    | 14   | 14   |  |
| 156                                     | -                     | 4                                   | 93     | 20      | 5       | 20              |      |      |      |      |  |
|                                         |                       | 6                                   | 96     | 85      | 18      | 18              | 3    | 4    | 13   | 16   |  |
| 313                                     | -                     | 4                                   | 93     | 16      | 5       | 15              |      |      |      |      |  |
|                                         |                       | 3                                   | 110    | 102     | 12      | 14              | 2    | 4    | 18   | 17   |  |
| 625                                     | -                     | 4                                   | 75     | 25      | 3       | 16              |      |      |      |      |  |
|                                         |                       | 6                                   | 70     | 73      | 21      | 23              | 4    | 4    | 14   | 15   |  |
| 1250                                    | -                     | 4                                   | 77     | 17      | 4       | 20              |      |      |      |      |  |
|                                         |                       | 3                                   | 51     | 64      | 15      | 16              | 1    | 3    | 16   | 18   |  |
| 2500                                    | -                     | 324                                 | 55     | 14      | 0       | 9               |      |      |      |      |  |
|                                         |                       | 399                                 | 62     | 59      | 8       | 12              | 0    | 0    | 10   | 10   |  |
| 5000                                    | -                     | 7                                   | 26     | 26      | 0       | 3               |      |      |      |      |  |
|                                         |                       | 8                                   | 172    | 99      | 11      | 19              | 0    | 0    | 5    | 4    |  |
| Solvent control (DMSO)                  | +                     | 8                                   | 88     | 19      | 6       | 17              |      |      |      |      |  |
|                                         |                       | 7                                   | 82     | 26      | 4       | 16              |      |      |      |      |  |
|                                         |                       | 4                                   | 91     | 87      | 21      | 22              | 9    | 6    | 27   | 21   |  |
| 78                                      | +                     | 4                                   | 104    | 16      | 7       | 24              |      |      |      |      |  |
|                                         |                       | 9                                   | 95     | 100     | 17      | 17              | 7    | 7    | 25   | 23   |  |
| 156                                     | +                     | 4                                   | 78     | 23      | 8       | 21              |      |      |      |      |  |
|                                         |                       | 1                                   | 93     | 86      | 16      | 20              | 9    | 9    | 25   | 23   |  |
| 313                                     | +                     | 5                                   | 84     | 18      | 4       | 18              |      |      |      |      |  |
|                                         |                       | 2                                   | 82     | 88      | 24      | 21              | 8    | 6    | 27   | 23   |  |
| 625                                     | +                     | 5                                   | 76     | 16      | 6       | 26              |      |      |      |      |  |
|                                         |                       | 3                                   | 76     | 77      | 25      | 21              | 2    | 4    | 27   | 27   |  |
| 1250                                    | +                     | 3                                   | 68     | 22      | 1       | 27              |      |      |      |      |  |
|                                         |                       | 3                                   | 70     | 69      | 12      | 17              | 2    | 2    | 19   | 23   |  |
| 2500                                    | +                     | 3                                   | 83     | 30      | 9       | 20              |      |      |      |      |  |
|                                         |                       | 2                                   | 54     | 59      | 20      | 23              | 1    | 5    | 20   | 20   |  |
| 5000                                    | +                     | 9                                   | 45     | 11      | 2       | 14              |      |      |      |      |  |
|                                         |                       | 2                                   | 52     | 49      | 27      | 19              | 0    | 1    | 7    | 11   |  |

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| Positive control -53 | Name     | ENNG     | AF2       | AF2       | KR191    | AF2  |      |      |     |     |
|----------------------|----------|----------|-----------|-----------|----------|------|------|------|-----|-----|
|                      | µg/plate | 3.0      | 0.01      | 0.01      | 1.0      | 0.1  |      |      |     |     |
| colonies/plate       | 63       | mean 450 | mean 80   | mean 3440 | mean 238 |      |      |      |     |     |
|                      | 47       | 55       | 468       | 459       | 78       | 79   | 3792 | 3616 | 236 | 237 |
| Positive control 459 | Name     | ZAA      | ZAA       | ZAA       | ZAA      | ZAA  |      |      |     |     |
|                      | µg/plate | 2.0      | 1.0       | 20.0      | 2.0      | 0.5  |      |      |     |     |
| colonies/plate       | 104      | mean 496 | mean 1128 | mean 93   | mean 233 | mean |      |      |     |     |
|                      | 101      | 103      | 558       | 527       | 884      | 1006 | 89   | 91   | 223 | 228 |

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**Study title:** Chromosomal aberration test of TEI-6720 in cultured Chinese hamster cells

**Key findings:** TMX-67 induced chromosomal aberration (break and exchange) in Chinese hamster lung fibroblast (V79) cell line in the absence and presence of S-9 mixtures.

