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RESEARCH**

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 200796

Submission receipt date: 4/27/2010

Drug: azilsartan medoximil

Applicant: Takeda Pharmaceuticals North America

Indication: Hypertension

Reviewing Division: Division of Cardiovascular and Renal Products

Comments: The pharm/tox reviewers and supervisor found the nonclinical information submitted for azilsartan medoximil to be sufficient to support the use indicated above.

The proposed pregnancy section of labeling states that azilsartan medoximil is Pregnancy Category C (first trimester) and D (second and third trimesters). This appears appropriate because it is consistent with other drugs of this class (angiotensin receptor blockers).

The carcinogenicity of azilsartan medoximil and its primary metabolite (TAK-536 MII) was assessed in two-year studies in rats and six-month studies in transgenic Tg.rasH2 mice. The Executive Carcinogenicity Assessment Committee found these studies to be acceptable. The committee concluded that there were no drug-related tumors in any of these studies.

This drug moiety is an angiotensin II receptor blocker. There are several other approved members of this pharmacologic class. Most use the term "angiotensin II receptor blocker" in labeling as the Established Pharmacologic Class text phrase.

Conclusion:

I concur with the Division pharm/tox conclusion that the nonclinical data support approval of this NDA.

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/s/

PAUL C BROWN
02/24/2011

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA 200796
Supporting document/s:
Applicant's letter date: 4/22/2010
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Product: Edarbi™ (azilsartan medoximil; TAK-491)
Indication: Hypertension
Applicant: Takeda Pharmaceuticals North America
Review Division: CardioRenal Products DCRP
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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	3
1.1	RECOMMENDATIONS	3
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	4
2	DRUG INFORMATION	9
3	STUDIES SUBMITTED.....	12
4	PHARMACOLOGY OF TAK-491	13
4.1	PRIMARY PHARMACOLOGY	13
4.2	SECONDARY PHARMACOLOGY	19
4.3	SAFETY PHARMACOLOGY	24
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	30
5.1	PK/ADME.....	30
5.2	TOXICOKINETICS	48
6	GENERAL TOXICOLOGY.....	48
6.1	SINGLE-DOSE TOXICITY	48
6.2	REPEAT-DOSE TOXICITY	48
7	GENETIC TOXICOLOGY	72
8	CARCINOGENICITY	80
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	100
9.1	FERTILITY AND EARLY EMBRYONIC DEVELOPMENT	100
9.2	EMBRYONIC FETAL DEVELOPMENT	105
9.3	PRENATAL AND POSTNATAL DEVELOPMENT	113
10	SPECIAL TOXICOLOGY STUDIES.....	121
11	INTEGRATED SUMMARY AND SAFETY EVALUATION.....	160
12	APPENDIX/ATTACHMENTS	173

1 Executive Summary

1.1 Recommendations

No specific recommendations other than the proposed labeling changes are necessary.

1.1.1 Approvability

The new drug application for azilsartan medoxomil is approvable from a Pharmacology and Toxicology perspective following recommended changes in labeling (see below).

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

1.1.3.1 Under Section 13 Nonclinical Toxicology, subsection 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility: *Mutagenesis*.

[Sponsor's version]:

(b) (4)

The labeling should reflect the positive findings in the Chinese Hamster Lung Cytogenetic Assay.

Reviewer's proposed change:

Mutagenesis: Azilsartan medoxomil, azilsartan, and M-II were positive for structural aberrations in the Chinese Hamster Lung Cytogenetic Assay. Structural chromosomal aberrations were observed with the prodrug, azilsartan medoxomil without metabolic activation. The active moiety, azilsartan (TAK-536) was also positive in this assay both with and without metabolic activation. The major human metabolite, TAK-536 M-II was also positive in this assay during a 24 hr assay without metabolic activation.

Azilsartan medoxomil, azilsartan, and M-II were devoid of genotoxic potential in the Ames reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*, the *in vitro* Chinese hamster ovary cell chromosomal aberration assay, the *in vitro* mouse lymphoma (tk) gene mutation test, the *ex vivo* unscheduled DNA synthesis test, and the *in vivo* mouse and/or rat bone marrow micronucleus assay.

1.1.3.2 Under Section 13 Nonclinical Toxicology, subsection 13.2 Animal Toxicology and/or Pharmacology: *Reproductive Toxicology*

[Sponsor's version]:

(b) (4)

Reviewer's proposed change:

13.2 Animal Toxicology and/or Pharmacology

Reproductive Toxicology: In peri- and postnatal rat development studies, adverse effects on pup viability, delayed incisor eruption and dilatation of the renal pelvis along with hydronephrosis were seen when azilsartan medoxomil was administered to pregnant and nursing rats at 1.2 times the MRHD on a mg/m² basis. Reproductive toxicity studies indicated that azilsartan medoxomil was not teratogenic when administered to pregnant rats at oral doses up to 1000 mg /kg/day (122 times the MRHD on a mg/m² basis) or up to 50 mg /kg/day to pregnant rabbits (12 times the MRHD on a mg/m² basis). M-II also was not teratogenic in rats or rabbits at doses up to 3000 mg M-II/kg/day. Azilsartan crossed the placenta and was found in the fetuses of pregnant rats and was excreted into the milk of lactating rats.

1.2 Brief Discussion of Nonclinical Findings

Pharmacodynamics

Azilsartan medoxomil (TAK-491) is the pro-drug for TAK-536, a competitive reversible antagonist at angiotensin II (All) receptors (AT1). TAK-491 is hydrolyzed rapidly to TAK-536, by the action of aryl esterase primarily in the gastrointestinal tract and/or plasma, during absorption after oral administration. TAK-536 produces a dose-dependent decrease in arterial blood pressure in a variety of hypertensive animal models such as Spontaneously Hypertensive Rats (SHR, 0.1 to 1 mg/kg, p.o.) and renal hypertensive dogs (0.1 to 1 mg/kg, p.o.). It blocks the pressor effect of angiotensin II in rats (ID₅₀ = 0.12 mg/kg, p.o.) and has an antiproteinuric effect in rats with overt nephropathy (1 to 10 mg/kg, p.o.).

TAK-536 bound tightly to human receptors (hAT1) with an IC₅₀ value of 0.62 nmol/L. The binding affinity of TAK-536 to hAT1 was approximately 2-fold and 30-fold greater than that of olmesartan and All, respectively. TAK-536 selectively inhibited the binding of radiolabeled substrate to AT1 but not AT2 receptors with >10,000-fold selectivity. Scatchard plot analysis indicated that TAK-536 did not change the maximal number of binding sites (Bmax) in either the rabbit aorta or bovine adrenal cortex, suggesting that TAK-536 acts as a competitive antagonist in these preparations.

TAK-536 selectively and potently inhibited the vasoconstriction produced by AII, in isolated rabbit aortic preparations, but had no effect on the constriction produced by KCl, norepinephrine, serotonin, endothelin or prostaglandin F₂α.

The effect of repeated doses of TAK-491 on systolic blood pressure (measured by tail cuff method) was assessed in conscious SHR after oral administration of 0.1, 0.3 or 1.0 mg/kg/day for 14 days. Recovery of the pharmacological effect of TAK-491 was examined during a 14-day treatment withdrawal period. The oral administration of TAK-491 for 2 weeks significantly reduced blood pressure in a dose related manner. The minimally effective dose of TAK-491 was 0.1 mg/kg. Heart rate was not significantly affected. The antihypertensive effect of TAK-491 disappeared gradually after cessation of treatment; without rebound hypertensive effects.

In renal hypertensive dogs, with baseline systolic blood pressure over 200 mmHg, orally administered TAK-491, at doses of 0.1, 0.3 or 1 mg/kg, lowered systolic blood pressure in a dose-related manner without causing reflex tachycardia. The antihypertensive effect of TAK-491 at 1 mg/kg persisted for 24 hours.

Secondary pharmacologic effects were observed in studies examining binding to a wide spectrum of receptors, channels and enzymes (see Section 4.2). These effects were generally seen at much higher concentrations of free (non protein bound) compound than would be expected clinically, and are probably not of clinical relevance.

In GLP Safety Pharmacology studies, TAK-491 did not adversely affect CNS function in rats (up to 2000 mg/kg, p.o.), respiratory function in rats (up to 2000 mg/kg, p.o.) or cardiovascular (ECG, HR, QTc) parameters in conscious dogs given oral doses up to 300 mg/kg by gavage (other than the expected lowering of arterial blood pressure). *In vitro* assay for hERG channel current effects did not reveal potential for inhibition by TAK-536 or the primary human metabolite TAK-536 M-II.

PK/ADME

The absorption, distribution, metabolism, and excretion of TAK-491 were studied in rats, dogs, and monkeys following administration of both [¹⁴C]TAK-491 or [¹⁴C]TAK-536. TAK-491 was rapidly hydrolyzed by gut and plasma aryl esterase to form the active moiety TAK-536 *in vivo* after oral administration of [¹⁴C]TAK-491.

The systemic bioavailability of TAK-536, after oral dosing of TAK-491, was estimated to be 12.4% in rats and 53.9% in dogs. The respective bioavailability of oral TAK-536 in fed rats and dogs was 14.6% and 24.3% and was increased to 40.9% (rats) and 39.2% (dogs) when animals were fasted. In fasted animals, drug half-lives were approximately 5.2 hours in rats, 2.8 hours in dogs, and 1.4 hours in monkeys. C_{max} was achieved at 0.7 hours in rats and dogs and 1.7 hours in monkeys after oral dosing under fasted conditions.

Following oral administration of [¹⁴C]TAK-536 to rats, total radioactivity was distributed widely to tissues, with relatively high concentrations in the liver.

[¹⁴C]TAK-491 and its related compounds were highly bound to plasma protein (>99.9% in rats and >97.1% in dogs) and poorly distributed into blood cells (<2% in rats and <3% in dogs) after single oral administration to rats and dogs. [¹⁴C]TAK-536 exhibited high *in vitro* plasma/serum protein binding in plasma of mice (99.8%), rats (≥99.8%), dogs (≥98.8%), and humans (99.5%), and protein binding was broadly concentration-independent in all species.

After a single oral administration of [¹⁴C]TAK-491 to pregnant rats, radioactivity was gradually transferred to the fetuses via the placenta. Following administration of [¹⁴C]TAK-491 to lactating rats, plasma radioactivity was distributed into milk. The majority of the radioactivity in the plasma and milk was from TAK-536.

The biotransformation of [¹⁴C]TAK-491 was investigated *in vitro* in rats, dogs, and humans and *in vivo* in rats and dogs. Most of the radioactivity in plasma was as the active moiety TAK-536. *In vitro*, TAK-491 was rapidly hydrolyzed to TAK-536 in the plasma from rats, dogs, monkeys, and humans and in the incubation mixture with human hepatic and intestinal S9 fractions. TAK-536 was either further decarboxylated to form the pharmacologically inactive metabolite, TAK-536 M-I, primarily by CYP2C8, or was O-dealkylated to form another pharmacologically inactive metabolite, TAK-536 M-II, primarily by CYP2C9. Incubation of [¹⁴C]TAK-536 with microsomes expressing human CYP isoforms indicated that CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 all were capable of metabolizing TAK-536. All human Phase I metabolites of TAK-536 were present in rat and dog excreta.

Both TAK-491 and TAK-536 have low potency to induce CYP3A4 activity *in vitro*. TAK-491 inhibited some CYP isoforms *in vitro*, but because it is converted rapidly to TAK-536 *in vivo* during the absorption phase, this activity is unlikely to be clinically significant. TAK-536 itself had no inhibitory activity (IC₅₀ values >100 μmol/L) against human hepatic CYP isoforms. Based on *in vitro* studies, TAK-491 and TAK-536 are unlikely to have clinically significant potential for drug-drug interactions at anticipated clinical exposures.

The biotransformation of TAK-536 was investigated *in vitro* in mice, rats, dogs, monkeys, and humans and both TAK-536 M-I and TAK-536 M-II was formed. All metabolites formed by human hepatic microsomes were also formed by hepatic microsomes from animal species.

Following oral administration of [¹⁴C]TAK-491 to rats and dogs, TAK-536 was the main component in plasma (93.7% and 90.0%, respectively). TAK-536 was metabolized and then excreted mainly into feces. In a 0-120 hour postdose period, 95.0% and 96.8% of the dosed radioactivity were recovered in rat and dog feces, respectively. Only 2% and 5.4% of the dosed radioactivity were excreted in rat and dog urine, respectively in the same time collection period. More than 80% of the radioactivity in rat and dog feces was

TAK-536 M-I. TAK-536 M-II, a major metabolite in humans, was a minor metabolite in animals.

Following oral administration of [¹⁴C]TAK-536 to rats, dogs, and monkeys only a small amount of unchanged TAK-536 was detected in urine and feces indicating that TAK-536 is almost completely metabolized before being eliminated. In biliary-cannulated rats, 22.2% and 32.4% of total ¹⁴C in bile was composed of unchanged TAK-536 and TAK-536 M-I, respectively.

Toxicology

The toxicology program for TAK-491 included assessment of this compound and/or the active moiety, TAK-536, in preliminary and definitive single (rat, ≤ 2000 mg/kg; dog ≤ 30 mg/kg) and repeat-dose toxicity studies in rats (up to 26 weeks, ≤ 2000 mg/kg) and dogs (up to 26 weeks with TAK-491 (≤ 60 mg/kg) and up to 52 weeks with TAK-536 (≤ 300 mg/kg), rodent carcinogenicity studies, genotoxicity studies, and reproduction and developmental toxicity studies. Recovery from changes occurring after 4 weeks of dosing TAK-491 was examined in dogs. The major human metabolite, TAK-536 M-II, was examined in rat and dog repeat-dose toxicity studies (up to 13 weeks in duration), in 6-month transgenic mouse and 2-year rat carcinogenicity assays, and in genotoxicity and reproduction/developmental studies. A full listing of all Toxicology studies conducted is presented in the Appendix. NOAELs, AUCs and human exposure ratios for pivotal repeat-dose toxicity studies are presented in 2 Tables (based on AUC for TAK-536 and for TAK-536 M-II) at the end of the Integrated Summary and Safety Evaluation.

Toxicologic findings of dark red foci with corresponding observation of erosion in the glandular stomach were noted in the 26-week rat study with azilsartan medoxomil. Ulceration in various sections of the GI tract was also seen in dogs.

Renal histopathological findings of hypertrophy of the juxtaglomerular cells were also observed in this 26-week study in rats with azilsartan medoxomil (TAK-491) and azilsartan (TAK-536) in dogs at 26 and 52 weeks. Again, these effects are consistent with the pharmacological effect of blocking angiotensin II receptors chronically. These renal effects are also commonly seen with other ARB's including olmesartan and valsartan, and with ACE inhibitors.

Histopathological effects were observed in the adrenal gland in this 26-week study. Minimal or mild atrophy of the zona glomerulosa was observed in 7/15 females in the 2 mg/kg group, 14/15 males and 15/15 females in the 20 mg/kg group and 15/15 animals in both sexes in the 200 and 2000 mg/kg groups. Again, these effects appear in many toxicology studies using ARB's and ACE inhibitors.

The renal and adrenal effects in rats, and renal effects in dogs, occur at AUC levels equal to or lower than human exposure at the MRHD of 80 mg, whereas the GI effects occur at approximately 20-fold higher AUC in rats and 4 to 5-fold higher AUC in dogs. Olmesartan, the other angiotension receptor blocker (ARB) that has a medoxomil side

chain, produced ulceration in the stomach of dogs in a 3-month repeat dose toxicity study. This toxicity is a known class effect of ARB's and ACE inhibitors.

These effects on the kidney and adrenal gland may be pharmacologically mediated and a response to prolonged antagonism of the Renin-Angiotensin-Aldosterone system. In 13-week repeat-dose toxicity studies in rats, using the metabolite TAK-536 M-II, the toxicity of the kidney, adrenal gland and stomach were not observed. NOAELs of 300 mg/kg/day (male) and 3000 mg/kg/day (female) were reported. Similarly, GI, renal and adrenal effects were also not observed in 13-week studies of this metabolite in dogs, where NOAELs of 2000 mg/kg/day were reported. These findings again suggest that the majority of the observed toxicities with TAK-491 and TAK-536 may be pharmacologically mediated as TAK-536 M-II is relatively devoid of pharmacological activity.

Genetic toxicology

Structural chromosomal aberrations were observed in the Chinese Hamster Lung Cytogenetic Assay with the prodrug, azilsartan medoxomil (TAK-491) without metabolic activation. The active moiety, azilsartan (TAK-536) was also positive in this assay both with and without metabolic activation. Finally, the major human metabolite, TAK-536 M-II was also positive in this assay during a 24 hr assay without metabolic activation. Other genetic toxicity assays were negative. These findings should be addressed in labeling, as proposed above.

Carcinogenicity

Both TAK-491 and TAK-536 M-II (the major metabolite in human) were separately evaluated for carcinogenicity in 26-week Tg.rasH2 mouse studies or in 24-month rat studies. TAK-536 was evaluated in 24-month studies in both rat and mouse. All dose selections were evaluated and approved by the Executive CAC, and no evidence of carcinogenicity was apparent in any of the studies.

TAK-491 was not carcinogenic at the highest oral gavage dose of 450 mg/kg/day (MTD basis) in a 26-week Tg.rasH2 mouse study or at the highest oral gavage dose of 600 mg/kg/day (MTD basis) in a 24-month rat study. Exposure ratios, as measured by TAK-536, were 7X and 17X human exposure, in male and female mice, respectively. In rats, human exposure ratios were 25X and 28X, in male and female rats, respectively. These exposure margins are based on a MRHD of 80 mg/day of TAK-491.

Similarly, dosing with azilsartan (TAK-536) was also not carcinogenic at the highest dietary dose of 100 mg/kg/day (MTD basis) in a 24-month mouse study or at the highest dietary dose of 300 mg/kg/day (MTD basis) in a 24-month rat study. These findings are discussed further in the Toxicology summary, but are not relevant to approval of TAK-491, as actual exposures to TAK-536 were higher in the above TAK-491 studies.

Finally, TAK-536 M-II was not carcinogenic at the highest dietary concentration of 5% (MFD basis) in a 26-week Tg.rasH2 mouse study, or at the highest oral gavage doses of 1000 mg/kg/day (MTD, male) or 3000 mg/kg/day (MFD, female) in a 24-month rat study. Exposure margins were 21X and 38X for male and female mice, and 7.4X for both male and female rats, respectively, relative to human AUC for TAK-536 M-II at the MRHD of 80 mg/day of TAK-491.

Reproductive Toxicology

In the rat and rabbit embryo-fetal development studies, there were minor variations in fetal development observed in both species at the highest dose administered [1000 mg/kg/day in rats (122 x MRHD) and 50 mg/kg/day (12 x MRHD) in rabbits] where maternal toxicity (such as decrease in body weight and food consumption) was evident, even at the lowest dose administered (10 mg/kg/day). The relevance of these fetal effects in the context of maternal toxicity is unclear.

There were adverse effects seen on pup viability in the peri- and postnatal rat development studies at a dose 1.2X the MRHD on a mg/m² basis. In addition, delayed incisor eruption and dilatation of the renal pelvis along with hydronephrosis was also observed at the lowest (10 mg/kg/day) dose. This dose also produced maternal toxicity (decrease in body weight and food consumption). Use of TAK-491 is clearly contraindicated in pregnancy as are all members of this drug class.

Summary and Conclusions

In conclusion, azilsartan medoxomil does not appear to have any major unique toxicities compared to other ARB's currently approved by the FDA. The observed toxicity targets were the kidney (juxtaglomerular hypertrophy), adrenal gland (atrophy of the zona glomerulosa) and GI tract (gastric erosion). Olmesartan medoxomil, the only other approved ARB with a medoxomil sidechain, has similar toxicities. Tasosartan (not approved) and losartan also exhibited similar toxicologic profiles. The renal and adrenal effects are clearly related to the primary pharmacology and are consistently seen with drugs affecting the RAAS.

2 Drug Information

2.1 Drug

Edarbi™

2.1.1 CAS Registry Number

863031-24-7

2.1.2 Generic Name

Azilsartan medoximil potassium

2.1.3 Code Name

TAK-491

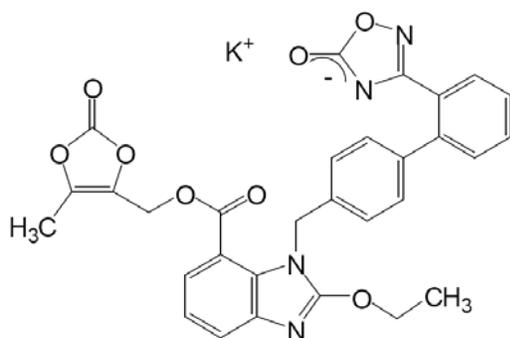
2.1.4 Chemical Name

(5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 2-ethoxy-1-[[2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl]1-*H*-benzimidazole-7-carboxylate monopotassium salt

2.1.5 Molecular Formula/Molecular Weight

C₃₀H₂₃KN₄O₈; 606.62

2.1.6 Structure



2.1.7 Pharmacologic class

Angiotensin II A1 receptor antagonist

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 71,867 (TAK-491); IND (b) (4) (TAK-536); (b) (4) (TAK-491/chlorthalidone)
DMF (b) (4)

2.3 Clinical Formulation

Table 1. Composition of TAK-491 Tablets

Component	Reference to Quality Standards	Function	Quantity per Tablet (mg)		
			20 mg tablets	40 mg tablets	80 mg tablets
TAK-491 ⁽¹⁾ (As the free acid)	In-house standard	Active ingredient	(b) (4)	42.68 ⁽¹⁾ (40)	85.36 ⁽¹⁾ (80)
Mannitol	Ph.Eur., USP	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Fumaric Acid	NF				
Sodium Hydroxide	Ph.Eur., NF				
Hydroxypropyl cellulose	Ph.Eur., NF				
Croscarmellose sodium	Ph.Eur., NF				
Microcrystalline cellulose (b) (4)	Ph.Eur., NF				
Magnesium stearate (b) (4)	Ph.Eur., NF				
	Ph.Eur., USP				
<i>Tablet weight</i>					

2.3.1 Drug Formulation

See above

2.3.2 Comments on Novel Excipients

None.

2.3.3 Comments on Impurities/Degradants of Concern

(b) (4), an impurity formed in the process of synthesizing the prodrug, azilsartan medoxomil, was tested for toxicity and none was found.

2.4 Proposed Clinical Population and Dosing Regimen

The proposed indication for azilsartan medoxomil is for the treatment of hypertension, either alone or in combination with other antihypertensive agents. The recommended starting dose in adults is 40 mg taken once daily. The dose may be increased to a maximum of 80 mg once daily when additional blood pressure reduction is required.

2.5 Regulatory Background

Azilsartan medoxomil (TAK-491) is the prodrug of the active moiety azilsartan (TAK-536) which is a potent and selective competitive reversible antagonist at angiotensin II (AT1) receptors. (b) (4)

(b) (4) This drug has a human metabolite named TAK-536 M-II that is not generated to any great extent in the rat or dog. A number of studies, including additional genotoxicity, reproductive toxicity and carcinogenicity studies have been performed to qualify this metabolite.

3 Studies Submitted

A comprehensive listing of submitted studies is provided in the Appendix.

3.1 Studies Reviewed

For azilsartan medoxomil (**TAK-491**), the prodrug:

Pharmacodynamics/ Pharmacokinetics

- a. Effects Related to the Therapeutic Indication
- b. Ancillary Pharmacodynamic Studies
- c. Safety Pharmacology
- d. Absorption and Pharmacokinetics
- e. Distribution
- f. Metabolism
- g. Excretion

Toxicity Studies

- h. Single Dose Toxicity
- i. 4-Week Repeated Oral (Gavage) Dose Toxicity Study in Rats
- j. 4-Week Repeated Oral (Gavage) Dose Toxicity Study in Rabbits
- k. 26-Week Repeated Oral (Gavage) Dose Toxicity Study in Rats
- l. 52-Week Repeated Oral (Gavage) Dose Toxicity Study in Dogs (TAK-536)
- m. Ames Test
- n. Chinese Hamster Lung Cytogenetic Assay (Chromosomal Aberration)
- o. Unscheduled DNA Synthesis Assay
- p. Mouse Micronucleus Assay
- q. Rat Micronucleus Assay
- r. Two-Year Rat Carcinogenicity Study
- s. 26-Week transgenic ras-Mouse Carcinogenicity Study
- t. Fertility and Early Embryonic Development Study in Rats
- u. Embryofetal Development Study in Rats.
- v. Embryofetal Development Study in Rabbits
- w. Study for Effects of TAK-491 on Pre- and Postnatal Development, Including Maternal Function, in Rats

For **TAK-536 M-II**, the major human metabolite:

Toxicity Studies

- a. 13-Week Repeated Oral (Gavage) Dose Toxicity Study in Rats

- b. 13-Week Repeated Oral (Gavage) Dose Toxicity Study in Dogs
- c. Ames Test
- d. Chinese Hamster Lung (CHL) Cytogenetic Assay
- e. Two-Year Rat Carcinogenicity Study
- f. 26-Week transgenic ras-Mouse Carcinogenicity Study
- g. Fertility and Early Embryonic Development Study in Rats
- h. Embryofetal Development Study in Rats
- i. Embryofetal Development Study in Rabbits

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

Previous Pharmacology/Toxicology reviews, written by A. Proakis and D. Jensen under IND 71,867 are included in the body of the manuscript and identified where used.

4 Pharmacology of TAK-491

Terminology used:

TAK-491 (pro-drug) is the K⁺ salt of azilsartan medoximil.

TAK-491F is the free acid form of the pro-drug.

TAK-536 (azilsartan, is the major circulating active form)

TAK-536 MI is the major (relatively inactive) animal metabolite

TAK-536 M-II is the major (relatively inactive) human metabolite

4.1 Primary Pharmacology

Azilsartan medoxomil is a competitive reversible antagonist at angiotensin II (AT1) receptors (IC_{50} = 0.62 to 2.6 nmol/L). It produces a dose-dependent decrease in arterial blood pressure in a variety of hypertensive animal models such as SHR (0.1 to 1 mg/kg, p.o.) and renal hypertensive dogs (0.1 to 1 mg/kg, p.o.). It blocks the pressor effect of angiotensin II in rats (ID_{50} = 0.12 mg/kg, p.o.) and has an antiproteinuric effect in rats with overt nephropathy (1 to 10 mg/kg, p.o.).

4.1.1 *In Vitro* Studies

The following is excerpted from the sponsor's submission:

TAK-491 is a prodrug that is hydrolyzed rapidly to TAK-536, primarily in the gastrointestinal tract and/or plasma, during absorption after oral administration. Since TAK-491 has never been detected in plasma after oral administration to humans and

TAK-536 is the active moiety producing antihypertensive effects, the *in vitro* studies described below were conducted with TAK-536.

4.1.1.1 Specific Binding of TAK-536 to Angiotensin AT1 Receptors

4.1.1.1.1 Inhibitory Effects of TAK-536 on the Specific Binding of [¹²⁵I]-Sar¹-Ile⁸-All to Human Angiotensin AT1 Receptors, Report Number TAK-491-00053-001R

The binding of TAK-536 to human AT1 (hAT1) receptors was evaluated by measuring its inhibitory effects on the specific binding of [¹²⁵I]-Sar¹-Ile⁸-All to hAT1 receptors coated on membranes. Various concentrations of TAK-536, olmesartan, and All were examined. IC₅₀ values were determined by non-linear logistic regression analysis.

IC₅₀ Values for TAK-536, Olmesartan, and All for the Specific Binding of [¹²⁵I]-Sar¹-Ile⁸-All to Human AT1 Receptors

Compound	Mean IC ₅₀ Value (nmol/L) (a)
TAK-536	0.62 (0.50-0.78)
Olmesartan	1.2 (0.88-1.5)
All	20 (10-35)

(a) Numbers in parentheses indicate the 95% confidence intervals of the IC₅₀ values (triplicate determinations).

TAK-536 bound tightly to hAT1 receptors with an IC₅₀ value of 0.62 nmol/L. The binding affinity of TAK-536 to hAT1 was approximately 2-fold and 30-fold greater than that of olmesartan and All, respectively.

4.1.1.1.2 Inhibitory Effects of TAK-536 on the Specific Binding of [¹²⁵I]All or [¹²⁵I]-Sar¹-Ile⁸-All to Rabbit and Bovine AT1 or AT2 Receptors, Report Number TAK-536-C-46-00057-002A

The binding and selectivity of TAK-536 to AT1 and AT2 receptors were examined using All receptors in rabbit and bovine tissue membrane preparations. Crude membrane preparations from freshly isolated rabbit aorta or bovine adrenal cortex were used as sources of AT1 receptors, and a membrane preparation of bovine cerebellum was used as the source of AT2 receptors. The inhibitory effect of TAK-536 on the binding of [¹²⁵I]All or [¹²⁵I]-Sar¹-Ile⁸-All to AT1 and AT2 receptors was compared to that of several positive controls, i.e., EXP3174 [the active metabolite of losartan (DuP753)], and PD123177 (a selective AT2 antagonist).

IC₅₀ Values for TAK-536, EXP3174, and PD123117 for the Binding of [¹²⁵I]All or [¹²⁵I]-Sar¹-Ile⁸-All to Rabbit and Bovine All Receptors

Compound	Rabbit Aorta AT1 Receptors IC50 Value (nmol/L)	Bovine Adrenal Cortex AT1 Receptors IC50 Value (nmol/L)	Bovine Cerebellum AT2 Receptors IC50 Value (nmol/L)
TAK-536	12	140	>10,000
EXP3174	43	220	>10,000
PD123177	>10,000	>10,000	300

As shown above, TAK-536 and EXP3174 selectively and potently inhibited the binding of radiolabeled substrate to AT1 but not AT2 receptors. In both AT1 binding experiments, the IC₅₀ values for TAK-536 were lower than those for EXP3174. As expected, PD123177 only bound to AT2 receptors.

The mode of AT1 receptor antagonistic action of TAK-536 was analyzed using Scatchard plot analysis by increasing the concentration of radiolabeled All in the assay mixture. TAK-536 did not change the maximal number of binding sites (B_{max}) in either the rabbit aorta or bovine adrenal cortex, suggesting that TAK-536 acts as a competitive antagonist in these preparations.

4.1.1.1.3 Slow Dissociation of TAK-536 from Human AT1 Receptors, Report Number TAK-536-10013

Using a method similar to that described above, the dissociation of compounds from AT1 receptors was determined for TAK-536 and compared to that of positive controls olmesartan, telmisartan, valsartan, and irbesartan. IC₅₀ values for each compound were evaluated after 90 minutes of incubation with AT1 receptors expressed in Chinese hamster ovary (CHO) cells with or without washout of the test compound from the media.

Inhibitory Effects of TAK-536 and Other AT1 Antagonists on the Specific Binding of [¹²⁵I]-Sar¹-Ile⁸-All to Human AT1 Receptor: Comparison Between Effects With and Without Washout

Compound	IC ₅₀ (95% confidence interval) (nmol/L)		Ratio (Washout/Without Washout)
	Without Washout	Washout	
TAK-536	2.6 (1.7-4.1)	7.4 (3.9-14.2)	3
Olmesartan	6.7 (3.8-10.8)	242.5 (91.0-1056.8)	36
Telmisartan	5.1 (3.0-8.1)	191.6 (124.1-303.2)	37
Valsartan	44.9 (30.5-64.7)	>10,000	>223
Irbesartan	15.8 (8.5-29.7)	>10,000	>635

Values show the mean concentrations of the test compound (nmol/L) required for 50% inhibition of the specific binding of [¹²⁵I]-Sar¹-Ile⁸-All (IC₅₀). The values are calculated from 4 independent experiments performed in duplicate. After the membranes were pre-incubated for 90 minutes with compounds, the wells were divided into 2 groups (with or without washout) and incubated with [¹²⁵I]-Sar¹-Ile⁸-All for 5 hours.

Pre-incubation of AT1 receptors with TAK-536 potently inhibited radiolabeled All binding to AT1 receptors; in this study the IC₅₀ value was 2.6 nmol/L. The inhibitory effect of TAK-536 on radiolabelled All binding was only slightly reduced after the compound was washed from the incubation medium (IC₅₀, 7.4 nmol/L). Olmesartan, telmisartan, valsartan, and irbesartan also potently inhibited the specific binding of [¹²⁵I]-Sar¹-Ile⁸-All, with IC₅₀ values of 6.7, 5.1, 44.9, and 15.8 nmol/L, respectively. However, these inhibitory effects were attenuated greatly after the compounds were washed from the medium with IC₅₀ values of 242.5, 191.6, >10,000, and >10,000 nmol/L, respectively. These results suggest that TAK-536 tightly binds to AT1 receptors and that it dissociates slowly from them.

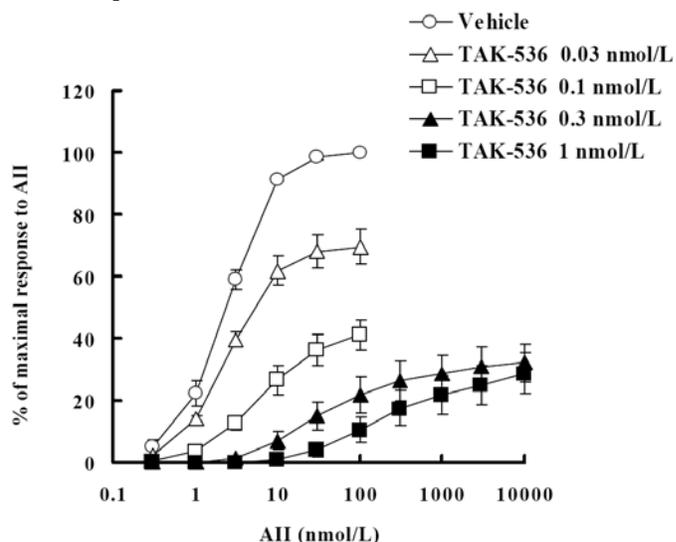
4.1.1.2 Inhibitory Effects of TAK-536 on All-Induced Contraction of Rabbit Aorta

4.1.1.2.1 Inhibitory Effects of TAK-536 on the All-Induced Contraction of Rabbit Aorta, Report Number TAK-491-00054-001R

The effects of TAK-536 on All-induced vessel contraction were examined using isolated rabbit aortic preparations. Thoracic aorta strips from Japanese white male rabbits were mounted at a resting tension of 2 grams in organ baths containing a Krebs-Henseleit solution. Contractile tension was amplified and recorded on a polygraph.

After control concentration-contractile response curves for All were obtained, and after repeated tissue washout, tissues were incubated with TAK-536, olmesartan, or vehicle [0.1% dimethyl sulfoxide (DMSO)] for 90 minutes. Concentration-response curves for All were then re-determined in the presence of the test compounds or vehicle. TAK-536 potently inhibited All-induced constriction of aortic strips in a concentration dependent fashion and was as potent as olmesartan (the logarithm of the molar concentration required to produce 50% of the maximal obtainable effect [pD'2] values for both compounds was 9.9; data not shown for olmesartan).

Inhibitory Effects of TAK-536 on All-Induced Contraction of Rabbit Aorta



Mean±SEM.

To determine the selectivity of TAK-536 for All-induced effects, aortic strip constriction in response to potassium chloride (KCl) (60 mmol/L), norepinephrine (1 μ mol/L), serotonin (1 μ mol/L), or prostaglandin F₂ α (2 μ mol/L) was measured in the presence and absence of TAK-536 (10 μ mol/L). TAK-536 had no effect on the activity of these vasoconstrictors.

Thus, TAK-536 selectively and potently inhibited the vasoconstriction produced by All, but had no effect on the constriction produced by KCl, norepinephrine, serotonin, or prostaglandin F₂ α .

4.1.1.2.2 Inhibitory Effects on All-Induced Contraction of Rabbit Aorta, Report Number TAK-536-C-46-00057-002A

In another experiment using a protocol similar to the above study, the inhibitory effects of TAK-536 on All-induced contraction of rabbit aorta were evaluated and compared with those of losartan (DuP753) and its active metabolite, EXP3174. The results of these studies indicate that TAK-536 was more potent than losartan and EXP3174 in inhibiting aorta contractions produced by All; relative pD'₂ or pA₂ (logarithm of the dissociation constant K_b when the slope of the Schild's plot is exactly 1) values were 9.93, 8.25, and 8.95, respectively. In addition, TAK-536 (10 μ mol/L) had no effect on aortic contractions in response to KCl, norepinephrine, serotonin, prostaglandin F₂ α , or endothelin, demonstrating the selectivity of TAK-536 for inhibiting the effects of All in this model.

4.1.1.3 Binding of TAK-536 Metabolites to Angiotensin AT1 receptors

4.1.1.3.1 *Inhibitory Effects of TAK-536 M-I and TAK-536 M-II on the Specific Binding of [¹²⁵I]-Sar¹-Ile⁸-All to the hAT1 Receptors, Report Number TAK-491-00138*

This study investigated the binding potency of TAK-536 M-I and TAK-536 M-II to AT1 receptors and compared their potencies to that of TAK-536 using the same assay as that described in Section 4.1.1.1. TAK-536 M-I and TAK-536 M-II demonstrated weak inhibition of the specific binding of [¹²⁵I]-Sar¹-Ile⁸-All to hAT1 receptors, with IC₅₀ values of 2300 nmol/L and 1100 nmol/L, respectively. In contrast, the IC₅₀ value for TAK-536 was 1.3 nmol/L. These results indicate that TAK-536 M-I and TAK-536 M-II are approximately 1770 and 850 times less potent than TAK-536, respectively, in binding to hAT1 receptors.

4.1.1.3.2 *Inhibitory Effects of TAK-536 M-I on the Specific Binding of [¹²⁵I]All to Bovine AT1 Receptors, Report Number TAK-536-C-46-00171*

The inhibitory effect of TAK-536 M-I on the specific binding of [¹²⁵I]All to AT1 receptors in bovine adrenal cortex membrane preparations was compared to that of TAK-536. TAK-536 M-I produced a slight (4%) inhibition of binding at 1000 nmol/L (ie, IC₅₀ was much greater than 1000 nmol/L) whereas the IC₅₀ value for TAK-536 was 140 nmol/L, indicating, again, that TAK-536 M-I is a much weaker AT1 receptor antagonist than TAK-536.

4.1.2 *In Vivo* Studies

4.1.2.1 *Effects Related to Therapeutic Indication*

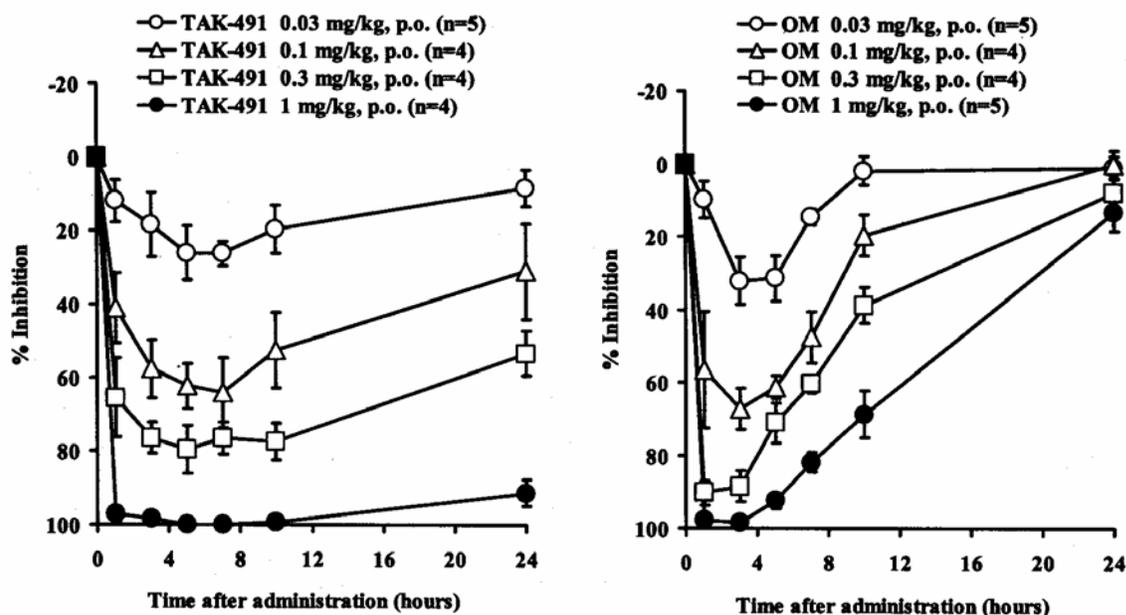
4.1.2.1.1 *Inhibitory Effects of TAK-491 on Angiotensin II-Induced Pressor Response in Conscious Rats*

From review by A. Proakis, 8/2/05

The pressor response produced by an IV infusion of angiotensin II was assessed in conscious rats after oral treatment with TAK-491 and compared to the reference angiotensin II antagonist olmesartan (OM). Pressor responses were induced by the IV administration of angiotensin II (100 ng/kg) before and after oral (gavage) administration of TAK-491 or OM at doses of 0.03 to 1 mg/kg. Blood pressures were measured directly from an indwelling catheters placed in femoral arteries.

Oral administration of TAK-491 inhibited angiotensin II-induced pressure response in a dose-related manner (Figure 1). The dose that produced 50% inhibition (ID₅₀) for TAK-491 (0.12 mg/kg) was approximately 4 times lower than that of OM (0.55 mg/kg). Also, the inhibitory effect of TAK-491 persisted longer than that of olmesartan.

Figure 1. Inhibitory Effects of TAK-491 or Olmesartan on Angiotensin II-Induced Pressor Responses

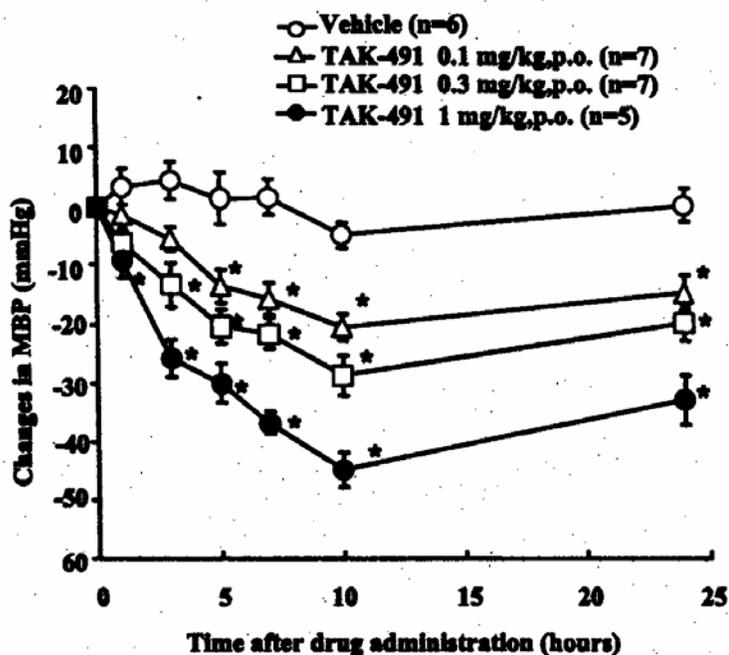


4.1.2.1.2 Antihypertensive Effects of Oral TAK-491 in Spontaneous Hypertensive Rats (SHR)

From review by A. Proakis, 8/2/05

The antihypertensive effects of orally administered TAK-491 were assessed in spontaneously hypertensive rats (SHR). TAK-491 doses of 0.1, 0.3 or 1 mg/kg were administered orally to SHR and blood pressures were measured for 24 hours postdose directly from indwelling arterial cannulas. Heart rates were obtained from the blood pressure pulse. TAK-491 produced dose-dependent reductions in mean arterial blood pressure without significant effects on heart rate (Figures 2-3). The reductions in blood pressure with these doses persisted for 24 hours postdose.