**Study no.:** S054W0C900

**Volume# 4.2.3.3.1.3, module 4, and page #: 1**

**Conducting laboratory and location:** Safety Research Department, Teijin Ltd., 4-3-2 Asahigaoka, Hino-shi, Tokyo, Japan

**Date of study initiation:** June 7, 1994

**GLP compliance:** Yes

**QA reports:** yes (x ) no ( )

**Drug, lot # 50729, and % purity:** 99.4%

#### **Methods**

Strains/species/cell line: Chinese hamster lung fibroblast cell line (V79) obtained from the NIH was used in the assay.

Doses used in definitive study: Doses used for 6-hour incubation in the absence of S-9 mixtures were 0.593, 0.888, 1.33 and 2.0 mM. Doses used in the absence of S-9 mixtures were 0.175, 0.263, 0.395 and 0.593 mM for 24 hour incubation. Doses used for the 48 hour incubation were 0.078, 0.117, 0.175 and 0.263 mM in the absence of S-9 mixtures.

Doses used in the presence:

Doses used in the presence of S-9 mixtures were 0.593, 0.888, 1.336 and 2.0 mM. The incubation period was 6 hours.

Basis of dose selection: Cytotoxicity was determined in the preliminary assay by monitoring the effect of the test substance on cell proliferation. Cells were treated with the test substance for 24 or 48 hours in the absence of S-9 mixtures. Cell proliferation and relative growth were determined. The concentrations used for the cytotoxicity assay in the absence of S-9 mixtures were 0.0078, 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 mM. Above concentrations were chosen after conducting a preliminary assay in which about 79% and 50% inhibition of growth was noted at 0.25 and 0.5 mM in 24-hour incubation, respectively, in the absence of S-9 mixtures. About 69% and 27% inhibition

of relative growth was noted at 0.25 and 0.5 mM in the absence of S-9 mixtures with 48 hours incubation in the preliminary assay.

Concentrations used in the cytotoxicity assay in the presence of S-9 mixtures were 0.0195, 0.0391, 0.0781, 0.156, 0.313, 0.625, 1.25, 2.5 and 5.0 mM. The concentrations were selected from a preliminary assay in which about 70 and 46% inhibition of growth was noted in the presence of S-9 mixtures at 2.5 and 5 mM, respectively. Drug substance was incubated for 6 hours in the presence of S-9 mixtures. The medium was replaced and further incubated for another 18 hours.

At the end of the incubation period, plates were removed from the incubator and cells were fixed with 3.5% formaldehyde. Cells were washed and stained with 0.1% crystal violet. Cell proliferation was measured with a monolayer cell densitometer. Cytotoxicity was measured by plotting optical absorbance versus concentration plot. The  $IC_{50}$  for inhibition of optical absorbance was considered to be 50% cytotoxic dose. The experiment was conducted using duplicate cultures for each concentration. Untreated and vehicle controls were used in the experiment. Doses for the chromosome aberration study were selected on the basis of the result of cytotoxicity test.

Negative controls: Media alone and DMSO were used as untreated and solvent controls, respectively.

Positive controls: N-methyl-N-nitro-N-nitrosoguanidine (MNNG) was used as a positive control in the absence of S-9 mixtures at 1.25 and 2.5  $\mu\text{g/ml}$ . Benzo- $\alpha$ -pyrene (BP) was used at 10 and 20  $\mu\text{g/ml}$  in the presence of S-9 mixtures.

Incubation and sampling times: The culture was incubated with the appropriate control, test substance or positive control for 24 or 48 hours in the absence of S-9 mixtures.

Cultures were treated with the control, drug substance or positive control for 6 hours in the presence of metabolic activation system (S-9- mixtures). A similar set of experiment was conducted in the absence of S-9 mixtures concurrently for comparison. Cells were washed with the fresh media and reincubated for another 18 hours.