Figure 2. Effect of Oral TAK-491 on Mean Blood Pressure in SHR

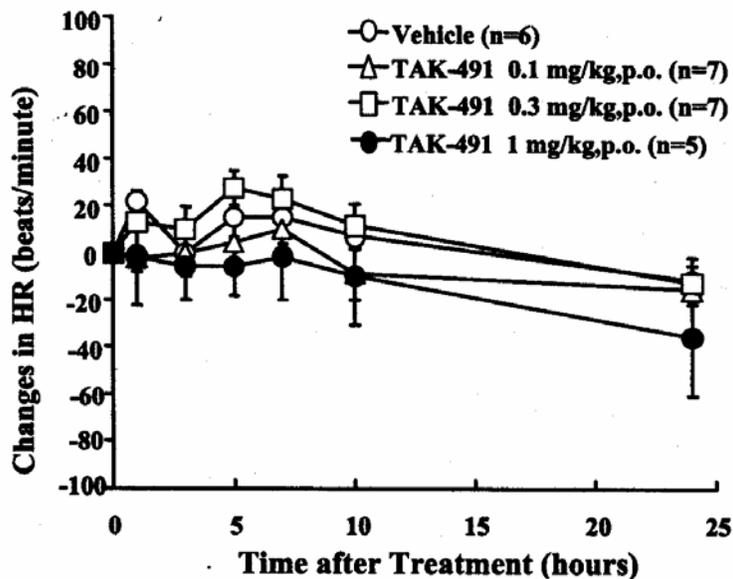


Source: TAK-491/00056.001R

Data are mean±SEM.

* p<0.025 vs vehicle (1-tailed Williams' test).

Figure 3. Effect of Oral TAK-491 on Heart Rate in SHR

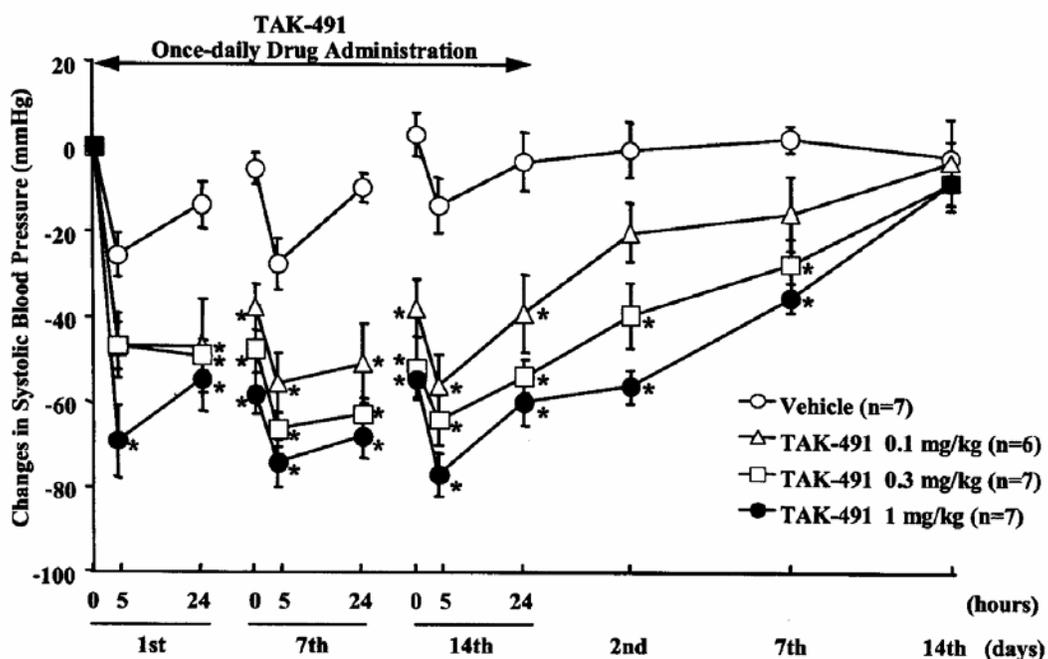


4.1.2.1.3 Effect of Repeated Oral Administration of TAK-491 on Systolic Blood Pressure in SHR

From review by A. Proakis, 8/2/05

The effect of TAK-491 on systolic blood pressure (measured by tail cuff method) was assessed in conscious SHR after oral administration of 0.1, 0.3 or 1.0 mg/kg/day for 14 days. Recovery of the pharmacological effect of TAK-491 was examined during a 14-day treatment withdrawal period. The oral administration of TAK-491 for 2 weeks significantly reduced blood pressure in a dose related manner (Figure 4). The minimally effective dose of TAK-491 was 0.1 mg/kg. Heart rate was not significantly affected. The antihypertensive effect of TAK-491 disappeared gradually after cessation of treatment; a rebound hypertensive effect was not observed.

Figure 4. Effect of Repeated Oral Administration of TAK-491 on Systolic Blood Pressure in Conscious SHR.



4.1.2.1.4 Antihypertensive Effect of TAK-491 in Renal Hypertensive Dogs

From review by A. Proakis, 8/2/05

The effect of orally administered TAK-491 on systolic blood pressure was assessed in renal hypertensive dogs. Approximately 4 weeks after surgery to reduce left renal arterial blood flow, dogs with baseline systolic blood pressure (SBP) over 200 mmHg were administered TAK-491 orally by gavage at doses of 0.1, 0.3 or 1 mg/kg. SBP and heart rate (obtained from the blood pressure pulse) were measured at varying intervals up to 24 hours post dose. Oral administration of TAK-491 at doses from 0.1 to 1 mg/kg

lowered systolic blood pressure in a dose-related manner without causing reflex tachycardia (Figures 5-6). The antihypertensive effect of TAK-491 at 1 mg/kg persisted for 24 hours.

Figure 5. Effect of Oral TAK-491 on Systolic Blood Pressure in Conscious Renal Hypertensive Dogs

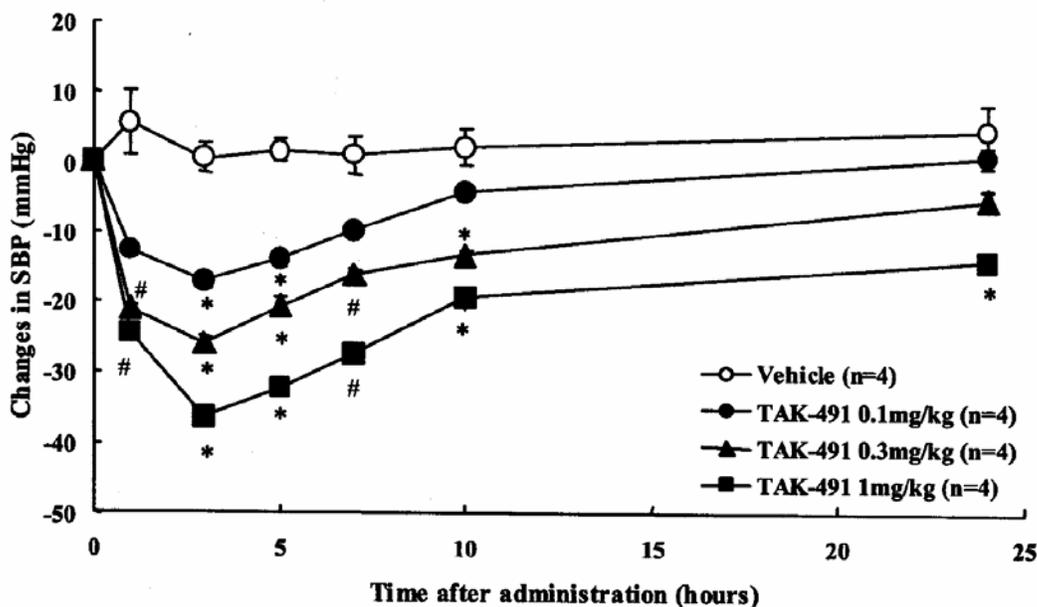
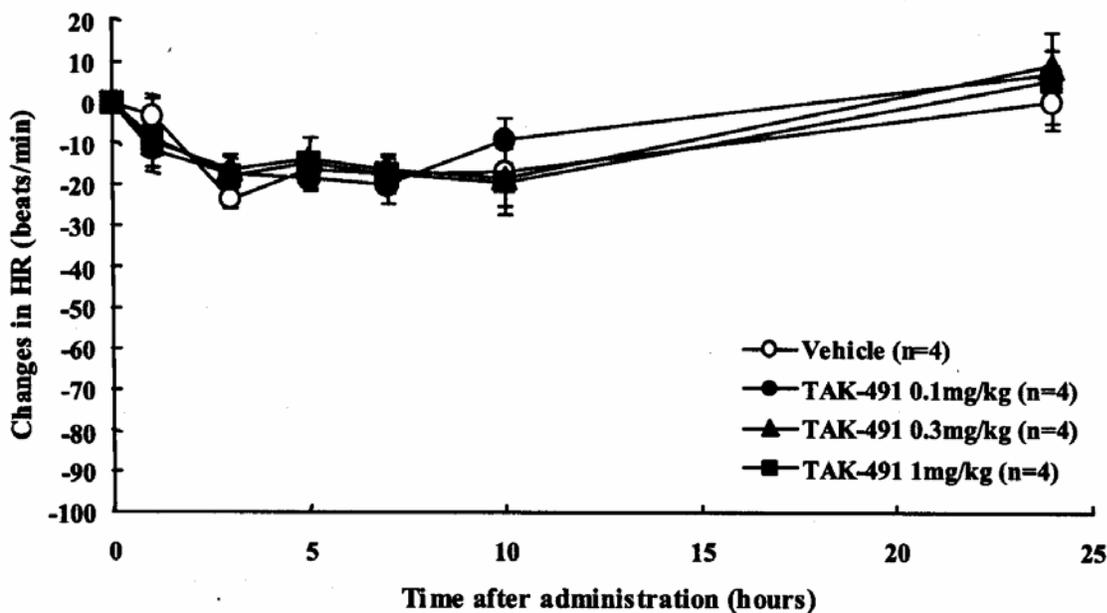


Figure 6. Effect of Oral TAK-491 on Heart Rate in Conscious Renal Hypertensive Dogs



4.1.2.2 Ancillary Pharmacodynamic Studies

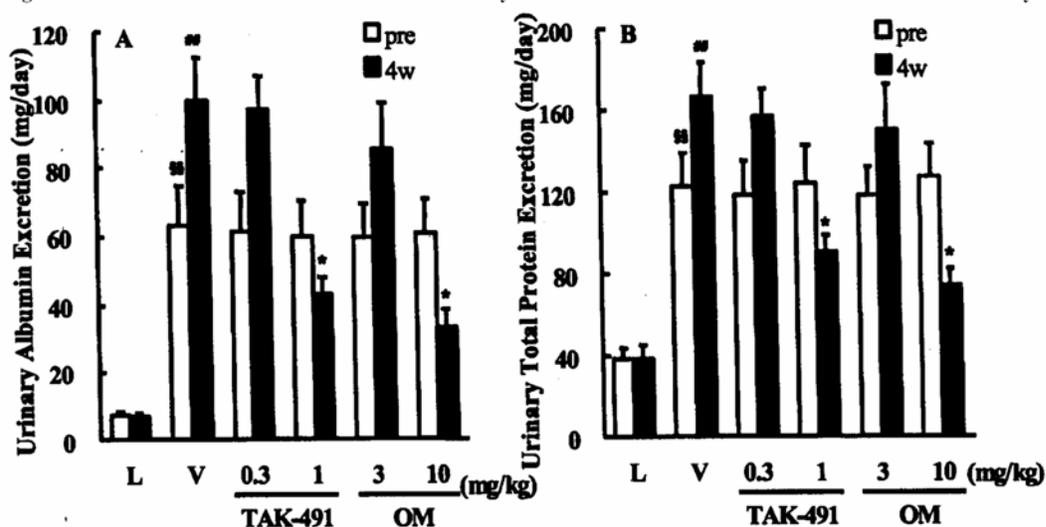
4.1.2.2.1 Antiproteinuric Effect of TAK-491 in Rats with Overt Nephropathy

From review by A. Proakis, 8/2/05

The progression of proteinuria was examined in 26-week old Wistar fatty rats, an animal model of non-insulin-dependent diabetes mellitus with overt nephropathy. TAK-491 was administered daily by oral gavage for 4 weeks at doses of 0.3 or 1 mg/kg/day. At the end of treatment, the rats were placed in metabolic cages, provided with food and water and 24-hour urine samples were collected.

Prior to initiation of treatment, the rats showed marked urinary excretion of albumin and total protein compared to Wistar lean rats. TAK-491 at 1 mg/kg significantly inhibited the progression of albuminuria and proteinuria in fatty rats when compared to vehicle treatment; this effect was similar to that seen with 10 mg/kg of olmesartan (Figure 7).

Figure 7. Effect of TAK-491 or Olmesartan on Urinary Excretion of Albumin and Total Protein in Wistar Fatty Rats.



Source: TAK-491/00060.001R

L and V indicate Wistar lean rats and vehicle, respectively.

Values are mean±SEM. Drugs and vehicle were given to Wistar fatty rats for 4 weeks (n = 7). Vehicle was given to Wistar lean rats for 4 weeks (n = 4).

*P<0.025 vs vehicle group (1-tailed Williams' test).

##P<0.01 vs Wistar lean rats (Aspin-Welch test).

##P<0.01 vs pre-value in Wistar lean rats (Aspin-Welch test).

4.2 Secondary Pharmacology

The following is excerpted from the sponsor's submission:

4.2.1 *In Vitro* Studies

4.2.1.1 *In Vitro* Pharmacological Diversity Profile

TAK-491 (pro-drug, K⁺ salt of azilsartan medoximil), TAK-491F (K⁺-free form), TAK-536 (azilsartan), and TAK-536 M-II (unique human metabolite) each were screened for their potential binding to a broad range of receptors and ion channels and effects on enzyme activities using standard in vitro assay protocols. A concentration of 10 µmol/L was used for each compound in the initial screening. Inhibition or facilitation of substrate binding or enzymatic activity ≥50% was considered a significant response. Follow-up assays were conducted to determine the IC₅₀ values for some of the activities meeting this significance criterion.

4.2.1.1.1 *In Vitro* Pharmacological Diversity Profile of TAK-491F and TAK-491, Report Number TAK-491-00185 and TAK-491-00200

TAK-491F was screened for activity at 73 receptor or ion channel binding sites and at 45 enzymes in support of the safety of personnel involved in the manufacturing of TAK-491. Significant responses were observed in the following assays: AT1 receptor (100% inhibition at 10 µmol/L), somatostatin sst2 receptors (IC₅₀, 3.98 µmol/L), thromboxane A2 receptors (60% inhibition at 10 µmol/L), 5-lipoxygenase (78% inhibition at 10 µmol/L, IC₅₀, 1.84 µmol/L), phosphodiesterase (PDE) 3 (52% inhibition at 10 µmol/L), PDE4 (86% inhibition at 10 µmol/L, IC₅₀, 0.835 µmol/L), PDE6 (54% inhibition at 10 µmol/L), protein serine/threonine kinase p38α (59% inhibition at 10 µmol/L), and thromboxane synthetase (61% inhibition at 10 µmol/L).

In another screening study, TAK-491 at 10 µmol/L was tested for activity at 74 receptor or ion channel binding sites and at 55 enzymes. Similar to what was seen with TAK-491F, significant responses were observed in the following assays: AT1 receptor (98% inhibition), thromboxane A2 receptor (59% inhibition), 5-lipoxygenase (87% inhibition), PDE3 (59% inhibition), PDE4 (84% inhibition), PDE6 (66% inhibition), and protein serine/threonine kinase CDC2/CCB1 (cdk1/cyclin B) (62% activation). To investigate further the effect of TAK-491 on cdk1/cyclin B kinase activity, a subsequent study was conducted using a [³²P]ATP direct phosphorylation method (as compared to using enzyme-linked immunosorbent assay [ELISA]) to detect the phosphorylated protein in the screening assay. In this assay, TAK-491 (1 and 10 µmol/L) inhibited, rather than stimulated, enzyme activity by 12% and 23%, respectively; these changes were not considered biologically significant. Although there is no obvious reason for the discrepant test results between studies, data from the follow-up study are deemed more reliable since this assay method is more sensitive than the previous method, and 2 concentrations of TAK-491 were examined.

In summary, nonspecific activity was detected for TAK-491F and TAK-491 at various receptor sites and at various enzymes when the compounds were examined at 10 $\mu\text{mol/L}$. Aside from effects at AT1 receptors, the most potent effect of TAK-491F was inhibition of PDE4 activity, with an IC_{50} value of 0.835 $\mu\text{mol/L}$. TAK-491 also demonstrated inhibition of PDE4 activity. However, since TAK-491 is not detectable in human plasma after oral administration of TAK-491 at doses up to 320 mg, this nonspecific activity of TAK-491 is considered not to be of clinical relevance.

4.2.1.1.2 In Vitro Pharmacological Diversity Profile of TAK-536, Report Number TAK-536-C-46-00434 and TAK-536-C-46-00440

Two screening studies were conducted for TAK-536. In one study, TAK-536 (0.1 nmol/L-1000 $\mu\text{mol/L}$) was tested in 77 *in vitro* assays, which included 29 receptor or ion channel binding sites and 4 enzyme activity assays. As expected, TAK-536 bound potently to AT1 receptors (IC_{50} , 0.00157 $\mu\text{mol/L}$). TAK-536 demonstrated some weak activity at AT2 receptors (IC_{50} , 17.2 $\mu\text{mol/L}$) and TAK-536 at 1 $\mu\text{mol/L}$ enhanced the binding of prostaglandin E2 to prostanoid EP1 receptors by 59%. The mean C_{max} of TAK-536 in humans dosed orally with TAK-491 at 80 mg is 4.377 $\mu\text{g/mL}$. Of this, 99.5% is protein bound, as determined *in vitro* (vs 99.9%, determined *in vivo*; therefore, the C_{max} unbound plasma concentration of TAK-536 would be 0.048 $\mu\text{mol/L}$ (0.022 $\mu\text{g/mL}$) after an 80 mg dose of TAK-491. This concentration is more than 20-fold lower than the TAK-536 concentrations that had effects *in vitro* on AT2 and prostanoid EP1 receptors (ie, 1 $\mu\text{mol/L}$). Therefore, TAK-536 would not be expected to affect AT2 or prostanoid EP1 receptors at therapeutic doses.

In the second study, TAK-536 (10 $\mu\text{mol/L}$) was assayed for activities at 74 receptor or ion channel binding sites and at 55 enzymes. As expected, TAK-536 demonstrated activity at AT1 receptors (103% inhibition at 10 $\mu\text{mol/L}$); no significant activity was seen in any other assays, including AT2 and prostanoid EP1 receptors.

4.2.1.1.3 In Vitro Pharmacological Diversity Profile of TAK-536 M-II, Report Number TAK-536-C-46-00435-001A and TAK-536-C-46-00441

Two screening studies were conducted for TAK-536 M-II (10 $\mu\text{mol/L}$). In one study, TAK-536 M-II was tested for activity at 73 receptor or ion channel binding sites and at 45 enzymes. TAK-536 M-II demonstrated activity at AT1 receptors (68% inhibition), which is in agreement with data shown in Section 4.1.1.3.1; the IC_{50} value for TAK-536 M-II binding to AT1 receptors was 1.1 $\mu\text{mol/L}$. TAK-536 M-II showed no significant activity in any other assays.

In the second study, TAK-536 M-II was tested for activity at 74 receptor or ion channel binding sites and at 55 enzymes. In addition to binding to AT1 receptor (57% inhibition), TAK-536 M-II also showed activity at serotonin 5-hydroxytryptamine (5-HT)₃ receptors (56% inhibition). This moderate effect, which was not seen previously, is unlikely to be

of biological significance. In clinical use, TAK-536 M-II is unlikely to affect 5-HT₃ receptors since the mean C_{max} for the unbound fraction in plasma is less than 0.0022 µmol/L (0.9294 ng/mL) in humans dosed orally with TAK-491 at 80 mg (using *in vivo* plasma binding of >99.9%).

4.3 Safety Pharmacology

The effects of TAK-491 or TAK-536 on the central nervous, cardiovascular, and respiratory systems were evaluated in both *in vitro* and *in vivo* safety pharmacology studies. Additionally, TAK-536 M-II was evaluated for its effect on the hERG channel since it is a major metabolite in humans. The vehicle used in the *in vitro* studies was 0.1% DMSO. In the *in vivo* evaluations, TAK-491 was suspended in 0.5% methylcellulose solution and administered by oral gavage. The *in vivo* doses of TAK-491 are expressed as the weight of TAK-491F.

Azilsartan did not affect CNS function in rats (up to 2000 mg/kg, p.o.), respiratory function in rats (up to 2000 mg/kg, p.o.) or cardiovascular (ECG, HR) parameters in conscious dogs given oral doses up to 300 mg/kg by gavage (other than the expected lowering of arterial blood pressure). See review by A. Proakis, 08/02/2005 below.

4.3.1 Effect of Oral TAK-491 on CNS Function in Rats

The effects of TAK-491 on CNS function were assessed in rats following oral (gavage) administration of single doses of 0, 20, 200, or 2000 mg/kg. Neurobehavioral assessments (Irwin test) were conducted predose and at 1, 2, 4, 8 and 24 hours postdose. The physical and behavioral responses in TAK-491 treated animals were comparable to those of control.

4.3.2 Effect of TAK-491 on the Cardiovascular System in Dogs

TAK-491 was administered to conscious male dogs at oral (gavage) doses of 0, 3, 30 or 300 mg/kg to evaluate its effects on blood pressure, heart rate and the electrocardiogram. Mean blood pressures were slightly lower than predose or vehicle control values after 3 mg/kg, 30 mg/kg, and 300 mg/kg at 1 to 8 hours postdose: the blood pressure lowering effect after 300 mg/kg persisted for 24 hours (Table 1). There were no TAK-491 related effects on heart rate or on ECG parameters (PR, QRS, QT or QTc intervals).

Table 1. Effect of Oral TAK-491 on Mean Blood Pressure in Conscious Dogs

Test substance	Dose ¹⁾ (mg/kg p.o.)	Number of animals	Mean blood pressure (mmHg)				
			Before ²⁾	Time after administration (hour)			
				-1	-0.75	-0.5	0.5
Vehicle ³⁾	--	4	108 ± 7	109 ± 10	112 ± 7	102 ± 5	98 ± 7
TAK-491	3	4	103 ± 3	107 ± 5	98 ± 7	103 ± 3	96 ± 9
	30	4	104 ± 13	103 ± 9	106 ± 14	103 ± 18	102 ± 15
	300	4	94 ± 20	91 ± 20	93 ± 24	97 ± 19	84 ± 19

Test substance	Dose ¹⁾ (mg/kg p.o.)	Number of animals	Mean blood pressure (mmHg)				
			Time after administration (hour)				
			1	2	4	8	24
Vehicle ³⁾	--	4	100 ± 5	100 ± 7	101 ± 4	102 ± 13	104 ± 9
TAK-491	3	4	88 ± 5	89 ± 11	89 ± 7	96 ± 6	101 ± 11
	30	4	94 ± 13	98 ± 12	85 ± 7 *	85 ± 7 *	102 ± 18
	300	4	76 ± 14 *	80 ± 15	95 ± 7	82 ± 9 *	84 ± 12 *

Each value represents the mean±S.D.

1) Dose levels as TAK-491F

2) The mean of 3 time points, 1, 0.75, and 0.5 hours before administration, was regarded as the before value and used in the statistical analysis.

3) 0.5 w/v% methylcellulose solution containing citric acid at the same concentration as that in the TAK-491F 60 mg/mL dosing suspension

* : Significantly different from the control substance group by contrast analysis for the effect of dose with the Holm correction, $p < 0.05$.

4.3.3 Effects of TAK-536 on hERG Currents, Report Number TAK-491-00035

TAK-536 was evaluated for its ability to block the hERG ion channel expressed in human embryonic kidney (HEK)293 cells using the whole cell clamp method. The residual currents after treatment with the vehicle and with TAK-536 at 0.6, 6, and 60 µg/mL (1, 10 and 100 µM) were 92.9%, 95.6%, 93.2%, and 91.7% of pretreatment values, respectively; the effects of TAK-536 did not differ significantly from those of vehicle. The positive control substance, E-4031 at 0.1 µmol/L, caused marked inhibition of hERG currents with a residual current value of 8.1%.

4.3.4 Effects of TAK-536 M-II on hERG Currents, Report Number TAK-536-C-46-00375

TAK-536 M-II was evaluated for its ability to block the hERG ion channel expressed in HEK293 cells using the whole cell clamp method. The residual currents after treatment with the vehicle and with TAK-536 M-II at 0.6, 6, and 60 µg/mL were 97.8%, 96.1%, 95.5%, and 96.3% of pretreatment values, respectively; the effects of TAK-536 M-II did not differ significantly from those of vehicle. The positive control substance, E-4031 caused marked inhibition of hERG currents at 0.1 µmol/L with a residual current value of 7.8%.

4.3.5 Effect of Oral TAK-491 on Respiratory Function in Rats

The potential of TAK-491 to affect pulmonary function was evaluated in conscious male rats at oral (gavage) doses of 0, 20, 200 or 2000 mg/kg. There were no TAK-491 related effects on respiratory rate, tidal volume or minute volume when measured up to 24 hours postdose (Tables 2-4).

Table 2. Effect of Oral TAK-491 on Respiratory Rate in Rats

Test substance	Dose ¹⁾ (mg/kg p.o.)	Number of animals	Respiratory rate (breaths/min)					
			Before	Time after administration (hour)				
				1	2	4	8	24
Vehicle ²⁾	—	8	115 ± 12	111 ± 6 ³⁾	110 ± 11	107 ± 9	110 ± 14	114 ± 7
TAK-491	20	8	113 ± 14	112 ± 12	106 ± 7	105 ± 14	105 ± 15	112 ± 12 ³⁾
	200	8	112 ± 10	116 ± 10	110 ± 12	107 ± 7	103 ± 11	112 ± 12 ³⁾
	2000	8	108 ± 6	103 ± 7	101 ± 10	99 ± 3	104 ± 4	108 ± 11

Each value represents the mean±S.D.

Since 1 values in each group was affected by body movement of animals, the values were excluded from evaluation.

1) Dose levels as TAK-491F

2) 0.5 w/v% methylcellulose solution containing citric acid at the same concentration as that in the TAK-491F 200 mg/mL dosing suspension

3) n=7

Table 3. Effect of Oral TAK-491 on Respiratory Tidal Volume in Rats

Test substance	Dose ¹⁾ (mg/kg p.o.)	Number of animals	Tidal volume (mL/breath)					
			Before	Time after administration (hour)				
				1	2	4	8	24
Vehicle ²⁾	—	8	1.07 ± 0.10	1.04 ± 0.05 ³⁾	1.06 ± 0.12	1.03 ± 0.10	1.07 ± 0.11	1.01 ± 0.07
TAK-491	20	8	1.06 ± 0.18	1.07 ± 0.13	1.00 ± 0.13	1.07 ± 0.16	1.14 ± 0.20	1.02 ± 0.18 ³⁾
	200	8	1.03 ± 0.09	1.01 ± 0.09	1.03 ± 0.13	1.08 ± 0.12	1.18 ± 0.12	1.07 ± 0.18 ³⁾
	2000	8	1.14 ± 0.21	1.08 ± 0.12	1.08 ± 0.14	1.11 ± 0.13	1.19 ± 0.14	1.08 ± 0.14

Each value represents the mean±S.D.

Since 1 values in each group was affected by body movement of animals, the values were excluded from evaluation.

1) Dose levels as TAK-491F

2) 0.5 w/v% methylcellulose solution containing citric acid at the same concentration as that in the TAK-491F 200 mg/mL dosing suspension

3) n=7

Table 4. Effect of Oral TAK-491 on Respiratory Minute Volume in Rats

Test substance	Dose ¹⁾ (mg/kg p.o.)	Number of animals	Minute volume (mL/min)					
			Before	Time after administration (hour)				
				1	2	4	8	24
Vehicle ²⁾	---	8	122.3 ± 13.9	115.2 ± 5.5 ³⁾	115.7 ± 6.6	109.4 ± 6.6	116.6 ± 11.1	115.8 ± 9.8
TAK-491	20	8	118.4 ± 12.2	118.9 ± 12.2	105.3 ± 9.3	111.2 ± 8.6	118.0 ± 17.1	112.0 ± 10.4 ³⁾
	200	8	114.2 ± 8.7	117.0 ± 6.5	111.7 ± 11.6	115.4 ± 14.5	120.2 ± 10.9	118.6 ± 17.4 ³⁾
	2000	8	122.0 ± 19.3	110.4 ± 7.8	108.2 ± 11.0	109.3 ± 13.6	123.8 ± 13.4	115.6 ± 18.7

Each value represents the mean ± S.D.

Since 1 value in each group was affected by body movement of animals, the values were excluded from evaluation.

1) Dose levels as TAK-491F

2) 0.5 w/v% methylcellulose solution containing citric acid at the same concentration as that in the TAK-491F 200 mg/mL dosing suspension

3) n=7

5 Pharmacokinetics/ADME/Toxicokinetics

Azilsartan medoxomil (TAK 491) is converted rapidly to the active moiety, azilsartan through the action of gut or plasma aryl esterase. Thus, the PK of TAK-536 is noted in the review. The bioavailability after p.o. administration was 54% in dogs. The T_{1/2} of TAK-536 is 5.8 hr in rats and 4 hr in dogs. Distribution of the compound was throughout the entire body. The major route of excretion was via the feces in rat and dog ($\geq 95\%$). The major metabolite in mice, rats, dogs and monkeys is TAK-536 M-I. The major metabolite in humans is TAK-536 M-II which is formed in animals but at relatively low levels compared to humans.

5.1 PK/ADME

5.1.1 Methods of Analysis

Plasma concentrations of TAK-491F, TAK-536, TAK-536 M-I, and TAK-536 M-II from a number of nonclinical toxicology studies in mice, rats, rabbits, and dogs were determined using validated liquid chromatography tandem mass spectrometry (LC/MS/MS) methods. The LC/MS/MS assays were able to quantify the following analytes: TAK-491F, TAK-536, TAK-536 M-I, and TAK-536M-II. Validated bioanalytical methods were used to support all Good Laboratory Practice nonclinical bioanalytical assays in the toxicokinetic studies.

5.1.2 Absorption and Pharmacokinetics

5.1.2.1 Absorption

The following summaries are excerpted from the sponsor's submission:

The absorption of TAK-536 after oral dosing with TAK-491 and IV dosing with TAK-536 was determined in fed rats and dogs. In these studies, [¹⁴C]TAK-491 was used for oral dose administration. TAK-491 is a pro-drug and it is rapidly converted to the active moiety TAK-536 in rats and dogs after oral administration. Since most plasma concentrations of TAK-491 were at or below the limit of quantitation (LOQ) at all time points, systemic exposure to TAK-491 was virtually negligible and pharmacokinetic parameters of TAK-491 were not calculated.

Systemic bioavailability of TAK-536 after oral dosing with [¹⁴C]TAK-491 was calculated based on the TAK-536 equivalent, on the dose adjusted ratio of AUC values of TAK-536 obtained after oral dosing of [¹⁴C]TAK-491 versus dose normalized AUC values of TAK-536 after IV dosing of [¹⁴C]TAK-536. Key pharmacokinetic parameters of TAK-536 after oral administration of [¹⁴C]TAK-491 in rats and dogs are summarized below.

Pharmacokinetic Parameters of TAK-536 Following Oral Administration of [¹⁴C]TAK-491 and IV Administration of [¹⁴C]TAK-536 in Male Rats and Male Dogs

Species	Route	Compound	Dose (mg/kg)	Cmax (ng/mL)	Tmax (hr)	AUC(0-24) (ng-hr/mL)	T1/2 (hr)	BA (%)	Report Number
Rat	Oral	TAK-491	1.33	563	2.67	5679	5.83	12.4	491-00079
Dog	Oral	TAK-491	1.33	440	1.88	2807	3.93	53.9	491-00080
Rat	IV	TAK-536	0.2	-	-	9137	5.93	-	491-00079
Dog	IV	TAK-536	0.2	-	-	1045	1.33	-	491-00080

n=3-4.

-=not calculated, AUC(0-24)=area under the plasma concentration-time curve from time 0 to 24 hours, BA=bioavailability, T1/2=terminal elimination half-life, Tmax=time at which Cmax occurred.

The absorption of TAK-536 was also studied in rats, dogs, and monkeys. Pharmacokinetic parameters of TAK-536 in animals after oral administration of [¹⁴C]TAK-536 are summarized below.

Pharmacokinetic Parameters of TAK-536 Following Oral and IV Administration of [¹⁴C]TAK-536 in Rats, Dogs, and Monkeys

Species	Sex	Dose (mg/kg) (Route)	Food	Cmax (µg/mL)	Tmax (hr)	AUC(0-24) (µg-hr/mL)	T1/2 (hr)	BA (%)	Report Number
Rat	M	1 (Oral)	fed	0.901	1.7	7.26	5.2	14.6	536-C-46-00292
	M	1 (Oral)	fasted	3.231	0.7	20.4	5.2	-	
	M	0.2 (IV)	fed	-	-	9.97	-	-	
Dog	M	1 (Oral)	fed	0.247	0.8	1.18	2.9	24.3	536-C-46-00292
	M	1 (Oral)	fasted	0.842	0.7	1.90	2.8	-	
	M	0.2 (IV)	fed	-	-	0.97	-	-	
Monkey	M	3 (Oral)	fasted	2.264	1.7	6.71	1.4	13.6	536-C-46-00414
	M	0.2 (IV)	fasted	3.637	-	3.36	1.6	-	

n=3.

-=not determined, AUC(0-24)=area under the plasma concentration-time curve from time 0 to 24 hours, BA=bioavailability, T1/2=terminal elimination half-life, Tmax=time at which Cmax occurred.

The transcellular transport of [¹⁴C]TAK-491 (10 µmol/L) at 37°C was examined using Caco-2 cells. [¹⁴C]antipyrine (a reference compound for high permeability [10 µmol/L]), [¹⁴C]mannitol (a reference compound for low permeability [10 µmol/L]), and [³H]digoxin (a positive control for P-glycoprotein (P-gp) substrate [3 µmol/L]) were used as controls. The apical to basal permeability (Papp) of [¹⁴C]TAK-491 across Caco-2 cells after incubation at 37°C for 1 and 2 hours was 2.73×10^{-6} and 2.80×10^{-6} cm/sec, respectively; the basal to apical Papp was value of [¹⁴C]TAK-491 from apical to basal was approximately 4 times higher than that of [¹⁴C]mannitol but was 1/20 lower than that of [¹⁴C]antipyrine, suggesting that TAK-491 would be a low permeability drug. However, the Papp ratios might be underestimated due to rapid conversion to TAK-536 during the experiment. The Papp ratios of [³H]digoxin at 1 and 2 hours were 3.7 and 4.6,

respectively. Because the Papp ratios of [¹⁴C]TAK-491 were much lower than those for [³H]digoxin, it is concluded that TAK-491 is not a P-gp substrate.

In a similar Caco-2 study with [¹⁴C]TAK-536 (10 μmol/L), the apical to the basal Papp of [¹⁴C]TAK-536 across Caco-2 cells after incubation at 37°C for 1 and 2 hours was 0.0305×10^{-6} and 0.0181×10^{-6} cm/sec, respectively; the basal to apical Papp was 0.0876×10^{-6} and 0.105×10^{-6} cm/sec, respectively. The Papp values of [¹⁴C]TAK-536 were lower than those of [¹⁴C]mannitol suggesting that TAK-536 is a low permeability drug. The Papp ratios of [¹⁴C]TAK-536 at 1 and 2 hours were 2.9 and 5.8, respectively. The Papp ratios of [³H]digoxin at 1 and 2 hours were 3.7 and 4.6, respectively. Although the Papp ratio of [¹⁴C]TAK-536 was similar to that of [³H]digoxin, the amounts of [¹⁴C]TAK-536 transported in both directions were extremely low. Therefore, it was difficult to evaluate the involvement of P-gp in the transport of TAK-536.

The inhibitory effect of TAK-491 on P-gp substrate [³H]digoxin (3 μmol/L) transport across Caco-2 cell monolayers was also examined. After incubation at 37°C for 2 hours with TAK-491 (at 0, 20, 100, and 250 μmol/L), the apical to basal Papp values for [³H]digoxin across Caco-2 cell monolayers were 1.03, 1.51, 3.29, and 3.92×10^{-6} cm/sec, respectively; the basal to apical Papp values were 7.18, 7.36, 5.37, and 4.33×10^{-6} cm/sec, respectively. The Papp ratios were 7.0, 4.9, 1.6, and 1.1, respectively. These results show that TAK-491 had an inhibitory effect on P-gp-mediated efflux activity.

The inhibitory effect of TAK-536 on P-gp substrate [³H]digoxin (3 μmol/L) transport across Caco-2 cell monolayers was also examined. After incubation at 37°C for 2 hours with TAK-536 (at 0, 20, 100, and 500 μmol/L), the apical to basal Papp values for [³H]digoxin across Caco-2 cell monolayers were 1.20, 1.07, 1.24, and 1.15×10^{-6} cm/sec, respectively; the basal to apical Papp values were 7.62, 7.49, 8.24, and 7.23×10^{-6} cm/sec, respectively. The Papp ratios were 6.4, 7.0, 6.6, and 6.3, respectively. These results show that TAK-536 had no inhibitory effect on P-gp-mediated efflux activity. Because TAK-491 is converted rapidly to TAK-536 and because TAK-536 is not a P-gp inhibitor, clinically significant drug-drug interactions based on TAK-491 inhibitory effect on P-gp are unlikely.

Rat

One group of male rats was administered an oral dose of 1.33 mg/kg of [¹⁴C]TAK-491 and a separate group of male rats was administered an IV dose of 0.2 mg/kg [¹⁴C]TAK-536. Based on the ratio of the relative TAK-536 dose equivalent normalized area under the plasma concentration-time curve from time 0 to 24 hours ($AUC_{(0-24)}$) values of TAK-536 obtained in the oral group and dose normalized $AUC_{(0-24)}$ values from the IV administration groups, the bioavailability of TAK-536 after an oral dose of [¹⁴C]TAK-491 was estimated to be 12.4% and the terminal elimination half-life ($T_{1/2}$) of TAK-536 was 5.83 hours in rats. Conversion from TAK-491 to TAK-536 was rapid. Plasma concentrations of TAK-491 were at or below LOQ, and the radioactivity in the plasma was mainly attributed to TAK-536, which accounted for 93.7% of the $AUC_{(0-24)}$ value of

the total radioactivity. TAK-491 only accounted for about 0.2% of the $AUC_{(0-24)}$ value of the total radioactivity, suggesting that systemic exposure to TAK-491 was indeed negligible after oral dose administration of [^{14}C]TAK-491.

The linearity in the pharmacokinetics of TAK-491 and TAK-536 was investigated after a single oral administration of TAK-491 at doses equivalent to 0.3, 1, and 3 mg/kg of TAK-536. Plasma concentrations of TAK-491 were at or below LOQ at all time points. Therefore, the pharmacokinetic parameters of TAK-491 were not obtained. The increases in C_{max} and area under the plasma concentration-time curve from time 0 to 48 hours ($AUC_{[0-48]}$) values for TAK-536 were greater than dose-proportional.

[^{14}C]TAK-491-related radioactivity was mostly absorbed from the jejunum, duodenum, and ileum and was poorly absorbed from the stomach and colon of fasted male rats after a single administration of 1.33 mg/kg [^{14}C]TAK-491 into the stomach, duodenum, jejunum, ileum, or colon. Dosed radioactivity was moderately absorbed and transported via the portal vein route to the systemic circulation in fasted male rats after a single administration of [^{14}C]TAK-491 into the jejunal loop. After a single oral administration of [^{14}C]TAK-491 to thoracic duct-cannulated male rats, [^{14}C]TAK-491-related radioactivity was poorly recovered in the lymph, suggesting that lymphatic absorption is not important for this compound.

The absorption of [^{14}C]TAK-536 was also studied after oral administration of [^{14}C]TAK-536. The bioavailability of [^{14}C]TAK-536 in fed rats was 14.6% and was increased to 40.9% when animals were fasted. In fasted animals, [^{14}C]TAK-536 $T_{1/2}$ was approximately 5.2 hours and C_{max} was achieved 0.7 hours after oral dosing of [^{14}C]TAK-536.

Dog

Male dogs were administered an oral dose of 1.33 mg/kg of [^{14}C]TAK-491 or an IV dose of 0.2 mg/kg [^{14}C]TAK-536. TAK-491 was rapidly hydrolyzed to TAK-536. Based on the ratio of the relative TAK-536 equivalent dose normalized $AUC_{(0-24)}$ values of TAK-536 obtained in the oral group and dose normalized $AUC_{(0-24)}$ values from the IV administration groups, the bioavailability of TAK-536 after an oral dose of [^{14}C]TAK-491 was 53.9%. Following oral administration of [^{14}C]TAK-491, the $T_{1/2}$ of TAK-536 was 3.93 hours. Plasma concentrations of TAK-491 were at or below LOQ, and the radioactivity in the plasma was mainly attributed to TAK-536, which accounted for 90.0% of the $AUC_{(0-24)}$ value of the total radioactivity. In contrast, TAK-491 only accounted for about 0.2% of the $AUC_{(0-24)}$ value of the total radioactivity, suggesting that systemic exposure to TAK-491 was minimal in dogs.

The linearity in the pharmacokinetics of TAK-491 and TAK-536 was investigated after a single oral administration of TAK-491 at doses equivalent to 0.3, 1, and 3 mg/kg of TAK-536. Plasma concentrations of TAK-491 were also at or below LOQ at all time points. Therefore, the pharmacokinetic parameters of TAK-491 were not obtained. The increases in C_{max} and $AUC_{(0-48)}$ values for TAK-536 were greater than dose-

proportional. The absorption of [¹⁴C]TAK-491 associated radioactivity was enhanced under fasted conditions. The AUC₍₀₋₂₄₎ value of total radioactivity after oral administration of [¹⁴C]TAK-491 to fasted dogs was 1.7 times higher than that obtained under fed conditions.

The absorption of [¹⁴C]TAK-536 also was studied after oral administration of [¹⁴C]TAK-536 to dogs. The bioavailability of TAK-536 in fed dogs was 24.3% and was increased to 39.2% in fasted dogs. In fasted animals, drug half-lives were approximately 2.8 hours in dogs and C_{max} was achieved 0.7 hours in dogs after oral dosing. Absorption of radioactivity was higher after oral dosing of [¹⁴C]TAK-491 (AUC_[0-24] values: 5.310 µg·hr/mL) compared with oral dosing of [¹⁴C]TAK-536 (1.827 µg·hr/mL) in dogs, demonstrating that absorption was indeed improved by the prodrug approach.

Monkey

The absorption of TAK-536 was studied in fasted male monkeys. The bioavailability of TAK-536 in monkeys was 13.6%. C_{max} was 2.264 µg/mL at 1.7 hours after oral administration and the T_{1/2} was approximately 1.4 hours. [¹⁴C]TAK-536 was the predominant material detected in the plasma and accounted for the majority of the total plasma radioactivity after a single oral administration of 3 mg/kg of [¹⁴C]TAK-536 to fasted monkeys.

Repeat-Dose Pharmacokinetics

Following repeated once-daily oral doses of 1.33 mg/kg [¹⁴C]TAK-491 to rats for 14 days, slight increases were noted in C_{max} and AUC₍₀₋₂₄₎ values. The C_{max}, minimum observed plasma concentration (C_{min}), and AUC₍₀₋₂₄₎ parameters attained steady state within 4 days in rats.