Two hour before harvest, cells were treated with colcemid (0.2  $\mu\text{g/ml}$ ) to arrest cell division in metaphase. Cells were treated with a hypotonic salt solution (not specified which salt and concentration used), fixed and stained with 1.5% Giemsa stain for chromosome aberration analysis. Two hundred metaphase cells were score at each concentration.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Duplicate cultures were used for each concentration tested. Total number of cells observed, number of cells with numerical and structural abnormality were determined.

Structural aberration with or without gap was determined and expressed as percent of total cells analyzed. Statistical analysis was conducted using a trend test. The slides were blinded for analysis. The criteria for selection of the high dose were either 50% inhibition of cell growth or 10 mM. However, the initial cytotoxicity assay showed that the high dose could be selected on the basis of cytotoxicity to the drug.

Study outcome:

IC<sub>50</sub> for inhibition of cellular growth was 0.635 and 0.275 mM at 24- and 48-hour incubation, respectively, in the absence of S-9 mixtures. The IC<sub>50</sub> for growth inhibition was 2.1 in the presence of S-9 mixtures when cells were treated for 6 hours.

Chromosomal analysis data for TMX-67 in the absence of S-9 mixtures at 24 and 48 hour incubation are shown in the table below.

| Treatment     | Dose, mM      | Cells analyzed | Treatment, hr | % Polyploidy | Total aberration with gap | % aberration without gap |
|---------------|---------------|----------------|---------------|--------------|---------------------------|--------------------------|
| No treatment- | -             | 200            | 24            | 1.0          | 0                         | 0                        |
| DMSO          | -             | 200            | 24            | 0.5          | 1                         | 0.5                      |
| TEI-6720      | 0.175         | 200            | 24            | 0.5          | 2                         | 0.5                      |
| TEI-6720      | 0.263         | 200            | 24            | 0            | 2                         | 1                        |
| TEI-6720      | 0.395         | 200            | 24            | 2            | 9                         | 3.5                      |
| TEI-6720      | 0.593         | 200            | 24            | 0            | 5                         | 1.5                      |
| MNNG          | 1.25<br>µg/ml | 200            | 24            | 0            | 105                       | 50                       |
| MNNG          | 2.5 µg/ml     | 200            | 24            | 1            | 182                       | 90                       |
| No treatment  | -             | 200            | 48            | 0            | 0                         | 0                        |
| DMSO          | -             | 200            | 48            | 0            | 1                         | 0.5                      |
| TEI-6720      | 0.078         | 200            | 48            | 0            | 0                         | 0                        |
| TEI-6720      | 0.117         | 200            | 48            | 0            | 2                         | 1                        |
| TEI-6720      | 0.175         | 200            | 48            | 0            | 5                         | 1.5                      |
| TEI-6720      | 0.263         | 200            | 48            | 0            | 0                         | 0                        |
| MNNG          | 1.25<br>µg/ml | 200            | 48            | 0            | 67                        | 32                       |
| MNNG          | 2.5 µg/ml     | 200            | 48            | 1.5          | 110                       | 54                       |

IC<sub>50</sub> for cell growth inhibition in the chromosomal aberration assay was about 0.4 and 0.2 mM at 24 and 48 hour incubation, respectively in the absence of S-9 mixtures.

The sponsor provided historical control data for spontaneous polyploidy and % aberrant cells without gaps. In the absence of S-9 mixtures, polyploid and aberrant cells were

0.46% and 0.67%, respectively. In the presence of S-9 mixtures, 0.52% and 0.87% cells showed polyploidy and aberration without gaps, respectively.

Based on the historical control data, an increase in % aberrant cells was noted at 0.39 and 0.59 mM following 24-hour incubation in the absence of S-9 mixtures. The increase was statistically significant. The maximum dose used in the assay showed higher than 50% inhibition of cell growth.

A slight increase in the % aberrant cells was also noted at 0.117 and 0.175 mM following 48 hour incubation. However, it was statistically not significant.