Pharmacokinetic Parameters of Total Radioactivity after 14-Day Repeated Once-a-day Oral Administrations of 1.33 mg/kg [¹⁴C]TAK-491 to Male Rats

Days on Drug (Day)	T _{max} (hr)	C _{max} (µg equivalent of TAK-536/mL)	C _{min} (µg equivalent of TAK-536/mL)	T _{1/2} (hr)	AUC(0-24) (µg equivalent of TAK-536·hr/mL)
1	3.67	0.855	0.125	7.09	11.674
4	1.50	1.901	0.126	6.10	15.918
7	2.00	1.247	0.138	6.80	14.820
11	1.67	1.266	0.103	6.02	13.193
14	2.67	1.303	0.109	5.86	14.053

Source: 491-10034.

n=3.

T_{max}=time at which C_{max} occurred.

Similar results were obtained following repeated once-daily oral doses of 1 mg/kg [¹⁴C]TAK-536 to rats for 14 days. The C_{max}, C_{min}, and AUC₍₀₋₂₄₎ parameters attained steady state on the 4th to 7th day in rats.

Pharmacokinetic Parameters of Total Radioactivity after 14-Day Repeated Once-a-day Oral Administrations of 1 mg/kg [¹⁴C]TAK-536 to Male Rats

Days on Drug (Day)	Tmax (hr)	Cmax (µg equivalent of TAK-536/mL)	Cmin (µg equivalent of TAK-536/mL)	T1/2 (hr)	AUC(0-24) (µg equivalent of TAK-536·hr/mL)
1	1.3	0.769	0.063	6.3	6.57
4	1.3	1.027	0.075	6.0	8.86
7	1.7	1.116	0.080	5.8	10.15
11	1.2	1.150	0.066	5.6	9.16
14	1.2	1.000	0.059	5.6	8.12

Source: 536-C-46-00292.

n=3.

Tmax=time at which Cmax occurred.

Comparison of Cmax, Cmin, and AUC₍₀₋₂₄₎ values from rats dosed with [¹⁴C]TAK-491 or [¹⁴C]TAK-536 has shown that the absorption was improved by using the prodrug approach. Although no other repeat-dose pharmacokinetic studies of TAK-491 were conducted, results from toxicokinetic studies of TAK-491 indicate that TAK-491 was converted rapidly to TAK-536 under repeat-dose administration conditions. Plasma concentrations of TAK-536 increased with TAK-491 doses approximately proportionally. No obvious sex-related differences were observed in toxicokinetic studies. No apparent changes were noted in any toxicokinetic parameter after repeated dosing.

5.1.2.2 Distribution

Following an oral dose of 1.33 mg/kg of ¹⁴C-TAK-491 to male rats, radioactivity was absorbed and widely distributed throughout various tissues reaching maximum concentrations about 3 hours post dose. The tissues with the highest levels of radioactivity were GI tract, liver, kidneys, plasma, lungs, pituitary gland and heart. The radioactivity was below detection amounts in many tissues by 72 hours postdose and, by 168 hours postdose, the radioactivity was undetectable in almost all tissues (Table below).

Tissue Distribution of Radioactivity in Male Rats Following an Oral Dose of 1.33 mg/kg [¹⁴C]TAK-491

Tissue	Mean µg equivalent [¹⁴ C]TAK-536/g tissue at post dose (hr)					
	0.5	3	8	24	72	168
Blood (a)	0.205	0.641	0.384	0.074	0.001	LOQ
Plasma (a)	0.305	1.002	0.597	0.111	0.001	0.000
Brain	0.002	0.008	0.005	0.001	LOQ	LOQ
Spinal cord	0.002	0.005	0.004	0.001	LOQ	LOQ
Hypophysis	0.054	0.189	0.128	0.026	LOQ	LOQ
Eye	0.003	0.020	0.019	0.004	LOQ	LOQ
Harderian gland	0.011	0.056	0.049	0.009	LOQ	LOQ
Submaxillary gland	0.027	0.099	0.060	0.012	0.000	LOQ
Thyroid	0.018	0.098	0.062	0.023	LOQ	LOQ
Thymus	0.004	0.027	0.031	0.006	LOQ	LOQ
Heart	0.028	0.125	0.081	0.016	0.000	LOQ
Lung	0.049	0.197	0.142	0.038	0.002	LOQ
Liver	0.171	1.103	1.383	0.170	0.007	0.003
Spleen	0.024	0.065	0.040	0.011	0.001	LOQ
Pancreas	0.024	0.079	0.051	0.011	0.000	LOQ
Adrenal gland	0.042	0.114	0.051	0.023	LOQ	LOQ
Kidney	0.089	0.245	0.187	0.053	0.010	0.003
Testis	0.009	0.096	0.072	0.015	0.000	LOQ
Skeletal muscle	0.003	0.024	0.032	0.008	LOQ	LOQ
Skin	0.005	0.053	0.100	0.029	0.001	LOQ
Fat	0.002	0.020	0.035	0.012	LOQ	LOQ
Bone marrow	0.024	0.085	0.053	0.010	LOQ	LOQ
Abdominal aorta	0.004	0.033	0.029	0.004	LOQ	LOQ
Inferior vena cava	0.008	0.057	0.073	0.012	LOQ	LOQ
Stomach	0.775	0.421	0.213	0.085	0.001	LOQ
Intestine	0.273	0.785	0.548	0.050	0.001	LOQ

Source: 491-00052.

LOQ=below the lower limit of quantitation (twice of background or 0.001 µg TAK-536 equivalent/g).

(a) µg equivalent [¹⁴C]TAK-536/mL.

The tissues with the highest mean C_{max} values, excluding the gastrointestinal tract tissues, were liver, plasma, kidney, lung, hypophysis, and heart. The tissues with the lowest C_{max} values were spinal cord, brain, eye, and thymus. Radioactivity was eliminated rapidly in all tissues and in plasma. By 168 hours postdose, concentrations of radioactivity in most tissues were below the LLOQ. The C_{max} values of radioactivity in the eyeball of pigmented rats were similar to those obtained in albino rats, and the elimination of total radioactivity was also rapid in pigmented rats, suggesting that [¹⁴C]TAK-491-derived material does not have any appreciable affinity for melanin.

After oral administration of [¹⁴C]TAK-536 (1 mg/kg) to rats, radioactivity was widely distributed in tissues. The distribution of radioactivity in tissues was similar to that dosed with [¹⁴C]TAK-491 with the lowest levels detected in the spinal cord and brain.

Radioactivity was distributed in the bone marrow in the studies of mice and rats. Levels of radioactivity 24 hours after dosing increased slightly in most tissues during once daily

oral dosing of 1 mg/kg [¹⁴C]TAK-536 to rats for 14 days; steady state was achieved in 4 to 7 days. The one exception was the kidney, where concentrations of radioactivity increased across the 14 days of treatment.

Tissue Distribution of Radioactivity in Male Rats Given an Oral Dose of 1 mg/kg/day [¹⁴C]TAK-536 for 14 Days

Tissue	Mean µg equivalent [¹⁴ C]TAK-536/g tissue (Days)				
	1	4	7	11	14
Plasma (a)	0.051	0.085	0.072	0.086	0.093
Brain	0.001	0.001	0.001	0.001	0.001
Spinal cord	0.001	0.002	0.002	0.001	0.002
hypothesis	0.011	0.021	0.024	0.023 (b)	0.022
Eye ball	0.002	0.003	0.004	0.004	0.004
Harderian gland	0.004	0.006	0.007	0.007	0.007
Submaxillary gland	0.006	0.009	0.008	0.009	0.010
Thyroid	0.008	0.015	0.019	0.015	0.013
Thymus	0.003	0.004	0.005	0.005	0.005
Heart	0.007	0.012	0.009	0.011	0.013
Lung	0.020	0.034	0.025	0.030	0.032
Liver	0.075	0.136	0.104	0.123	0.111
Spleen	0.007	0.009	0.009	0.010	0.010
Pancreas	0.005	0.008	0.007	0.008	0.009
Adrenal gland	0.017	0.019	0.018	0.019	0.020
Kidney	0.030	0.054	0.079	0.089	0.104
Testis	0.006	0.011	0.009	0.011	0.011
Skeletal muscle	0.003	0.005	0.005	0.006	0.006
Skin	0.015	0.024	0.014	0.017	0.021
Epididymal fat	0.008	0.011	0.013	0.011	0.010
Abdominal aorta	0.004	0.007	0.009	0.006	0.008
Inferior vena cava	0.006	0.014	0.017	0.014	0.010
Stomach	0.021	0.037	0.037	0.041	0.032
Intestine	0.036	0.048	0.051	0.054	0.048

Source: 536-C-46-00292.

n=3.

(a) µg/mL.

(b) n=2.

Protein Binding and Distribution in Blood Cells

TAK-491 and related compounds were highly bound to plasma proteins after a single oral administration of [¹⁴C]TAK-491 to animals. *In vivo* plasma protein binding ratio of radioactivity in animal blood after a single oral administration of [¹⁴C]TAK-491 at a dose equivalent to 1 mg of [¹⁴C]TAK-536/kg to rats and dogs was determined using ultracentrifugation. The plasma concentrations of radioactivity were 0.156, 0.483, and 0.799 µg equivalents of TAK-536/mL in rats and 0.421, 0.262, and 0.065 µg equivalents of TAK-536/mL in dogs at 0.5, 3, and 8 hours after dose administration, respectively.

The ratio of plasma protein binding to free radioactivity was >99.9% in rats and ≥97.1% in dogs at every time point.

The *in vitro* studies indicate that TAK-536 is highly protein bound in the mouse, rat, dog, and human plasma. The protein binding of TAK-536 in the plasma of mice (99.8%), rats (99.8%-99.9%), dogs (98.8%-98.9%), and humans (99.5%) was determined using ultracentrifugation. The protein binding ratio of TAK-536 was independent of the drug concentrations from 0.3 to 30 µg/mL in all the species.

In human plasma the major binding protein of TAK-536 was albumin. There were no obvious binding ratio differences between animal species and humans and binding was broadly concentration independent. [¹⁴C]TAK-491 and related compounds were poorly distributed into the blood cells of rats and dogs after a single oral administration of 1.33 mg/kg [¹⁴C]TAK-491. At 0.5, 3, and 8 hours after dose administration blood concentrations of radioactivity were 0.099, 0.305, and 0.481 µg equivalent of TAK-536/mL in rats and 0.213, 0.141, and 0.035 µg equivalent of TAK-536/mL in dogs, respectively. The calculated distribution of radioactivity into rat and dog blood cells was less than 2% and 3%, respectively, at every time point.

The *in vitro* distribution into blood cells of [¹⁴C]TAK-536 spiked blood, at concentrations of 0.3, 3, and 30 µg/mL were 0.7%, 0.0%, and 0.0% in rats, 5.0%, 2.9%, and 2.4% in dogs, and 2.4%, 2.0%, and 1.6% in humans, respectively. These results indicate that distribution of [¹⁴C]TAK-536 into blood cells in animals and in humans is very limited.

Placental Transfer

The concentrations of the radioactivity in tissues of pregnant rats on gestation day 18 (GD 18) were determined after single oral administration of 1.33 mg/kg [¹⁴C]TAK-491 to determine the fetal-placental transfer of radioactivity. The time to reach C_{max} in fetal plasma and fetal tissue was delayed compared to that in the maternal plasma suggesting that [¹⁴C]TAK-491-related radioactivity gradually transferred into the fetuses.

Concentrations of Radioactivity in the Tissues of Pregnant Rats at GD 18 After Single Oral Administration of 1.33 mg/kg [¹⁴C]TAK-491

Sample/Tissue	Mean Concentration (µg equivalent of TAK-536g /g or mL) GD/Postdose Time				
	18/0.5 hr	18/3 hr	18/8 hr	19/24 hr	20/48 hr
Maternal plasma	1.693	1.330	0.962	0.188	0.030
Placenta	0.282	0.324	0.302	0.112	0.042
Fetal plasma	0.002	0.097	0.327	0.377	0.243
Fetal tissue	LOQ	0.012	0.085	0.160	0.114
Amniotic fluid	LOQ	LOQ	0.001	0.023	0.079

Source: 491-10046.
n=3.

Metabolite profiles were determined in maternal and fetal plasma after a single oral administration of 1.33 mg/kg [¹⁴C]TAK-491 to pregnant rats. The major radioactive component in maternal and fetal plasma was TAK-536 which transferred gradually into the fetuses via the placenta.

Radioactivity Percentage of TAK-491F and Metabolites in the Plasma of Pregnant Rats and Fetuses at GD 18 After a Single Oral Dose of 1.33 mg/kg [¹⁴C]TAK-491

Sample	Sampling Time (hr)	Concentration (µg equivalent of TAK-536 g/mL)	% in Sample				
			TAK-491F	TAK-536	TAK-536 M-I	TAK-536 M-II	Others
Maternal Plasma	0.5	1.693	0.1	95.9	1.7	0.9	1.4
	3	1.330	0.0	96.9	1.2	1.1	0.8
	8	0.962	0.0	96.9	1.1	1.1	0.9
	24	0.188	0.0	96.3	1.1	1.1	1.5
	48	0.030	0.0	93.3	0.0	0.0	6.7
Fetal Plasma	3	0.097	0.0	75.3	20.6	1.0	3.1
	8	0.327	0.0	78.3	19.3	0.9	1.5
	24	0.377	0.0	77.5	19.6	1.1	1.8
	48	0.243	0.0	76.5	19.8	1.6	2.1

Source: 491-10047.
n=3.

5.1.2.3 Metabolism

In Vivo Studies

Rat

After an oral administration of 1.33 mg/kg [¹⁴C]TAK-491 to male rats, most of the radioactivity in plasma was in the form of TAK-536, which accounted for 93.7% of the AUC₍₀₋₂₄₎ total radioactivity value. TAK-491 was barely detectable and accounted for only about 0.2% of the AUC₍₀₋₂₄₎ total radioactivity value. TAK-536 M-I, TAK-536 M-II, and other metabolites accounted for 1.7%, 1.8%, and 2.6% of the AUC₍₀₋₂₄₎ total radioactivity value, respectively. In the same study, after IV administration of 0.2 mg/kg [¹⁴C]TAK-536, most of the radioactivity in plasma was in the form of TAK-536, which accounted for 95.1% of the AUC₍₀₋₂₄₎ total radioactivity value. TAK-536 M-I, TAK-536 M-II, and other metabolites accounted for 1.8%, 1.5%, and 1.6% of the AUC₍₀₋₂₄₎ total radioactivity value, respectively. Together, these results indicate a rapid and efficient conversion of TAK-491 to its active moiety TAK-536 *in vivo*.

After a single administration of 1.33 mg/kg [¹⁴C]TAK-491 into the jejunal loop, the 0 to 0.5 hour, 0.5 to 1 hour, 1 to 1.5 hour and 1.5 to 2 hours postdose portal vein plasma samples were profiled. Most radioactivity was in the form of TAK-536, which accounted

for about 68.0%-76.8% of total radioactivity. Concentrations of TAK-491 were low, which accounted for about 1.8%-3.7% of total radioactivity. Both TAK-536 M-I and TAK-536 M-II were present in portal vein plasma samples as minor components, they accounted for about 9.5% and 0.6% total radioactivity, respectively. These results are consistent with a rapid conversion of prodrug in the gut and during hepatic first pass metabolism.

TAK-491, TAK-536, TAK-536 M-I, TAK-536 M-II, and other metabolites accounted for 0.1%, 0.6%, 76.7%, 1.8%, and 15.0% of the dosed radioactivity in feces, respectively. Most of the radioactivity in rat feces was from TAK-536 M-I, which accounted for 76.7% of administered total radioactivity. Only 0.1% of the administered dose was detected as TAK-491 in the feces. TAK-536 M-I was also a major metabolite in rat bile. In biliary-cannulated rats, 22.2% and 32.4% of the total ^{14}C in bile was composed of TAK-536 and TAK-536 M-I, respectively, whereas most of the radioactivity in intestinal contents was from TAK-536 M-I after dosing with [^{14}C]TAK-536. These results suggest that TAK-536 is metabolized in the liver and intestine.

Dog

After an oral administration of 1.33 mg/kg [^{14}C]TAK-491 to male dogs most of the radioactivity in plasma was in the form of TAK-536 which accounted for 90.0% of the $\text{AUC}_{(0-24)}$ value for total radioactivity. TAK-491, TAK-536 M-I, TAK-536 M-II, and other metabolites accounted for 0.2%, 2.2%, 1.1%, and 6.5% of the total $\text{AUC}_{(0-24)}$ for radioactivity, respectively. These data indicate a rapid conversion of TAK-491 to TAK-536 in dogs. The majority of radioactivity was recovered as TAK-536 M-I in dog feces, which accounted for 77.6% of the total radioactivity administered.

Monkey

The composition of radioactivity in monkey plasma was investigated after a single oral administration of 3 mg/kg [^{14}C]TAK-536 to fasted monkeys. TAK-536 was the predominant material detected and accounted for 71% to 91% of the radioactivity in plasma. The ratios of the area under the concentration-time curve from time 0 to 8 hours ($\text{AUC}_{[0-8]}$) values for TAK-536, TAK-536 M-I, and TAK-536 M-II relative to the dosed radioactivity $\text{AUC}_{(0-8)}$ were 86.7%, 0.5%, and 0.5%, respectively.

Metabolism Comparison Across Species

A comparison of metabolites across species is presented below. Although there was no unique human metabolite, levels of TAK-536 M-II were relatively low in animals. Therefore, additional pharmacodynamic and toxicology studies, including rodent carcinogenicity studies, were conducted with this metabolite.

Comparison of Metabolites Across Species

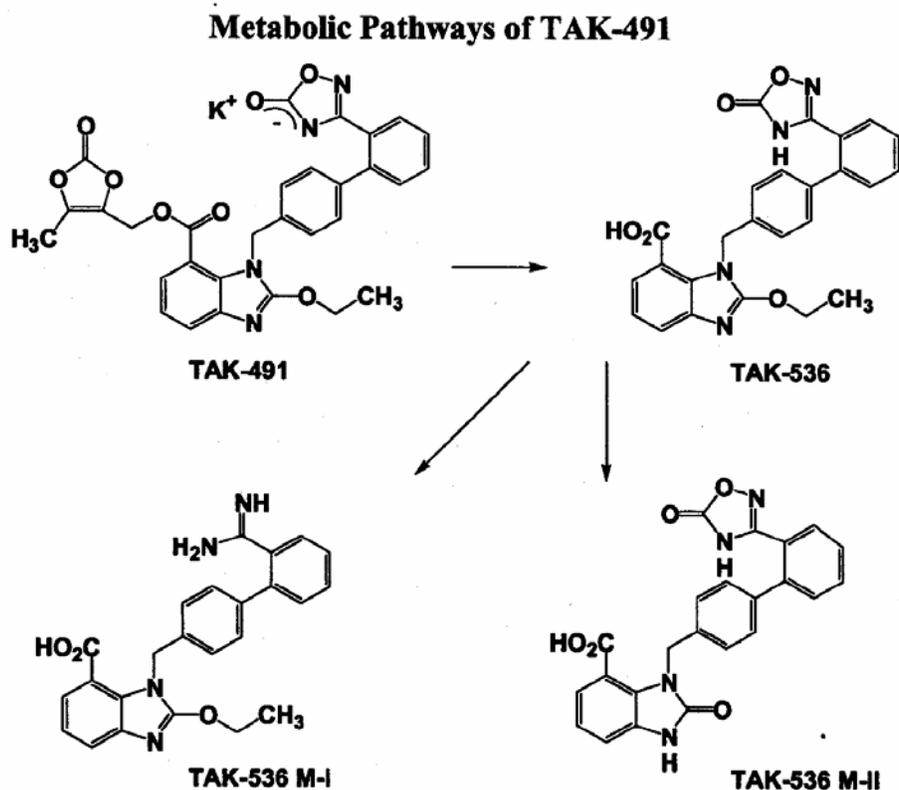
Compound	Rats	Dogs	Monkeys	Humans
TAK-491F	P, F	P, F	(a)	(b)
TAK-536	P, B, U, F	P, U, F	P, U, F	P, U
TAK-536 M-I	P, B, U, F	P, U, F	P, U, F	P, U, F
TAK-536 M-II	P, B, U, F	P, F	P, U, F	P, U, F
Report Number:	491-00079 491-00076	491-00080 491-00077	536-C-46-00416 536-C-46-00417	Study 012

P=plasma (serum used for human metabolite analysis), U=urine, F=feces, B=bile.

(a) Monkeys were dosed with TAK-536. There was no TAK-491 expected in the samples.

(b) TAK-491F concentration was below detection limit.

TAK-491 was rapidly hydrolyzed in the plasma from rats, dogs, monkeys, and humans and in the incubation mixture with human hepatic and intestinal S9 fractions. Both TAK-536 M-I and TAK-536 M-II were present in rats and dogs after an oral administration of [¹⁴C]TAK-491 and were present after incubation of [¹⁴C]TAK-536 with human hepatic microsomes. TAK-491 is converted to TAK-536 via hydrolysis. The pharmacologically inactive metabolites, TAK-536 M-I and TAK-536 M-II, are formed by decarboxylation and dealkylation of TAK-536, respectively. Plasma and feces samples were used to elucidate the structures of TAK-491 metabolites after oral dosing of rats with [¹⁴C]TAK-491; samples from incubation of [¹⁴C]TAK-536 with human hepatic microsomes also were used. The identification of the metabolites was performed by LC/MS/MS. The metabolic pathway of TAK-491 is shown below.



***In Vitro* Studies**

***In Vitro* Metabolism**

When [¹⁴C]TAK-491 was incubated at 37°C in the plasma from rats, dogs, and humans, most of TAK-491 was hydrolyzed to TAK-536 within 5 minutes. Human serum albumin could contribute to the hydrolysis of TAK-491 to TAK-536 as the amount of TAK-491 was lower in the presence of human serum albumin. The enzyme that catalyzes the hydrolysis of TAK-491F in human plasma would be aryl esterase, the same enzyme that is responsible for the hydrolysis of olmesartan medoxomil. Plasma hydrolytic activity of TAK-491 was not affected by the addition of diisopropyl fluorophosphate.

[¹⁴C]TAK-491 also was rapidly hydrolyzed to TAK-536 by hepatic and intestinal S9 fractions from rats, dogs, and humans, with the exception of relatively slow conversion from TAK-491 to TAK-536 by dog intestinal S9 fractions. *In vitro* hydrolysis studies of TAK-491 with hepatic and intestinal S9 fractions from rats and humans showed that TAK-491 was mainly biotransformed to TAK-536, and that the production of the medoxomil alcohol was not observed for up to 30 minutes. These results are similar to those seen with olmesartan medoxomil. Both TAK-536 M-I and TAK-536 M-II were formed when [¹⁴C]TAK-536 was incubated with hepatic microsomes from mice, rats, dogs, monkeys, and humans. An unknown metabolite, UK-A, was the major metabolite in mice. TAK-536 M-I was the major metabolite in rats and dogs. TAK-536 M-II was the major metabolite in human hepatic microsomes. A small amount of metabolite TAK-536 M-II was also observed in monkey hepatic microsomes. All metabolites formed by human hepatic microsomes were also formed by hepatic microsomes from animal species.

Human CYP isoforms involved in the metabolism of TAK-536 were identified in a correlation study using human hepatic microsomes and in a metabolism study of TAK-536 using microsomes from baculovirus-infected insect cells expressing human CYP isoforms. Incubation of [¹⁴C]TAK-536 with microsomes expressing human CYP isoforms indicated that CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 all were capable of metabolizing TAK-536; among them, CYP2C9 showed the highest activity in metabolizing TAK-536 to TAK-536 M-II. In the correlation study, TAK-536 was metabolized to TAK-536 M-I, TAK-536 M-II, and other unidentified metabolites. The elimination rate of TAK-536 and formation rate of TAK-536 M-II correlated with CYP2C9, CYP2B6, and CYP2C8 activity. The formation rate of TAK-536 M-I correlated with CYP2C8 activity. Based on these results, it was concluded that TAK-536 was metabolized to TAK-536 M-II primarily by CYP2C9.

CYP Induction

CYP3A induction was studied *in vitro* in primary human hepatocytes. Based on the measurement of testosterone 6β-hydroxylation activity, TAK-491 and TAK-536 had

virtually no effect on the activity of CYP3A in human hepatocytes at free drug concentrations up to 100 µmol/L and 30 µmol/L, respectively. This result indicates that TAK-536 is unlikely to induce CYP3A activity. Since the expected plasma concentrations of TAK-491 in humans would be below LOQ, CYP3A induction by TAK-491 in humans is even more unlikely.

CYP Inhibition

The *in vitro* inhibitory effects of TAK-491 and TAK-536 on the marker activities of CYP isoforms were examined in human liver microsomes and results are summarized in a Table below. Inhibition of CYP isoforms (IC_{50} values) by TAK-491 were determined for CYP2C8 (3.5 µmol/L), CYP2C9 (4.7 µmol/L) CYP3A4 (midazolam 1'-hydroxylation, 29 µmol/L), CYP2B6 (50 µmol/L), CYP3A4 (testosterone 6β-hydroxylation, 44 µmol/L), CYP1A2 (65 µmol/L), and CYP2C19 (66 µmol/L). TAK-491 did not inhibit CYP2D6 or CYP2E1 activities ($IC_{50} > 100$ µmol/L). Because intestinal CYP2C activity is limited and TAK-491 is hydrolyzed extensively to TAK-536 during intestinal absorption, clinical drug-drug interactions based on CYP inhibition are very unlikely. In this study, TAK-536 did not show any inhibitory effect on any CYP isoform studied ($IC_{50} > 100$ µmol/L).

The *in vitro* inhibitory effects of TAK-536 at concentrations of 3, 10, 30, and 100 µmol/L also were examined using human recombinant CYP isoforms; CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C9*2, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Two markers were used for assay the CYP3A4 activity, one is midazolam and is referred as CYP3A4(M) in the Table, the other is testosterone and is referred as CYP3A4(T) in the same table. TAK-536 decreased the activities of recombinant CYP2C9 at 30 and 100 µmol/L but had no inhibitory effect on other CYP isoforms at 100 µmol/L. TAK-536 inhibited more than 50% of recombinant CYP2C9 activity compared to control activity at a concentration of 100 µmol/L. TAK-536 also slightly inhibited recombinant CYP2C8 activity at a concentration of 100 µmol/L. Based on these recombinant CYP isoform data, it was estimated that the IC_{50} values for TAK-536 were about 50 µmol/L for inhibiting recombinant CYP2C9 activity and >100 µmol/L for inhibiting the activity of other recombinant isoforms. Based on these two studies, it is concluded that TAK-536 is not expected to have any clinically significant drug-drug interactions when co-administrated with drugs that are metabolized by CYP2C9.

Inhibitory Effects of TAK-491 and TAK-536 on CYP Isoform Activities in Human Liver Microsomes

CYP Isoform	Remaining Marker Activity (%)					Remaining Marker Activity (%)				
	TAK-491 (µmol/L)				Positive Control	TAK-536 (µmol/L)				Positive Control
	3	10	30	100		3	10	30	100	
CYP1A2	100.7	98.5	79.4	32.6	5.6	99.6	98.9	100	94.9	6.4
CYP2B6	100.6	88.7	69.8	28.5	24.3	98.9	97.9	96.3	99.5	18.9
CYP2C8	55.4	23.9	<7.8	<7.8	15.9	101.3	96.2	97.5	89.9	18.4
CYP2C9	65.4	29.9	10.1	<3.0	10.2	99.0	98.0	85.6	62.1	11.3
CYP2C19	96.9	75.5	70.0	40.1	13.0	98.4	98.4	98.0	96.0	12.4
CYP2D6	99.7	100.3	85.7	56.9	15.2	98.1	96.9	96.9	94.9	17.7
CYP2E1	100.2	104.1	105.1	91.1	17.7	99.5	100.3	101.3	100.8	15.3
CYP3A4(M)	100.0	80.7	49.7	21.1	6.2	100.5	100.2	103.2	101.2	6.6
CYP3A4(T)	95.7	82.9	61.5	29.5	4.8	101.4	99.5	100.0	100.9	6.1

Source: 491-10051, 491-10052.

Marker activity: CYP1A2: Phenacetin *O*-deethylation, CYP2B6: Bupropion hydroxylation, CYP2C8: Paclitaxel 6 α -hydroxylation, CYP2C9: Diclofenac 4'-hydroxylation, CYP2C19: (*S*)-Mephenytoin 4'-hydroxylation, CYP2D6: Bufuralol 1'-hydroxylation, CYP2E1: Chlorzoxazone 6-hydroxylation, CYP3A4(M): Midazolam 1'-hydroxylation, CYP3A4(T): Testosterone 6 β -hydroxylation.

CYP/Isoform substrate / Incubation period (minutes): CYP1A2/Phenacetin/30, CYP2B6/Bupropion/30, CYP2C8/Paclitaxel/20, CYP2C9/Diclofenac/5, CYP2C19/(*S*)-Mephenytoin/30, CYP2D6/Bufuralol/20, CYP2E1/Chlorzoxazone/30, CYP3A4/Midazolam/5, CYP3A4/Testosterone/10.

Positive control (concentration): CYP1A2, α -Naphthoflavone (0.2 µmol/L); CYP2B6, Ticlopidine (1 µmol/L); CYP2C8, Quercetin (25 µmol/L); CYP2C9, Sulfaphenazole (5 µmol/L); CYP2C19, Tranilcypropramine (25 µmol/L); CYP2D6, Quinidine (1 µmol/L); CYP2E1, Diethylthiocarbamate (200 µmol/L); CYP3A4, Ketoconazole (0.5 µmol/L).

5.1.2.4 Excretion

Radioactivity excretion profiles following administration of single oral doses of [¹⁴C]TAK-491 or [¹⁴C]TAK-536 to rats and dogs are summarized below. The metabolite profiles in bile (rat only), urine, and feces are summarized in the second Table.

Excretion of Total Radioactivity in Male Rats, Dogs, and Monkeys Following a Single Oral Dose of [¹⁴C]TAK-491 or [¹⁴C]TAK-536

Species	Test Article	Route	Food	Dose (mg/kg)	Collection period (hr)	% of Dose Radioactivity			Report Number
						Urine	Feces	Total	
Rat	[¹⁴ C]TAK-491	Oral	Fed	1.33	0-168	2.0	95.0	97.0	[491-00044]
Rat	[¹⁴ C]TAK-536	Oral	Fed	1	0-72	1.4	98.5	99.9	[536-C-46-00292]
Dog	[¹⁴ C]TAK-491	Oral	Fed	1.33	0-120	5.4	96.8	102.2	[491-00051]
Dog	[¹⁴ C]TAK-536	Oral	Fed	1	0-96	2.5	95.2	97.7	[536-C-46-00292]
Monkey	[¹⁴ C]TAK-536	Oral	Fasted	3	0-96	10.2	87.3	97.4	[536-C-46-00413]
Monkey	[¹⁴ C]TAK-536	Oral	Fed	3	0-72	4.5	91.1	95.6	[536-C-46-00413]

n=2-4.

Percent of Radioactivity in Urine, Feces, and Rat Bile After Oral Administration of 1.33 mg/kg [¹⁴C]TAK-491 to Male Rats and Dogs

Compound Collection time	Percent of dose radioactivity (%)				
	Rat (a)			Dog (b)	
	Urine (0-24 hr)	Feces (0-48 hr)	Bile (0-24 hr)	Urine (0-48 hr)	Feces (0-48 hr)
Total ¹⁴ C	1.9	94.2	32.8	5.4	94.2
TAK-491F	ND	0.1	ND	ND	0.8
TAK-536	0.1	0.6	5.6	3.4	0.1
TAK-536 M-I	0.3	76.7	14.1	1.1	77.6
TAK-536 M-II	0.1	1.8	0.5	ND	0.4
Others	1.4	15.0	12.6	0.9	15.3

Source: 491-00076, 491-00077.

n=3-4.

ND=not detected.

(a) Urine and feces of nonfasted male rats were collected after oral administration [491-00044] and bile of nonfasted male bile duct-cannulated rats was collected after intraduodenal administration [491-00045].

(b) Urine and feces of nonfasted male dogs were collected after oral administration [491-00051].

Rat

Following a single oral dose of 1.33 mg/kg of [¹⁴C]TAK-491 to nonfasted male rats, the feces was the major route of excretion, and accounted for 95.0% of administered total radioactivity. About 2.0% of the dose was excreted in the urine. The radioactivity recovered in trapped, expired air was negligible, suggesting that the ¹⁴C labeling site was metabolically stable. Excretion of total radioactivity was almost complete 72 hours after dosing. The mean total recovery of the radioactive dose was 97.0%, with 78.1% of the dose excreted within 24 hours after dosing.

After dosing with [¹⁴C]TAK-536 almost all radioactivity was excreted in the feces (98.5%) via the hepatobiliary route and was eliminated from the body mostly within 48 to 72 hours. [¹⁴C]TAK-536 M-I accounted for most of radioactivity in rat feces. Biliary excretion of [¹⁴C]TAK-491 was examined in bile duct-cannulated, fed male rats that were given a single, intraduodenal dose of [¹⁴C]TAK-491 at 1.33 mg/kg. Fractions of the administered radioactive dose excreted into bile, urine, and feces within 24 hours were 32.8%, 2.8%, and 37.4%, respectively, while 0.0%, 22.6%, and 3.5% of the dose remained in the gastric contents, intestinal contents, and carcass, respectively. These data indicate that most of the absorbed [¹⁴C]TAK-491 radioactivity was excreted into the bile after intraduodenal administration. The extent of re-absorption of [¹⁴C]TAK-491-derived radioactivity in the bile was estimated to be about 7.1%. [¹⁴C]TAK-491-derived radioactivity undergoes minimal enterohepatic recirculation.

Dog

Following a single oral dose of 1.33 mg/kg of [¹⁴C]TAK-491 to fed male dogs, the major route of excretion was via the feces. Urinary and fecal excretion of radioactivity was

almost complete 72 hours after dosing, and the excretion ratios into the urine and feces at that time point were 5.4% and 96.7% of the dose, respectively. The mean total recovery of the radioactive dose during the 120 hour postdose period was 102.2%, with >99% of the dose excreted within 48 hours after dosing. Of the dosed radioactivity, the respective excretion ratios for TAK-491F, TAK-536, TAK-536 M-I, TAK-536 M-II, and other metabolites were 0.0%, 3.4%, 1.1%, 0.0%, and 0.9% in the urine, and 0.8%, 0.1%, 77.6%, 0.4%, and 15.3% in the feces within 48 hours. TAK-491 was metabolized almost completely before being eliminated and the dosed radioactivity was excreted mainly into the feces after oral administration. Most of the dosed radioactivity was excreted in the feces (95.0%) and was mostly eliminated from the body within 48 to 72 hours after dosing with [¹⁴C]TAK-536. TAK-536 M-I was the major metabolite (89.2%) in the feces.

Monkey

The excretion of dosed radioactivity was almost complete 72 hours after a single oral administration of 3 mg/kg [¹⁴C]TAK-536 to fasted/fed monkeys. Respective urinary and fecal excretion of dosed radioactivity was 10.2% and 87.3% in fasted animals and 4.5% and 91.1% in fed animals. Of the 9.7% radioactivity excreted into the urine 24 hours postdose, 63.9% was TAK-536. The excretion ratios of TAK-536, TAK-536 M-I, TAK-536 M-II, and other metabolites were 6.2%, 0.7%, 0.2%, and 2.8% of the dosed radioactivity, respectively. TAK-536 M-I was the major component in feces; of the 87.0% dosed radioactivity excreted into the feces 72 hours after dosing, 92.0% was TAK-536 M-I. The excretion ratios of TAK-536, TAK-536 M-I, TAK-536 M-II, and other metabolites were 0.2%, 80.0%, 0.2%, and 6.7% of the dose, respectively. The major component in excreta was TAK-536 M-I, which was excreted predominantly into the feces and accounted for about 80% of the dose.

Rat Lactal Secretion

The excretion of radioactivity into the breast milk of lactating rats on lactation day 14 was investigated. Concentrations of radioactivity in the plasma and milk of lactating rats after a single oral administration of [¹⁴C]TAK-491 at a dose equivalent to 1 mg [¹⁴C]TAK-536/kg are listed below. [¹⁴C]TAK-491-related compounds were excreted into milk at concentrations lower than those in plasma.

Plasma and Milk Total Radioactivity Following a Single Oral Administration of 1.33 mg/kg [¹⁴C]TAK-491 to Lactating Rats

Lactation Day/Time	Concentration of μg [¹⁴ C]TAK-536 Equivalents/mL of Plasma or Milk				
	14/2 hr	14/4 hr	14/8 hr	15/24 hr	16/48 hr
Plasma	1.908	1.38	0.736	0.099	0.006
Milk	0.064	0.119	0.137	0.017	0.001

Source: 491-10049.

n=3.

The metabolic composition in the plasma and milk of lactating rats were determined and results are presented below. The major radioactive component in the plasma and milk was TAK-536.

Composition of Total Radioactivity in Plasma and Milk Following a Single Oral Dose of 1.33 mg/kg [¹⁴C]TAK-491 to Lactating Rats

Sample	Sampling Time (hr)	Concentration (µg equivalent of TAK-536/mL)	Percentage of TAK-491F and Metabolites in Sample				
			TAK-491F	TAK-536	TAK-536 M-I	TAK-536 M-II	Others
Plasma	2	1.908	0.1	95.2	3.0	0.7	1.0
	4	1.380	0.1	96.5	1.9	0.7	0.8
	8	0.736	0.0	97.0	1.4	0.7	0.9
	24	0.099	0.0	83.8	4.0	1.0	11.2
Milk	2	0.064	0.0	89.1	0.0	0.0	10.9
	4	0.119	0.0	81.5	0.0	0.8	17.7
	8	0.137	0.0	78.8	0.7	0.7	19.8
	24	0.017	0.0	52.9	0.0	5.9	41.2

Source: 491-10050.
n=3.

Pharmacokinetic Drug Interactions

The effect of pioglitazone, on the pharmacokinetics of TAK-536 was studied in monkeys. After IV administration of 0.2 mg/kg of TAK-536 in combination with oral 36 mg/kg of pioglitazone, the AUC₍₀₋₂₄₎ value of TAK-536 was 8.27 µg·hr/mL, which was higher than the corresponding value of 3.36 µg·hr/mL for TAK-536 alone; T_{1/2} of TAK-536 in combination with pioglitazone was 2.3 hours and was longer than the corresponding value of 1.6 hours for TAK-536 alone. These results suggest that pioglitazone may inhibit the elimination of TAK-536.

After oral administration of 3 mg/kg of TAK-536 in combination with oral 36 mg/kg of pioglitazone, the AUC₍₀₋₂₄₎ value of TAK-536 was only 4.83 µg·hr/mL and was lower than the corresponding AUC₍₀₋₂₄₎ value of 6.71 µg·hr/mL for TAK-536 alone. This result suggests that concomitant dosing with pioglitazone reduces the absorption of TAK-536.

Other Pharmacokinetic Studies

Chlorthalidone is a diuretic agent and is used in several TAK-491/chlorthalidone combination studies. Several *in vitro* studies were conducted with chlorthalidone. The results indicate that chlorthalidone was not metabolized by CYP isoforms and was not a CYP inhibitor. A Caco-2 study showed that the Papp of [¹⁴C]chlorthalidone from the basal to apical side was higher than that from the apical to the basal side, and the efflux activity of [¹⁴C]chlorthalidone was inhibited by quinidine and verapamil. These results suggest that chlorthalidone is a substrate for P-gp.

5.2 Toxicokinetics

(presented and discussed in context with toxicology studies)

6 General Toxicology

6.1 Single-Dose Toxicity

From review by A. Proakis, 08/02/2005:

The single-dose toxicity of TAK-491 was evaluated in rats (oral and IV) in a single-dose study design and in dogs (oral) using an escalating dose study design. No deaths occurred in rats at oral doses up to 2000 mg/kg and in dogs at oral cumulative doses up to 30 mg/kg. The study results and noteworthy findings are summarized in Table 8.

Table 8

Single-Dose Toxicity Studies – Noteworthy Findings

Species/ Strain	Vehicle	Number/ Sex/Group	Dose (mg/kg)(a,b)	Observed Maximum Non-lethal Dose (mg/kg)(a)	Approximate Lethal Dose (mg/kg)(a)
Oral Gavage Single-Dose Toxicity Study in Rats					
F344/Jcl rats	0.5% MC 0.25% to 1% CA	5	0, 500, and 2000	2000	>2000
Noteworthy findings: 2000 mg/kg: gray-colored stool.					
Intravenous Single-Dose Toxicity Study in Rats					
F344/Jcl rats	NND	5	0 (saline), 0 (vehicle), 8, 40, and 200	8 M 40 F	8 to 40 M 40 to 200 F
Noteworthy findings: 0 (vehicle): ↓ locomotor activity, tremors, chromaturia, and purpura at injection site; 8 mg/kg: ↓ locomotor activity, tremors, chromaturia, and purpura at injection site; 40 mg/kg/day: mortality (1M), prone position, tonic convulsions and dyspnea (male that died), ↓ body weight, ↓ locomotor activity, tremors, chromaturia, bradypnea, prone position, and purpura at injection site; 200 mg/kg: mortality (5M/5F), lateral position, prone position, tonic convulsions, and dyspnea.					
Oral Gavage Escalating Dose Toxicity Study in Dogs					
Beagle dogs	0.5% MC 0.015% to 1% CA	2	0, 30, 100, 300, 1000, and 2000	2000	>2000
Noteworthy findings: ≥30 mg/kg: vomiting; ≥100 mg/kg: diarrhea.					
Oral Gavage Escalating Dose Toxicity Study in Dogs					
Beagle dogs	0.5% MC 0.0015% to 0.015% CA	2	0, 3, 10, and 30	30	>30
Noteworthy findings: 30 mg/kg: vomiting and diarrhea.					

M: male; F: female; CA: citric acid; MC: methylcellulose; NND: 5% *N,N*-dimethylacetamide, 80% polyethylene glycol 400, and saline (approximate pH=5); ↓: decreased.
(a) TAK-491.
(b) Unless specified, control (0 mg/kg) animals received the vehicle.

6.2. Repeat-Dose Toxicity**6.2.1 4-week repeated oral (gavage) dose toxicity study in rats**

From reviewed by A. Proakis dated 8/05/05:

4-Week Repeated Oral (Gavage) Dose Toxicity Study in Rats

Study Facility: (b) (4)

Study No.: B-5355

Study Dates: Initiation of dosing, 7/21/04; Necropsy, 8/18/04

GLP Compliance: Compliance with GLP regulations attested.

QA Report: Yes

Animals: Male and female F344/Jcl rats (M, 85-118 gm; F 95-121 gm at time of dosing) were housed individually and maintained on a pellet diet of CE-2 (CLEA, Japan) and tap water *ad libitum*.

Drug Administration: TAK-491 (Lot # M491-001) was suspended in 0.5% methylcellulose and administered orally by gavage. Control animals received the methylcellulose solution. Dose Levels: 0 (vehicle), 2, 20, 200 and 2000 mg/kg/day (10/sex/dose group); additional 7/sex/drug treated group for satellite toxicokinetics.

Observations/Measurements: Animals were observed daily for survival and clinical signs of toxicity. Body weights were measured on days 1, 4 and 7 of treatment and then twice weekly at 3 or 4 day intervals. Food consumption was measured on days 1, 4 and 7 of treatment and then every 3 to 4 days.

Ophthalmologic examinations were performed prior to dosing and during week 4 of treatment. Urine was collected during week 4 of treatment for urinalysis. Blood samples were obtained from the abdominal aorta at time of necropsy for hematology and clinical chemistry analyses. Animals were necropsied at the end of the 4 week treatment period and the following organs removed and weighed: brain, pituitary, adrenals, thymus, spleen heart, lungs liver, kidneys, testes prostate and ovaries. For all main study animals, sections of the following organs and tissues were placed onto slides, fixed and examined microscopically. The paired organs, indicated by an asterisk (*), were examined bilaterally; those marked with # were examined unilaterally.

cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves#, eyeballs*, optic nerves*, Harderian glands*, pituitary, thyroids*, parathyroids*, adrenals*, thymus, spleen, submandibular lymph node, mesenteric lymph node, heart, thoracic aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands*, sublingual glands*, liver, pancreas, kidneys*, urinary bladder, testes*, epididymides*, prostate, seminal vesicles*, ovaries*, uterus (both horns)*, vagina, mammary glands (inguinal region, both sides)#, sternum (including bone marrow), femurs (including knee joint and bone marrow)#, femoral skeletal muscles# and skin (inguinal, both sides)#

Blood samples were obtained from satellite animals on day 1 and during week 4 of treatment for measurement of plasma drug levels of TAK-491, TAK-536 and TAK-536 metabolites.

Results

Mortality and Clinical Signs of Toxicity

No animals died during the study. No clinical signs of toxicity were observed.

Body Weight

Lower than control mean body weight was noted at study termination for males in the 20, 200 and 2000mg/kg/day dose groups and for females in him 2000 mg/kg /day dose group (Table 9, Figure 7).

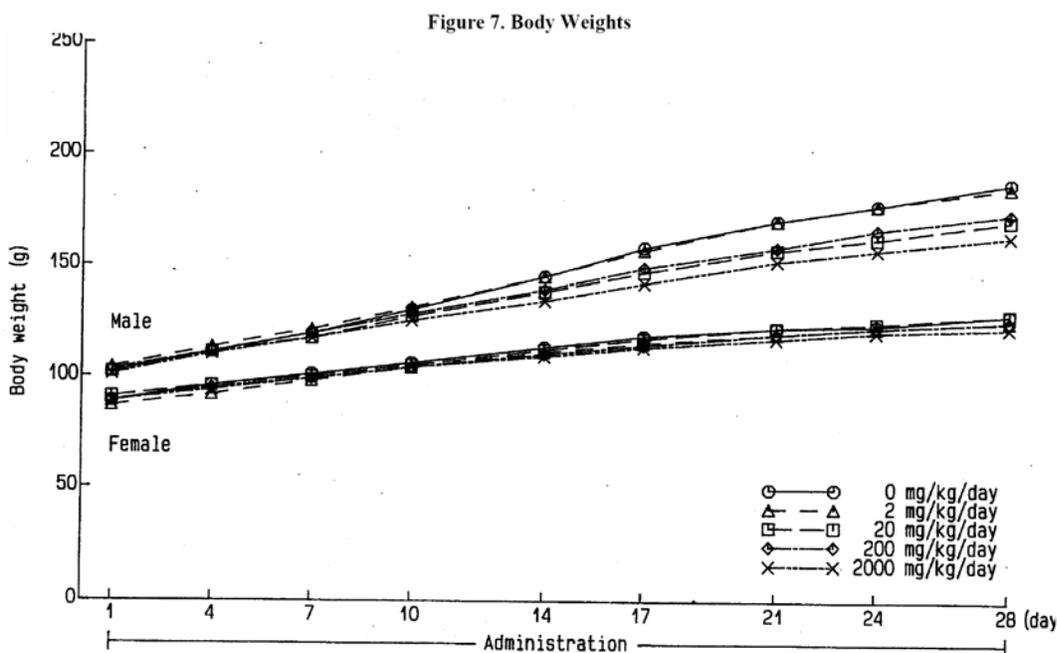
Table 9. Mean Body Weight and Body Weight Gains

Sex	Male				Female				
	Dosage(mg/kg/day)	2	20	200	2000	2	20	200	2000
No. of animals	10	10	10	10	10	10	10	10	10
Mean body weight on day 28	N	-9%*	-7%*	-13%*	N	N	N	-5%*	
Body weight gain (day 1-28)	N	-21%*	-18%*	-27%*	N	N	N	-18%*	

Values in the table indicate percentage of change against the control mean (-: decrease).

N: No remarkable changes

* p<0.05 (significantly different from the control group)



Food Consumption

Lower than control food consumption was observed beginning on day 10 of treatment for the male 20, 200 and 2000 mg/kg/day groups and the female 2000 mg/kg/day group.

Ophthalmologic Examination

No treatment-related ocular effects were observed.

Urinalysis

Higher than control urine volume was noted for males and females in the 2000 mg/kg/day group (39% higher and 26% higher, respectively).

Hematology

Slightly lower than control levels of RBC counts, hemoglobin concentration and hematocrit were noted in the 20, 200 and 2000 mg/kg/day dose groups (Table 10). But the largest difference from control was increased WBC which was limited to high dose females.

Table 10. Hematology Results

Sex	Male				Female				
	Dosage(mg/kg/day)	2	20	200	2000	2	20	200	2000
No. of animals	10	10	10	10	10	10	10	10	10
RBC	N	-4%*	-4%*	-4%*	N	-4%*	-3%*	-4%*	
Hb	N	-4%*	-2%*	-2%*	N	-4%*	-3%*	-3%*	
Ht	N	-4%*	-4%*	-4%*	N	-4%*	-4%*	-4%*	
WBC	N	N	N	N	N	N	N	+27%*	
Monocyte ratio	N	N	N	↑*	N	N	↓*	↓*	

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).

N: No remarkable changes

↑, ↓: High or low values (Percentage of change against the control mean for monocyte ratio was not calculated, because control or treated group mean value was zero.)

* $p \leq 0.05$ (significantly different from the control group)

Clinical Chemistry

Significantly higher than control levels of blood urea nitrogen was noted for all treated male groups (+14%, +43%, +71% and +100% for the 2, 20, 200 and 2000 mg/kg/day groups, respectively). Blood urea nitrogen was unaffected in treated females. Higher than control serum creatinine was noted for males (+17%) and females (+14%) treated with 2000 mg/kg/day.

Organ Weights

Differences in thymus, heart, liver, spleen and adrenal weights between control and treated groups were observed (Table 11). Differences in organ weights between treated and control groups were more prevalent in males than in females and appeared to reflect treatment related effects on mean body weight.

Table 11. Organ Weights

Sex	Male				Female				
	Dosage(mg/kg/day)	2	20	200	2000	2	20	200	2000
No. of animals	10	10	10	10	10	10	10	10	10
Body weight at necropsy	N	-9%*	-7%*	-13%*	N	N	N	N	-5%
Thymus									
absolute	N	N	N	-26%*	N	N	N	N	-14%*
relative	N	N	N	-15%*	N	N	N	N	-10%*
Heart									
absolute	-7%*	-22%*	-19%*	-27%*	-11%*	-11%*	-16%*	-16%*	-23%*
relative	-6%*	-14%*	-11%*	-17%*	-8%*	-11%*	-14%*	-14%*	-16%*
Liver									
absolute	N	-14%*	-11%*	-16%*	N	N	N	N	N
relative	N	-6%*	-4%*	-3%*	N	N	N	N	N
Spleen									
absolute	N	-12%*	-7%*	-20%*	N	N	-6%*	-9%*	-9%*
relative	N	N	N	-8%*	N	N	N	N	N
Adrenal									
absolute	N	N	N	+11%*	N	N	N	N	+8%
relative	N	+13%*	+9%*	+26%*	N	N	N	N	+12%*

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).

N: No remarkable changes

* $p \leq 0.05$ (significantly different from the control group)

Macroscopic Pathology

Dark red foci were observed in the glandular stomach in 4/10 males treated with 2000 mg/kg/day of TAK-491 (Table 12).

Table 12. Necropsy Findings

Sex	Male					Female					
	Dosage(mg/kg/day)	0	2	20	200	2000	0	2	20	200	2000
No. of animals	10	10	10	10	10	10	10	10	10	10	10
Stomach											
Dark red focus in glandular stomach	0	0	0	0	4	0	0	0	0	0	0

Values in the table indicate the number of animals.

Microscopic Pathology

Treatment-related findings (Table 13) were observed in the kidney (both sexes), adrenal gland (both sexes) and stomach (males). Hypertrophy of the JG cells of the kidney and atrophy of the adrenal zona glomerulosa are considered to be pharmacological responses to blockade of the renin-angiotensin system by TAK-491 and not regarded as toxicologically relevant. Erosion of the glandular stomach seen in 5/10 males is considered to be a treatment-related toxicity (4 of these males also showed macroscopic dark red foci in the stomach).

Table 13. Histopathology Findings

Sex	Male					Female					
	Dosage (mg/kg/day)	0	2	20	200	2000	0	2	20	200	2000
No. of animals	10	10	10	10	10	10	10	10	10	10	10
Kidney											
Hypertrophy of juxtaglomerular cell	0	0	0	1	6	0	0	0	1	3	
Eosinophilic body of tubular cell	10	9	4	2	2	0	0	0	0	0	
Adrenal											
Atrophy of zona glomerulosa	0	0	7	10	10	0	0	5	8	9	
Stomach											
Erosion in glandular stomach	0	0	0	0	5	0	0	0	0	0	

Values in the table indicate the number of animals.

The no-observed-adverse-effect-level (NOAEL) was considered to be 200 mg/kg/day for male rats (AUC of active metabolite, TAK-536, 762 ug.hr/ml) and 2000 mg/kg/day for female rats (AUC for TAK-536, 1348 ug.hr/ml).

Toxicokinetics

TAK-491 was not detected in the plasma at any dosage levels after the 1st and 27th doses of TAK-491. The C_{max} and AUC values for TAK-536, TAK-536 MI and TAK-536 M-II increased with increasing doses of TAK-491. There were no apparent gender differences in any toxicokinetic parameter (Table 14).

Table 14. Toxicokinetic Summary

Sex	Male (n=3)				Female (n=3)				
	Dosage(mg/kg/day)	2	20	200	2000	2	20	200	2000
TAK-491F									
T_{max} (h)									
Day 1 (1st dose)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Week 4 (27th dose)	NA	NA	NA	NA	NA	NA	NA	NA	NA
C_{max} (ng/mL)									
Day 1 (1st dose)	0	0	0	0	0	0	0	0	0
Week 4 (27th dose)	0	0	0	0	0	0	0	0	0
AUC_{0-24h} (ng·h/mL)									
Day 1 (1st dose)	0	0	0	0	0	0	0	0	0
Week 4 (27th dose)	0	0	0	0	0	0	0	0	0
TAK-536									
T_{max} (h)									
Day 1 (1st dose)	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Week 4 (27th dose)	1.0	1.0	1.0	0.5	0.5	0.5	0.5	0.5	0.5
C_{max} (ng/mL)									
Day 1 (1st dose)	1779	29321	158680	207459	3185	35170	161910	190556	190556
Week 4 (27th dose)	3016	33834	127002	267194	4532	63873	149597	304599	304599
AUC_{0-24h} (ng·h/mL)									
Day 1 (1st dose)	13774	188346	923566	1703642	18981	195931	888030	1961386	1961386
Week 4 (27th dose)	17447	174012	761562	1639305	25020	235466	689245	1347978	1347978
TAK-536 M-I									
T_{max} (h)									
Day 1 (1st dose)	2.0	2.0	0.5	2.0	1.0	1.0	1.0	2.0	2.0
Week 4 (27th dose)	1.0	1.0	1.0	1.0	1.0	0.5	1.0	0.5	0.5
C_{max} (ng/mL)									
Day 1 (1st dose)	60	1616	1419	1427	151	576	1386	1617	1617
Week 4 (27th dose)	173	983	2025	2655	221	1425	2863	2252	2252
AUC_{0-24h} (ng·h/mL)									
Day 1 (1st dose)	509	8992	18784	22863	1085	5810	16964	19110	19110
Week 4 (27th dose)	1191	6289	21419	25569	1210	8140	17667	21243	21243
TAK-536 M-II									
T_{max} (h)									
Day 1 (1st dose)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Week 4 (27th dose)	0.5	1.0	1.0	1.0	0.5	0.5	0.5	0.5	0.5
C_{max} (ng/mL)									
Day 1 (1st dose)	2	79	459	628	4	73	425	578	578
Week 4 (27th dose)	2	64	282	607	7	93	260	450	450
AUC_{0-24h} (ng·h/mL)									
Day 1 (1st dose)	9	431	2630	4204	10	359	2271	4390	4390
Week 4 (27th dose)	3	297	1557	3038	8	317	1116	1901	1901

NA: Not applicable (Not calculated)

TAK-491F is the free acid form of TAK-491

6.2.2 4-week repeated oral (gavage) dose toxicity study in dogs

Attached reviewed by A. Proakis dated 8/05/05:

4-Week Repeated Oral (Gavage) Dose Toxicity Study in Dogs

Study Facility: (b) (4)

Study No.: B-5342

Study Dates: Initiation of dosing, 8/05/04; Necropsy, 9/02/04

GLP Compliance: Compliance with GLP regulations attested.

QA Report: Yes

Animals: Male and female Beagle dogs (M, 8.0 – 10.9 kg; F 7.6 – 9.7 kg at time of dosing) were housed individually and maintained on 300 gm/day of pellet diet (DS-A; Oriental Yeast Co., Japan) and tap water *ad libitum*.

Drug Administration: TAK-491 (Lot # M491-001) was suspended in 0.5% methylcellulose and 0.2% w/v of citric acid and administered orally by gavage. Control animals received the drug vehicle.

Dose Levels: 0 (vehicle), 3, 12, 60 and 300 mg/kg/day (3/sex/dose group).

Observations/Measurements: Animals were observed 3 times daily during treatment period for survival and clinical signs of toxicity. Body weights were measured on day 1 of treatment and weekly thereafter. Food consumption was measured daily. Ophthalmologic examinations were performed prior to dosing and during week 4 of treatment. Electrocardiograms were obtained prior to dosing and during week 4 of treatment. Urine was collected prior to dosing and during week 4 of treatment for urinalysis. Blood samples were obtained from a cephalic vein prior to dosing and during week 4 of treatment for hematology and clinical chemistry analyses. Blood samples were also obtained from a cephalic vein at varying intervals (up to 24 hours post dose) after dosing on day 1 and after the 28th dose for measurement of plasma drug levels of TAK-491, TAK-536 and TAK-536 metabolites. Animals were necropsied at the end of the 4 week treatment period and organs were removed and weighed (Table 15). Sections of organs and tissues from all animals were placed onto slides, fixed and examined microscopically (Table 15).

Table 15. Pathological Examination

Organ/tissue	Examination	Weighing	Organ/tissue	Examination	Weighing
	H-E			g	
Cerebrum	O	} O	Jejunum	O	
Cerebellum	O		Ileum	O	
Medulla oblongata	O		Cecum	O	
Spinal cord	O		Colon	O	
Optic nerves ^{a)}	O		Rectum	O	
Sciatic nerve ^{b)}	O		Submandibular gland ^{b)}	O	O
Eyeballs ^{a)}	O		Sublingual gland ^{b)}	O	
Lacrimal gland ^{b)}	O		Liver	O	} O
Pituitary	O	O	Gallbladder	O	
Thyroid gland ^{a)}	O		Pancreas	O	O
Parathyroid ^{a)}	O		Kidney ^{a)}	O	O
Adrenal ^{a)}	O	O	Urinary bladder	O	
Thymus	O	O	Testis ^{a)} / Ovary ^{a), c)}	O	
Spleen	O	O	Epididymis ^{a), d)} / Uterus ^{e)}	O	O / O
Cervical lymph node ^{b)}	O		Prostate/ Vagina	O	O /
Mesenteric lymph node	O		Mammary gland ^{b)}	O	
Heart	O	O	Sternum (including bone marrow) ^{b)}	O	
Aorta (aortic arch)	O		Femur (including bone marrow) ^{b)}	O	
Trachea	O		Femoral skeletal muscle ^{b)}	O	
Lung (including bronchial tubes)	O	O	Skin (Abdominal) ^{b)}	O	
Tongue	O		Macroscopical lesions	O	
Esophagus	O		Parts for identification (auricle)	(preservation only)	
Stomach	O				
Duodenum	O				

Items with O were examined.

H-E: Hematoxyline/Eosin Stain

- a) examined both sides
- b) excised bilaterally but examined left side only
- c) Salpingitis were fixing only
- d) Cranial and caudal
- e) Uterine horn (left side only) and cervix

Results

Survival and Clinical Signs of Toxicity

One male in the 300 mg/kg/day group was found dead on study day 15 and one female in the same dose group was sacrificed *in extremis* on study day 16. The male showed salivation, frequent vomiting, bloody stool and hematemesis. The female showed vomiting, salivation, hypothermia, decrease in food consumption and decrease in spontaneous movement prior to sacrifice. Vomiting, salivation, whitish stool and decreased body weight and food consumption was seen in 300 mg/kg/day males and females that survived the 4 week treatment.

Body Weight and Food Consumption

Body weights of the male that died and the female that was sacrificed in the 300 mg/kg/day group were, respectively, 1.1 kg and 0.8 kg lower after 2 weeks than on study day 1. For the survivors, the body weight of one female in the 300 mg/kg/day group in study week 4 was 0.6 kg lower than on study day 1. No remarkable body weight effects were observed for the other survivors.

Lower than control food consumption was noted for the male and female decedents and for the additional female in the 300 mg/kg/day group that showed body weight loss. Food consumption among other survivors was comparable to control.

Ophthalmologic Examination

No treatment-related ocular effects were observed in any dose groups.

ECG

The female dog in the 300 mg/kg/day group that was sacrificed prior to study termination showed elevation of the T-wave, decreased heart rate and prolongation of the QT and QTc interval before moribund sacrifice on Day 16. No treatment-related effects on the ECG were noted for the survivors in any dose group.

Urinalysis, Hematology and Clinical Chemistry

No treatment-related effects on urinalysis or hematology parameters were noted in any of the surviving dogs in any dose group. Higher than control urea nitrogen and creatinine were observed in 1 male in the 60 mg/kg/day group, in 1 male survivor and 1 female survivor in the 300 mg/kg/day group and in the male and female in the 300 mg/kg/day group that died prior to scheduled sacrifice. No other treatment-related effects on clinical chemistry parameters were observed.

Macroscopic Examination

Small thymus and red discoloration in the mucosa of the stomach was noted in the 300 mg/kg/day group male that died and the female sacrificed *in extremis*. The male also showed red discoloration of the mucosa of the esophagus and the female showed dark red focus of the mucosa of the gallbladder. No treatment-related findings were noted for surviving dogs.

Organ Weights

No significant differences were detected in absolute or relative organ weights between treated and control groups (organ weights from non-survivors were excluded from statistical evaluation).

Histopathology

Multiple pathologic findings were observed in the male dog that died and female that was killed *in extremis* involving the kidneys (tubular basophilia, tubular dilatation), heart (myocardial necrosis, neutrophilic cell infiltration, myocardial mineralization), stomach (hemorrhage, mucosal erosion), adrenals (cell necrosis), thymus (atrophy), ileum (atrophy of Peyer's patches), gallbladder (hemorrhage and ulcer) and esophagus (atrophy of esophageal glands). Renal tubular basophilia was noted in 1 male dog in the 60 mg/kg group; the same dog that had higher than control serum urea nitrogen and creatinine.

No treatment-related pathological findings were observed in the other surviving dogs.

The NOAEL was considered to be 12 mg/kg/day for male dogs (AUC for TAK-536, 26.8 ug.hr/ml) and 60 mg/kg/day for females dogs (AUC for TAK-536, 259.4 ug.hr/ml).

Toxicokinetics

The concentration of TAK-491 in dog plasma was below the level of detection (5 ng/ml). The C_{max} and AUC for TAK-536, TAK-536 MI and TAK-536 M-II metabolites increased with increasing dose level and these values after the 1st dose were almost comparable to those after the 28th dose. There were no gender differences in the pharmacokinetic parameters of TAK-491-related compounds (Table 16).

Table 16. Toxicokinetic Parameters of TAK-491 Related Compounds

Dose (mg/kg/day)	Analyte	Number of dosing		Male (N=3)			Female (N=3)			Total (N=6)		
		1st	28th	Thax (h)	Cmax (ng/mL)	AUC 0-24h (ng·h/mL)	Thax (h)	Cmax (ng/mL)	AUC 0-24h (ng·h/mL)	Thax (h)	Cmax (ng/mL)	AUC 0-24h (ng·h/mL)
3	TAK-491F	1st		- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)
		28th		- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)
	TAK-536	1st	0.5 (0.0)	2540 (673)	0.5 (0.0)	3066 (610)	0.5 (0.0)	2449 (775)	5109 (1355)	0.5 (0.0)	2495 (651)	4338 (1116)
		28th	0.5 (0.0)	2835 (1386)	0.7 (1.3)	3711 (1393)	0.7 (0.3)	1568 (273)	3856 (846)	0.6 (0.2)	1962 (831)	3076 (978)
	TAK-536 N-I	1st	0.7 (0.3)	467 (97)	1.0 (0.0)	1147 (86)	1.0 (0.0)	516 (107)	1371 (360)	0.8 (0.3)	491 (95)	1269 (364)
		28th	0.7 (0.3)	576 (211)	0.8 (0.3)	1324 (382)	0.8 (0.3)	591 (114)	1610 (226)	0.8 (0.3)	584 (138)	1487 (315)
	TAK-536 N-II	1st	0.5 (0.0)	14 (4)	15 (6)	15 (6)	0.8 (0.3)	25 (8)	49 (14)	0.7 (0.3)	20 (8)	33 (21)
		28th	0.5 (0.0)	15 (9)	12 (8)	12 (8)	1.3 (0.6)	13 (2)	17 (10)	0.9 (0.6)	14 (6)	15 (6)
	TAK-491F	1st	- (-)	0 (0)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)
		28th	- (-)	0 (0)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)
	12	TAK-536	1st	0.8 (0.3)	13418 (2165)	27984 (4390)	0.8 (0.3)	14772 (1154)	33080 (5235)	0.8 (0.3)	14095 (1720)	30032 (4111)
			28th	0.8 (0.3)	14784 (7695)	26529 (10656)	0.5 (0.0)	16926 (8763)	34350 (10486)	0.7 (0.3)	16960 (7466)	30576 (10307)
TAK-536 N-I		1st	0.8 (0.3)	1139 (61)	3379 (522)	1.0 (0.0)	1181 (126)	3684 (637)	0.9 (0.2)	1159 (96)	5532 (481)	
		28th	1.3 (0.6)	1170 (266)	3889 (1647)	1.2 (0.8)	1095 (114)	4294 (819)	1.3 (0.6)	1155 (138)	4092 (3854)	
TAK-536 N-II		1st	0.5 (0.0)	98 (44)	157 (63)	0.7 (0.3)	106 (64)	172 (35)	0.6 (0.2)	104 (49)	164 (44)	
		28th	0.8 (0.3)	60 (55)	111 (50)	1.0 (0.0)	78 (32)	166 (89)	0.9 (0.2)	79 (39)	158 (47)	
TAK-491F		1st	- (-)	0 (0)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)
		28th	- (-)	0 (0)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)
60		TAK-536	1st	1.3 (0.6)	50965 (8445)	157678 (7010)	2.3 (1.5)	59210 (21967)	248634 (53957)	1.8 (1.2)	54638 (15705)	203156 (54451)
			28th	1.7 (0.6)	57583 (10275)	217516 (132691)	1.7 (0.6)	72596 (23245)	259435 (114464)	1.7 (0.5)	64974 (17740)	238470 (113191)
		TAK-536 N-I	1st	1.0 (0.0)	1944 (254)	6815 (1387)	1.2 (0.8)	1215 (355)	9782 (1190)	1.1 (0.5)	1280 (285)	8299 (1994)
			28th	1.3 (0.6)	1243 (276)	6008 (134)	1.2 (0.8)	1284 (147)	7679 (2146)	1.3 (0.6)	1263 (199)	6844 (1658)
	TAK-536 N-II	1st	1.5 (0.9)	719 (119)	1853 (280)	2.0 (1.7)	460 (276)	1927 (396)	1.8 (1.2)	580 (338)	1890 (310)	
		28th	1.7 (0.6)	332 (208)	917 (539)	1.2 (0.8)	361 (90)	1261 (428)	1.4 (0.7)	352 (147)	1088 (470)	
	TAK-491F	1st	- (-)	0 (0)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)
		28th	- (-)	0 (0)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)	- (-)	0 (0)	0 (0)
	300	TAK-536	1st	2.2 (1.8)	76715 (23577)	393760 (232373)	4.0 (0.0)	128980 (41718)	1063199 (573912)	3.1 (1.5)	102788 (41644)	727980 (536539)
			28th	0.5 (-)	123124 (-)	417106 (-)	6.0 (-)	114795 (-)	1149310 (-)	3.3 (3.6)	116969 (14853)	783108 (643220)
		TAK-536 N-I	1st	0.7 (0.3)	1241 (121)	10229 (3701)	3.5 (4.0)	1203 (100)	14056 (3480)	2.1 (3.0)	1222 (101)	12143 (3536)
			28th	1.3 (-)	1223 (-)	8543 (-)	4.5 (-)	1506 (-)	15689 (-)	2.9 (8.5)	1363 (257)	13216 (6928)
TAK-536 N-II		1st	2.2 (1.8)	412 (182)	2126 (1077)	3.0 (1.7)	732 (144)	4426 (2343)	2.6 (1.6)	572 (229)	3276 (2681)	
		28th	1.3 (-)	404 (-)	1338 (-)	6.0 (-)	806 (-)	5448 (-)	3.6 (3.3)	605 (402)	3353 (2461)	

Mean (S.D.)
 - : Not calculated
 Parameters for 300 mg/kg/day after the 28th dose: Male(N=2), Female(N=2), Total(N=4)

6.2.3 26-week repeated oral (gavage) dose toxicity study in rats

Study Facility: [REDACTED]

(b) (4)

Study Number: B-5470

Study Dates: 3/11/05-4/27/06

GLP Compliance: There was a statement indicating that this study was conducted under GLP conditions.

Animals: F344/Jcl SPF rats (4 weeks old at start of dosing)

Drug Administration: TAK-491 was dissolved in 0.5 w/v% methylcellulose solution and administered by oral gavage for 26 weeks in the following manner: 0 (vehicle control) and TAK-491 (2, 20, 200 and 2000 mg/kg/day). In the control group, there were 15 animals/sex. In the TAK-491 groups there were also 15 animals/sex. Toxicokinetic parameters were measured in satellite group animals (n=3) in the TAK-491 groups only on day 1 and in week 26 of treatment.

Observations/Measurements: Mortality and clinical signs were monitored 3X a day (before dosing, immediately after dosing and 2 hr after dosing). Body weight was measured 3X/week during week 1, 2X/week up to week 13 and then 1X/week thereafter. Food consumption was measured 2X in week 1 and then 1X/week thereafter. Ophthalmological examinations were performed once prior to drug administration and once in week 25 of treatment. Urinalysis (including water intake) was performed twice: once in week 13 and once in week 26. Hematological examination was performed at the time of necropsy. Blood chemical examination was performed at the same time as hematology. Careful examination of all organs/tissues in the cephalic, thoracic and abdominal cavities was made at necropsy.

The following organs were weighed at necropsy:

brain, pituitary, adrenals, thymus, spleen, heart, lungs (including bronchus), salivary glands (submandibular + sublingual glands), liver, kidneys, testes, prostate, ovaries and uterus

Histological examination of the following tissues was performed:

cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves, eyeballs, optic nerves, Harderian glands, pituitary, thyroids, parathyroids, adrenals, thymus, spleen, submandibular lymph node, mesenteric lymph node, heart, thoracic aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands, sublingual glands, parotid glands, liver, pancreas, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, ovaries, uterus (both horns), vagina, mammary glands (inguinal region, both sides), sternum (including bone marrow), femurs (including bone marrow), femoral skeletal muscles and skin (inguinal, both sides)

Toxicokinetics: Plasma concentrations (T_{max} , C_{max} and AUC_{0-24h}) of the compound were measured in the TAK treated group after the first dose at 0.5, 1, 2, 8 and 24 hr on day 1 of treatment. Measurements were also made immediately before the 181st dosing and 0.5, 1, 2, 8 and 24 hr after dosing.

Results: No animals died in any group. Body weight: In males, body weight decreased in all but the lowest dose treatment group; however, in females, body weight decreased in only the highest dose group. Food consumption: In males, food consumption decreased significantly in the 2 highest dose groups (200 and 2000 mg/kg/day); in females, food consumption decreased only in the highest dose group (2000 mg/kg/day). There were no ophthalmological changes in the treatment groups. UA/water intake: week 13: In males, a significant decrease in osmotic pressure was observed in the 2 mg/kg and higher groups. A significant decrease in output of sodium was observed in the 20 mg/kg and higher groups. Significant increases in water intake and urine volume were observed in the 2000 mg/kg group. In females, significant decreases in osmotic pressure, output of sodium and output of chloride were observed in the 2000 mg/kg group. Week 26: In males, a significant decrease in osmotic pressure was observed in the 2 mg/kg and higher groups. A significant decrease in output of sodium was observed in the 200 and 2000 mg/kg groups. Significant increases in water intake, urine volume and output of potassium was observed in the 2000 mg/kg group. In females, significant decreases in osmotic pressure, output of sodium and output of chloride were observed in the 2000 mg/kg group. Decreased urinary sodium excretion would be consistent with an expected decrease in systemic blood pressure.

Hematological examination: Males:

In the erythrocyte parameters, significant decreases in red blood cell count, hemoglobin concentration and hematocrit value were observed in the 2 mg/kg and higher groups. A significant increase in reticulocyte ratio was observed in the 2000 mg/kg group.

Significant increases in MCV and MCH were observed in the 200 and 2000 mg/kg groups.

In the leukocyte parameters, a significant increase in white blood cell count was observed in the 2000 mg/kg group. In differential leukocyte count, a significant increase in segmented neutrophil ratio and a significant decrease in lymphocyte ratio were observed in the 2000 mg/kg group. Calculation of the actual number of the lymphocytes and neutrophils based on the white blood cell count and their ratios revealed an increase in the neutrophils, while the number of the lymphocytes was comparable to that of the control group.

In platelet count, a significant increase was observed in the 2000 mg/kg group.

Females:

In the erythrocyte parameters, significant decreases in red blood cell count, hemoglobin concentration and hematocrit value were observed in the 2 mg/kg and higher groups. A significant increase in MCV was observed in the 2000 mg/kg group.

In the leukocyte parameters, a significant increase in white blood cell count was observed in the 2000 mg/kg group.

In platelet count, a significant increase was observed in the 200 and 2000 mg/kg groups.

Blood chemical examination: Males:

A significant increase in BUN level was observed in the 2 mg/kg and higher groups. Significant decreases in AST, ALT, LDH, phospholipids, sodium, total protein and albumin values and significant increases in creatinine and potassium values were observed in the 20 mg/kg and higher groups. Significant decreases in total cholesterol and calcium levels and a significant increase in A/G ratio were observed in the 2000 mg/kg group. Females: Significant decreases in AST and ALT activities, total protein and albumin values were observed in the 2 mg/kg and higher groups. A significant decrease in LDH activity was observed in the 200 and 2000 mg/kg groups. Significant decreases in total cholesterol, phospholipids, sodium and calcium levels were observed in the 2000 mg/kg group.

Organ Weight:

Treatment-related changes were observed in the thymus (both sexes), heart (both sexes), spleen (males) and adrenal (both sexes). Thymus: In both sexes in the 2000 mg/kg group, significant decreases in the absolute and relative weights were observed. In males, a significant decrease in the absolute weight was observed in the 200 mg/kg group; however, it was not judged to be treatment-related, since no apparent changes were found in the relative weight. Heart: In both sexes in the 2 mg/kg and higher groups, significant decreases in the absolute and relative weights were observed. Spleen: In males in the 2000 mg/kg group, significant decreases in the absolute and relative weights were observed. A significant decrease in the absolute weight was observed in males in the 20 and 200 mg/kg groups and in females in the 2000 mg/kg group; however, it was not judged to be attributable to low body weight at necropsy. A significant difference in the absolute and relative weights was recorded in females in the 200 mg/kg group; however, it was judged to be incidental, as there was a lack of dose dependency. Adrenal: In both sexes in the 2000 mg/kg group, significant increases in the absolute and relative weights were observed. A significant increase in the relative weight was observed in males in the 200 mg/kg group; however, it was not judged to be attributable to low body weight at necropsy.

Histopathological examination:

Treatment-related changes were observed in both sexes in the kidney, adrenal and stomach.

Kidney: Minimal or mild hypertrophy of the juxtaglomerular cells was observed in 5/15 males and 6/15 females in the 2 mg/kg group, 14/15 males and 15/15 females in the 20 mg/kg group and 15/15 animals in both sexes in the 200 and 2000 mg/kg groups. Minimal to moderate intimal proliferation of the interlobular artery, revealed by special staining with Elastica van Gieson stain, was observed in 14/15 males and 10/15 females in the 20 mg/kg group, 15/15 males and 10/15 females in the 200 mg/kg group and 15/15 males and 12/15 females in the 2000 mg/kg group. Adrenal: Minimal or mild atrophy of the zona glomerulosa was observed in 7/15 females in the 2 mg/kg group, 14/15 males and 15/15 females in the 20 mg/kg group and 15/15 animals in both sexes in the 200 and 2000 mg/kg groups. Stomach: Minimal to moderate erosion in the

glandular stomach was observed in 3/15 males in the 200 mg/kg group and 14/15 males and 10/15 females in the 2000 mg/kg group. Minimal erosion was also observed in a few females in the control, 2 and 20 mg/kg groups; however, they were judged to be incidental from their incidence or histopathological characteristics. Mild ulcer in the glandular stomach was observed in 1/15 males in the 2000 mg/kg group.

TOXICOKINETICS

The T_{max} values for TAK-536, TAK-536 M-I and TAK-536 M-II were 0.5 to 2.0 hours after the 1st dosing in both sexes. After the 1st and 181st doses, the C_{max} and AUC_{0-24h} values for TAK-536, TAK-536 M-I and TAK-536 M-II increased mostly dose-dependently, except for the C_{max} value for TAK-536 M-I after the 1st dose in females in the 2000 mg/kg group. There were no apparent gender differences in any TK parameter. With repeated dosing, the C_{max} and AUC_{0-24h} values for each analyte tended to increase in both sexes in the 2 and 20 mg/kg groups, except for the C_{max} value for TAK-536 M-I in females in the 2 mg/kg group. Those for all analytes in both sexes in the 200 and 2000 mg/kg were comparable except for TAK-536 M-I and TAK-536 M-II in females in the 200 mg/kg group. The C_{max} and AUC_{0-24h} values for each analyte were higher in order of TAK-536 > TAK-536 M-I > TAK-536 M-II. In addition, any analyte was not detected in the control group.

Conclusion: Twenty-six week oral administration of TAK-491 at 2, 20, 200 and 2000 mg/kg/day was associated with stomach toxicity in males at the 200 mg/kg/day dose. This toxicity included erosion in the glandular stomach and dark red focus in glandular stomach. Thus, the NOAEL in males is 20 mg/kg/day. In females, these effects on the stomach were seen only in the 2000 mg/kg/day dose. Thus, the NOAEL in females was 200 mg/kg/day.

Twenty-six-week oral gavage toxicity study of TAK-491 in rats (B-5470)

Animal	F344/Jcl rats, 6 weeks of age				
Test article	Control*	TAK-491			
Dosage level (mg/kg/day)	0	2 ^{b)}	20 ^{b)}	200 ^{b)}	2000 ^{b)}
Dosage volume (mL/kg/day)	10	10	10	10	10
No. of animals (M:F) ^{b)}	15:15	15:15	15:15	15:15	15:15
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0
Clinical signs	-	-	-	-	-
Body weight	-	-	↓ (M: -5%)	↓ (M: -6%)	↓ (M: -17%#, F: -5%)
Food consumption	-	-	-	↓ (M)	↓
Ophthalmology	-	-	-	-	-
Urinalysis (including water intake) [Week 13]	-	↓ Osmotic pressure (M)			
		↓ Na (M)			↑ Water intake (M), ↑ Urine volume (M), ↓ Osmotic pressure (F), ↓ Na (F), ↓ Cl (F)
Urinalysis (including water intake) [Week 26]	-	↓ Osmotic pressure (M)			
		↓ Na (M)			↑ Water intake (M), ↑ Urine volume (M), ↓ Osmotic pressure (F), ↓ Na (F), ↑ K (M), ↓ Cl (F)
Hematology	-	↓ RBC, ↓ Hb, ↓ Ht			
		↑ MCV (M), ↑ MCH (M)			
		↑ Platelet (F)	↑ MCV (F), ↑ Reticulocyte (M), ↑ Platelet#, ↑ WBC#, ↑ Seg ratio (M)#		
Blood chemistry	-	↓ AST (F), ↓ ALT (F), ↑ BUN (M)			
		↓ TP (F), ↓ Albumin (F)	↓ PL (M), ↓ TP, ↓ Albumin		↓ TP#, ↓ Albumin#, ↓ PL#, ↓ T. cho.#, ↓ Ca, ↓ Na (F), ↑ A/G (M)
			↓ AST (M), ↓ ALT (M), ↓ LDH (M), ↓ Na (M), ↑ K (M), ↑ Creatinine (M)		
			↓ T. bilirubin (F), ↓ ID. bilirubin (F), ↓ LDH (F)		

*: 0.5 w/v% methylcellulose solution containing 1.0 w/v% citric acid

M: Male, F: Female

a): as TAK-491F, free acid of TAK-491

b): Additional 8 (dose group) or 4 (control) animals/sex/group were used as satellite groups for toxicokinetics.

-: No treatment-related effects, ↑: Increase, ↓: Decrease

#: Toxicologically significant change.

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(continued)

Animal	F344/Jcl rats, 6 weeks of age				
Test article	Control*	TAK-491			
Dosage level (mg/kg/day)	0	2 ^{a)}	20 ^{a)}	200 ^{a)}	2000 ^{a)}
Dosage volume (mL/kg/day)	10	10	10	10	10
No. of animals (M:F) ^{b)}	15:15	15:15	15:15	15:15	15:15
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0
Organ weights	-	↓ Heart ↓ Thymus, ↓ Spleen (M), ↑ Adrenal#			
Gross pathology	-	-	-	Stomach: Dark red focus in glandular stomach (M)# Stomach: Dark red focus in glandular stomach (F)#, Content of grayish mass#	
Histopathology	-	Kidney: Hypertrophy of juxtaglomerular cell Adrenal: Atrophy of zona glomerulosa (F) Kidney: Intimal proliferation of interlobular artery Adrenal: Atrophy of zona glomerulosa (M) Stomach: Erosion in glandular stomach (M)# Stomach: Erosion (F) and ulcer (M) in glandular stomach#			

*: 0.5 w/v% methylcellulose solution containing 1.0 w/v% citric acid

M: Male, F: Female

a): as TAK-491F, free acid of TAK-491

b): Additional 8 (dose group) or 4 (control) animals/sex/group were used as satellite groups for toxicokinetics.

-: No treatment-related effects, ↑: Increase, ↓: Decrease

#: Toxicologically significant change.

(continued on the next page)

(continued)

Animal	F344/Jcl rats, 6 weeks of age				
Test article	Control*	TAK-491			
Dosage level (mg/kg/day)	0	2 ^{a)}	20 ^{a)}	200 ^{a)}	2000 ^{a)}
Dosage volume (mL/kg/day)	10	10	10	10	10
No. of animals (M:F) ^{b)}	15:15	15:15	15:15	15:15	15:15
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0
Toxicokinetic parameters (n=3, mean, M/F)					
TAK-536					
T _{max} (h)	Day 1	1.0/1.0	1.0/1.0	1.0/0.5	0.5/1.0
	Week 26	0.5/1.0	0.5/0.5	1.0/1.0	0.5/0.5
C _{max} (ng/mL)	Day 1	1533/1678	28114/34379	163292/145610	204430/243192
	Week 26	3507/4008	49989/42399	135014/179958	241814/224436
AUC _{0-24h} (ng·h/mL)	Day 1	9975/ 13599	168030/ 189358	1068046/ 1041488	2471320/ 2355414
	Week 26	24491/ 26293	237496/ 223418	923247/ 930937	1987221/ 1980129
TAK-536 M-I					
T _{max} (h)	Day 1	1.0/2.0	0.5/2.0	1.0/2.0	2.0/2.0
	Week 26	1.0/2.0	1.0/1.0	1.0/0.5	2.0/2.0
C _{max} (ng/mL)	Day 1	133/166	955/797	1878/3792	2261/2279
	Week 26	226/162	1700/1232	2295/1551	2641/1845
AUC _{0-24h} (ng·h/mL)	Day 1	734/981	5143/7402	23943/30298	36731/34042
	Week 26	1359/1416	8506/8645	20167/16073	24608/23946
TAK-536 M-II					
T _{max} (h)	Day 1	NA/2.0	1.0/0.5	2.0/1.0	2.0/0.5
	Week 26	0.5/1.0	0.5/0.5	1.0/1.0	0.5/0.5
C _{max} (ng/mL)	Day 1	0/2	45/52	321/314	395/460
	Week 26	7/7	97/104	330/533	529/594
AUC _{0-24h} (ng·h/mL)	Day 1	0/7	272/220	2031/1762	4069/3749
	Week 26	29/22	424/488	1992/2358	4099/4644
Conclusion	Non-toxic dosage level: 20 mg/kg/day for males and 200 mg/kg/day for females				

*: 0.5 w/v% methylcellulose solution containing 1.0 w/v% citric acid

M: Male, F: Female

a): as TAK-491F, free acid of TAK-491

b): Additional 8 (dose group) or 4 (control) animals/sex/group were used as satellite groups for toxicokinetics.

NA: Not applicable (Not calculated)

Determination of plasma drug concentrations was also conducted on the control group (1 hour after dosing on day 1 and in week 26), and the concentrations of any analyte were less than the quantification limit (5 ng/mL for each analyte).

6.2.4 52-week repeat dose oral (gavage) dose toxicity study in dogs (TAK-536)

Results: (copied from review) Four animals in the 100 and 300 mg/kg/day groups died or were sacrificed in moribund condition. These animals exhibited renal lesions (tubular dilatation and regeneration), hepatic lesions, occasional ulcerations at various locations in the GI tract, atrophy of salivary gland acinar cells and atrophy of lymph nodes, spleen and thymus. There was a dose-dependent hypertrophy of renal juxtaglomerular cells in drug-treated groups. The animals sacrificed at the end of the study in the 300 mg/kg/day group exhibited increased severity of renal tubular regeneration. No NOAEL was specified.

Review by D. Jensen dated 4/24/07.

52-Week Oral Gavage Toxicity Study of TAK-536 in Beagle Dogs

Testing Facility: (b) (4)

Study Number: B-2534

Study Date(s): January 17, 1994 – September 29, 1995

GLP Compliance: Yes

QA Report: Yes

Key Findings: Four animals in 100 and 300 mg/kg/day groups died or were sacrificed in moribund condition. These animals exhibited renal lesions (tubular dilation and regeneration), hepatic lesions, occasional ulcerations at various locations in the GI tract, atrophy of salivary gland acinar cells, and atrophy of lymph nodes, spleen and thymus. There was dose-dependent hypertrophy of renal juxtaglomerular cells in drug-treated groups. 300 mg/kg/day animals sacrificed as scheduled at 52 weeks had increased severity of renal tubular regeneration.

Purpose: To examine the toxicity of the test article following daily administration for 26 weeks (interim sacrifice group) and for 52 weeks (main study group) in beagle dogs.

Methods: The 26-week study consisted of three beagle dogs/sex/group and the 52-week study consisted of four beagle dogs/sex/group. Animals were dosed daily with an oral feeding catheter with vehicle control or with 10, 30, 100 or 300 mg/kg/day of TAK-536. Powdered drug was suspended in a 5% solution (w/v) of gum Arabic in water. Drug concentrations in the oral suspensions delivered to the four drug-treated groups were 0.33%, 1.0%, 3.3% and 10.0% (w/v), respectively. A 5% solution (w/v) of gum Arabic in water was used as the vehicle control.