Chromosomal analysis data in the absence and presence of 6-hour incubation are shown in the table below.

| Treatment    | Dose, mM | Cells analyzed | S-9 | % Polyploidy | Total aberration with gap | % aberration without |
|--------------|----------|----------------|-----|--------------|---------------------------|----------------------|
| No treatment | -        | 200            | -   | 0            | 1                         | 0.5%                 |
| DMSO         | -        | 200            | -   | 0            | 0                         | 0                    |
| TEI-6720     | 0.593    | 200            | -   | 0            | 3                         | 1.5                  |
| TEI-6720     | 0.888    | 200            | -   | 0            | 0                         | 0                    |
| TEI-6720     | 1.336    | 200            | -   | 0            | 22                        | 10.5                 |
| TEI-6720     | 2.0      | 200            | -   | 0.5          | 79                        | 38.5                 |
| BP           | 10 µg/ml | 200            | -   | 0            | 1                         | 0.5                  |
| BP           | 20 µg/ml | 200            | -   | 0            | 3                         | 1.0                  |
| No treatment | -        | 200            | +   | 0.5          | 3                         | 1.0                  |
| DMSO         | -        | 200            | +   | 0.5          | 4                         | 0.5                  |
| TEI-6720     | 0.593 mM | 200            | +   | 0.5          | 6                         | 2.5                  |
| TEI-6720     | 0.888 mM | 200            | +   | 1.5          | 6                         | 3.0                  |
| TEI-6720     | 1.336 mM | 200            | +   | 1.5          | 64                        | 31                   |
| TEI-6720     | 2.0      | 200            | +   | 0            | 72                        | 35                   |
| BP           | 10 µg/ml | 200            | +   | 3.0          | 81                        | 40.5                 |
| BP           | 20 µg/ml | 200            | +   | 0.5          | 130                       | 64.0                 |

In 6-hour incubation, TEI-6720 showed a statistically significant increase in aberrant cells at 1.33 and 2.0 mM in the absence and presence of S-9 mixtures. Increased chromatid gaps, breaks and exchanges were noted in the absence and presence of S-9 mixtures. The IC<sub>50</sub> for cell viability in the absence and presence of S-9 mixtures was

greater than 2 mM because a 50% inhibition of cell growth was not achieved up to 2 mM (see figure 4, page 37 of the report).

Data suggest that TMX-67 is mutagenic in chromosomal aberration assay in the absence and presence of S-9 mixtures. TMX-67 induced chromatid break and chromatid exchange. Concentrations used for 24- and 48-hour incubation were lower than those used in the 6 hour incubation due to cytotoxicity. As a result, chromosomal aberration was not higher at 24- and 48-hour incubation.

ICH guidelines S2A, 1996 suggest that the desired level of toxicity for in vitro cytogenetic tests using cell lines should be greater than 50% reduction in cell number. However, a significant increase in the aberration was noted at 6-hour incubation at or slightly lower than 50% inhibition of cell growth. Therefore, chromosomal aberration due to TMX-67 treatment was not resulted from its cytotoxicity as suggested by the sponsor b(4)

It is concluded that TMX-67 induced chromosomal aberration in the absence and presence of S-9 mixtures.

**Study title:** Micronucleus test on TEI-6720 in BDF<sub>1</sub> mice (intraperitoneal administration)

**Key findings:** TMX-67 is not mutagenic in mouse micronucleus test in vivo.

**Study no.:** S054Z0M200

**Volume #4.2.3.3.1.5 and page #:** 1

**Conducting laboratory and location:** Safety Research department, Teijin Ltd., Asahigaoka, Tokyo, Japan

**Date of study initiation:** Aug 29, 1994

**GLP compliance:** Yes

**QA reports:** yes ( x ) no ( )

**Drug, lot # 50729, and % purity:** 99.4%

#### **Methods**

**Strains/species/cell line:** Male and female BDF<sub>1</sub> mice used in the preliminary study. Animals were 8 weeks old at the beginning of the dosing. Male mice weighed about 22-25 g and female mice weighed about 17-20 g.

Based on the preliminary experiment, only male mice were used in the study. Mice weighed 21.16-25.8 g at the beginning of the treatment.

Doses used in definitive study: Doses used in the definitive study were 50, 100 and 200 mg/kg. The drug substance was suspended in methyl cellulose.