Treatment groups are summarized below in the sponsor's Table.

Test Groups	Dose Levels (mg/kg)	Sex	No of Animals		Animal No.	
			26-week group	52-week group	26-week group	52-week group
Control	0	M	3	4	1001 - 1003	1004 - 1007
		F	3	4	1101 - 1103	1104 - 1107
Low	10	M	3	4	2001 - 2003	2004 - 2007
		F	3	4	2101 - 2103	2104 - 2107
Intermediate	30	M	3	4	3001 - 3003	3004 - 3007
		F	3	4	3101 - 3103	3104 - 3107
High	100	M	3	4	4001 - 4003	4004 - 4007
		F	3	4	4101 - 4103	4104 - 4107
Highest	300	M	3	4	5001 - 5003	5004 - 5007
		F	3	4	5101 - 5103	5104 - 5107

Animals were approximately nine months old when treatment was initiated. Animals were housed individually. Animals were supplied with 250 g of pellet diet (CD-5, CLEA Japan, Inc.) at 2:00 PM each day with any remaining food removed at 9:00 AM the following morning.

Water was provided ad libitum via an automatic watering system except on days when water intake was measured. On days when water intake was measured, 1000 mL of water was supplied in a plastic container and the amount of water remaining in the container after approximately 22 hours was measured.

Animals were observed daily for one week prior to dosing and three times daily during the dosing period for clinical signs, abnormal behavior, color of oral mucosa and appearance of stools. Body weights were measured once prior to dosing, on the first day of dosing, once per week for the first three weeks of dosing, and once every two weeks thereafter. Daily food consumption was determined from the amount of food remaining each morning and was used to calculate mean food consumption for each group for each one-week interval during the first three months of the study and mean food consumption for each group for each two-week interval thereafter. Water intake for each animal was measured once per week during the first three months of the study and once every two weeks thereafter.

Urinalysis parameters, hematology parameters, clotting parameters and clinical chemistry parameters were evaluated prior to initiation of dosing and at 3, 6, 9 and 12 months. Urinalysis parameters included: pH, protein, glucose, ketones, occult blood, urobilinogen, bilirubin, urinary sediments, specific gravity, color, volume, sodium, potassium, chloride and creatinine.

Hematology parameters included: RBCs, WBCs, platelet count, differential leukocyte count, hemoglobin, hematocrit, MCV, MCH, MCHC and reticulocyte ratio. Clotting parameters included: prothrombin time, activated partial thromboplastin time and fibrinogen. Clinical chemistry parameters included: alkaline phosphatase, glucose, BUN, bilirubin, cholesterol, total protein, protein fractions, albumin/globulin ratio, creatinine, uric acid, triglyceride, phospholipid, non-esterified fatty acid, calcium, sodium, potassium, chloride, inorganic phosphorus, SGOT, (AST), SGPT (ALT), LDH, CPK, and leucine aminopeptidase.

Ophthalmoscopy exams were completed prior to dosing and at 6 and 12 months. Resting electrocardiograms were recorded on conscious animals prior to dosing and at 6 and 12 months. Calculated ECG parameters included: heart rate, P-R interval, Q-T interval, QRS interval, QTc (Bazett's formula) and QRS electrical axis. Body temperature was recorded prior to dosing and at 6 and 12 months.

Blood samples for determination of toxicokinetic parameters were collected on dosing days 1, 87, 179 and 365 from all surviving animals in each of the four drug-treated groups. Blood samples were collected prior to dosing, at 30 minutes post-dosing and at 1, 2, 4, 8, and 24 hours post-dosing on each day.

Gross necropsies were completed on animals following scheduled sacrifice at 26 or 52 weeks. Organ weights were determined for brain, pituitary, thyroids, submandibular glands, thymus, heart, lungs, liver, kidneys, spleen, pancreas, adrenals, testes, epididymides, prostate, ovaries and uterus. Histological exams were completed on tissue samples from cerebrum, cerebellum, medulla oblongata, pituitary, thyroids, parathyroids, thymus, trachea, lungs, heart, liver, gallbladder, kidneys, pancreas, adrenals, testes, epididymides, ovaries, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, prostate, uterus, vagina, submandibular glands, sublingual glands, parotid glands, spleen, spinal cord, eyeballs, optic nerve, lacrimal glands, urinary bladder, mesenteric lymph nodes, submandibular lymph nodes, sternum, femur, femoral skeletal muscle, sciatic nerve, aorta, mammary glands, skin, tongue and larynx.

Results: One female in the 300 mg/kg/day group died on day 277 of dosing. One male and one female in the 300 mg/kg/day group plus one female in the 100 mg/kg/day group were sacrificed in moribund states on days 30, 79 and 139 of dosing, respectively. Loose, and/or fatty stools sometimes containing mucous were observed in animals dosed with 100 or 300 mg/kg/day.

There were no differences in body weight changes, food consumption or water intake among treatment groups.

Isolated statistically significant differences in urinalysis parameters were noted between treated animals and controls: Specific gravity was increased in 30 mg/kg/day females compared to controls at 6 months (1.044 vs. 1.026) and at 12 months (1.043 vs. 1.022) and 100 mg/kg/day females compared to controls at 12 months (1.044 vs. 1.022). Urine creatinine was decreased in 10 and 30 mg/kg/day females compared to controls at 6 months (360.2 and 356.1 vs. 421.2). The differences are judged to be incidental.

Significant differences in hematology parameters in drug treated animals compared to controls were limited to increased MCHC in 30 mg/kg/day males compared to controls at 3 months (33.3% vs. 32.5%). Significant differences in clotting parameters in drug treated animals compared to controls were limited to reduced activated partial thromboplastin times in 10 and 30 mg/kg/day females at 12 months (12.2 and 12.2 sec. vs. 14.7 sec.) and in 100 and 300 mg/kg/day females at 9 months (11.5 and 11.0 sec. vs. 13.3 sec.). For these hematology and clotting parameters, the differences are judged to be incidental.

BUN was modestly but significantly elevated in some drug-treated groups compared to controls at some timepoints: in 300 mg/kg/day males at 6 months (15.8 mg/dL vs. 11.0 mg/dL), in 10, 30, 100 and 300 mg/kg/day males at 6 months (11.1, 10.7, 10.9 and 13.7 mg/dL vs. 8.0 mg/dL), in 300 mg/kg/day males at 9 months (21.0 mg/dL vs. 10.0 mg/dL), in 300 mg/kg/day females at 3 months (12.7 mg/dL vs. 10.0 mg/dL) and in 30 and 300 mg/kg/day females at 6 months (12.4 and 12.6 mg/dL vs. 8.4 mg/dL). Creatinine was modestly but significantly elevated compared to controls in 30 and 100 mg/kg/day females at 3 months (0.94 and 0.93 mg/dL vs. 0.84 mg/dL), in 30 mg/kg/day females at 6 months (0.95 mg/dL vs. 0.86 mg/dL), and in 30 and 100 mg/kg/day

females at 12 months (0.99 and 0.95 mg/dL vs. 0.83 mg/dL). The few other differences in clinical chemistry parameters in drug-treated animals compared to controls were more isolated than the differences observed for BUN and creatine and were all modest in magnitude. All differences in clinical chemistry parameters are judged to be incidental.

Results of ophthalmoscopy, electrocardiography and body temperature measurements were unremarkable.

On gross necropsy, each of the four animals found dead or sacrificed in a moribund condition (all from 100 or 300 mg/kg/day groups) demonstrated paleness in the cortical regions of the kidneys. The male sacrificed on day 30 also demonstrated reddish areas on the mucosa of the stomach, jejunum and colon. The female sacrificed on day 79 also demonstrated yellowish discoloration of the liver and reddish areas on the mucosa of the stomach. The female sacrificed on day 139 demonstrated reddish areas on the mucosa of the trachea, esophagus and stomach.

The female that died on day 277 demonstrated distention of the gallbladder. Necropsy findings in animals sacrificed as scheduled at 26 and 52 weeks are judged by the study pathologist and by this reviewer to be incidental.

Absolute brain weights were lower in 100 mg/kg/day females sacrificed at 52 weeks compared to controls (69.7 g vs. 79.9 g), but there was no difference in brain weight to body weight ratio between these groups. There were no other differences in absolute or relative organ weights between drug-treated animals and controls.

Histological lesions in the four animals found dead or sacrificed in moribund condition that had a greater incidence or severity than in control animals included: renal lesions (variously: moderate tubular dilation, severe tubular regeneration, moderate hypertrophy of juxtaglomerular cells); atrophy or necrosis of hepatocytes (severity not specified); erosions and/or necrosis at the locations of gross GI tract lesions; atrophy of acinar cells in submandibular, parotid and sublingual glands; atrophy of mesenteric lymph nodes; atrophy of white pulp of spleen in 3 of 4 animals; and atrophy of thymus.

Histological lesions in animals sacrificed as scheduled at 26 or 52 weeks and that had a greater incidence or severity than in control animals included: mild-to-severe regeneration of renal tubules (300 mg/kg/day animals at 52 weeks) and mild or moderate hypertrophy of renal juxtaglomerular cells (generally more severe in animals treated with 100 or 300 mg/kg/day than in animals treated with 10 or 30 mg/kg/day). Other lesions in animals sacrificed at 26 or 52 weeks are judged by this reviewer to be incidental. Individual animals at 26 weeks are listed as 1, 2 or 3 by the sponsor. Individual animals at 52 weeks are listed as 4, 5, 6 or 7 by the sponsor.

The study report does not specify a NOAEL dose.

Toxicokinetics results: Results of the toxicokinetics substudy are summarized below in the sponsor's Table. Exposures were similar between the sexes. The increase in exposure with increasing dose was roughly dose-proportional, except for a less than dose-proportional exposure increase between the two highest doses. Exposures were lower at later timepoints than on day 1 for the three higher doses in males, but not in females.

Plasma drug concentration (Pharmacokinetic parameters of TAK-536 in dogs)

Dose (mg/kg/day)	No. of dosing	Male (N=7)				Female (N=7)				Total (N=14)			
		Tmax (h)	Cmax (µg/ml)	AUC 0-24h (µg·h/ml)	Tmax (h)	Cmax (µg/ml)	AUC 0-24h (µg·h/ml)	Tmax (h)	Cmax (µg/ml)	AUC 0-24h (µg·h/ml)	Tmax (h)	Cmax (µg/ml)	AUC 0-24h (µg·h/ml)
10	1	1	7.41 ± 3.82	27.7 ± 10.9	2	4.15 ± 3.01	22.9 ± 9.3	1	5.67 ± 3.94	25.3 ± 10.1			
	87	1	5.57 ± 3.32	25.1 ± 5.6	1	4.60 ± 4.84	24.2 ± 11.4	1	5.08 ± 4.02	24.6 ± 8.6			
179	2	5.44 ± 2.40	25.4 ± 10.3	1	7.58 ± 3.71	27.2 ± 9.1	1	6.34 ± 3.19	26.3 ± 9.3				
	365(a)	2	5.85 ± 0.81	25.5 ± 4.9	1	5.10 ± 3.77	31.4 ± 15.1	2	5.13 ± 1.97	28.4 ± 10.9			
30	1	1	16.63 ± 8.29	90.2 ± 19.1	2	14.47 ± 7.59	92.8 ± 32.6	2	14.62 ± 7.06	91.5 ± 25.7			
	87	2	9.82 ± 3.06	76.8 ± 28.8	2	11.57 ± 2.58	72.3 ± 24.8	2	10.70 ± 2.87	74.5 ± 25.9			
179	1	13.85 ± 5.56	60.8 ± 18.7	2	12.89 ± 5.52	69.1 ± 22.6	1	12.76 ± 7.06	65.0 ± 20.4				
	365(a)	1	9.18 ± 6.02	78.0 ± 23.7	2	14.33 ± 3.47	81.7 ± 25.9	2	11.48 ± 4.09	79.8 ± 23.1			
100	1	4	29.67 ± 6.99	291.1 ± 50.3	4	28.70 ± 10.93	251.5 ± 71.4	4	29.19 ± 8.83	271.3 ± 62.8			
	87	2	32.87 ± 12.72	296.0 ± 72.5	4	38.95 ± 18.29	337.3 ± 86.9	4	31.60 ± 15.77	316.6 ± 79.8			
179	2	30.48 ± 9.76	181.6 ± 61.8	2	40.16 ± 7.65(d)	277.3 ± 69.3(d)	2	34.95 ± 9.86(e)	225.7 ± 79.9(e)				
	355(a)	4	20.52 ± 13.18	189.7 ± 63.6	2	36.10 ± 12.93	233.1 ± 106.7	2	27.65 ± 12.56	201.4 ± 88.1			
300	1	4	45.18 ± 15.95	463.5 ± 204.4	4	51.29 ± 12.38	491.1 ± 166.7	4	48.24 ± 14.08	477.3 ± 179.8			
	87(b)	4	40.37 ± 9.78	438.1 ± 87.0	2	52.22 ± 23.79	428.9 ± 177.0	2	45.85 ± 17.99	433.5 ± 133.1			
179(b)	4	32.12 ± 13.17	289.7 ± 90.2	2	54.01 ± 16.05	379.4 ± 109.9	2	42.71 ± 16.60	334.6 ± 106.7				
	365(c)	4	37.45 ± 23.25	352.9 ± 110.8	2	58.82 ± 27.80	486.7 ± 156.7	4	44.63 ± 22.31	419.8 ± 141.8			

Mean ± S.D. (a): Male and Female; N=4, Total, N=8 (b): Male and Female; N=6, Total, N=12 (c): Male and Female; N=3, Total, N=6 (d): N=6 (e): N=13

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Key Finding: Concentrations of TAK-491 up to 5000 ug/plate did not increase the incidence of revertant mutant colonies. See attached review by A. Proakis dated 8/05/2005.

Bacterial Mutagen (Ames) Assay

Study Facility: [REDACTED] (b) (4)

Study No: B040637

Study Dates: 7/27/04 - 7/30/04

GLP Compliance: Compliance with GLP regulations attested.

QA Report: Yes

Bacterial Stains: *Salmonella typhimurium* strains TA98, TA100, TA 1535, TA1537 and *Escherichia coli* strain WP2uvrA.

Procedure: TAK-491 (Lot # M491-001) was dissolved in DMSO and added to culture plates containing the bacterial tester strains in the presence and absence of metabolic activation (S-9 liver fraction obtained from Aroclor 1254 treated rats) at TAK-491 concentrations of 0 (vehicle) 39.1, 78.1 156, 313, 625, 1250, 2500 and 5000 ug/plate. Sodium azide (NaN₃, 0.5 ug/plate), 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2; 0.01 ug/plate), 2-aminoanthracene (2-AA, 0.5-10 ug/plate) and 9-aminoacridine (9-AA, 80 ug/plate) were used as positive controls. The mean number of revertant colonies was determined for each concentration (2 plates/conc.) of TAK-491 and for positive controls in the presence and absence of metabolic activation and compared to the vehicle control. The test agent was considered to be positive for mutagenic activity when the mean number of revertant colonies on the treated plates were dose-dependent and more than twice that of the concurrent negative control.

Results: For all test strains, the number of revertant colonies on the TAK-491 treated plates were less than twice that of the corresponding vehicle control in the presence or absence of metabolic activation (Table). The positive controls used in the assay showed clear positive responses by the respective tester strains. Thus, TAK-491 is judged to be negative in this gene mutation assay.

With (+) or without (-) S9 mix	Dose level (µg / plate) †	Number of revertants (number of colonies / plate)				
		Base-pair change type			Frameshift type	
		TA100	TA1535	WP2 _{uvrA}	TA98	TA1537
S9 mix (-)	Negative control	100 118 (109)	9 10 (10)	25 29 (27)	21 23 (22)	11 13 (12)
	39.1	95 96 (96)	8 10 (9)	28 23 (26)	19 21 (20)	11 9 (10)
	78.1	90 119 (105)	10 9 (10)	26 31 (29)	19 17 (18)	10 10 (10)
	156	92 99 (96)	9 8 (9)	31 22 (27)	21 18 (20)	11 10 (11)
	313	113 125 (119)	11 10 (11)	38 35 (37)	24 21 (23)	11 9 (10)
	625	105 104 (105)	9 11 (10)	33 33 (33)	22 21 (22)	11 14 (13)
	1250	119 104 (112)	10 9 (10)	39 36 (38)	19 22 (21)	9 12 (11)
	2500	126 110 (118)	8 11 (10)	32 26 (29)	19 26 (23)	11 9 (10)
	5000 p	120 103 (112)	9 * 7 * (8)	33 28 (31)	19 17 (18)	11 7 (9)
	S9 mix (+)	Negative control	103 96 (100)	10 11 (11)	30 34 (32)	26 24 (25)
39.1		117 101 (109)	11 10 (11)	30 32 (31)	25 21 (23)	15 19 (17)
78.1		97 110 (104)	11 14 (13)	31 42 (37)	23 29 (26)	15 15 (15)
156		117 108 (113)	10 12 (11)	36 34 (35)	24 23 (24)	20 21 (21)
313		125 113 (119)	15 14 (15)	39 39 (39)	26 25 (26)	18 25 (22)
625		91 121 (106)	12 13 (13)	44 43 (44)	26 23 (25)	23 18 (21)
1250		143 117 (130)	11 10 (11)	58 57 (58)	25 23 (24)	14 15 (15)
2500		126 * 126 * (126)	9 * 9 * (9)	58 * 53 * (56)	23 * 28 * (26)	11 * 11 * (11)
5000		136 * 105 * (121)	10 * 7 * (9)	57 * 50 * (54)	29 * 26 * (28)	15 * 10 * (13)
Positive control S9 mix (-)		Name	AF-2	NaN ₃	AF-2	AF-2
	Dose (µg/plate)	0.01	0.5	0.01	0.1	80
	Number of revertants	560 577 (569)	565 562 (564)	188 216 (202)	729 711 (720)	194 192 (193)
Positive control S9 mix (+)	Name	2-AA	2-AA	2-AA	2-AA	2-AA
	Dose (µg/plate)	1	2	10	0.5	2
	Number of revertants	1230 1183 (1207)	201 208 (205)	1548 1843 (1696)	354 398 (376)	157 179 (168)

(Note) * : Microbial toxicity was observed. p: Precipitation was observed. (Mean)

†: as TAK-491F

Negative control : DMSO

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, NaN₃: sodium azide, 9-AA: 9-aminoacridine hydrochloride, 2-AA: 2-aminoanthracene

7.2 In Vitro Chromosomal Aberration Assays in Mammalian Cells

Chinese Hamster Lung Cytogenetic Assay

Key finding: Azilsartan medoxomil was negative for inducing chromosomal aberrations the presence of metabolic activation but *positive* in the absence of metabolic activation in this assay. In the 6 hr test, the incidences of structural aberrant cells were 8%, 21% and 31.5% at the 700, 850 and 1000 ug/ml concentrations, respectively, in the absence of metabolic activation. In the 24 hr test, the incidences of aberrant cells were 2%, 4.5%

and 21% at the 200, 300 and 400 ug/ml concentrations, respectively, also in the absence of metabolic activation. See attached review by A. Proakis dated 8/05/2005:

Cytogenetic Assay in Chinese Hamster Lung Cells

Study Facility: [REDACTED] (b) (4)

Study No: B040640

Study Dates: 6/25/04 – 8/05/04

GLP Regulations: Compliance with GLP regulations attested
QA Report: Yes

Cell Culture: Chinese Hamster lung cells

Procedure: TAK-491 (Lot #M491-001) was dissolved in DMSO and added to cell cultures at concentrations of 700, 850 and 1000 ug/ml in the absence of metabolic activation and 700, 800 and 900 ug/ml in the presence of metabolic activation (S-9 fraction from livers of Aroclor 1254 treated rats). Dose selection was based on preliminary studies conducted to determine the degree of drug-induced cytotoxicity. The lowest dose at which cell growth index was less than 50% was selected as the highest dose for each assay. DMSO was used as the vehicle control. Mitomycin C (0.05 and 0.1 ug/ml) and benzo(α)pyrene (20 ug/ml) were used as the positive controls. In the short-term treatment assay, the cell cultures were incubated with TAK-491 (with and without S-9 fraction) for 6 hours and then the medium was replaced and cells further incubated with drug-free medium for 18 hours. In the long-term assay, the cells were incubated with TAK-491 for 24 hours in the absence of metabolic activation. Two hours before the cells were harvested, colcemid was added to the medium to arrest cell division. The cells were fixed, placed onto glass slides and stained for microscopic examination and counting. A portion of the cell suspension was used for cell counting with a hemocytometer and for determination of cell growth index (relative cell density (%) of treated sample compared to average cell density of vehicle control). The cells in metaphase (100 metaphases/plate; 200 metaphases/concentration) were examined microscopically for chromosomal aberrations (breaks, exchanges, fragments). According to the study report, a test substance is considered to have tested positive in this assay when aberrant cells occur at an incidence of 10% or more with at least one concentration and the incidence increases dose-dependently.

Results: The lowest concentration at which cell growth index was reduced to less than 50% was 1000 ug/ml in the absence of S-9 and 900 ug/ml in the presence of S-9 in the short term assay and 400 ug/ml in the 24 hr long-term assay in the absence of metabolic activation; these concentrations of TAK-491 were selected as the highest concentrations for the main test.

In the short term (6 hr) test, the incidences of structural aberrant cells were 8%, 21% and 31.5% at the 700, 850 and 1000 ug/ml concentrations, respectively, in the absence of metabolic activation (Table). In the presence of metabolic activation, the incidence of structural aberrant cells was less than 5% for each of the concentrations tested. In the 24 hr long-term assay (without S-9 fraction), the incidences of structural aberrant cells were 2%, 4.5% and 21% at the 200, 300 and 400 ug/ml concentrations of TAK-491, respectively (Table). In the positive control groups, the incidences of structural aberrant cells were 28% (without S-9) and 50.5% (with S-9) in the short-term test and 30% (without S-9) in the 24-hr long-term assay, whereas the incidences were $\leq 1\%$ for the negative control groups.

It is concluded that TAK-491 was negative for inducing chromosomal aberrations in the presence of metabolic activation but positive in the absence of metabolic activation in this assay.

Treatment	Conc. (ug/ml)	S-9	Cell Growth Index (%)	Total # Aberrant Cells/200 Cells Examined (%)
Vehicle (DMSO) Control		-	100	0 (0%)
TAK-491	550	-	68.5	7 (3.5%)
	700	-	61.2	16 (8.0%)
	850	-	51.4	42 (21%)
	1000	-	41.1	63 (31.5%)
Positive Control (Mitomycin C)	0.1	-	N.D.	56 (28.%)
Vehicle (DMSO) Control		+	100	2 (1.0%)
TAK-491	700	+	57.9	3 (1.5%)
	800	+	55.6	1 (0.5%)
	900	+	35.1	1 (0.5%)
Positive Control (Benzo(a)pyrene)	20	+	N.D.	101 (50.5%)

N.D. Not Determined

Table 29. Chromosomal Aberration Assay (24-Hr Exposure)

Treatment	Conc. (ug/ml)	S-9	Cell Growth Index (%)	Total # Aberrant Cells/200 Cells Examined (%)
Vehicle (DMSO) Control		-	100	0 (0%)
TAK-491	200	-	76.5	4 (2.0%)
	300	-	57.8	9 (4.5%)
	400	-	46.5	42 (21.5%)
Positive Control (Mitomycin C)	0.1	-	N.D.	60 (30.0%)

N.D. Not Determined

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Key Findings: Orally administered TAK-491 tested negative in mouse (500-2000 mg/kg, po) and rat (500-2000 mg/kg) micronucleus assays. See attached reviews by A. Proakis dated 8/5/2005:

Micronucleus Assay in Mice

Study Facility: [REDACTED] (b) (4)

Study No: B040638

Study Dates: 7/13/04 – 7/26/04

GLP Regulations: Compliance with GLP regulations attested

QA Report: Yes

Animals: Male ICR (Crj:CD-1) mice (32.3-36.0 gm at start of dosing). The animals were housed 5/cage and maintained on a pellet diet (MF, Oriental yeast Co., Japan) and tap water *ad libitum*.

Drug Administration: TAK-491 (Lot#491-001) was suspended in 0.5% methylcellulose containing 1%w/v citric acid and administered orally by gavage. Control mice received the

vehicle by the same route, whereas the positive control, cyclophosphamide, was administered IP.

Procedure: TAK-491 (Lot # M491-001) or vehicle was administered at daily doses of 0 (vehicle), 500, 1000 or 2000 mg/kg (6 mice/dose group) for 2 consecutive days. A separate group of 6 mice received the positive control, cyclophosphamide, as a single IP dose of 40 mg/kg. Twenty-four hours after dosing, the mice were killed, both femurs were removed and bone marrow cells were collected and fixed onto glass slides. . Since no deaths occurred, cell specimens from only 5 animals (in order of animal number) were selected for evaluation. One thousand erythrocytes per animal were examined to determine the ratio of polychromatic erythrocytes (PCEs) to total erythrocytes. A total of 2000 PCEs were examined for presence of micronuclei. The frequencies of MNPCEs for TAK-491 treated animals were compared to that of vehicle control. The test agent was considered positive in this assay if it induced a statistically significant and dose-dependent increase above control frequency of MNPCEs.

Results: No significant difference was observed in the PCE percentage among total erythrocytes between the TAK-491 and vehicle control groups (Table). No statistically significant increases in MNPCEs were observed when the groups treated with TAK-491 were compared to the vehicle control group (Table). In contrast, the positive control, cyclophosphamide, produced a significant increase above control MNPCE frequency. Thus, TAK-491 is judged to be negative in this mouse micronucleus assay.

Treatment	Dose Group mg/kg	No. Erythrocytes Scored	PCEs/PCEs + NCEs (%)
Vehicle Control	-	5000	52.3
TAK-491,	500	5000	51.4
	1000	5000	51.7
	2000	5000	52.4
Cyclophosphamide	40	5000	50.2

PCEs= polychromatic erythrocytes, NCEs= normochromatic erythrocytes

No discernable systemic toxicity or cytotoxicity of TAK-491 was observed in this assay and the sponsor does not provide evidence of systemic exposure to the drug. However, it is known from studies in rats, rabbits and dogs that TAK-491 is rapidly hydrolyzed and the active metabolite, TAK-536, is found in the blood shortly after oral administration.

In a 3-month rangefinding study in ICR (Crj:CD-1) mice, oral (dietary) doses of 3, 30, 300 and 3000 mg TAK-536/kg produced dose-dependent C_{max} and AUC levels of TAK-536 (Review and Evaluation of Carcinogenicity Protocols and Dose-Rangefinding Studies, dated 8/26/94). The AUCs (M, 133.6 ug.h/ml and F, 221.1 ug.h/ml) for TAK-536 from the 300 mg/kg dose are 27X and 45X (M and F, respectively) the AUC (4.9ug.h/ml) from the anticipated human dose of 5 mg/day. Also, the sponsor had conducted a study to determine the presence of TAK-536-equivalent material found in mouse bone marrow after the oral administration of radiolabelled TAK-536. TAK-536-equivalent radioactive material was evident in mouse bone marrow within 15 min after oral dosing with TAK-536 (Review and Evaluation of Toxicology Data, dated 2/02/94).

Present reviewer's comment

[This indicates that systemic and bone marrow exposure of the active product TAK-536, is achieved in mice.]

Title (Study No.)	Micronucleus Assay with TAK-491 in Mice (B040638)
Test substance	TAK-491
Negative control	0.5 w/v% Methylcellulose (MC) containing 1 w/v% citric acid
Positive control	Cyclophosphamide monohydrate (CP)
Test system	Male ICR (Crj: CD-1) mice (7 weeks old)

Compound	Dose ¹ (mg/kg/day)	No. of doses	Route	Sampling time after the final dose	No. of mice		% MNPCEs (range)
					evaluated	treated	
0.5 w/v% MC containing 1 w/v% citric acid	10 mL/kg/day	2	p.o.	24 h	5	6	0.13 (0.05-0.25)
TAK-491	500	2	p.o.	24 h	5	6	0.17 (0.10-0.25) NS
	1000	2	p.o.	24 h	5	6	0.15 (0.10-0.20) NS
	2000	2	p.o.	24 h	5	6	0.10 (0.05-0.15) NS
CP	40	1	i.p.	24 h	5	6	5.07 (2.70-9.40)**
Conclusion: TAK-491 did not induce micronuclei under the conditions of this study.							

MNPCEs: micronucleated polychromatic erythrocytes

NS: no significant increase; **: $p \leq 0.01$ (Conditional Binomial test)

Micronucleus Assay in Rats

Study Facility: (b) (4)

Study No: B040639

Study Dates: 7/0/04 - 7/29/04

GLP Regulations: Compliance with GLP regulations attested

QA Report: Yes

Animals: Male Fischer (F344/Jcl) rats (117-131 gm at start of dosing). The animals were housed 3/cage and maintained on a pellet diet (MF, Oriental yeast Co., Japan) and tap water *ad libitum*.

Drug Administration: TAK-491 (Lot#491-001) was suspended in 0.5% methylcellulose containing 1%w/v citric acid and administered orally by gavage. Control rats received the vehicle by the same route, whereas the positive control, cyclophosphamide, was administered IP.

Procedure: TAK-491 (Lot # M491-001) or vehicle was administered at daily doses of 0 (vehicle), 500, 1000 or 2000 mg/kg (6 rats/dose group) for 2 consecutive days. A separate group of 6 rats received the positive control, cyclophosphamide, as a single IP dose of 10 mg/kg. Twenty-four hours after dosing, the rats were killed, both femurs were removed and bone marrow cells were collected and fixed onto glass slides. Since no deaths occurred, cell specimens from only 5 animals (in order of animal number) were selected for evaluation. One thousand erythrocytes per animal were examined to determine the ratio of polychromatic erythrocytes (PCEs) to total erythrocytes. A total of 2000 PCEs were examined for the presence of micronuclei. The

frequencies of MNPCEs for TAK-491 treated animals were compared to that of vehicle control. The test agent was considered positive in this assay if it induced a statistically significant and dose-dependent increase above control frequency of MNPCEs.

Results: No significant difference was observed in the PCE percentage among total erythrocytes between the TAK-491 and vehicle control group (Table 33). No statistically significant increases in MNCPEs were observed when the groups treated with TAK-491 were compared to the vehicle control group (Table). In contrast, the positive control, cyclophosphamide, produced a significant increase above control MNCPE frequency. Thus, TAK-491 is judged to be negative in this rat micronucleus assay.

Treatment	Dose Group mg/kg	No. Erythrocytes Scored	PCEs/PCEs + NCEs (%)
Vehicle Control	-	5000	51.9
TAK-491,	500	5000	52.9
	1000	5000	50.8
	2000	5000	53.3
Cyclophosphamide	40	5000	46.9 *

PCE= polychromatic erythrocytes, NCE= normochromatic erythrocytes

* Significantly different from vehicle control (p<0.05)

Title (Study No.)	Micronucleus Assay with TAK-491 in Rats (B040639)
Test substance	TAK-491
Negative control	0.5 w/v% Methylcellulose (MC) containing 1 w/v% citric acid
Positive control	Cyclophosphamide monohydrate (CP)
Test system	Male Fischer (F344/Jcl) rats (7 weeks old)

Compound	Dose (mg/kg/day)	No. of doses	Route	Sampling time after the final dose	No. of rats		% MNPCEs (range)
					evaluated	treated	
0.5 w/v% MC containing 1 w/v% citric acid	10 mL/kg/day	2	p.o.	24 h	5	6	0.08 (0.00-0.15)
TAK-491	500	2	p.o.	24 h	5	6	0.10 (0.05-0.15) NS
	1000	2	p.o.	24 h	5	6	0.05 (0.00-0.15) NS
	2000	2	p.o.	24 h	5	6	0.08 (0.05-0.15) NS
CP	10	1	i.p.	24 h	5	6	1.89 (1.65-2.05)**
Conclusion: TAK-491 did not induce micronuclei under the conditions of this study.							

MNPCEs: micronucleated polychromatic erythrocytes

NS: no significant increase; **: p<0.01 (Conditional Binomial test)

7.4 Other Genetic Toxicity Studies

Unscheduled DNA Synthesis Assay

Azilsartan medoxomil (1000-2000 mg/kg, p.o.) was negative in this assay. See attached review by A. Proakis dated 8/05/2005:

Unscheduled DNA Synthesis Assay in Rat Hepatocytes

Study Facility: (b) (4)

Study No: B040641

Study Dates: 7/22/04 – 9/01/04

GLP Regulations: Compliance with GLP regulations attested

QA Report: Yes

Animals: Male Fischer F344/Jcl rats (7 weeks old at start of dosing). The animals were housed 4/cage and maintained on a pellet diet (MF, Oriental yeast Co., Japan) and tap water *ad libitum*.

Drug Administration: TAK-491 (Lot#491-001) was suspended in 0.5% methylcellulose containing 1%w/v citric acid and administered orally as a single dose by gavage. Dimethylnitrosamine (DMN) and 2-acetylaminofluorene (2-AAF) were used as positive controls. Dose Levels: 0 (vehicle), 1000 and 2000 mg/kg (8 rats/ group).

Procedure: TAK-491, vehicle or positive controls were administered as a single dose and 3 rats/group were sacrificed at 2 hours and 16 hours post treatment. The livers were removed, hepatocytes were isolated with collagenase treatment and the cells collected by centrifugation. The isolated hepatocytes were suspended in medium, and cell viability was calculated from numbers of viable and dead cells (cell viability was 63%-86% in vehicle control group). After a 2-hr incubation period, ³H-thymidine was added to each hepatocyte-containing tube and incubated for 18 hours at 37°C. After incubation the cells were fixed onto slides, air-dried, coated with photographic emulsion and placed in a dark refrigerator for radioactive exposure. The autoradiograph was subjected to nuclear staining of the hepatocytes. The slides were examined microscopically and the numbers of net grains (NG; nuclear grains minus the grains in an equivalent area of the cytoplasm adjacent to the nucleus) and hepatocytes with 5 or more net grains (counted as cells in repair) were calculated. For each animal, 2 slides were examined and 150 hepatocytes (75 cells/slide) were observed and the mean NG was calculated. The percentage of cells in repair as a function of the total hepatocytes examined was determined.

Results: The mean number of net grains and the mean repair cell rate in each of the TAK-491 treated groups was similar to the net grain number and repair cell rate in the vehicle control group. In contrast, the mean number of net grains was larger and the mean repair cell rates were higher in both positive control groups when compared to the vehicle control (Table 30). Thus, TAK-491 did not induce unscheduled DNA synthesis in rat hepatocytes and is judged to be negative in this assay.

Title (Study No.)	<i>In vivo/in vitro</i> Unscheduled DNA Synthesis (UDS) Assay with TAK-491 in Rat Hepatocytes (B040641)
Test substance	TAK-491
Negative control	0.5 w/v% Methylcellulose (MC) containing 1 w/v% citric acid
Positive controls	Dimethylnitrosamine (DMN), 2-Acetylaminofluorene (2-AAF)
Test system	Male Fischer (F344/Jcl) rats (7 weeks old)

Compound	Dose (mg/kg)	No. of doses	Route	Duration of treatment	No. of rats		Net grains per nucleus Mean±SD	Repair cell rate(%) Mean±SD
					evaluated	treated		
0.5 w/v% MC containing 1 w/v% citric acid	10 mL/kg	1	p.o.	2 h	3	4	-0.30±0.32	3.8±1.0
TAK-491	1000	1	p.o.	2 h	3	4	-0.25±0.05	2.4±0.8
	2000	1	p.o.	2 h	3	4	-0.50±0.26	3.6±1.4
DMN	10	1	p.o.	2 h	3	4	11.91±0.47	96.7±1.2
0.5 w/v% MC containing 1 w/v% citric acid	10 mL/kg	1	p.o.	16 h	3	4	-0.17±0.37	3.3±0.7
TAK-491	1000	1	p.o.	16 h	3	4	-0.07±0.38	4.9±0.8
	2000	1	p.o.	16 h	3	4	-0.05±0.29	3.8±1.6
2-AAF	50	1	p.o.	16 h	3	4	5.83±0.31	74.9±6.0

8. Carcinogenicity

Reviewed by P. Gatti on 9/23/10.

Key Findings: TAK-491 tested negative for carcinogenicity in the 2-year rat carcinogenicity study. Doses tested were considered to be adequate by the Executive CAC. See attached review.

Study Title: Twenty-four-month Oral Gavage Carcinogenicity Study of TAK-491 in Rats

Study Facility: (b) (4)

Study Number: B-5471

Study Dates: Jan. 29, 2007- Nov. 26, 2009

GLP Compliance: Yes

QA Report: Yes

Methods

Dosing: Male and female F344/Fcl SPF rats (50/sex/group) were given vehicle (2 control groups received 0.5 w/v% methylcellulose solution containing 0.3 w/v% citric acid), 60 mg/kg/day, 200 mg/kg/day and 600 mg/kg/day of the drug via oral gavage once per day in a volume of 10 ml/kg/day. For both control groups, 5 animals/sex were utilized for TK analysis. For treatment groups, 8 animals/sex were utilized for TK analysis.

Basis for Dose Selection: In a 13-week oral gavage range-finding toxicity study of TAK-491, dosage levels of 0, 200, 600 and 2000 mg/kg/day were studied. At the 2000 mg/kg/day dose, there was a suppression of body weight gain in males (-12%), an increase in white blood cell count in both sexes, decreases in total cholesterol (female), phospholipids (female) and total protein (both sexes) and erosion in the glandular stomach in almost all animals at this dose. At the 600 mg/kg/day dose, only increases in adrenal weight and white cell count were seen in males. Thus, this dose was chosen as the highest dose in this carcinogenicity study.

Clinical Signs: Animals were examined 3X daily for clinical signs and once per week for any palpable masses.

Body Weights: Animals were weighed twice in the first week of treatment and weekly up to week 14 and once every 2 weeks thereafter until study termination/completion.

Food Intake: Food intake was measured twice in the first week of treatment and weekly up to week 14 and once every 2 weeks thereafter until study termination/completion.

Water Intake: Not reported.

Hematology: Blood was collected at the time of necropsy.

Clinical Chemistry: Not reported.

Urinalysis: Not reported.

Histopathology: After 24 months, all remaining animals from all the groups were subjected to a necropsy. External appearance of all the organs/tissues in the cranial, thoracic and abdominal cavities were examined and the results recorded. Microscopic examination was performed on the following tissues:

cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves, eyeballs, optic nerves, Harderian glands, pituitary, thyroids, parathyroids, adrenals, thymus, spleen, submandibular lymph node, mesenteric lymph node, heart, thoracic aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands, sublingual glands, liver,

pancreas, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, ovaries, uterus (both horns), vagina, mammary glands (inguinal region), sternum (including bone marrow), femurs (including bone marrow and knee joint), femoral muscles, skin (inguinal region) and gross lesions

Toxicokinetics: Blood was taken from the additional 5-8 satellite animals/ group for toxicokinetic analysis at day 1 and at week 52.

Statistical Analysis: Tumors that occurred with high incidence (10 or more animals in total for each sex, >10) were evaluated using survival-adjusted Peto's test to assess increasing trend of incidence to dose level for all groups and to compare the incidence between the control groups (combined with control group-I and -II) and each dose group. Tumors that occurred with low incidence (less than 10 animals in total for each sex, <10) were evaluated using exact permutation trend test to assess increasing trend of incidence to dose level for all groups and to compare the incidence between the control groups (combined with control group-I and -II) and each dose group. For incidental tumors, the analysis intervals were: Weeks 1 through 52, 53 through 78, 79 through 92 and 93 through 104 and the period scheduled necropsy of the live phase. Analysis of positive trend in incidence was conducted at the significance levels of 0.01 (1 tailed-level) for common tumors and 0.05 (1 tailed-level) for rare tumors. Pairwise comparison was conducted at the significance levels of 0.01 (1 tailed-level) for common tumors and 0.05 (1 tailed-level) for rare tumors. Common tumors were defined as those with a historical incidence in controls (F344/Crlj rats) in the (b) (4) of more than 1% (>1%) and rare tumors as 1% or less (<1%). The incidences of tumors were analyzed with Peto's survival-adjusted tumor analysis.

Results

Mortality

1) Males

The survival rate in the 60 mg/kg and higher groups was slightly higher than that of the control group, and statistical significance was noted in the 200 mg/kg group (Table and Fig. 1 below).

2) Females

A statistically significant trend of increase in the survival rate with increasing dosage levels was observed. The survival rate in the 200 and 600 mg/kg groups was slightly higher than that of the control group, and statistical significance was noted in the 600 mg/kg group (Table and Fig. 1 below).

Text Table 1. Summary of mortality and survival rate

Sex	Male					Female				
	0 ^{a)}	0 ^{b)}	60	200	600	0 ^{a)}	0 ^{b)}	60	200	600
Dosage (mg/kg/day)	0 ^{a)}	0 ^{b)}	60	200	600	0 ^{a)}	0 ^{b)}	60	200	600
No. of animals used	50	50	50	50	50	50	50	50	50	50
Week 1 - 26	0	0	0	0	2	0	0	0	0	0
Week 1 - 52	0	0	1	0	2	0	1	2	0	0
Week 1 - 78	3	1	3	0	4	0	2	2	1	0
Week 1 - 105	20	19	13	4	12	16	11	14	8	6
No. of survivors	61 ^{c)}		37	46	38	73 ^{c)}		36	42	44
Survival rate (%)	61.0 ^{c)}		74.0	92.0*	76.0	73.0S ^{c)}		72.0	84.0	88.0*

Values in the table indicate the cumulative number of the animals that died or were sacrificed as moribund.

a): Control group-I, b): Control group-II, c): Combined value of with control group-I and -II

*: p<0.05 (significantly different from the control group, log-rank test)

S: p<0.05 (statistically significant trend, Tarone's test)

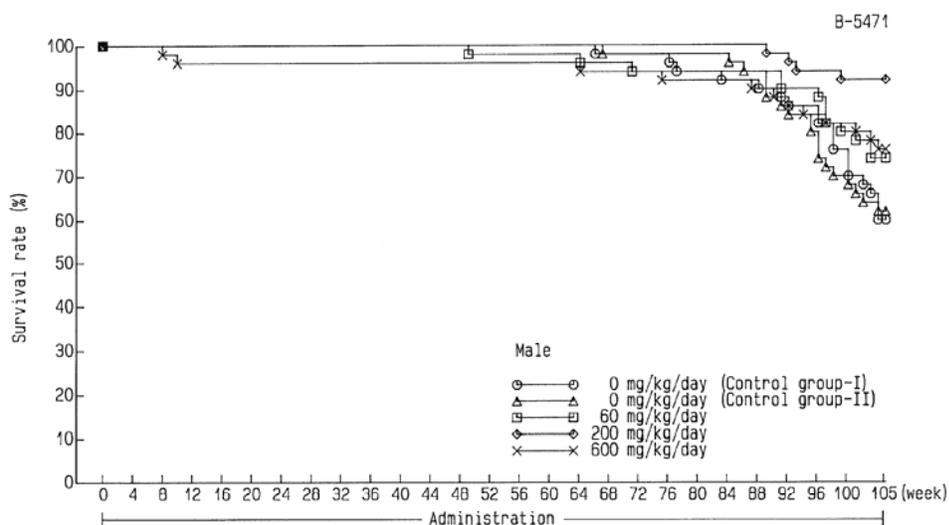


Fig.1 Twenty-four-month oral gavage carcinogenicity study of TAK-491 in rats

— Survival rate —

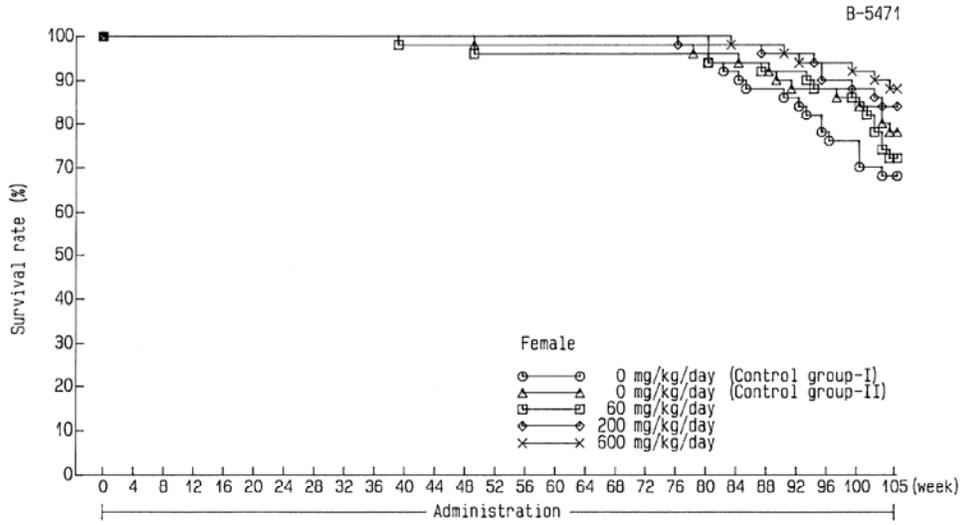


Fig.2 Twenty-four-month oral gavage carcinogenicity study of TAK-491 in rats

— Survival rate —

Body Weights

Males:

There were no treatment-related changes in the 60 mg/kg/day group. In the 200 and 600 mg/kg/day groups, the mean body weight at the end of treatment was 4% and 11% lower than in the control groups, respectively (Fig. 3)

Females

In the 60 mg/kg/day group, there was a significant decrease in body weight between weeks 3 and 78, but after that period, the weight was comparable to control animals. In the 200 and 600 mg/kg/day groups, the mean body weight at the end of treatment was 6% and 15% lower than in the controls, respectively (Fig. 4).

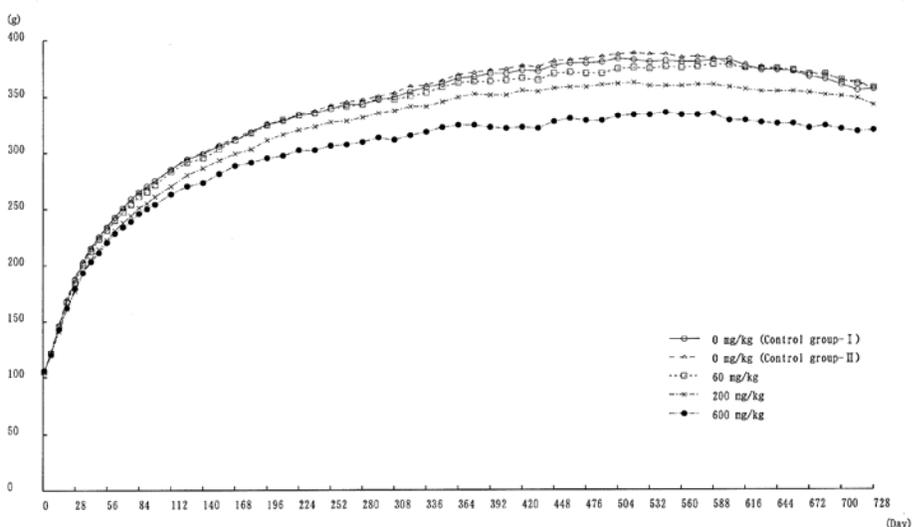


Fig. 3 Twenty-four-month oral gavage carcinogenicity study of TAK-491 in rats
Body Weight - Male

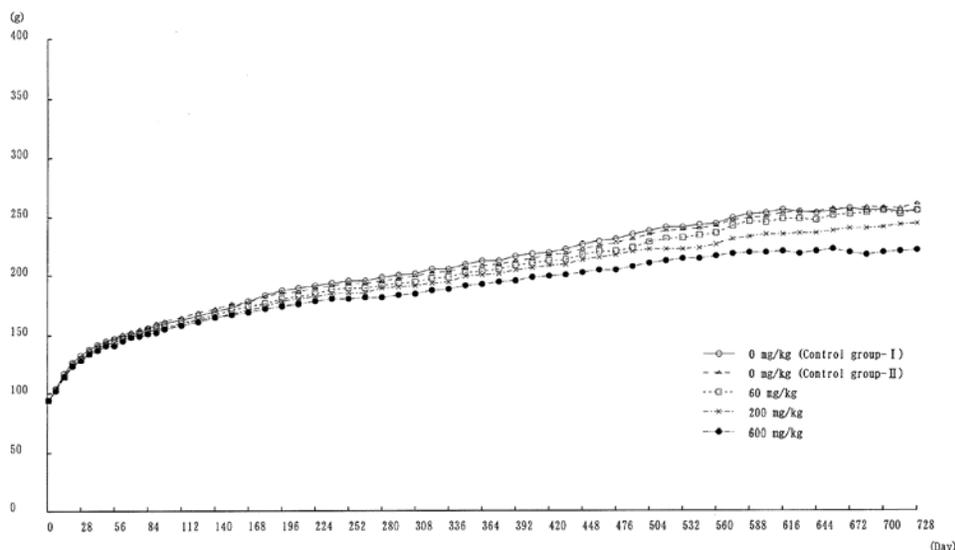


Fig. 4 Twenty-four-month oral gavage carcinogenicity study of TAK-491 in rats

Body Weight - Female

Hematology

Males:

Statistically significant decreases in reticulocyte ratio and platelet count were observed in all treatment groups. A statistically significant increase in red blood cell count was observed in the 200 and 600 mg/kg/day groups and statistically significant increases in hemoglobin concentration and hematocrit value was observed only at the 600 mg/kg/day group (Table 2)

Females

No significant effects on hematological parameters were observed.

Table 2. Summary of Hematology (Sponsor's table)

Sex	Male				Female			
	0	60	200	600	0	60	200	600
Dosage (mg/kg/day)	100	50	50	50	100	50	50	50
No. of animals used	61	37	46	38	73	36	42	44
RBC ($10^3/\mu\text{L}$)	817 ^{a)}	853 (N) ^{b)}	875* (+7%)	925* (+13%)	793 ^{a)}	818 (N)	808 (N)	791 (N)
HGB (g/dL)	15.0 ^{a)}	15.5 (N)	15.9 (N)	16.6* (+11%)	15.0 ^{a)}	15.5 (N)	15.2 (N)	15.0 (N)
HCT (%)	41.4 ^{a)}	42.6 (N)	43.7 (N)	45.6* (+10%)	41.3 ^{a)}	42.8 (N)	42.2 (N)	41.7 (N)
Reticulocyte ratio (%)	4.3 ^{a)}	3.2* (-26%)	2.7* (-37%)	2.2* (-49%)	3.2 ^{a)}	2.3 (N)	2.4 (N)	3.5 (N)
Platelet ($10^3/\mu\text{L}$)	89.0 ^{a)}	79.9* (-10%)	79.4* (-11%)	79.5* (-11%)	72.4 ^{a)}	69.3 (N)	69.7 (N)	72.0 (N)
Eosinophil ratio (%)	1.1 ^{a)}	1.5* (+36%)	1.8* (+64%)	1.7* (+55%)	1.1 ^{a)}	1.4* (+27%)	1.5* (+36%)	1.2* (+9%)
Monocyte ratio (%)	3.3 ^{a)}	2.8* (-15%)	3.2 (N)	2.9 (N)	3.6 ^{a)}	3.4 (N)	3.6 (N)	3.4 (N)

a): Mean value of double control groups (calculated from individual values of control group-I and -II)

b): Values in parentheses indicate percentage of change against the double control mean (-: decrease, +: increase).

N: No remarkable changes

*: $p \leq 0.05$ (significantly different from the control groups)

Non-Neoplastic Changes

Treatment-related non-tumor lesions that showed an increase/decrease in incidence and/or severity were observed in the adrenal, kidney, spleen and heart. (Table 3).

Table 3. Summary of Non-Tumor Lesions (Sponsor's table)

Sex	Male					Female				
	0 ^{a)}	0 ^{b)}	60	200	600	0 ^{a)}	0 ^{b)}	60	200	600
Dosage (mg/kg/day)										
Effective No. of animals	50	50	50	50	50	50	50	50	50	50
Adrenal										
Atrophy, zona glomerulosa, diffuse (total)	5	0	49	50	48	0	0	8	12	20
(±/+)	5	0	1	0	0	0	0	8	12	19
(++/+++)	0	0	48	50	48	0	0	0	0	1
Hyperplasia, zona fasciculata, diffuse										
(total, ±/+)	0	0	44	50	49	0	0	0	1	1
Hyperplasia, cortical, focal (total, ±)										
(total, ±)	17	7	4	8	4	15	18	22	23	31
Hypertrophy, cortical cell, focal (total, ±/+)										
(total, ±/+)	5	6	12	9	7	17	5	26	35	23
Hypertrophy, zona glomerulosa, focal										
(total, ±)	3	4	0	0	1	11	5	15	18	23
Angiectasis (total, ±/+)										
(total, ±/+)	20	10	23	21	31	39	40	31	32	39

a): Control group-I, b): Control group-II

Number in the table indicates the number of animals with respective lesions.

±: Minimal, +: Mild, ++: Moderate, +++: Severe

(Continued to the next page)

Sex		Male					Female				
		0 ^{a)}	0 ^{b)}	60	200	600	0 ^{a)}	0 ^{b)}	60	200	600
	Dosage (mg/kg/day)										
	Effective No. of animals	50	50	50	50	50	50	50	50	50	50
Kidney											
	Thickening, arterial wall (total, ± to ++)	0	0	50	50	48	0	0	46	49	49
	Hypertrophy, juxtaglomerular cell (total, ±/+)	0	0	50	49	50	0	0	48	50	49
	Chronic progressive nephropathy (total)	50	50	49	50	47	44	44	41	45	44
	(±)	16	19	25	27	39	30	28	30	41	37
	(+)	26	20	19	18	7	12	16	8	2	6
	(++)	7	11	4	4	1	2	0	1	2	1
	(+++)	1	0	1	1	0	0	0	2	0	0
	Cyst (total, ± to ++)	1	2	3	9	9	1	1	4	1	3
Spleen											
	Pigmentation (total)	20	22	36	41	41	35	32	45	41	41
	(±)	19	21	32	40	35	27	23	23	26	16
	(+)	1	1	3	1	6	8	9	21	15	25
	(++)	0	0	1	0	0	0	0	1	0	0
Heart											
	Cardiomyopathy (total)	50	49	47	49	44	43	48	43	45	44
	(±)	21	18	38	43	37	42	46	42	42	44
	(+)	29	31	9	6	7	1	2	1	3	0

a): Control group-I, b): Control group-II

Number in the table indicates the number of animals with respective lesions.

±; Minimal, +; Mild, ++: Moderate, +++: Severe

Neoplastic Changes

There was no treatment-related increase in either the number of tumors or tumor-bearing animals in either sex (Table 4). There was a tendency for a decrease in total number of tumors in males at the 600 mg/kg/day dose.

Table 4. Number of tumors and Tumor Bearers (Sponsor's table)

Sex	Male					Female				
	0 ^{a)}	0 ^{b)}	60	200	600	0 ^{a)}	0 ^{b)}	60	200	600
Dosage (mg/kg/day)										
No. of animals used	50	50	50	50	50	50	50	50	50	50
Total No. of tumors	167	180	169	162	134	73	67	64	75	81
No. of benign tumors	140	146	154	141	120	49	50	49	57	58
No. of malignant tumors	27	34	15	21	14	24	17	15	18	23
Total No. of tumor bearing animals	49	49	47	50	47	43	39	37	40	40
No. of benign tumor bearers	48	47	46	50	46	35	31	33	35	35
No. of malignant tumor bearers	26	28	14	20	13	21	14	15	18	21
No. of multiple tumor bearers	44	46	45	47	43	21	21	21	22	28

a): Control group-I, b): Control group-II

Number in the table indicates the number of tumors or animals.

Tumors that demonstrated a change in incidence are summarized in Table 5.

Table 5. Incidence Summary of Major Tumors (Sponsor's table)

Sex	Male					Female				
	0 ^{a)}	0 ^{b)}	60	200	600	0 ^{a)}	0 ^{b)}	60	200	600
Dosage (mg/kg/day)										
No. of animals used	50	50	50	50	50	50	50	50	50	50
Adrenal										
Adenoma, cortical cell	0	1	0	1	0	0	0	4	2	2
Carcinoma, cortical cell	0	0	0	0	0	0	0	0	1	0
Adenoma + Carcinoma, cortical cell	0	1	0	1	0	0	0	4	3	2
Hemolymphoreticular										
Sarcoma, histiocytic	1	1	2	2	1	1S	0S	1	1	4#
Leukemia, large granular lymphocytic	21	20	7	10	6	12	11	8	9	11
Pancreas										
Adenoma, islet cell	10	9	10	7	6	0	0	1	1	2
Carcinoma, islet cell	0S	0S	0	0	2	0	0	0	0	0
Adenoma + Carcinoma, islet cell	10	9	10	7	8	0	0	1	1	2

a): Control group-I, b): Control group-II

Number in the table indicates the number of animals with respective lesions.

#: p<0.05 (significantly different from the control groups, rare tumor, Peto's test)

S: p<0.05 (significantly difference for positive trend among control groups and dose groups, rare tumor, Peto's test)

In the adrenal gland, incidence of cortical cell adenoma showed a tendency for increase in females at all doses tested compared to the control groups. In females but not males, there was an overall increase in incidence of cortical cell tumors (adenoma and

carcinoma) in all doses compared to control. Carcinoma was observed in only 1/50 females in the 200 mg/kg/day group. The incidence of histiocytic sarcoma showed a statistically significant increase in females at the 600 mg/kg/day group ($p < .05$), and also showed a statistically significant positive trend ($p < .05$). The incidence of large granular lymphocytic leukemia was decreased in males in all treatment groups. In the pancreas, islet cell carcinoma was observed in 2/50 males in the 600 mg/kg/day group and showed a statistically positive trend ($p < .05$).

Toxicokinetics

In all dose groups, TAK-491F at any time point in both sexes was not detected. The C_{max} and AUC 0-24h values for TAK-536 and metabolite TAK-536 M-II in both sexes after the first and last doses increased with increment of dosage levels of TAK-491. For the metabolite TAK-536 MI, the AUC 0-24h values in both sexes increased after the first dose of TAK-491, but there was no tendency to increase in C_{max} after the first and last doses and the AUC 0-24h after the last dose (Table 6).

Table 6. Toxicokinetics of TAK-491 (Sponsor's table)

Sex	Male (n=3)			Female (n=3)		
	Dosage (mg/kg/day)	60	200	600	60	200
TAK-491F						
T _{max} (h)						
Day 1 (1st dose)	NC	NC	NC	NC	NC	NC
Week 52 (363rd dose)	NC	NC	NC	NC	NC	NC
C _{max} (ng/mL)						
Day 1 (1st dose)	0	0	0	0	0	0
Week 52 (363rd dose)	0	0	0	0	0	0
AUC _{0-24h} (ng-h/mL)						
Day 1 (1st dose)	0	0	0	0	0	0
Week 52 (363rd dose)	0	0	0	0	0	0
TAK-536						
T _{max} (h)						
Day 1 (1st dose)	0.5	1.0	1.0	1.0	1.0	0.5
Week 52 (363rd dose)	1.0	1.0	0.5	1.0	1.0	0.5
C _{max} (ng/mL)						
Day 1 (1st dose)	89290	144177	200643	90102	181702	191548
Week 52 (363rd dose)	108724	137575	190765	126965	145647	217982
AUC _{0-24h} (ng-h/mL)						
Day 1 (1st dose)	497566	1076554	1385116	420904	897918	1089110
Week 52 (363rd dose)	500236	823230	1012748	557356	726105	1127893

NC: Not calculated

Table 6 continued

Sex	Male (n=3)			Female (n=3)		
	60	200	600	60	200	600
TAK-536 M-I						
<i>T</i> _{max} (h)						
Day 1 (1st dose)	2.0	2.0	1.0	0.5	0.5	0.5
Week 52 (363rd dose)	1.0	2.0	0.5	1.0	1.0	1.0
<i>C</i> _{max} (ng/mL)						
Day 1 (1st dose)	1655	1994	1845	1356	2554	2003
Week 52 (363rd dose)	6518	4861	3069	4362	2593	2804
AUC _{0-24h} (ng·h/mL)						
Day 1 (1st dose)	12991	16964	17470	11999	16805	22271
Week 52 (363rd dose)	37562	39515	38773	23570	20602	28165
TAK-536 M-II						
<i>T</i> _{max} (h)						
Day 1 (1st dose)	1.0	1.0	1.0	1.0	1.0	1.0
Week 52 (363rd dose)	1.0	1.0	1.0	1.0	1.0	1.0
<i>C</i> _{max} (ng/mL)						
Day 1 (1st dose)	208	366	703	131	323	376
Week 52 (363rd dose)	191	279	343	191	250	370
AUC _{0-24h} (ng·h/mL)						
Day 1 (1st dose)	952	2258	3279	534	1375	1802
Week 52 (363rd dose)	705	1209	1634	626	777	1236

Plasma drug concentrations were also measured for the control group-I and -II (1 hour after dosing on Day 1 and in Week 52), and the concentrations of each analyte were less than the quantification limit (5 ng/mL for each analyte).

SUMMARY

Carcinogenicity of TAK-491 was assessed in F344/Jcl rats. Doses administered by gavage were 60, 200 and 600 mg/kg/day. Dose selection was approved by the Executive CAC based on MTD. The high dose was chosen based on a 13-week toxicity study that demonstrated that this dose did not significantly affect weight gain in the animals.

Results:

There was no decrease in survival rate in either species. In fact, rates were statistically significantly increased in males at 200 mg/kg/day and females at 600 mg/kg/day.

- 1- Body weights in treated animals decreased in both sexes at 200 and 600 mg/kg/day doses.
- 2- Hematological effects in treated animals were seen only in males. At the 200 and 600 mg/kg/day doses, there was a significant increase in red blood cell count. At the

600 mg/kg/day dose, significant increases in hemoglobin concentration and hematocrit value were observed.

- 3- Histopathology: Treatment-related changes were seen in the adrenal, kidney and spleen. Diffuse atrophy of the zonal glomerulosa was observed in both sexes in all dose groups. In males at all doses, there was a significant increase in the incidence of atrophy in the zona glomerulosa in all groups but a decrease in the incidence of focal cortical hyperplasia and no effect on the incidence of cortical tumors. Females exhibited an increase in the incidence of focal cortical hyperplasia at the 600 mg/kg/day dose. A small increase in the incidence of cortical tumors was seen in females at the lowest (60 mg/kg/day) dose. This incidence did not increase with increasing doses in females. Also in females, there was an increase in the incidence of focal hypertrophy of cortical cells at the 200 mg/kg/day dose. In the kidney, thickening of the arterial wall and hypertrophy of the juxtaglomerular cells were observed in both sexes at all doses. In the spleen, increased incidence and/or severity of pigmentation in the red pulp was observed in both sexes at all doses.
- 4- Toxicokinetics: Systemic exposure to the metabolites of TAK-491 was demonstrated. The C max and AUC 0-24h values for TAK-536 and TAK-536 M-II in both sexes after the first and last doses increased with increment of dosage levels of the prodrug TAK-491. For the TAK-536 MI metabolite, the AUC 0-24h values in both sexes after the first dose increased with increment of TAK-491 dosages, but there was no tendency to increase in the C max values after the first and last dosages and the AUC 0-24h values after the last (363rd) dose. The C max and AUC 0-24h values for TAK-536 and TAK-536 M-II in both sexes after the last dose were mostly comparable to the first dose indicating no repeated dose effect. The C max and AUC 0-24h values for TAK-536 MI in both sexes after the last dose were mostly higher than those values after the first dose. There were no apparent gender differences in the C max and AUC 0-24h values for each analyte at any doses after the first and last doses.

In conclusion, a 24-month administration of TAK-491 did not cause any tumors in rats. Major non-neoplastic toxicologic pathology included diffuse atrophy of the zona glomerulosa in both sexes, diffuse hyperplasia of zona fasciculata in males and focal hypertrophy of cortical cells and focal hyperplasia of cortical cells in females in the adrenal cortex, thickening of the arterial wall and hypertrophy of the juxtaglomerular cells in both sexes in the kidney and hyperpigmentation in both sexes in the spleen.

Mouse Carcinogenicity Studies

Study Title: Twenty-six-week Oral Gavage Carcinogenicity Study of TAK-491 in Tg.rasH2 Mice

Study Facility: [REDACTED] (b) (4)
Study Number: 07-241/CO
Study Dates: Oct. 25, 2007- July 23, 2009
GLP Compliance: Yes
QA Report: Yes

Key Finding: TAK-491 tested negative for carcinogenicity in a 6-month transgenic ras mouse carcinogenicity study. Dosages tested were considered to be adequate by the Executive CAC.

Methods

Dosing: Male and female Tg.rasH2 mice (25/sex/group) were given either vehicle (0.5w/v% methylcellulose with 1w/v% citric acid) or TAK-491 via gavage. Mice received 50 mg/kg/day, 150 mg/kg/day or 450 mg/kg/day in a volume of 10 ml/kg/day. For treatment groups, 44 animals/sex/group (non-transgenic mice) were utilized for TK analysis.

Basis for Dose Selection: In a 4-week oral gavage range-finding toxicity study of TAK-491, dosage levels of 0 (vehicle control), 200, 600, 2000 or 3000 mg/kg/day of TAK-491 were studied. There were high levels of premature mortality (mostly prior to day 11) in both the 2000 and 3000 mg/kg/day groups. In addition, in the TK substudy in non-transgenic mice, 5/52 animals receiving the 600 mg/kg/day dose died. The ECAC recommended doses of 50, 150 and 450 mg/kg/day based on the MTD (mortality at 600 mg/kg/day observed in the TK substudy and at 2000 mg/kg/day observed in both the main study and the TK substudy).

Clinical Signs: Animals were examined 2X daily for clinical signs and once per week for any palpable masses.

Body Weights: Animals were weighed once per week in the first 13 weeks of treatment and then biweekly until study termination/completion.

Food Intake: Food intake was measured once per week in the treated animals only.

Water Intake: Not reported.

Hematology: Not reported.

Clinical Chemistry: Not reported.

Urinalysis: Not reported.

Organ Weights: The brain, heart, liver, kidneys, lung, spleen, thymus and testes or ovaries were weighed at terminal necropsy.

Histopathology: After 26 weeks, all remaining animals from all the groups were subjected to a necropsy. Microscopic examination was performed on the following tissues:

Adrenal Glands	Parathyroid Glands
Aorta	Pituitary Gland
Bone (femur and sternum)	Prostate Gland
Bone Marrow (femur and sternum)	Salivary Gland
Brain	Sciatic Nerve
Epididymides	Seminal Vesicles
Esophagus	Skeletal Muscle (thigh)
Eyes	Small Intestine (duodenum, jejunum, and ileum)
Gall Bladder	Spinal Cord (cervical, thoracic, and lumbar)
Gross Lesions	Spleen
Harderian Glands	Stomach
Heart	Testes
Kidneys	Thymus
Large Intestine (cecum, colon, rectum)	Thyroid Glands
Liver	Tongue
Lungs and Bronchi	Trachea
Lymph Nodes (mesenteric, mediastinal and mandibular)	Urinary Bladder
Mammary Gland with adjacent skin	Uterus
Nasal Cavity	Vagina
Ovaries	
Pancreas	

Toxicokinetics: Eighteen TK animals per sex and dose (3/sex/dose/timepoint) were bled at 0.5, 1, 2, 4, 8 and 24 hr after the first dose on Day 1. Twenty-one TK animals (3/sex/dose/timepoint) after 177 treatments during week 26 were bled at pre-dose (female mice only-protocol deviation), 0.5, 1, 2, 4, 8 and 24 hours after the last dose in week 26. Three vehicle control TK animals per sex were bled 1 hour post dosing on day 1 and during day 177.

Statistical Analysis: The following table lists the tests used to perform the statistical analysis of the data:

Table 1. Statistical Analyses and Mathematical Functions (from Sponsor)

Statistical Analyses and Mathematical Functions.

Parameter	Statistical Test or Mathematical Function	Program
Mortality	Fisher's Exact Test Generalized Wilcoxon tests*	Microsoft® Excel 2003 ¹ SAS Version 8.2
Selected Clinical Observations	Fisher's Exact Test	Microsoft® Excel 2003 ¹
Tumor Incidence	Fisher's Exact Test Peto Analysis Test*	Microsoft® Excel 2003 ¹ SAS Version 8.2
Body Weights, Body Weight Gain and Weekly Food Consumption, Organ Weights (Absolute and Relative)	Means and Standard Deviations, ANOVA, Dunnett's t-test	Provantis™ Version 6.5.0.1
Absolute (Total) Body Weight Gain, Total Food Consumption	ANOVA, Dunnett's t-test	Minitab Version 15.1.0.0

¹ Excel worksheet templates are validated for functionality, accuracy and worksheet security according to (b) (4) SOPs ODQP2725 and OPIS2108.

Note: A probability level of $p < 0.05$ was used to determine statistical significance.

Results

Mortality

1) Males Three males in the vehicle control group died before study end and one in the middle dose group. Nine urethane treated animals died during the study.

2) Females Two female animals in the low dose and one in the high dose group died before the end of the study. Eleven urethane treated animals died during the study.

Clinical Signs

1) Males Clinical signs of toxicity in test-article treated groups that were statistically significantly increased compared to vehicle treated controls included decreased motor activity, hunched posture and rapid and shallow breathing in groups treated with 150 and 450 mg/kg/day. Also, in the 450 mg/kg/day group, the animals exhibited lethargy and dyspnea.

2) Females Clinical signs of toxicity in test-article treated groups that were statistically significantly increased compared to vehicle treated controls included hunched posture in all groups, decreased motor activity, lethargy and dyspnea in the 150 and 450 mg/kg/day groups. Lastly, in the 450 mg/kg/day group, rapid and shallow breathing was observed.

Body Weights

1) Males:

Group body weight at the end of the administration period for the 450 mg/kg/day dose was significantly decreased compared to control on day 15 and significantly decreased in a dose-dependent manner in the mid-dose and high dose males from day 22 to day 127 and in the low dose to high dose males from day 141 to day 183 when compared to vehicle treated mice (Table 2).

2) Females

Group body weight was significantly decreased in mid and high dose groups on day 15 and statistically significantly decreased in a dose-dependent manner in all test article treated groups from day 22 to day 183 when compared to vehicle treated mice (Table 2).

Food Consumption

1) Males

No statistically significant changes in food consumption were observed in males comparing treated and control mice for the whole study.

2) Females

No statistically significant changes in food consumption were observed in females comparing treated and control mice for the whole study.

Organ Weights

1) Males

Heart weights were significantly decreased in all treated groups compared to control mice. Thymus weight was significantly decreased in mid and high dose groups compared to control (Table 2).

2) Females

Heart weights were significantly decreased in all treated groups compared to control mice. Thymus weight was significantly decreased in the high dose group compared to control (Table 2).

Non-Neoplastic and Neoplastic Changes

1) Males

Degenerative, inflammatory and hyperplastic lesions were seen in the nasal cavity of vehicle and test-article treated male mice. The severity of these lesions was increased in the test-article treated groups. An adenoma was observed in 1 vehicle treated male and an adenocarcinoma was noted in 1 low-dose male and one mid-dose male (Table 2)

2) Females

Degenerative, inflammatory and hyperplastic lesions were seen in the nasal cavity of vehicle and test-article treated female mice. The severity of these lesions was increased in the test-article treated groups. Adenocarcinoma was noted in 1 mid-dose female (Table 2).

Toxicokinetics

Plasma levels of TAK-491 were not detected in all dose groups on day 1 of dosing. Plasma levels of the pro-drug were also not detected on day 177 except in a few instances where the C max was very low (10 ng/ml). There were much greater levels of all 3 metabolites in the plasma of all treated groups. This suggests very rapid metabolism of the prodrug in mice. The C max and AUC 0-24h values of the major metabolite TAK-536 were much higher than those of TAK-536 MI and TAK-536 M-II in both sexes. Increases in TAK-491 dose resulted in a less than dose-proportional increases in C max and AUC 0-24h values for all 3 metabolites at all doses in both sexes (Table 2).

SUMMARY

Carcinogenicity of TAK-491 was assessed in Tg.rasH2 mice. Dosages administered by gavage were 0 (vehicle), 50, 150 and 450 mg/kg/day.

In conclusion, after 26-weeks of administration, TAK-491 did not cause a statistically significant increase in incidence of tumors in transgenic mice.

Tabulated Summary

Tabulated Summary

(b) (4)

26-Week Oral Gavage Carcinogenicity Study of TAK-491 in Tg.rasH2 mice (Study No. AB39LX.7G8R)					
Main Study: Tg.rasH2 mice, approximately 7-9 weeks of age at study start					
Animal	Control ¹	Positive control (urethane) ²	TAK-491		
Dosage (mg/kg/day)	0	1000	50	150	450
No. of animals (M:F)	25:25	25:25	25:25	25:25	25:25
Mortality (M:F)	3:0	9:11	0:2	1:0	0:1
Clinical signs (Cageside)	-	Decreased motor activity, lethargy, rapid and shallow breathing, ataxic (F), prostrate (F).	Hunched posture (F)	Decreased motor activity, hunched posture, rapid and shallow breathing (M), lethargy (F), labored/dyspnea (F) Rapid and shallow breathing (F), lethargy (M), labored/dyspnea (M)	
Clinical signs (Hands-on)	-	Rapid and shallow breathing, mass ⁴ (M), eschar (M), thin appearance (F)	Thin appearance (F)	Rapid and shallow breathing (M) Thin appearance (F)	
Body weights	-	↓(M; Days 8 & 15) ↑(M; Day 57-, F; Day 36-)	↓(M-7%, F-11%)	↓(M-17%, F-12%)	↓(M-13%, F-12%)
Body weight gains	-	↓(M; Day 1 to 15)	↓	↓	↓
Food consumption	-	↓(Day 1 to 8)	-	-	-
Necropsy	-	Pulmonary and splenic lesions	-	-	-
Organ weights	-	-	↓ Heart ↓ Thymus (M) ↓ Thymus		
Histopathology	-	Alveolar bronchiolar adenomas, (single and multiple) alveolar bronchiolar carcinomas and splenic hemangiosarcomas (single and multiple)	Hyperplasia of juxtaglomerular cells, tubular regeneration# and nephrosis# Increased incidence and severity of degenerative, inflammatory and hyperplastic lesions in the nasal cavity#		
TK Study: CByB6F1 mice, approximately 7-9 weeks of age at study start					
Animal	Control ¹	TAK-491			
Dosage (mg/kg/day)	0	50	150	450	
No. of animals (M:F)	10:10	44:44	44:44	44:44	
Mortality (M:F)	0:0	0:1 ³	0:0	1:0	
Plasma concentration [M:F, Mean (n=3)]					
Tmax (h)	TAK-491 F	1st	-	-	-
		177th	-	1.0:0.5	1.0:4.0
	TAK-536	1st	0.5:0.5	0.5:0.5	0.5:0.5
		177th	0.5:0.5	1.0:0.5	1.0:0.5
	TAK-536 M-I	1st	0.5:0.5	0.5:0.5	0.5:0.5
		177th	0.5:1.0	0.5:0.5	0.5:1.0
	TAK-536 M-II	1st	0.5:0.5	1.0:0.5	1.0:1.0
		177th	1.0:0.5	1.0:1.0	1.0:1.0

- = No treatment-related effects, M: Male, F: Female, ↑: Increase, ↓: Decrease, #: Toxicologically significant changes, ¹: 0.5 w/v% methylcellulose with 1 w/v% citric acid, ²: urethane in saline at dose of 1000 m/kg in 3 intraperitoneal injections (one each on Day 1, 3 and 5), ³: Animal was accidentally killed, ⁴: Mass on the ventral side of the body. % : Compared to the vehicle control on Day 183.

Tabulated Summary - Continued

26-Week Oral Gavage Carcinogenicity Study of TAK-491 in Tg.rasH2 mice (Study No. AB39LX.7G8R (b) (4))

Animal		TK Study (CByB6F1) mice, approximately 7-9 weeks of age at study start				
Test article		Control ¹	TAK-491			
Dosage (mg/kg/day)		0	50	150	450	
No. of animals (M:F)		10:10	44:44	44:44	44:44	
Mortality (M:F)		0:0	0:1 ²	0:0	1:0	
Plasma concentration [M:F, Mean (n=3)]						
C _{max} (ng/mL)	TAK-491 F	1st	0:0	0:0	0:0	
		177th	0:0	8:0	10:7	
	TAK-536	1st	61596:75836	126149:141824	155234:192142	
		177th	103445:93265	74065:198048	143744:276236	
	TAK-536 M-I	1st	1025:975	1542:1405	1901:1929	
		177th	4141:2861	1157:3965	1616:3734	
	TAK - 536 M-II	1st	845:875	2251:1405	3998:3284	
		177th	1125:1184	1308:5437	7259:11480	
	AUC _{0-24h}	TAK-491 F	1st	0:0	0:0	0:0
			177th	0:0	6:0	17:21
TAK-536		1st	96032:132101	234697:328773	344111:605394	
		177th	143516:176374	161252:482053	292441:693142	
TAK-536 M-I		1st	2556:4130	7244:6602	8845:8634	
		177th	6084:5732	4243:10058	9290:9439	
TAK-536 M-II		1st	1493:1356	4039:3087	7696:8186	
		177th	2189:1534	2590:10983	8186:17753	
Conclusion: Daily oral gavage treatment with TAK-491 produced no treatment-induced hyperplastic or neoplastic changes in any organ except for renal juxtaglomerular cell hyperplasia.						

M: Male, F: Female, ¹: 0.5 w/v% methylcellulose with 1 w/v% citric acid, ²: Animal was accidentally killed.

9. Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Effects of TAK-491 on Fertility and Early Embryonic Development to Implantation in Rats

Key findings: Dosages ≤ 1000mg/kg produces maternal toxicity without effects on fertility or early embryonic development.

Study Facility: [REDACTED]

(b) (4)

Study Number [REDACTED] 010-085

Study Dates: 7/26/07 – 2/29/08

GLP Compliance: Statement indicates that this study was performed in compliance with

GLP regulations.

Animals: Crl:CD(SD) rats (M=13 weeks old, F=12 weeks old at initiation of dosing)

Drug Administration: TAK-491 was suspended in distilled water containing citric acid (0.05 w/v %) and methylcellulose (0.5 w/v %) and administered to males by gavage from 14 days before mating, through the mating period and until necropsy (about 8 weeks total). The compound was administered to females from 14 days before mating, through the mating period and until day 6 of gestation. They were then necropsied on day 15 of gestation.

Dose Levels: 0 (vehicle), 10, 100 and 1000 mg/kg/day. There were 20 animals/group/sex for the toxicity studies and 16 animals/group/sex for the satellite (PK) studies.

Observations/Measurements: **Mating**: On day 14 of treatment, males and females were paired on a one-to-one basis in the same group in each male cage. Copulation was confirmed by the presence of a copulatory plug or the presence of sperm in a vaginal smear. The day copulation was confirmed was designated as day 0 of gestation. The mating period was set at a maximum of 3 weeks. After the initiating of mating, the number of days until the confirmation of mating was determined. The copulatory index (# of copulated females/# of females used X 100) and the fertility index (# of pregnant females/ # of copulated females X 100) were calculated. Copulatory intervals were also measured. Mating was not conducted in the satellite group. **Females**: All dams in the toxicity group were euthanized and necropsied on Day 15 of gestation. The numbers of corpora lutea and implantations were recorded and the preimplantation loss rate [(number of corpora lutea- number of implantations)/ number of corpora lutea X 100] and implantation rate [(number of implantations/number of corpora lutea) X 100] were calculated. The estrous cycle was analyzed. The ovaries, uterus and stomach were removed for macroscopic analysis. **Males**: On Day 15 of gestation, all males in the toxicity group were weighed and euthanized. The testes, epididymes, prostate and seminal vesicles were removed and weighed. The T_{max} , C_{max} and AUC_{0-24h} were measured from plasma samples of the satellite animals on days 0 (0.5, 1, 2, 4, 8 and 24 hr after dosing) and 13 (before, 0.5, 1, 2, 4, 8 and 24 hr after dosing). The metabolites TAK-536, TAK-536 MI and TAK-536 M-II were measured.

Results: **Males**: No males died in any group. In all groups, body weight gain and food consumption were significantly decreased. No treatment-related changes were noted in gross pathological findings or organ weights in any treated group. **Females**: No females died in any group. In the 100 and 1000 mg/kg/day groups, body weight gain and food consumption were significantly decreased. In the 10 mg/kg/day group, there were significant decreases in both body weight and body weight gain. There were no treatment-related changes noted in estrous cycles, gross pathological findings, or the numbers of corpora lutea or implantations in any treated group. At the highest dose, the numbers of corpora lutea and implantations were statistically smaller than control but the sponsor asserts that these values were within the historical control range for their

laboratory. There were no treatment related changes in copulatory index, fertility index or mean copulatory interval in any treatment group. No treatment-related changes were noted in the number of live embryos, number of postimplantation losses, preimplantation loss rate or postimplantation loss rate in any group. At the highest dose, the number of live embryos was statistically smaller than in the control group, but was within the historical control range of the lab.

TK

Mean C_{max} and AUC_{0-24h} for TAK-536, TAK-536 MI and TAK-536 M-II in both sexes on days 0 and 13 of treatment increased less than the dose increase, except for C_{max} for TAK-536 MI in males on day 13 and in females on day 0 of treatment. There were no clear differences in the mean values of C_{max} and AUC_{0-24h} for TAK-536 between day 0 and day 13 of treatment. The mean values of C_{max} and AUC_{0-24h} for TAK-536 MI and TAK-536 M-II decreased or tended to decrease by the repeated dose. No gender differences were noted in mean C_{max} or AUC_{0-24h} for any analyte. Mean T_{max} on days 0 and 13 of treatment in males were between 0.5 and 1.3 hours for TAK-536, between 1.3 and 2.5 hours for TAK-536 MI and 0.5-1.7 hours for TAK-536 M-II. Mean T_{max} on days 0 and 13 of treatment in females were between 0.5 and 1.2 hours for TAK-536, between 0.7 and 2.7 hours for TAK-536 MI and 0.5-1.2 hours for TAK-536 M-II.

Conclusion: There is no NOAEL for either sex regarding general toxicity. This conclusion is based on decreased body weight gain and food consumption at 10 mg/kg/day in both sexes. The NOAEL is 1000 mg/kg/day or above for reproductive functions in parental animals and for early embryonic development.

Table 3.

(No. 1)

Effects of TAK-491 on Fertility and Early Embryonic Development to Implantation in Rats

(Study No. (b) (4) 10-085)

Animal	Rat, CrI:CD(SD), 12-13-week-old males, 11-12-week-old females (at grouping)			
Treatment	Orally by gavage Dosing period (F ₀ males): 14 days before mating, through the mating period and until the day before necropsy (F ₀ females): 14 days before mating, through the mating period and until Day 6 of gestation			
Test article	Control Article ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	10	100	1000
Dosage volume (mL/kg/day)	10	10	10	10
No. of parental animals (M:F)	20:20	20:20	20:20	20:20
F ₀ males				
No. of deaths	0	0	0	0
Clinical signs	-	-	-	-
Body weight	-	↓\$(TD 31-52, Nec)#	↓\$(TD 24-52, Nec)#	↓\$(TD17-52, Nec)#
Body weight gain	-	↓\$(TD 7-10)#, ↓*(TD 28-31)#	↓@(TD 3-7) ↓\$(TD 7-10, 10-14, 21-24)# ↓*(TD 28-31)#	↓\$(TD 7-10, 10-14, 21-24, 24-28, 42-45)# ↓*(TD 28-31)#
Food consumption	-	↓\$(TD 7-8) ↓*(TD 13-14)#	↓*(TD13-14)#	↓\$(TD 0-1, 10-11) ↓*(TD 13-14)#
Gross pathological findings	-	-	Testis: Small and soft (1)	Testis: Small (1) Epididymis: Small or yellowish-white focus (1)
Organ weights ^{c)}				
Absolute weight	-	-	-	-
Relative weight	-	-	-	-

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Testes, epididymides, seminal vesicles (including coagulating glands), ventral prostate

-: No treatment-related effects, #: Adverse effects, ↓: Suppressed/decreased, ↑: Increased, TD: Treatment day,

Nec: Day of necropsy (necropsy was performed on Day 54, 55, 56 or 57 of treatment.),

-R: right, -L: left

\$: P<0.05 Significantly different from control by the Williams test

@: P<0.05 Significantly different from control by the Dunnett test

*: P<0.05 Significantly different from control by the Shirley-Williams test

§: P<0.05 Significantly different from control by the Steel test

(No. 2)

Effects of TAK-491 on Fertility and Early Embryonic Development to Implantation in Rats

(Study No. (b) (4) 010-085)

Test article	Control Article ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	10	100	1000
F ₀ females				
No. of deaths	0	0	0	0
Clinical signs	-	-	-	-
Body weight	-	↓\$(GD 6)#	↓\$(GD 6, 13)# ↓*(GD 10)#	↓\$(GD 5, 6, 13, 15)# ↓*(GD 10)#
Body weight gain	-	↓\$(GD 3-4)#	↓\$(GD 3-4)# ↓@(GD 5-6) ↓*(GD 6-10)# ↑\$(GD 10-13)	↑\$(GD 0-1, 10-13) ↓\$(GD 3-4)# ↓*(GD 6-10)#
Food consumption	-	-	↓\$(TD 10-11)# ↓*(GD 3-4)# ↓\$(GD 6-7)#	↓\$(TD 0-1, 10-11)# ↓*(GD 3-4)# ↓\$(GD 6-7, 10-11)#
Estrous cycles	-	-	-	-
No. of corpora lutea ^{c)}	16.0 ± 1.7	14.8 ± 2.4	15.1 ± 1.2	\$ 14.2 ± 2.0
No. of implantations ^{c)}	15.3 ± 2.1	13.4 ± 2.6	14.5 ± 1.6	\$ 13.5 ± 2.3
Gross pathological findings	-	-	-	-
Mating				
Copulatory index (%)	100.0	100.0	95.0	100.0
Copulatory interval (days) ^{c)}	3.0 ± 2.2	2.6 ± 1.7	2.9 ± 2.9	2.6 ± 1.4
Fertility index (%)	100.0	100.0	100.0	95.0
Embryos				
Preimplantation loss (%) ^{c)}	4.3 ± 5.0	9.3 ± 11.5	3.5 ± 6.5	4.9 ± 8.5
No. of postimplantation loss ^{c)}	0.8 ± 1.0	1.0 ± 1.1	1.1 ± 0.8	1.0 ± 1.4
Postimplantation loss (%) ^{c)}	5.5 ± 6.3	7.3 ± 8.6	7.2 ± 5.7	8.1 ± 11.1
No. of live embryos ^{c)}	14.5 ± 2.5	12.4 ± 2.7	13.5 ± 1.7	\$ 12.6 ± 2.9
Conclusion:	Males and females		General toxicity: less than 10 mg/kg/day	
No observed-adverse-effect dose level			Reproductive functions: 1000 mg/kg/day or above	
	Embryonic development		1000 mg/kg/day or above	

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Mean ± SD

-: No treatment-related effects, #: Adverse effects, ↓: Suppressed/decreased, ↑: Increased, TD: Treatment day,

GD: Gestation day

\$: P<0.05 Significantly different from control by the Williams test

@: P<0.05 Significantly different from control by the Dunnett test

*: P<0.05 Significantly different from control by the Shirley-Williams test

(No. 3)

Effects of TAK-491 on Fertility and Early Embryonic Development to Implantation in Rats

(Study No. (b) (4) 010-085)

Test article	Control Article ^{a)}	TAK-491			
Dosage level (mg/kg/day) ^{b)}	0	10	100	1000	
F ₀ males					
Toxicokinetic parameters (Day 0 / Day 13)					
TAK-491F	T _{max} (h)	ND	ND	ND	ND
	C _{max} (ng/mL)	ND	0 / 0	0 / 0	0 / 0
	AUC _{0-24h} (ng·h/mL)	ND	0 / 0	0 / 0	0 / 0
TAK-536	T _{max} (h)	ND	0.5 / 0.7	1.0 / 1.3	0.7 / 1.0
	C _{max} (ng/mL)	ND	32750 / 31597	118775 / 89389	180336 / 168542
	AUC _{0-24h} (ng·h/mL)	ND	160276 / 168284	931892 / 716624	1200994 / 1036457
TAK-536 M-I	T _{max} (h)	ND	1.7 / 1.7	2.0 / 1.5	2.0 / 2.3
	C _{max} (ng/mL)	ND	1680 / 871	1928 / 1023	2780 / 708
	AUC _{0-24h} (ng·h/mL)	ND	6564 / 6906	14297 / 5459	18111 / 6651
TAK-536 M-II	T _{max} (h)	ND	0.5 / 0.7	1.3 / 1.7	1.0 / 1.3
	C _{max} (ng/mL)	ND	105 / 20	246 / 84	453 / 307
	AUC _{0-24h} (ng·h/mL)	ND	447 / 66	1818 / 593	2748 / 1723
F ₀ females					
Toxicokinetic parameters (Day 0 / Day 13)					
TAK-491F	T _{max} (h)	ND	ND	ND	ND
	C _{max} (ng/mL)	ND	0 / 0	0 / 0	0 / 0
	AUC _{0-24h} (ng·h/mL)	ND	0 / 0	0 / 0	0 / 0
TAK-536	T _{max} (h)	ND	0.5 / 0.5	0.8 / 1.0	1.2 / 0.8
	C _{max} (ng/mL)	ND	42379 / 40763	123781 / 122541	219994 / 192147
	AUC _{0-24h} (ng·h/mL)	ND	196989 / 178687	905716 / 777884	1714730 / 1195531
TAK-536 M-I	T _{max} (h)	ND	2.2 / 0.7	1.0 / 1.7	2.7 / 0.8
	C _{max} (ng/mL)	ND	747 / 630	2537 / 1064	1837 / 1431
	AUC _{0-24h} (ng·h/mL)	ND	5606 / 3165	13198 / 6572	17940 / 7635
TAK-536 M-II	T _{max} (h)	ND	0.5 / 0.5	0.8 / 1.0	1.2 / 1.0
	C _{max} (ng/mL)	ND	110 / 23	217 / 148	424 / 268
	AUC _{0-24h} (ng·h/mL)	ND	428 / 51	1250 / 624	2624 / 1349

a) 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b) As TAK-491F (TAK-491 free acid)

ND: Not determined because the samples in the control group collected at 1 hour after dosing on Days 0 and 13 of treatment were analyzed and/or all the values determined were below the quantification limit (5 ng/mL)

Embryonic Fetal Development

Effects of TAK-491 on Embryo-Fetal Development in Rats

Study Facility: [REDACTED]

(b) (4)

Study Number (b) (4) 010-086

Study Dates: 8/8/07 – 2/22/08

GLP Compliance: Statement indicates that this study was performed in compliance with GLP regulations.