Basis of dose selection: A preliminary experiment was conducted at 50, 100 and 200 mg/kg single ip injection. Bone marrow cells were collected at 18, 24, 30, 48 and 72 hours after the treatment. Another preliminary test was also conducted at 12.5, 25, 50, 100 and 200 mg/kg in which animals were treated twice at 24 hour interval. The third study was conducted at 25, 50, 100, 200 mg/kg given twice at 24 hour interval. Animals were sacrificed 24 hours after the last injection. One animal that received two doses at 200 mg/kg each dose died. A statistically significant decrease in polychromatic erythrocytes was observed. Data suggested that two treatments at 24 hour interval induced cytotoxicity in the bone marrow cells. Based on the results of the preliminary study, the high dose was set at 200 mg/kg.

Negative controls: The control mice received 0.5% methyl cellulose as the vehicle.

Positive controls: Mitomycin C (MMC) was used as the positive control at 1 and 2 mg/kg doses given intraperitoneally. Erythrocytes were harvested at 24 hours only in MMC treated animals.

Incubation and sampling times: Mice were treated by single intraperitoneal injection with the test substance. Each treatment group had 6 mice per time point of sacrifice. Mice were sacrificed at 24, 48 and 72 hours after the treatment. Bone marrow cells were collected. The percent of PCE (polychromatic erythrocyte), micronucleated cells/1000 polychromatic erythrocytes were counted after appropriate staining of slides. Cells were stained with acridine-orange and Giemsa stains.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The percent of polychromatic erythrocytes (PCE), number of micronucleated (MN) cells per 1000 polychromatic or 1000 normochromatic (NCE) erythrocytes were determined. Mean values for six animals with standard deviation were calculated. Statistical analysis of micronucleated cells/1000 PCE per group was conducted for determination of a dose related change. The sponsor provided historical control data for BDF<sub>1</sub> male mice as shown below.

| PCE% ( mean ± SD) | MN/1000E, (PCE/NCE) | MN/1000PCE | MN/1000NCE |
|-------------------|---------------------|------------|------------|
| 50 ± 6.07         | 1.58 (0-8)          | 1.98 (0-9) | 1.18 (0-5) |

Study outcome:

Administration of 200 mg/kg dose showed a decrease in the locomotor activity in mice immediately after dosing and sustained up to next day. Data presented below do not show any increase in the micronucleated polychromatic cells. About 1000 PCE were scored for each mouse. A similar result was obtained when mice were treated with two injections at 24 hour interval. The positive control showed an increase in the micronucleated cells/1000 polychromatic cells. It is concluded that intraperitoneal injection of TMX-67 did not induce mutation in the maturing erythrocytes in the bone marrow.

Table 7. Results of micronucleus test : averages of micronucleated erythrocytes in male B6F<sub>1</sub> mice after single intraperitoneal administration of TEI-6720.

| Groups              | Dose (mg/kg) | Sampling time(hr.) | No. of animals | NO. of PCEs observed | MNPCEs/1000PCEs <sup>a)</sup> |           | MNWCEs/1000WCEs <sup>b)</sup> |         | PCEs        |
|---------------------|--------------|--------------------|----------------|----------------------|-------------------------------|-----------|-------------------------------|---------|-------------|
|                     |              |                    |                |                      | Mean                          | (range)   | Mean                          | (range) |             |
| Control<br>TEI-6720 | 0            | 24                 | 6              | 6000                 | 1.7                           | (0 - 4)   | 1.5                           | (0 - 2) | 49.9 ± 3.7  |
|                     | 50           | 24                 | 6              | 6000                 | 1.7                           | (0 - 4)   | 1.5                           | (0 - 3) | 54.2 ± 2.4  |
|                     | 100          | 24                 | 6              | 6000                 | 1.5                           | (0 - 4)   | 2.3                           | (0 - 3) | 47.8 ± 3.6  |
|                     | 200          | 24                 | 6              | 6000                 | 2.7                           | (1 - 4)   | 2.2                           | (0 - 3) | 50.8 ± 4.0  |
| Control<br>TEI-6720 | 0            | 48                 | 6              | 6000                 | 1.7                           | (0 - 4)   | 0.8                           | (0 - 2) | 48.6 ± 3.5  |
|                     | 50           | 48                 | 6              | 6000                 | 1.0                           | (0 - 4)   | 2.7                           | (0 - 4) | 58.8 ± 3.7  |
|                     | 100          | 48                 | 6              | 6000                 | 1.7                           | (0 - 4)   | 1.0                           | (0 - 2) | 56.3 ± 4.4  |
|                     | 200          | 48                 | 6              | 6000                 | 0.8                           | (0 - 2)   | 1.5                           | (0 - 2) | 47.9 ± 6.1  |
| Control<br>TEI-6720 | 0            | 72                 | 6              | 6000                 | 2.2                           | (1 - 3)   | 0.8                           | (0 - 2) | 48.8 ± 4.0  |
|                     | 50           | 72                 | 6              | 6000                 | 1.3                           | (0 - 3)   | 1.2                           | (0 - 3) | 50.2 ± 5.7  |
|                     | 100          | 72                 | 6              | 6000                 | 1.7                           | (1 - 2)   | 1.8                           | (0 - 4) | 46.7 ± 3.5  |
|                     | 200          | 72                 | 6              | 6000                 | 1.0                           | (0 - 3)   | 1.3                           | (0 - 2) | 46.7 ± 4.6  |
| MMC <sup>c)</sup>   | 1.0          | 24                 | 6              | 6000                 | 33.3*                         | (31 - 39) | 2.5                           | (0 - 5) | 49.4 ± 3.3  |
|                     | 2.0          | 24                 | 6              | 6000                 | 38.7*                         | (32 - 48) | 2.2                           | (0 - 4) | 47.7 ± 5.5  |
| Historical data     |              |                    | 354            | 354000               | 1.98                          | (0 - 9)   | 1.18                          | (0 - 5) | 50.0 ± 6.07 |

a) MNPCEs/1000PCEs : micronucleated PCEs per 1000 PCEs ( PCE : polychromatic erythrocyte )  
 b) MNWCEs/1000WCEs : micronucleated WCEs per 1000 WCEs ( WCE : nonchromatic erythrocyte )  
 c) MMC : mitomycin C

Control : 0.5% Methyl Cellulose

\* : significant difference from the historical control data ( p < 0.05 )

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Study title: Study on in vivo unscheduled DNA synthesis (UDS) in rat hepatocytes treated with TEI-6720

Key findings: TMX-67 was negative in the UDS test in vivo.

Study no.: 5L363

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Conducting laboratory and location: \_\_\_\_\_

b(4)

Date of study initiation: July 10, 1995

GLP compliance: Yes

QA reports: yes ( x ) no ( )

Drug, lot # 50729, and % purity: 99.4%

## Methods

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Strains/species/cell line: Eight week old male Fisher F344/Du rats were used in the study. The body weight of animals was 202-224 g at the beginning of study.

Doses used in definitive study: the experiment was conducted at 120 and 600 mg/kg/oral given as a single dose by oral gavage. The drug substance was suspended in 0.5% methylcellulose.

Basis of dose selection: A dose finding study was conducted at 150, 300 and 600 mg/kg. The sponsor stated that analysis of the dosing solution indicated that the actual doses were 139, 272 and 522 mg/kg, respectively. The drug substance was administered by oral gavage. Each group had 3 animals. General conditions and viability were observed for next three days. No mortality was observed in the treated rats. The sponsor selected 600 mg/kg as the highest dose because the basis of the maximum homogeneity achieved with the 60 mg/ml stock suspensions.

Negative controls: Untreated animals

Positive controls: DMN (dinitro-nitrosamine) was used as a positive control at 10 mg/kg/oral.

Incubation and sampling times: Rats were sacrificed at 2 and 16 hours after the treatment and hepatocytes from the liver were prepared after treating the liver with collagenase. The proportion of viable cells was determined by the trypan blue exclusion technique. Cells were suspended in William's medium at  $5 \times 10^5$  cells/ml. Cell suspensions were incubated with  $^3\text{H}$ -Thymidine for 4 hours. Hepatocytes were fixed with glacial acetic acid and ethanol (1:3) and air dried on the slide. Slides with hepatocytes were soaked with photographic emulsion for one week in dark for autoradiography. The grain number was determined under optical microscope. Nucleus of the cell was stained with hematoxylin to identify increase in grain numbers per cell. Number of grains in the nucleus of the cell and the number of grains in the cytoplasm were determined. The Difference was considered to be net gain per cell.

Fifty cells per animals were observed and mean net grain per animal was calculated.

The study design showed each group had three rats.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The mean net grain for each group of animals was determined. The result was considered to be positive if one or more groups showed a net grain of 5 or more. In the event if all groups show net grain less than 5, the effect was considered to be negative.