Animals: Crl:CD(SD) pregnant female rats (12-14 weeks old at initiation of dosing)

Drug Administration: TAK-491 was suspended in distilled water containing citric acid (0.05 w/v %) and methylcellulose (0.5 w/v %) and administered orally by gavage to pregnant female rats at dosage levels of 0, 10, 100 and 1000 mg/kg/day from Day 6 to Day 17 of gestation in order to assess adverse effects on dams and embryo-fetal development. Animals were euthanized and necropsied on Day 20 of gestation.

Dose Levels: 0 (vehicle), 10, 100 and 1000 mg/kg/day. There were 20 dams/group.

Observations/Measurements: **Dams**: Body weight, body weight gain and food consumption were measured in the dams. The numbers of corpora lutea and implantations were counted as were numbers of live fetuses and embryo-fetal deaths. **Fetuses**: Fetal body weights, placental weights, sex ratio, external and placental findings were recorded. Fetuses were examined for visceral and skeletal malformations.

Results: **Dams**: No dams died. Body weight was significantly decreased on day 20 of gestation. There was also a statistically significant decrease of body weight gain on days 6-12, 6-18, 18-20 and 0-20 of gestation and a statistically significant decrease in food consumption on days 19-20 of gestation in all groups. No treatment-related changes in the number of corpora lutea or implantations were seen in any group. **Fetuses**: No treatment-related effects were seen in the number of live fetuses, postimplantation loss rate, fetal body weight, placental weight, sex ratio, or in external or placental findings in any group. No treatment-related skeletal or visceral abnormalities were seen in any treated group. At the highest dose, there was a tendency toward high frequencies of short supernumerary rib as a skeletal variation and dilated renal pelvises as a visceral variation noted.

Conclusion: There is no NOAEL for general toxicity in dams based on adverse effects of TAK-491 on body weight, body weight gain and food consumption at 10 mg/kg/day. The NOAEL was 1000 mg/kg/day or above for reproductive toxicity in dams and 100 mg/kg/day for embryo-fetal development based on a short supernumerary rib and dilated renal pelvis, suggestive of pseudohydronephrosis, seen in the offspring of dams receiving 1000 mg/kg/day.

Table 4.

(No. 1)

Effects of TAK-491 on Embryo-Fetal Development in Rats

(Study No. (b) (4) 010-086)

Animal	Rat, CrI:CD(SD), 11-week-old males and 10-week-old females			
Treatment	Orally by gavage Dosing period: from Day 6 to Day 17 of gestation			
Test article	Control article ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	10	100	1000
Dosage volume (mL/kg/day)	10	10	10	10
No. of dams	20	20	20	19
Dams				
No. of deaths	0	0	0	0
Clinical signs	-	-	-	-
Body weight	-	↓(GD20)#	↓(GD20)#	↓(GD20)#
Body weight gain	-	↓(GD6 – 12, 6 – 18, 18 – 20, 0 – 20)#	↓(GD6 – 12, 12 – 18, 6 – 18, 18 – 20, 0 – 20)#	↓(GD6 – 12, 12 – 18, 6 – 18, 18 – 20, 0 – 20)#
Food consumption	-	↓(GD19)#	↓(GD19)#	↓(GD19)#
Gross pathological findings	-	-	-	-
No. of corpora lutea ^{c)}	15.3 ± 1.1	15.0 ± 1.4	15.3 ± 1.3	15.6 ± 1.9
No. of implantations ^{c)}	14.8 ± 1.3	14.0 ± 1.6	14.7 ± 1.3	14.8 ± 1.9
Preimplantation loss rate (%) ^{c)}	3.3 ± 4.1	6.7 ± 7.1	3.5 ± 5.6	4.9 ± 6.1
Placentae				
Placental weight (g): males ^{c)}	0.48 ± 0.05	0.51 ± 0.12	0.47 ± 0.05	0.51 ± 0.09
Placental weight (g): female ^{c)}	0.48 ± 0.05	0.50 ± 0.13	0.45 ± 0.04	0.49 ± 0.12
Placental abnormalities (%) ^{c)}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fetuses				
Postimplantation loss (%) ^{c)}	2.8 ± 4.1	4.4 ± 6.3	4.9 ± 6.0	7.9 ± 10.4
No. of live fetuses ^{c)}	14.4 ± 1.4	13.4 ± 1.9	14.0 ± 1.7	13.6 ± 2.1
Sex ratio (male/total) ^{c)}	0.459 ± 0.146	0.527 ± 0.150	0.516 ± 0.108	0.503 ± 0.160
Body weight (g): male ^{c)}	4.01 ± 0.22	4.09 ± 0.20	3.87 ± 0.27	3.86 ± 0.27
Body weight (g): female ^{c)}	3.77 ± 0.25	3.82 ± 0.25	3.67 ± 0.23	3.66 ± 0.29

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Mean ± SD

#: Adverse effects, -: No treatment-related effects,

↓: Suppressed/Decreased, GD: Gestation day

(No. 2)

Effects of TAK-491 on Embryo-Fetal Development in Rats

(Study No. (b) (4) 010-086)

Test article	Control article ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	10	100	1000
Fetuses				
External findings (%)				
Malformations (%) ^{c)}	0.00 ± 0.00	0.00 ± 0.00	0.34 ± 1.50	0.00 ± 0.00
Local edema	0.00	0.00	0.34	0.00
Visceral abnormalities (%) ^{c)}	0.56 ± 2.48	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
White focus in liver (%)	0.56	0.00	0.00	0.00
Visceral variations (%) ^{c)}	5.54 ± 11.39	8.94 ± 12.58	8.46 ± 14.84	13.48 ± 15.75
Main type (%)				
Thymic remnant in neck	1.97	1.25	2.38	3.14
Dilated renal pelvis	1.43	0.72	3.70	6.84#
Dilated ureter	1.43	5.42	3.82	4.89
Convolutated ureter	0.72	3.52	0.72	3.70
Skeletal abnormalities (%) ^{c)}	0.00 ± 0.00	0.00 ± 0.00	0.72 ± 3.20	0.00 ± 0.00
Main type (%)				
Absent rib	0.00	0.00	0.72	0.00
Skeletal variations (%) ^{c)}	17.76 ± 18.53	16.98 ± 19.76	17.12 ± 20.14	26.25 ± 25.19
Main type (%)				
Dumbbell-shaped thoracic centrum	5.30	1.27	4.65	1.51
Full supernumerary rib	0.00	0.63	0.00	0.75
Short supernumerary rib	12.46	13.97	11.23	24.74#
No. of ossified sacral and caudal vertebrae ^{c)}	8.16 ± 0.40	8.15 ± 0.38	7.99 ± 0.36	7.89 ± 0.59
Conclusion: No-observed-adverse-effect dose level	Dams	General toxicity: less than 10 mg/kg/day Reproductive toxicity: 1000 mg/kg/day or above		
	Embryo-fetal development	100 mg/kg/day		

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Mean ± SD
#: Adverse effects

Effects of TAK-491 on Embryo-Fetal Development in Rabbits

Key findings: Dosages ≤ 50 mg/kg produced maternal toxicity without substantial effects on the developing fetus. The NOAEL was ≥30 mg/kg/day for reproductive toxicity in dams based on high rates of postimplantation loss and lower number of live fetuses.

Study Facility: [REDACTED] (b) (4)

Study Number: (b) (4) 010-069

Study Dates: 6/28/07 – 2/27/08

GLP Compliance: Statement indicates that this study was performed in compliance with GLP regulations.

Animals: Kbl: JW pregnant female rabbits (19-20 weeks old at initiation of dosing)

Drug Administration: TAK-491 was suspended in distilled water containing citric acid

(0.05 w/v%) and methylcellulose (0.5 w/v%) and administered orally by gavage to pregnant female rabbits at dosage levels of 0, 10, 30 and 50 mg/kg/day from Day 6 to Day 18 of gestation in order to assess adverse effects on dams and embryo-fetal development. Animals were euthanized and necropsied on Day 28 of gestation.

Dose Levels: 0 (vehicle), 10, 30 and 50 mg/kg/day. There were 18-20 dams/group.

Observations/Measurements: **Dams:** Body weight, body weight gain and food consumption were measured in the dams. Blood chemistry was analyzed on Day 19 of gestation. Gross pathology of the dams was performed. The numbers of corpora lutea and implantations were counted as were number of live fetuses and embryofetal death. Post-implantation loss and postimplantation loss rate were measured and placental weights and fetal sex ratios determined. Plasma levels of TAK-491, TAK-536, TAK-536 MI and TAK-536 M-II were measured on days 6 and 18 of gestation. On day 6, levels were measured at 0.5, 1, 2, 4, 8 and 24 hr after dosing. On day 18, levels were measured before and at 0.5, 1, 2, 4, 8 and 24 after dosing.

Fetuses: Fetal body weights were determined and fetuses were examined for external, visceral and skeletal malformations.

Results: **Dams:** Seven dams died in the highest dose group between days 15 and 23 of gestation and 2 moribund dams in this 4th group were sacrificed on days 18 and 20 of gestation. Two dams in the 4th group aborted on days 22 and 25 of gestation. At this dose, there were significant decreases in body weight noted from day 14 to day 19 of gestation. There was also a statistically significant decrease of body weight gain on days 6-14, 14-19 and 6 to 19 of gestation. A statistically significant decrease in food consumption was noted from day 6-22 of gestation. With regard to blood chemistry, there was a significant increase or a tendency toward high values in blood urea nitrogen, creatinine, total cholesterol, glucose, sodium, potassium, inorganic phosphorus and creatinine phosphokinase. Also, there was a significant decrease in chloride and calcium. There was kidney discoloration in one dam that died and red focus in the stomach of another dam that died. In a dam that was sacrificed, there was liver discoloration.

At the intermediate dose (30 mg/kg/day), 3 dams died on days 20 and 23 and 3 moribund dams were sacrificed on days 17 and 20 of gestation. At this dose, there were significant decreases in body weight noted from day 14 to day 19 of gestation. There was also a statistically significant decrease of body weight gain on days 6-14 and 6 to 19 of gestation. A statistically significant decrease in food consumption was noted from day 8-18 of gestation. With regard to blood chemistry, there was a significant increase or a tendency toward high values in blood urea nitrogen, creatinine, sodium, inorganic phosphorus and creatinine phosphokinase. Also, there was a significant decrease in calcium levels. There was a soft kidney in one dam that died, and red focus in the stomach as well as kidney and liver discoloration in a dam that was sacrificed (see attached table).

At the lowest dose (10 mg/kg/day), 2 dams died on day 19 of gestation. There was a statistically significant decrease of body weight gain on days 6-14 and a statistically significant decrease in food consumption noted from day 8-16 of gestation. With regards

to blood chemistry, there was a significant increase in sodium and a significant decrease in calcium. Red focus in the stomach and kidney discoloration was observed in a dam that died.

No treatment-related changes in the number of corpora lutea or implantations were seen in any group. At the high dose, there was a tendency toward high rates of postimplantation loss and a lower number of live fetuses.

In toxicokinetics, TAK-491 was not detected at any time point on day 6 or 18 of gestation in any treated group. Mean AUC_{0-24h} for TAK-536, TAK-536 MI and TAK-536 M-II and mean C_{max} for TAK-536 and TAK-536 M-II increased dose-dependently. The mean C_{max} for TAK-536 MI increased less than the dose increase. The mean AUC_{0-24h} values for TAK-536, TAK-536 MI and TAK-536 M-II increased with repeated dosing at 30 and 50 mg/kg. There were no clear differences in the mean C_{max} values for TAK-536, TAK-536 MI and TAK-536 M-II between day 6 and day 18 of gestation.

Fetuses: No treatment-related effects were seen in the number of live fetuses or postimplantation loss rate in the 10 or 30 mg/kg/day groups. No treatment-related effects were seen in fetal body weight, placental weight, sex ratio, or in external or placental findings in any group. Treatment-related skeletal or visceral abnormalities were seen in the 50 mg/kg/day treatment group. This included an increased incidence of asymmetry of sternalbra and cataracts.

Conclusion: There is no NOAEL for general toxicity in dams based on number of deaths, decreases in body weight gain and food consumption, increase in plasma sodium, decrease in plasma calcium, kidney discoloration and red focus in the stomach at the lowest dose of 10 mg/kg/day. The NOAEL was 30 mg/kg/day or above for reproductive toxicity in dams based on high rates of postimplantation loss and lower number of live fetuses. The NOAEL for embryo-fetal development was also 30 mg/kg/day based on an increased incidence of asymmetry of sternalbra and cataracts at the 50 mg/kg/day dose.

Table 5.

(No. 1)

Effects of TAK-491 on Embryo-Fetal Development in Rabbits

(Study No. (b) (4) 010-069)

Animal	Rabbit, Kbl:JW, 39-week-old males and 18 to 19-week-old females (at the initiation of mating)			
Treatment	Orally by gavage, Dosing period: from Day 6 to Day 18 of gestation			
Test article	Control article ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	10	30	50
Dosage volume (mL/kg/day)	5	5	5	5
No. of dams	20	19	19	18
Dams				
No. of deaths	0	2#	3#	7#
No. of sacrifices due to moribundity	0	0	3#	2#
No. of abortions	1	0	0	2#
Clinical signs	-	-	DSA: 4# DA: 3# Prone position: 2#	DSA: 5# DA: 7# Mucous stool: 5# Prone position: 1#
Body weight	-	-	↓ (GD 14-19)#	↓ (GD 14-19)#
Body weight gain	-	↓ (GD 6-14)#	↓ (GD 6-14, 6-19)#	↓ (GD 6-14, 14-19, 6-19)#
Food consumption	-	↓ (GD 8-16)#	↓ (GD 8-18)#	↓ (GD 8-22)#
No. of corpora lutea ^{c)}	9.7 ± 2.1	9.5 ± 1.0	9.9 ± 1.4	10.1 ± 2.3
No. of implantations ^{c)}	8.0 ± 1.7	8.5 ± 1.7	9.1 ± 1.9	8.7 ± 2.4
Blood chemistry	-	↑\$(Na)#, ↓\$(Ca)#	↑\$ (BUN, Creat, Na)#, ↑(IP, CPK)#, ↓\$(Ca)#	↑\$ (BUN, Creat, Glu, T-chol, Na, K, CPK)#, ↑IP#, ↓\$(Ca, Cl)#
Gross pathological findings	-	Kidney: Discoloration: 1# Stomach: Red focus: 1#	Kidney: Soft: 1 Discoloration: 1# Liver: Discoloration: 1# Stomach: Red focus: 1#	Kidney Discoloration: 1# Liver: Discoloration: 1# Stomach: Dark red focus: 2#
Placentae				
Placental weight (g): male ^{c)}	5.25 ± 0.71	5.07 ± 0.77	5.14 ± 0.89	5.30 ± 1.03
Placental weight (g): female ^{c)}	5.06 ± 0.67	5.15 ± 0.71	4.94 ± 0.87	5.34 ± 0.42
Placental abnormalities (%) ^{c)}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Mean ± SD
#: Adverse effects, ↓: Suppressed/Decreased,

GD: Gestation day, BUN: blood urea nitrogen, Creat: creatinine, Glu: glucose, T-chol: total cholesterol, Na: sodium,
K: potassium, CPK: creatine phosphokinase, IP: inorganic phosphorus, Ca: calcium, Cl: chloride

DSA: Decrease in spontaneous activity, DA: Dirty around anus, -: No treatment-related effects

\$: Significantly different from the control (p<0.05, the Shirley-Williams test)

(No. 2)

Effects of TAK-491 on Embryo-Fetal Development in Rabbits

(Study No. (b) (4) 010-069)

Test article	Control article ^{a)}	TAK-491			
Dosage level (mg/kg/day) ^{b)}	0	10	30	50	
Toxicokinetic parameters (Day 6 / Day 18)					
TAK-491F	T _{max} (h)	ND	ND	ND	ND
	C _{max} (ng/mL)	ND	0 / 0	0 / 0	0 / 0
	AUC _{0-24h} (ng·h/mL)	ND	0 / 0	0 / 0	0 / 0
TAK-536 ^{c)}	T _{max} (h)	ND	0.8 / 0.5	0.5 / 0.7	0.7 / 1.2
	C _{max} (ng/mL)	ND	21867 / 27333	62125 / 65658	122362 / 94244
	AUC _{0-24h} (ng·h/mL)	ND	58515 / 66679	144369 / 367792	248504 / 690151
TAK-536 M-I ^{c)}	T _{max} (h)	ND	1.2 / 0.5	0.7 / 0.8	1.2 / 1.8
	C _{max} (ng/mL)	ND	280 / 254	318 / 328	536 / 548
	AUC _{0-24h} (ng·h/mL)	ND	700 / 753	1649 / 2751	2438 / 5905
TAK-536 M-II ^{c)}	T _{max} (h)	ND	0.5 / 0.5	0.5 / 0.5	0.5 / 1.8
	C _{max} (ng/mL)	ND	31 / 55	71 / 162	122 / 191
	AUC _{0-24h} (ng·h/mL)	ND	56 / 103	136 / 1521	262 / 1566
Fetuses					
No. of live fetuses ^{d)}	7.3 ± 1.9	8.1 ± 1.6	8.1 ± 2.0	5.9 ± 2.7#	
Postimplantation loss (%) ^{d)}	9.8 ± 14.9	4.4 ± 7.4	10.2 ± 13.9	27.4 ± 31.1#	
Sex ratio (male/total) ^{d)}	0.401 ± 0.185	0.510 ± 0.211	0.435 ± 0.154	0.579 ± 0.330	
Body weight (g): males ^{d)}	39.59 ± 5.43	38.37 ± 3.70	36.55 ± 6.30	35.41 ± 6.79	
Body weight (g): female ^{d)}	38.24 ± 4.42	38.70 ± 3.93	35.25 ± 6.01	37.16 ± 4.16	
External abnormalities (%) ^{d)}	1.24 ± 3.73	0.00 ± 0.00	0.00 ± 0.00	16.67 ± 37.27	
Main type (%)					
Cataract	0.00	0.00	0.00	14.29\$	
Thoracoschisis	0.00	0.00	0.00	2.39\$	
Absent claw	0.66	0.00	0.00	0.00	
Visceral abnormalities (%) ^{d)}	2.34 ± 7.92	0.00 ± 0.00	0.00 ± 0.00	2.39 ± 6.31	
Main type (%)					
Persistent truncus arteriosus	0.00	0.00	0.00	2.39\$	
Right-sided aortic arch	0.58	0.00	0.00	0.00	
Kidney: cyst	1.75	0.00	0.00	0.00	
Visceral variations (%) ^{d)}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid),

c): TAK-491F was not detected in any point in any group, d): Mean ± SD

ND: Not determined because the samples in the control group collected at 1 hour after dosing on Days 6 and 18 of gestation were analyzed and/or all the values determined were below the quantification limit (5 ng/mL)

#: Adverse effects

\$: Significantly different from the control (p<0.05, the Shirley-Williams test)

(No. 3)

Effects of TAK-491 on Embryo-Fetal Development in Rabbits

(Study No. (b) (4) 110-069)

Test article	Control article ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	10	30	50
Skeletal abnormalities (%) ^{c)}	1.99 ± 4.76	0.59 ± 2.43	0.00 ± 0.00	4.43 ± 7.59
Main type (%)				
Misshapen cervical arch and centrum	0.58	0.00	0.00	2.39
Absent phalanx	1.24	0.00	0.00	2.39
Absent lumbar vertebra	0.75	0.59	0.00	2.04
Skeletal variations (%) ^{c)}	29.34 ± 27.38	15.77 ± 15.00	24.40 ± 21.85	13.74 ± 12.22
Main type (%)				
Asymmetry of sternebra	0.00	0.00	0.00	2.39\$
Full supernumerary rib	6.67	1.49	5.93	0.00\$
Short supernumerary rib	21.80	12.34	20.40	6.94
No. of ossified Sacral and caudal vertebrae ^{c)}	18.83 ± 0.44	18.73 ± 0.48	18.82 ± 0.53	18.06 ± 1.44
Conclusion:	Dams	General toxicity: less than 10 mg/kg/day Reproductive toxicity: 30 mg/kg/day		
No-observed-adverse-effect dose level	Embryo-fetal development	30 mg/kg/day		

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Mean ± SD
\$: Significantly different from the control (p<0.05, the Shirley-Williams test)

Prenatal and Postnatal Development

Effects of TAK-491 Pre- and Postnatal Development Including Maternal Function in Rats

Key findings: 10 mg/kg doses were associated with maternal toxicity and decreases in numbers of live pups, live birth index and viability index and renal pelvic dilatation and hydronephrosis in pups.

Study Facility: (b) (4)

Study Number: (b) (4) 010-088

Study Dates: 1/28/08-11/20/08

GLP Compliance: There was a statement indicating that this study was conducted under GLP conditions.

Animals: Crl:CD (SD) rats (males were 12 weeks old and females were 11 weeks old at time of mating). Pregnant rats were dosed from Day 6 of gestation to Day 21 after delivery.

Drug Administration: TAK-491 was dissolved in 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid and administered by oral gavage to pregnant rats at the following dose levels: 0 (vehicle control), 0.1, 1 and 10 mg/kg/day from day 6 of

gestation to day 21 after delivery (during the period from implantation to weaning). There were 19-20 dams/group.

Observations/Measurements: Dams: Mortality and clinical signs were monitored 2X per day (before dosing and 1-2 hr after dosing) during the dosing period and 1X per day during the non-dosing period. Body weight was measured in the dams on Days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation and on days 0, 4, 7, 14 and 22 after delivery. Food consumption was measured on Days 6, 7, 9, 11, 13, 15, 17, 19 and 19 of gestation and days 5, 8, 15, and 20 after delivery. Dams were allowed to deliver naturally and their condition at delivery was observed 2X per day from day 21 of gestation until the completion of delivery or day 26 of gestation. The duration of gestation and the delivery index [(# of pregnant animals that delivered live pups/ # of pregnant animals) X 100] were calculated. F1 pups were nursed until day 22 after delivery. All dams were euthanized by exsanguination between day 22 and 24 after delivery and necropsied. External appearance and internal organs and tissues were observed macroscopically. The number of implantation sites was recorded. The stomachs from all dams were fixed as well as thoracic and abdominal (uterus, ovaries and mammary glands with skin) from 3 pregnant animals in the control group were collected and fixed for comparison.

F1 Pups: The total number of pups (live and dead) was counted and their sex and external appearances for live pups were observed. The live birth index [(# of live F1 pups at birth/ # of implantations) X 100], viability index [(# of live F1 pups at birth/ # of born) X 100] and the sex ratio at birth [(# of live F1 males at birth/ # of live F1 pups at birth) X 100] were calculated. All pups were observed for clinical signs (including nursing condition after delivery) and mortality once daily. On day 4 after birth, 4 males and 4 female pups were randomly selected from each litter (litters with less than 8 pups were not chosen for standardization). When there were fewer than 4 male or female pups in a litter, the total number of pups in that litter was adjusted to 8. The other pups were culled, euthanized by ether anesthesia and fixed. The viability index and sex ratio on day 4 were calculated (*vide supra*). Before weaning, all pups were weighed on days 0, 4 (before standardization of litter size), 7, 14 and 22 after birth. After weaning, all F1 animals were weighed on days 28, 42, 56 and 70 after birth and on the day of gross pathology. The developmental examination during the lactation period is tabulated below:

Examination of Physical Development

Examination	Number of Pups	Examination Period	Acceptance Criteria
Pinna detachment	All F1 pups after culling on Day 4 after birth	On day 4 after birth	Judged to be completed when pinna separates from auditory meatus and becomes elongated
Incisor eruption		On days 11 and 13 after birth and up to completion	Judged to be completed when the eruption of incisors from both the upper and lower jaws is observed
Eyelid opening		On days 14 and 16 after birth and up to completion	Judged to be completed when both eyelids open completely

Examination of Functional Development

Examination	Number of Pups	Examination Period	Acceptance Criteria
Righting reflex	All F1 pups	From day 1 after birth	Judged to be accomplished when animal returns to the normal position after being placed on its back
Negative geotaxis	2 males and 2 females from each litter	On days 5, 10 and 15 after birth	Animal placed as shown in Figure for 1 minute, and evaluated according to the negative geotaxis criteria (Table)
Pupillary reflex		On day 20 after birth	Judged to be normal when constriction of the pupil is observed after exposure to light following dark acclimation (approximately 30 seconds)
Preyer reflex			Judged to be normal when animal shows pinna reflex in response to exposure to sound at 5000 Hz and 15000 Hz
Pain response			Judged from the reaction to pinching the tail

One or 2 F1 males and F1 females from each dam were weaned on day 22 after birth, and were evaluated for behavior, learning and reproductive function. The weaning index [(# of live F1 pups on day 22 after birth/ # of live F1 pups after culling on day 4 after birth)] and sex ratio on day 22 after birth (# of live F1 male pups before weaning on day 22 after birth/ # of live F1 pups before weaning on day 22 after birth) were calculated. All pups (except for 1 or 2 males and females from each dam used for examination of behavior, learning and reproductive function) were euthanized on days 22, 23 or 24 after birth. External appearance and internal organs and tissues were observed macroscopically. The pups from the control and high dose groups were examined for skeletal abnormalities and variations and ossification condition by soft X-ray. The numbers of ossified sacral and caudal vertebrae were counted as an index of ossification. For males, all F1 animals were observed for cleavage of the balanopreputial gland from day 45 after birth to the completion of development. For females, all F1 animals were observed for vaginal opening on day 35 and day 40 after birth, and thereafter up to the completion of development. The following examinations were performed for 1 male and 1 female from each litter: open-field test and a water T-maze

test. When animals reached 11 or 12 weeks of age, 1 male and 1 female from each litter (animals not used for behavioral or learning examinations) were caged together for a maximum of 2 weeks on a one to one basis for mating. The copulation index [(# of copulated animals/ # of paired animals) X 100] and fertility index [(# of pregnant animals/ # of copulated animals) X 100] were calculated. All F1 dams were allowed to deliver naturally and their condition at delivery was observed. The date and time of delivery were recorded, and nursing condition was also observed. The duration of gestation period (from the day when copulation was confirmed to the time when the first pup was delivered) and the delivery index [(# of pregnant animals that delivered live pups/ # of pregnant animals) X 100] were calculated. F2 pups were nursed until days 7, 8 or 9 after delivery. After the reproductive performance test, all F1 males were weighed and euthanized. External appearance, and internal organs and tissues were examined macroscopically. Testes, epididymides and kidneys in which abnormalities were observed were fixed. Thoracic and abdominal organs from 3 males in the control group were collected and stored for comparison. All F1 females used for behavioral and learning examinations were weighed and euthanized. External appearance, and internal organs and tissues were examined macroscopically. Thoracic and abdominal organs from 3 females in the control group were collected and stored for comparison. The kidneys in which abnormalities were observed were fixed and stored. All F1 females used for reproductive performance test were weighed and euthanized. External appearance, and internal organs and tissues were examined macroscopically and the number of implantation sites was recorded. Thoracic and abdominal organs from 3 females in the control group were collected and stored for comparison. The kidneys in which abnormalities were observed were fixed and stored.

F2 Pups: F2 pups were observed until day 7, 8 or 9 after birth, and the data obtained were compiled per litter. The total number of pups (live or dead) was counted, and their sex, and external appearances for live pups were observed. The live birth index [(# of live F2 pups at birth/ # of implantations) X 100], viability index [(# of live F2 pups at birth/ # of born) X 100] and the sex ratio at birth [(# of live F2 males at birth/ # of live F2 pups at birth) X 100] were calculated. All pups were observed for clinical signs (including nursing condition after delivery) and mortality once daily at the observation of F1 dams. On day 4 after birth, 4 males and 4 female pups were randomly selected from each litter (litters with less than 8 pups were not chosen for standardization). When there were fewer than 4 male or female pups in a litter, the total number of pups in that litter was adjusted to 8. The viability index and sex ratio on day 4 were calculated. All F2 pups were weighed on days 0, 4 (before standardization of litter size) and day 7 after birth. The viability index and sex ratio on day 7 after birth were calculated. All F2 pups were euthanized between day 7 and day 9 after birth. External appearance, and internal organs and tissues were examined macroscopically. The thoracic and abdominal organs from the control group were fixed and stored for comparison. All F2 pups in the control and high dose groups were examined for skeletal abnormalities, variations and ossification condition by soft X-ray.

Results: Dams: No dams died in any group. No treatment related abnormalities in dams were noted in clinical signs, delivery or nursing conditions, duration of the gestation

period, delivery index or gross pathological findings including the number of implantations in any test article group. As toxicological effects on dams, a significantly low body weight, a suppression of body weight gain and a decrease in food consumption were noted during the gestation period and/or the lactation period in the 10 mg/kg/day group. Thus, the NOAEL in dams is 1 mg/kg/day.

F1 Pups: The number of live pups at birth and on day 4 after birth, live birth index and viability index on day 4 after birth tended to be decreased in the 10 mg/kg/day group. Also in this group, low body weight was noted during the lactation period and after weaning until day 56 after birth. In this group, delayed incisor eruption was observed as well as dilatation of the renal pelvis and hydronephrosis in the kidney (6 total). Thus, the NOAEL is 1 mg/kg/day in F1 pups. The hydronephrosis may be pseudo as blockage of the ureter was not reported.

F2 Pups: No treatment related effects were seen in any parameter.

Table 6.

(NO. 1)

Study for Effects of TAK-491 on Pre- and Postnatal Development, Including Maternal Function, in Rats

(Study No. (b)(4) 010-088)

Animal	Rat, CrI:CD(SD), 12-week-old males and 11-week-old females at the initiation of mating			
Treatment	Orally by gavage Dosing period: from Day 6 of gestation to Day 21 after delivery			
Test article	Control ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	0.1	1	10
Dosage volume (mL/kg/day)	5	5	5	5
No. of dams	20	19	20	20
F0 Dams				
Clinical signs (Gestation period)	-	-	-	-
Clinical signs (Lactation period)	-	-	-	Decrease in spontaneous activity (1) # Total litter loss (1) #
Body weight (Gestation and lactation periods)	-	-	-	↓(GD16-20) # ↓(LD0-22) #
Body weight gain (Gestation and lactation periods)	-	-	↓(GD 12-18)	↓(GD12-18, 6-18, 18-20, 0-20) #
Food consumption (Gestation and lactation periods)	-	↓(LD 19)	↓(GD 14) ↓(LD 19)	↓(GD12-18) # ↓(LD4-19) #
Duration of gestation (days) ^{c)}	21.75 ± 0.34	21.87 ± 0.37	21.73 ± 0.30	21.85 ± 0.29
Delivery index (%)	100.0	100.0	100.0	100.0
No. of implantations ^{c)}	15.8 ± 1.7	15.3 ± 1.4	15.2 ± 1.7	14.8 ± 2.2
Gross pathological findings	-	-	-	Stomach: Red focus (1) Mammary gland: Underdeveloped (1)
F1 pups				
Clinical signs	-	-	-	-
Body weight	-	-	-	↓(Male: AB7-22, Female: AB0-22) #
No. of live pups at birth ^{c)}	14.7 ± 1.5	14.2 ± 2.0	14.1 ± 1.8	12.8 ± 2.5 #
Live birth index (%) ^{c)}	93.31 ± 7.39	92.26 ± 7.94	92.96 ± 7.74	87.25 ± 15.12 #
No. of live pups on AB 4 ^{c)}	14.6 ± 1.5	14.0 ± 1.9	13.9 ± 1.9	11.8 ± 3.5\$ #
Viability index on AB 4 (%) ^{c)}	99.67 ± 1.50	98.99 ± 3.18	98.14 ± 3.33	89.61 ± 24.33\$ #
Weaning index (%) ^{c)}	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	98.49 ± 6.56
Sex ratio at birth (male/total) ^{c)}	0.51 ± 0.13	0.51 ± 0.14	0.50 ± 0.13	0.48 ± 0.09
Functional development ^{d)}	-	-	-	-
Physical development ^{e)}	-	-	Female: Delayed incisor eruption	Male, Female: Delayed incisor eruption #

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Mean ± SD, d): Righting reflex, negative geotaxis, pupillary reflex, preyer reflex and pain response, e): Pinna detachment, incisor eruption and eyelid opening, GD: Gestation day, LD: Lactation day, AB: Days after birth, -: No treatment-related changes, #: Adverse effects, ↓: Suppressed/low, \$: Significantly different from the control (p<0.05, the Shirley-Williams test)

The number in parentheses indicates the number of dams with the change.

(No. 2)

Study for Effects of TAK-491 on Pre- and Postnatal Development, Including Maternal Function, in Rats
(Study No. (b)(4) 010-088)

Test article	Control ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	0.1	1	10
Dosage volume (mL/kg/day)	5	5	5	5
F1 pups				
External findings				
Malformations ^{c)}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Visceral findings				
Abnormalities ^{c)}	1.25 ± 5.59	2.63 ± 7.88	1.25 ± 5.59	1.32 ± 5.74
Hydronephrosis (%)	1.25	0.00	0.00	0.00
Diaphragmatic hernia (%)	0.00	1.32	0.00	0.00
Dilated renal pelvis (%)	0.00	1.32	1.25	1.32
Skeletal findings				
Abnormalities ^{c)}	0.00 ± 0.00	NE	NE	0.00 ± 0.00
Variations ^{c)}	6.25 ± 13.75	NE	NE	2.63 ± 7.88
No. of ossified sacral and caudal vertebrae ^{c)}	32.15 ± 0.51	NE	NE	31.89 ± 0.61
F1 animals				
Clinical signs	-	-	-	-
Body weight	-	↓(Female: AB28)	↓(Female: AB28)	↓(Male: AB28-42, Female: AB28-56) #
Behavioral and learning tests	-	-	-	-
Morphological Development of Reproductive Organs	-	-	-	-
Mating performance	-	-	-	-
Gross pathology findings	-	Testis: Soft (2), Absent (1) Epididymis: Small (1), Absent (1)	Testis: Soft (1) Epididymis: Small (1) Hydronephrosis (1)	Testis: Soft (1), Small (1) Epididymis: Small (1) Hydronephrosis (2) #, Dilated renal pelvis (1) #

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Mean ± SD
 AB: Days after birth, NE: Not examined, -: No treatment-related changes, #: Adverse effects, ↓: Suppressed/low
 The number in parentheses indicates the number of pups with the change.

(No. 3)

Study for Effects of TAK-491 on Pre- and Postnatal Development, Including Maternal Function, in Rats
(Study No. (b)(4) 010-088)

Test article	Control ^{a)}	TAK-491		
Dosage level (mg/kg/day) ^{b)}	0	0.1	1	10
Dosage volume (mL/kg/day)	5	5	5	5
No. of F1 dams	16	16	15	16
F1 dams				
Clinical signs	Abnormal nursing behavior (1)	Abnormal nursing behavior (1) Total litter loss (1)	External genital bleeding (1)	-
Body weight				
Gestation and lactation periods	-	-	-	-
Body weight gain				
Gestation and lactation periods	-	-	-	-
Duration of gestation (days) ^{c)}	21.97 ± 0.34	22.19 ± 0.31	21.96 ± 0.37	22.00 ± 0.42
Delivery index (%)	94.1	100.0	93.3	100.0
No. of implantations ^{c)}	14.6 ± 2.2	13.9 ± 1.6	14.3 ± 1.0	14.4 ± 1.7
Gross pathology findings	Hydronephrosis (1)	-	-	Hydronephrosis (2) #, Dilated renal pelvis (1) #
F2 pups				
Clinical signs	-	-	-	-
Body weight				
No. of live pups at birth ^{c)}	12.9 ± 2.1	12.3 ± 2.6	13.3 ± 1.3	13.4 ± 2.1
Live birth index (%) ^{c)}	88.68 ± 10.61	87.67 ± 12.97	92.94 ± 5.76	93.33 ± 8.47
No. of live pups on Day 4 after birth ^{c)}	12.0 ± 2.3	12.2 ± 2.6	12.9 ± 1.4	13.0 ± 2.2
Viability index on Day 4 after birth (%) ^{c)}	94.25 ± 15.00	99.56 ± 1.78	97.04 ± 7.52	96.63 ± 4.95
Viability index on Day 7 after birth (%) ^{c)}	100.00 ± 0.00	93.75 ± 25.00	100.00 ± 0.00	100.00 ± 0.00
Sex ratio at birth (male/total) ^{c)}	0.49 ± 0.14	0.51 ± 0.16	0.51 ± 0.12	0.59 ± 0.14
External findings				
Malformations ^{c)}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Visceral findings				
Abnormalities ^{c)}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Skeletal findings				
Abnormalities ^{c)}	0.00 ± 0.00	NE	NE	0.00 ± 0.00
Variations ^{c)}	11.72 ± 24.35	NE	NE	15.63 ± 26.42
Conclusion	Dams	NOAEL: 1 mg/kg/day		
	F1/F2 animals	NOAEL: 1 mg/kg/day		

a): 0.05 w/v% citric acid / 0.5 w/v% methylcellulose solution, b): As TAK-491F (TAK-491 free acid), c): Mean ± SD, NE: Not examined, -: No treatment-related changes, #: Adverse effects
The number in parentheses indicates the number of pups with the change.

Conclusion: The NOAEL for TAK-491 was 1 mg/kg/day for dams and F1 animals.

10 Special Toxicology Studies

Studies Investigating the Potential Toxicity of TAK-536 M-II, the Unique Human Metabolite of TAK-491

10.1 13-Week Repeated Oral (Gavage) Dose Toxicity Study in Rats.

The NOAEL is 300 mg/kg/day. Exposure at the 1000 mg/kg/day dose was 10.8X greater than exposure to the metabolite in humans taking the maximum dose of 80 mg/day. Review by D. Jensen dated 4/3/2007 is below.

13-WEEK ORAL GAVAGE RANGE-FINDING STUDY IN RATS

The sponsor has provided draft data (various tables, plus plots of body weight and of food consumption) for this study. Neither a written study report nor a detailed description of study methods are provided, although the sponsor does provide brief discussions of some study results within the introductory portion of this submission. Histology results are not yet reported.

Methods: The sponsor's Table 3 below (which also summarizes some study results) represents the current primary source regarding study methods.

Table 2.e 13-Week Oral Gavage Toxicity Study of TAK-536 in Rats: Tabulated Summary

Animal	F344/Jcl rats, 6 weeks of age			
Test article	Control (a)	TAK-536 M-II		
Dosage level (mg/kg/day)	0	300	1000	3000
Dosage volume (mL/kg/day)	10	10	10	10
No. of animals (M:F) (b)	10:10	10:10	10:10	10:10
Mortality (M:F)	0:0	0:0	0:0	0:1 (c)
Clinical signs	-	-	-	-
Body weight (d)	-	-	↓ (M: -10%)	↓ (M: -12%)
Food consumption	-	-	-	↑ (F)
Ophthalmology	-	-	-	-
Urinalysis (including water intake)	-	-	-	-
Hematology	-	↓ RBC (M), ↓ HGB (M), ↓ HCT (M), ↑ Reticulocytes(M)		
Blood chemistry	-	-	↓ TP, ↓ ALB, ↓ TG (F) ↑ T. bilirubin (F), ↑ ID. bilirubin (F)	
Organ weights	-	-	↓ Liver (M)	↓ Prostate (M)
Gross pathology	-	-	-	-
Histopathology	-	On going		

Source: [9]. (a) Corn oil vehicle (b) An additional 8 animals/sex/dose were used as satellite groups for TK except for the control group (c) One female rat died due to a gavage error.

M:F=Male:Female, RBC=red blood cells, HGB=hemoglobin, HCT=hematocrit, TP=total protein, ALB=albumin, TG=triglycerides, T. bilirubin=total bilirubin, ID. bilirubin=indirect bilirubin.

–: No treatment-related effects, ↑: Increase, ↓: Decrease.

Results: One high-dose female died prematurely, with the death attributed (in Table 3 above) to gavage error. Per Table 3 above, clinical signs were negative for all treatment groups.

Toxicokinetics: Results of the toxicokinetics substudy are summarized in the sponsor's Table 4 below. Increases in exposure with increasing dose were less than dose proportional in females. In males, mean reported AUC at 90 days was greater in animals dosed with 1000 mg/kg/day than in animals dosed with 3000 mg/kg/day, potentially raising questions regarding study methods and/or assay validity. Table 4 is the only information provided regarding the toxicokinetics substudy. No information is provided regarding study methods or assay methods.

Clinical pharmacokinetic parameters for an 80 mg dose of TAK-491 (the highest anticipated clinical dose) and for two different doses of TAK-536 (studied for hypertension therapy under an earlier IND) are summarized below in the sponsor's Table 5. (There is one typographical error in Table 5. Units for all AUC₀₋₂₄ values should read $\mu\text{g}\cdot\text{h}/\text{mL}$, but one row has mistakenly been labeled $\text{ng}\cdot\text{h}/\text{mL}$ instead.) The first (upper) TAK-536 M-II AUC listed in the TAK-491 column of Table 5 ($26.2 \mu\text{g}\cdot\text{h}/\text{mL}$) was determined following a single dose of TAK-491 while the second TAK-536 M-II AUC listed lower in the same table ($22.8 \mu\text{g}\cdot\text{h}/\text{mL}$) was determined following multiple, once daily 80 mg doses of TAK-491. Compared to the mean AUC₀₋₂₄ for TAK-536 M-II in clinical subjects dosed with 80 of TAK-491 ($22.8 \mu\text{g}\cdot\text{h}/\text{mL}$), the mean AUC₀₋₂₄ at day 90 in male rats dosed with 1000 mg/kg/day of TAK-536 M-II ($142.9 \mu\text{g}\cdot\text{h}/\text{mL}$) was 10.8-fold greater and the mean AUC₀₋₂₄ at day 90 in female rats dosed with 3000 mg/kg/day of TAK-536 M-II ($245.7 \mu\text{g}\cdot\text{h}/\text{mL}$) was 6.3-fold greater.

Table 2.h 13-Week Oral Gavage Range-Finding Toxicity Study in Rats: Average C_{max} and AUC(0-24) Values of TAK-536 M-II

Dose (mg/kg/day)	Dose Number	Male (N=3)		Female (N=3)		Total (N=6)	
		C _{max} ($\mu\text{g}/\text{mL}$)	AUC ($\mu\text{g}\cdot\text{h}/\text{mL}$)	C _{max} ($\mu\text{g}/\text{mL}$)	AUC ($\mu\text{g}\cdot\text{h}/\text{mL}$)	C _{max} ($\mu\text{g}/\text{mL}$)	AUC ($\mu\text{g}\cdot\text{h}/\text{mL}$)
300	1	15.191	83.522	12.958	57.861	13.910	70.692
	90	13.765	152.850	25.294	69.978	19.530	111.414
1000	1	26.382	137.687	23.175	115.303	24.779	126.495
	90	22.079	245.742	28.485	113.779	25.282	179.761
3000	1	34.737	197.381	25.930	182.426	29.367	189.904
	90	16.028	157.646	18.487	142.919	17.258	150.283

Source: Study No. [9].

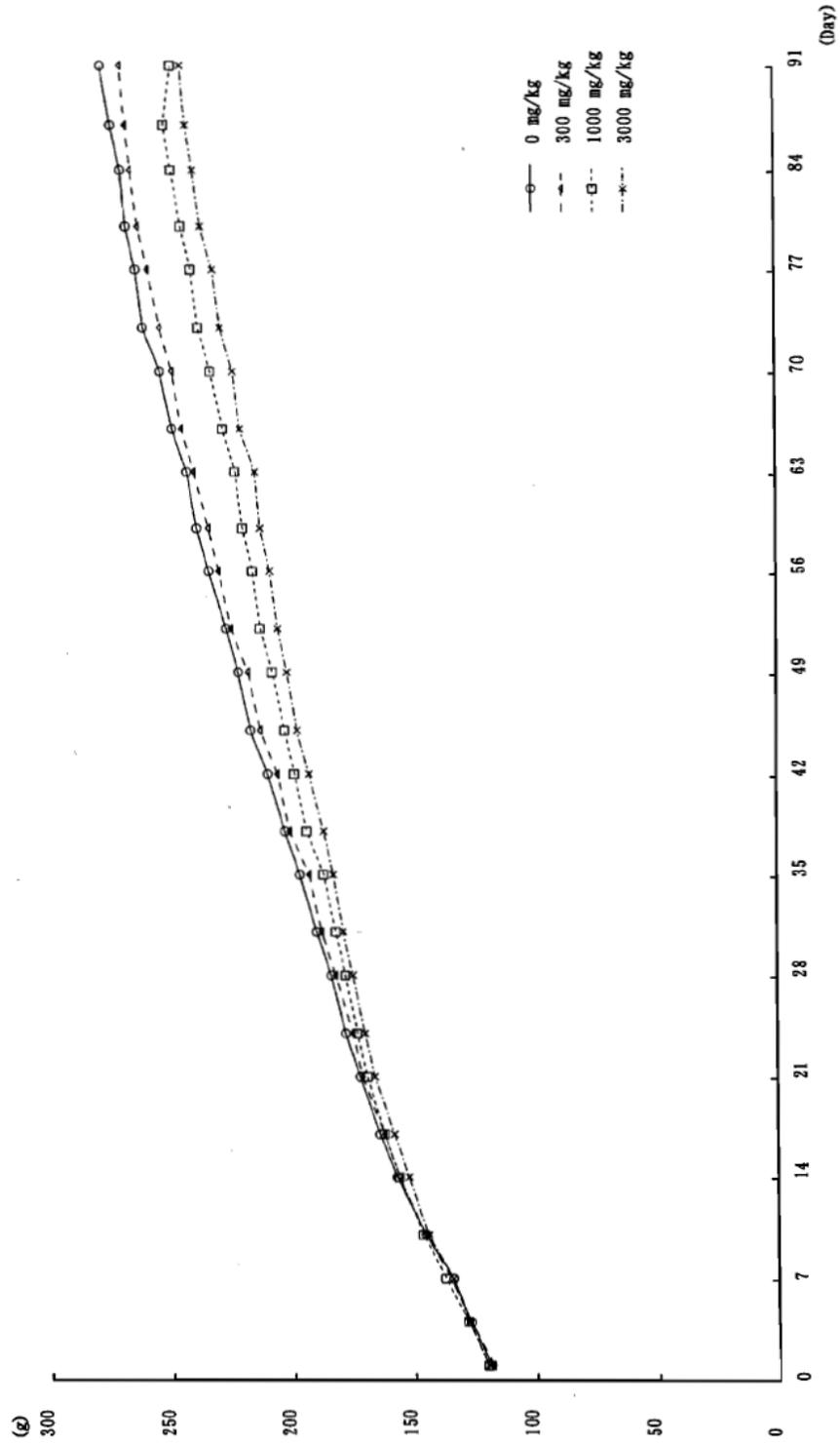
The dosing vehicle was corn oil. AUC(0-24) values were calculated from mean plasma concentrations. The total AUC(0-24) is expressed as the mean of the AUC(0-24) values in male and female rats.

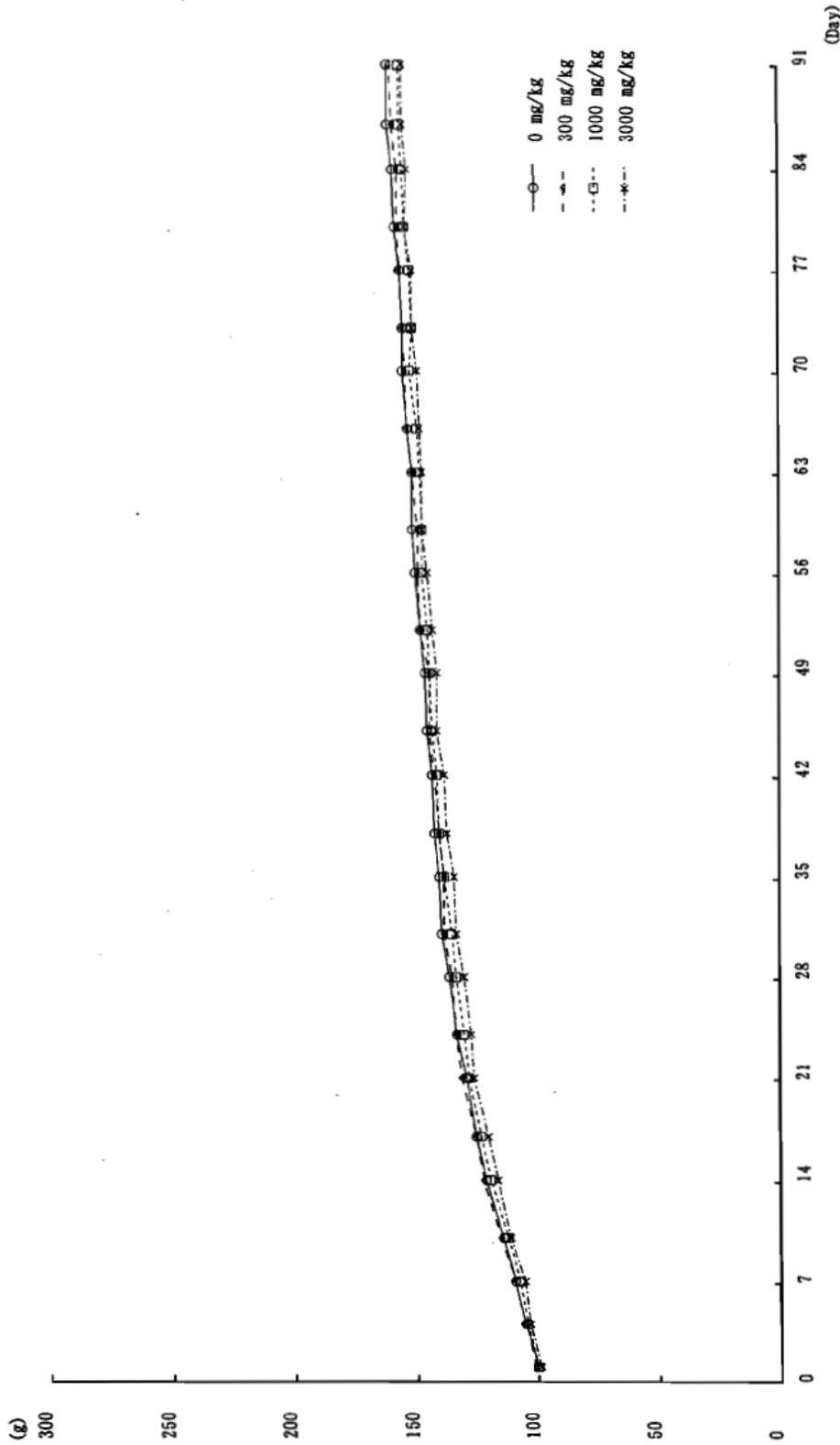
Table 2.i Pharmacokinetics of TAK-536 after Dosing With TAK-491 or TAK-536

Analyte	PK Parameter (Units)	Compound and Dosage		Study No.
		TAK-491 80 mg	TAK-536 80 mg	
TAK-536	AUC(0-inf) (µg·h/mL)	41.994 (28%)	68.183 (27%)	
	Cmax (µg/mL)	4.459 (19%)	9.691 (22%)	
TAK-536	AUC (0-inf)	26.209 (22%)	40.777 (27%)	01-07-TAK-491-017 [16]
M-II	Cmax (µg/mL)	0.961 (26%)	1.573 (27%)	
Metabolic Ratio (M-II AUC/TAK-536 AUC)		0.61	0.58	
TAK-536	AUC(0-24) (µg·h/mL)	42.908 (23%)	NA	01-05-TL-491-002 [17]
	Cmax (µg/mL)	4.617 (28%)		
TAK-536	AUC(0-24) (ng·h/mL)	22.793 (19%)	NA	
M-II	Cmax (µg/mL)	1.346 (21%)		
TAK-536 40 mg				
TAK-536	AUC(0-24) (µg·h/mL)	NA	34.963 (22%)	01-05-TL-536-011 [18]
	Cmax (µg/mL)		5.556 (22%)	
TAK-536	AUC(0-24) (µg·h/mL)	NA	19.886 (25%)	
M-II	Cmax (µg/mL)		1.465 (24%)	

Sources: [16,17,18]. Values are arithmetic means (% coefficient of variation).

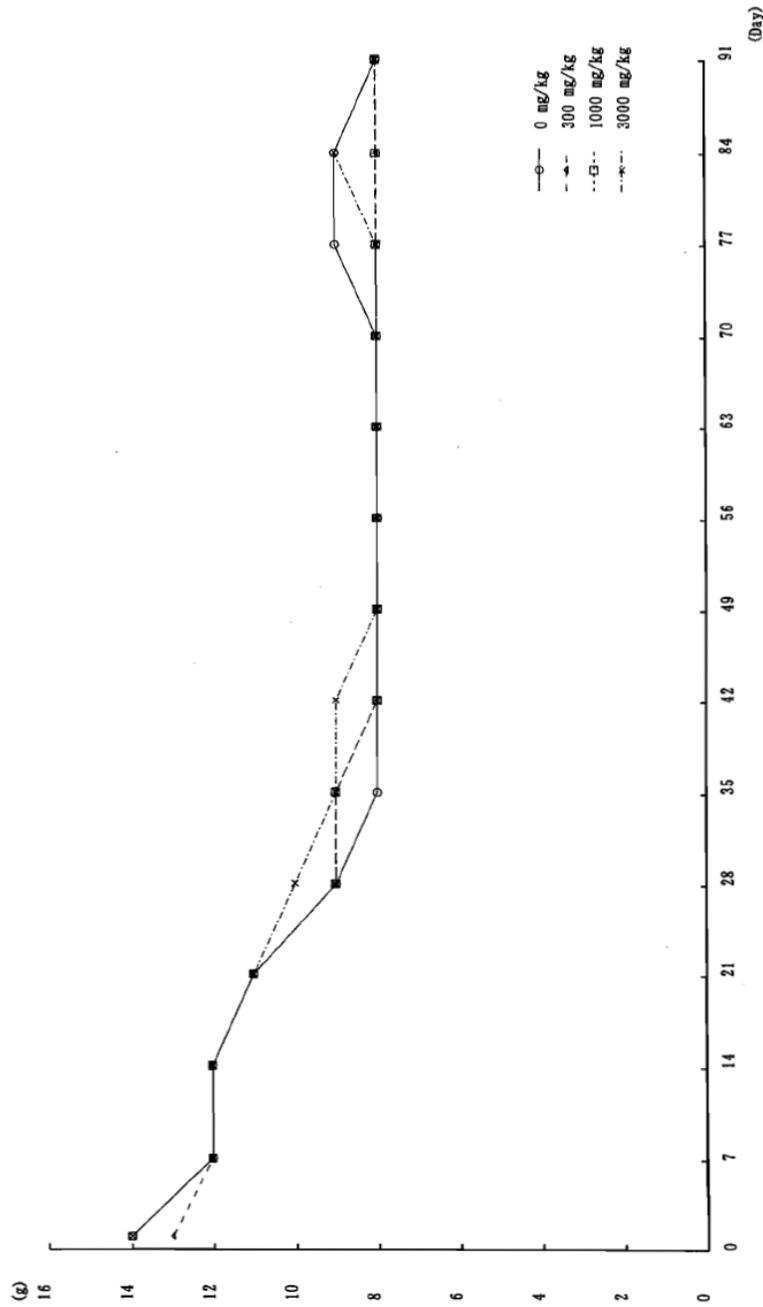
Body weight: Body weights were significantly lower than control in 1000 mg/kg/day males at 5 of 6 timepoints from day 73 through day 91 and in 3000 mg/kg/day males at all weighings from day 45 through day 91. On day 91, body weights were 10.4% lower than control in 1000 mg/kg/day males and 11.8% lower than control in 3000 mg/kg/day males. Body weights were significantly lower than control in 3000 mg/kg/day females on day 31. On day 91, body weights were 3.1% lower than control in 1000 mg/kg/day females and 3.7% lower than control in 3000 mg/kg/day females, but the differences were not statistically significant. Body weights are plotted below in the sponsors's Figures.

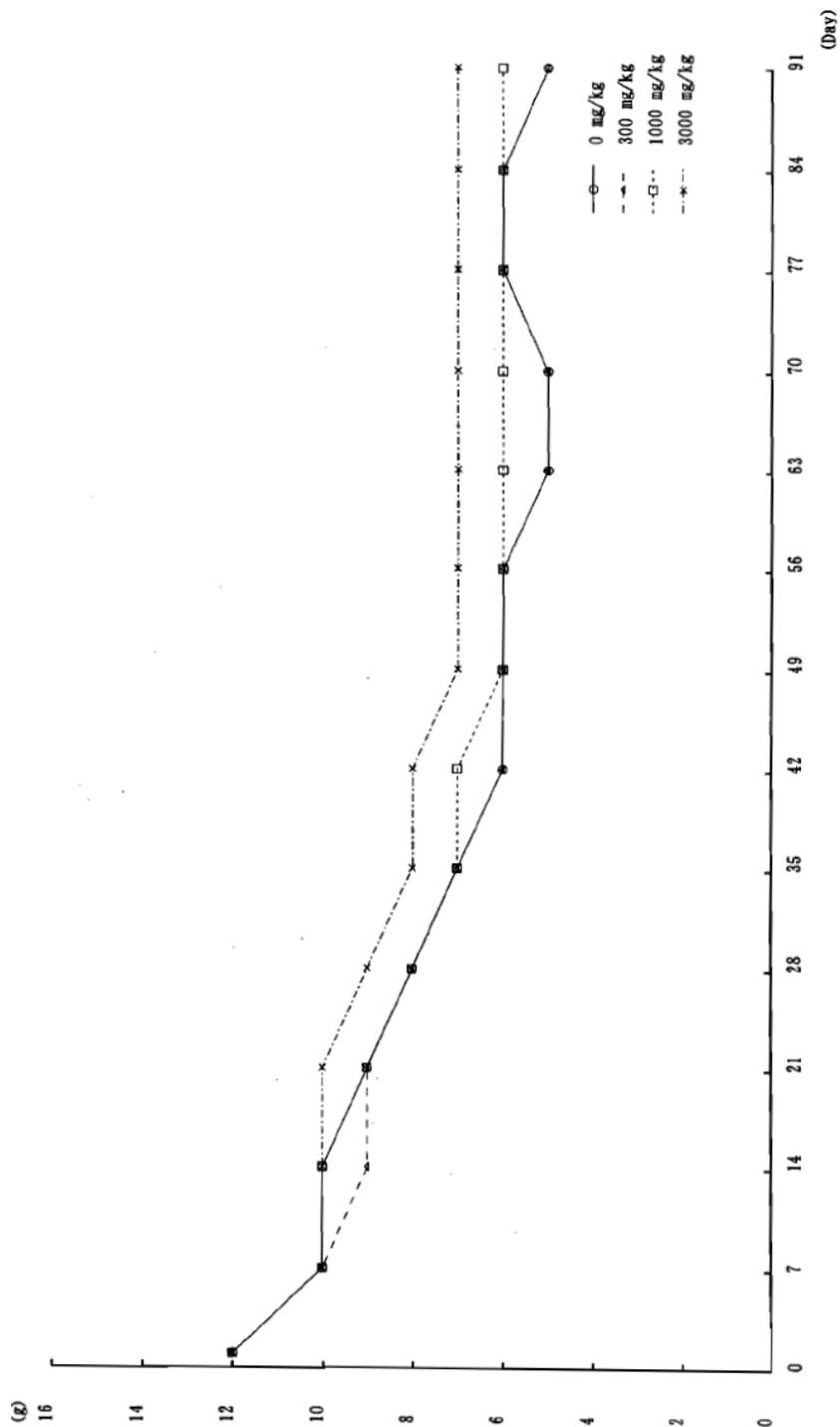




Food consumption: After day 1, there were no statistically significant differences in food consumption between drug treated animals and control. Food consumption was

modestly, but significantly, significantly higher than control in 3000 mg/kg/day females at each sampling period from day 28 through day 91. Food consumption in males and in females are plotted below in Figures 4 and 5, respectively.





Urinalysis: As noted in Table 3 above, there were no differences in urinalysis results between drug-treated animals and control.

Hematology, clinical chemistry and organ weights: Results for hematology, clinical chemistry and organ weights are usefully summarized in the sponsor's Table 6 below. A few, additional differences in hematology parameters, clinical chemistry parameters and

organ weights were noted for single treatment groups compared to control. None of the differences in hematology or clinical chemistry parameters between drug-treated animals and control are regarded as clinically significant by this reviewer. With regard to organ weights, given that body weights, compared to control, were reduced by more than 10% in 1000 mg/kg/day males and by nearly 12% in 3000 mg/kg/day males, it is most useful to compare organ weight/body weight ratios between these two treatment groups and control. As shown in Table 6 below, liver weight/body weight was reduced compared to control by 5% in 1000 mg/kg/day males and by 8% in 3000 mg/kg/day males. Prostate weight/body weight was 13.6% lower in 3000 mg/kg/day males than in control.

Table 2.f 13-Week Oral Gavage Range-Finding Toxicity Study in Rats: Summary of Changes

Dosage level (mg/kg/day)	0	300	1000	3000
No. of animals (M:F)	10:10	10:10	10:10	10:10
Body weight (g) (M:F) (a)	278:161	270:160	<u>249:156</u>	<u>245:155</u>
Food consumption (g/animal/day) (M:F) (b)	8:5	8:5	8:6	8:7
Hematology (M:F)				
RBC (10E4/uL)	932:865	<u>899:864</u>	<u>882:853</u>	<u>897:853</u>
HGB (g/dL)	16.5:16.2	<u>16.0:16.1</u>	<u>15.7:15.9</u>	<u>15.9:15.9</u>
HCT (%)	43.2:41.9	<u>42.0:41.8</u>	<u>41.5:41.3</u>	<u>41.7:41.0</u>
Reticulocyte (%)	1.9:2.1	<u>2.4:2.1</u>	<u>2.8:2.3</u>	<u>2.3:2.0</u>
Blood chemistry (M:F)				
TP (g/dL)	6.3:5.8	6.2:5.7	<u>6.0:5.6</u>	<u>5.9:5.6</u>
ALB (g/dL)	3.2:3.1	3.2:3.0	<u>3.1:2.9</u>	<u>3.0:2.9</u>
TG (mg/dL)	43:12	37:11	38:9	32:8
T. bilirubin (mg/dL)	0.0:0.0	0.0:0.0	0.0:0.0	0.0:0.1
ID. bilirubin (mg/dL)	0.0:0.0	0.0:0.0	0.0:0.0	0.0:0.1
Organ weights (M:F)				
Liver (g)	7.50:3.65	7.10:3.59	<u>6.46:3.54</u>	<u>6.04:3.45</u>
Liver (g/100g BW)	2.80:2.37	2.74:2.34	<u>2.65:2.33</u>	<u>2.57:2.33</u>
Prostate (g)	0.59	0.53	0.53	<u>0.45</u>
Prostate (g/100g BW)	0.22	0.21	0.22	<u>0.19</u>

Source: [9]. (a) Body weights at week 13 (b) Food consumption on Day 91.

M:F=Male:Female, RBC=red blood cells, HGB=hemoglobin, HCT=hematocrit, TP=total protein,

ALB=albumin, TG=triglycerides, T. bilirubin=total bilirubin, ID. bilirubin=indirect bilirubin.

Underlined values are significantly different from the control group (P<0.05).

Gross pathology: The sole report of gross pathology in this submission is the row of dashes (negatives) in Table 3 above.

Histopathology: This submission indicates that histopathology results are pending.

Thirteen-week Oral Gavage Study of TAK-536 M-II in DogsStudy Facility: (b) (4)Study Number: B-6113Study Dates: 2/8/07-12/20/07GLP Compliance: There was indicating that this study was conducted with GLP conditions.Animals: Beagle dogs (9 months old at time of dosing)Drug Administration: TAK-536 M-II was dissolved in distilled water containing 0.5% w/v methylcellulose and administered by oral gavage for 13 weeks at 200, 600 and 2000 mg/kg/day.Dosage Levels: 0 (vehicle control- 0.5% methylcellulose in water), 200, 600 and 2000 mg/kg/day for 13 weeks via oral gavage. There were 3 animals/sex/group.Observations/Measurements: Clinical signs were monitored 3X a day (before dosing, 1 hr and 6 hr after dosing). Body weight and food consumption were measured during the dosing period. Ophthalmological examinations were performed at weeks 2 and 13 of dosing. Water intake was measured once in week 1 and again in week 13. Electrocardiogram and body temperature were monitored once in week 1 and twice on one day in week 13. Hematology, urinalysis and blood chemistry were tested once in weeks 1 and 2 and once in week 13 before dosing. On day 91, animals were euthanized and necropsied. Organs such as brain (cerebrum, cerebellum and medulla oblongata), pituitary gland, thyroids, adrenals, thymus, spleen, heart, lungs (inc. bronchi), salivary glands, liver, kidneys, testes, prostate, seminal vesicles, ovaries and uterus spinal cord, sciatic nerve, lacrimal gland, cervical lymph node, gall bladder, abdominal skin, eyeballs, optic nerves, parathyroids, submandibular lymph node, mesenteric lymph node, thoracic aorta, trachea, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, urinary bladder, vagina, mammary glands, sternum, femurs (with marrow) and femoral skeletal muscles were weighed and histopathology was performed on the tissues.

Plasma concentrations (T_{max} , C_{max} and AUC_{0-24h}) of the compound were measured after the first dose at 0.5, 1, 2, 4, 8 and 24 hr. Measurements were also made on day 90 of week 13 before, and 0.5, 1, 2, 4, 8 and 24 hr after dosing.

Results: No animals died in any treatment group. In clinical observation, stool (or soft stool) contained test article material at all doses. Body weight, food consumption, water intake, ophthalmology, electrocardiography, body temperature, urinalysis, hematology, blood chemistry, organ weights, gross pathological or histopathological exams were not affected by the test article.

The mean T_{max} values ranged from 1.7 to 3.3 hr. The mean C_{max} and AUC_{0-24h} values in males increased with increment of dosage levels, but those values after the first dose were comparable between 200 and 600 mg/kg/day. The mean AUC_{0-24h} and C_{max} values in females after the first dose were the highest at 600 mg/kg/day, followed by 2000 and 200 mg/kg/day. Those values after the 90th dose increased with increment of dosage levels of the test article. The mean values of C_{max} and the AUC_{0-24h} in both sexes decreased by the repeated dose, except for the mean values of C_{max} in females at a dose level of 2000 mg/kg/day which increased by the repeated dose.

Results: Based on these results, the NOAEL was 2000 mg/kg/day or more for both sexes.

Table 7.

**Thirteen-week oral gavage toxicity study of TAK-536 M-II in dogs
(Study No. B-6113)**

Animal	Beagle dogs, 9 months of age			
Test article	Control *	TAK-536 M-II		
Dosage level (mg/kg/day)	0	200	600	2000
Dose volume (mL/kg/day)	10	10	10	10
Concentration (mg/mL)	0	20	60	200
No. of animals (M : F)	3 : 3	3 : 3	3 : 3	3 : 3
Mortality (M : F)	0 : 0	0 : 0	0 : 0	0 : 0
Clinical sign	–	Stool containing test article-like material (3 : 3)	Stool containing test article-like material (3 : 3) Soft stool containing test article-like material (1 : 0)	Stool containing test article-like material (3 : 3) Soft stool containing test article-like material (2 : 2)
Body weight	–	–	–	–
Food consumption	–	–	–	–
Water intake	–	–	–	–
Ophthalmology	–	–	–	–
Electrocardiography	–	–	–	–
Body temperature	–	–	–	–
Urinalysis	–	–	–	–
Hematology	–	–	–	–
Blood chemistry	–	–	–	–
Organ weight	–	–	–	–
Necropsy	–	–	–	–
Histopathology	–	–	–	–
Plasma concentration (M : F, mean) TAK-536 M-II				
T_{max} (h)	1st	2.3 : 2.0	2.3 : 3.3	2.0 : 3.0
	90th	2.7 : 2.7	2.7 : 2.7	2.0 : 1.7
C_{max} (ng/mL)	1st	13060 : 14256	16045 : 25918	27981 : 17391
	90th	6878 : 9006	13000 : 17480	21234 : 24007
AUC_{0-24h} (ng·h/mL)	1st	133448 : 120541	155488 : 260714	254027 : 217719
	90th	65114 : 89450	105461 : 138087	151010 : 185289
Conclusions		Non-toxic dosage level: 2000 mg/kg/day or above for both sex		

*: 0.5 w/v% methylcellulose solution, M: Male, F: Female
-: No treatment-related effects

Genotoxicity Studies of TAK-536 M-II

Ames Test

TAK-536 M-II was negative in this study. Review by D. Jensen dated 4/3/2007 is attached below.

Chinese Hamster Lung Cytogenetic Assay

Like azilsartan medoxomil, TAK-536 M-II was clastogenic without activation at 24 hr exposure. Review by D. Jensen dated 4/3/2007 is attached below:

Genetic toxicology: Both the prodrug TAK-491 and the hydrolysis product TAK-536 were highly clastogenic in the absence of metabolic activation during 6- and 24-hour chromosome aberration assays in CHO cells. Both compounds were negative during in-vitro chromosome aberration assays in the presence of metabolic activation and were also negative in other in-vitro and in-vivo genetic toxicology studies.

The metabolite, TAK-536 M-II, was recently evaluated in an Ames assay and in two sets of in-vitro chromosome aberration assays in Chinese hamster lung cells. Study reports for these assays were received concurrently with this submission and have undergone preliminary review. The Ames assay evaluated TAK-536 M-II concentrations of 156, 313, 625, 1250, 2500 and 5000 µg/plate in *S. typhimurium* TA100, TA1535, TA98 and TA1537 and in *E. coli* WP2uvrA. Ames assay results were negative, both in the presence and in the absence of metabolic activation. Study methods for the Ames assay appear to be valid.

A first set of in-vitro chromosome aberration assays for TAK-536 M-II, summarized below in the sponsor's Table 1, evaluated inadequate drug concentrations. There was no cytotoxicity during 6-hour assays with or without metabolic activation or during a 24-hour assay without metabolic activation. These assays are not considered to be valid. A second set of in-vitro chromosome aberration assays for TAK-536 M-II is summarized below in the sponsor's Table 2. Drug concentrations, as judged by a cell growth index (cell numbers relative to control), were valid for both 6-hour and 24-hour assays. Results were negative during 6-hour assays but there was an increased percentage of cells with structural aberrations at the highest drug concentration during a 24-hour assay without metabolic activation.

This signal for clastogenicity is much weaker than the signals observed for TAK-491 and TAK-536, where large percentages of aberrant cells were observed at multiple drug concentrations. Although the sponsor argues that the positive result was secondary to cytotoxicity, the percent reduction in cell numbers at the highest drug concentration is judged to be appropriate (e.g., per the recommendations of the ICH S2A guidance). Study methods for the second set of assays appear to be valid.

Title (Study No.)	Cytogenetic Assay with TAK-536 M-II in Chinese Hamster Lung (CHL) Cells (B050757)
Test substance	TAK-536 M-II
Negative control	Dimethyl sulfoxide (DMSO)
Positive controls	Mitomycin C (MMC), Benzo[a]pyrene (BP)
Test system	CHL/IU cells

Compound	Concentration (µg/mL)	Exposure-Recovery Time (h)	S9 mix	Cell Growth Index (%)	Relative Mitotic Index (%)	Cells with Structural Aberrations Excluding gap (%)	Judgment ¹
DMSO	10 µL/mL	6-18	-	100.0	100.0	0.0	-
TAK-536 M-II	25	6-18	-	100.3	78.9	0.5	-
	50			113.6	71.1	0.5	-
	100			108.5	73.7	1.5	-
MMC	0.1	6-18	-	N.D.	N.D.	49.5	+
DMSO	10 µL/mL	6-18	+	100.0	100.0	0.5	-
TAK-536 M-II	25	6-18	+	82.0	107.4	0.5	-
	50			84.2	103.7	0.5	-
	100			85.6	101.2	0.5	-
BP	20	6-18	+	N.D.	N.D.	73.0	+
DMSO	10 µL/mL	24-0	-	100.0	100.0	0.0	-
TAK-536 M-II	25	24-0	-	105.1	96.8	0.5	-
	50			95.4	98.4	0.5	-
	100			104.0	95.2	0.5	-
MMC	0.05	24-0	-	N.D.	N.D.	26.5	+

Conclusion: TAK-536 M-II did not induce chromosomal aberrations under the conditions employed in the present study.

1: Ishidate's method (-, negative; +, positive)

N.D.: Not determined

Title (Study No.)	Cytogenetic Assay with TAK-536 M-II in Chinese Hamster Lung (CHL) Cells (Supplement Study) (B061164)
Test substance	TAK-536 M-II
Negative control	Dimethyl sulfoxide (DMSO)
Positive controls	Mitomycin C (MMC), Benzo[a]pyrene (BP)
Test system	CHL/IU cells

Compound	Concentration (µg/mL)	Exposure-Recovery Time (h)	S9 mix	Cell Growth Index (%)	Relative Mitotic Index (%)	Cells with Structural Aberrations Excluding gap (%)	Judgment ¹
DMSO	10 µL/mL	6-18	-	100.0	100.0	1.0	-
TAK-536 M-II	2688	6-18	-	77.5	101.5	1.0	-
	3225			57.6	95.5	4.0	-
	3763			37.0	81.8	2.0	-
MMC	0.1	6-18	-	N.D.	N.D.	32.5	+
DMSO	10 µL/mL	6-18	+	100.0	100.0	0.0	-
TAK-536 M-II	1613	6-18	+	78.1	95.9	0.5	-
	2150			61.2	79.7	1.0	-
	2688			24.0	63.4	0.5	-
BP	20	6-18	+	N.D.	N.D.	78.0	+
DMSO	10 µL/mL	24-0	-	100.0	100.0	0.5	-
TAK-536 M-II	1613	24-0	-	80.6	61.8	2.0	-
	2150			75.1	53.9	3.0	-
	2688			42.9	34.2	10.5	+
MMC	0.05	24-0	-	N.D.	N.D.	30.0	+

Conclusion: TAK-536 M-II did not induce chromosomal aberrations in the -S9 mix assay or the +S9 mix assay. In the 24-hour assay, a positive response was noted only at the highest dose at which marked cytotoxicity was evident. Accordingly, the positive response at the highest dose was considered to be secondary to cytotoxicity from the test substance.

1: Ishidate's method (-, negative; +, positive)

N.D.: Not determined

Carcinogenicity Studies of TAK-536 M-II

Twenty-four Month Oral Gavage Carcinogenicity Study of TAK-536 M-II in Rats

TAK-536 M-II did not produce any drug-related neoplasms in the 2-year rat carcinogenicity study. Review dated 9/23/1010 is attached below:

Study Title: Twenty-four-month Oral Gavage Carcinogenicity Study of TAK-536 M-II in Rats

Study Facility: (b) (4)

Study Number: B-6177

Study Dates: June 12, 2007- Jan. 14, 2010

GLP Compliance: Yes

QA Report: Yes

Methods

Dosing: Male and female F344/Fcl SPF rats (50/sex/group) were given either vehicle (corn oil) or water via gavage. Male rats receive 100 mg/kg/day, male and female rats received 300 mg/kg/day and 1000 mg/kg/day, and female rats received a dose of 3000 mg/kg/day in a volume of 10 ml/kg/day. For treatment groups, 3 animals/sex were utilized for TK analysis.

Basis for Dose Selection: In a 13-week oral gavage range-finding toxicity study of TAK-536 M-II, dosage levels of 0, 300, 1000 or 3000 mg/kg/day of TAK-536 M-II were studied. Mean systemic exposures (AUC 0-24hr) at each dose were lower in females than in males. Compared to mean TAK-536 M-II AUC's observed in clinical subjects dosed with 80 mg/day of TAK-491 (the highest anticipated clinical dose), AUC's observed in high-dose female rats were 6X higher and AUC's in mid-dose male rats were 11X higher. The primary dose-limiting effect in male rats was a decrease in weight gain compared to control at the 1000 mg/kg/day dose (-10%) and the 3000 mg/kg/day dose (-12%). No dose-limiting effects were seen in females. Hence, females received doses of 300, 1000 and 3000 mg/kg/day and males received doses of 100, 300 and 1000 mg/kg/day.

Clinical Signs: Animals were examined 3X daily for clinical signs and once per week for any palpable masses.

Body Weights: Animals were weighed twice in the first week of treatment and weekly up to week 14 and once every 2 weeks thereafter until study termination/completion.

Food Intake: Food intake was measured twice in the first week of treatment and weekly up to week 14 and once every 2 weeks thereafter until study termination/completion.

Water Intake: Not reported.

Hematology: Blood was collected at the time of necropsy.

Clinical Chemistry: Not reported.

Urinalysis: Not reported.

Histopathology: After 24 months, all remaining animals from all the groups were subjected to a necropsy. External appearance of all the organs/tissues in the cranial, thoracic and abdominal cavities were examined and the results recorded. Microscopic examination was performed on the following tissues

cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves, eyeballs, optic nerves, Harderian glands, pituitary, thyroids, parathyroids, adrenals, thymus, spleen, submandibular lymph node, mesenteric lymph node, heart, thoracic

aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands, sublingual glands, liver, pancreas, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, ovaries, uterus (both horns), vagina, mammary glands (inguinal region), sternum (including bone marrow), femurs (including bone marrow and knee joint), femoral muscles, skin (inguinal region) and gross lesions

Toxicokinetics: Blood was taken from the additional 6 satellite animals/ group for toxicokinetic analysis at day 1 and at week 52.

Statistical Analysis: Tumors that occurred with high incidence (10 or more animals in total for each sex, >10) were evaluated using survival-adjusted Peto's test to assess increasing trend of incidence to dose level for all groups and to compare the incidence between the control groups (combined with control group-I and -II) and each dose group. Tumors that occurred with low incidence (less than 10 animals in total for each sex, <10) were evaluated using exact permutation trend test to assess increasing trend of incidence to dose level for all groups and to compare the incidence between the control groups (combined with control group-I and -II) and each dose group. For incidental tumors, the analysis intervals were: Weeks 1 through 52, 53 through 78, 79 through 92 and 93 through 104 and the period scheduled necropsy of the live phase.

Analysis of positive trend in incidence was conducted at the significance levels of 0.01 (1 tailed-level) for common tumors and 0.05 (1 tailed-level) for rare tumors. Pairwise comparison was conducted at the significance levels of 0.01 (1 tailed-level) for common tumors and 0.05 (1 tailed-level) for rare tumors. Common tumors were defined as those with a historical incidence in controls (F344/Crlj rats) in the (b) (4) of more than 1% (>1%) and rare tumors as 1% or less (<1%). The incidences of tumors were analyzed with Peto's survival-adjusted tumor analysis.

Results

Mortality

1) Males The survival rate in the 100 mg/kg and higher groups was slightly higher than that of the vehicle control group, and statistical significance was noted in the 100 mg/kg group (Table 1 and Fig. 1).

2) Females There were no effects of TAK-536 M-II on mortality (Table 1 and Fig. 2).

Text Table 1. Summary of mortality and survival rate

Sex	Male					Female				
	0 ^{a)}	100	300	1000	0 ^{b)}	0 ^{a)}	300	1000	3000	0 ^{b)}
Dosage (mg/kg/day)	0 ^{a)}	100	300	1000	0 ^{b)}	0 ^{a)}	300	1000	3000	0 ^{b)}
No. of animals used	50	50	50	50	50	50	50	50	50	50
Week 1 - 26	0	0	0	0	0	1	0	0	0	0
Week 1 - 52	7	1	2	1	0	1	0	1	0	0
Week 1 - 78	14	3	7	3	2	2	5	2	3	1
Week 1 - 105	24	14	16	15	20	7	12	13	11	6
No. of survivors	26	36	34	35	30	43	38	37	39	44
Survival rate (%)	52.0	72.0*	68.0	70.0	60.0	86.0	76.0	74.0	78.0	88.0

Values in the table indicate the cumulative number of the animals that died or were sacrificed as moribund.

a): Vehicle control group received corn oil, b): Negative control group received water for injection

*: $p \leq 0.05$ (statistically significant difference from the vehicle control group, log-rank test)

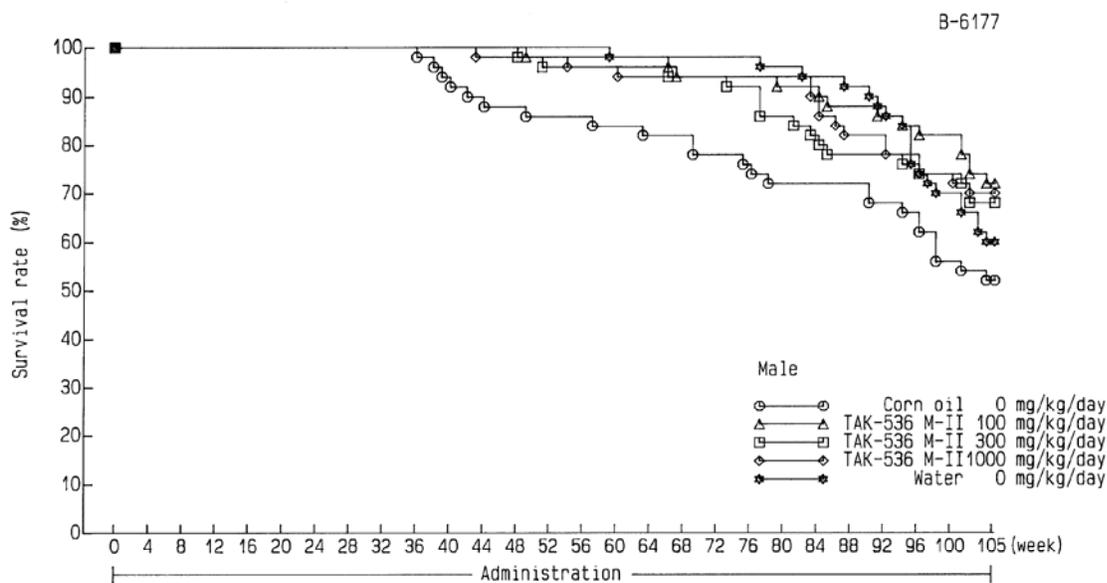


Fig.1 Twenty-four-month oral gavage carcinogenicity study of TAK-536 M-II in rats

— Survival rate —

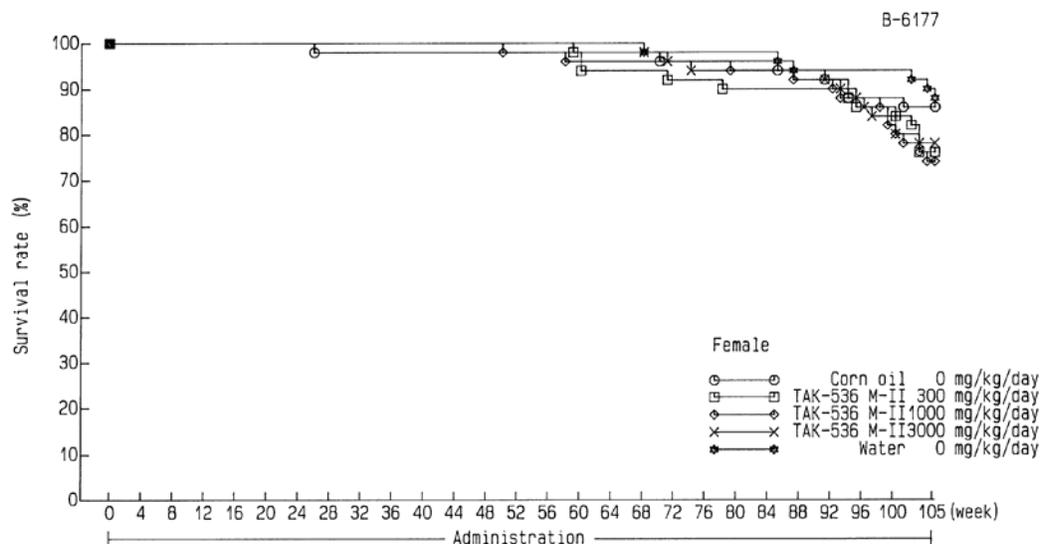


Fig.2 Twenty-four-month oral gavage carcinogenicity study of TAK-536 M-II in rats

— Survival rate —

Body Weights

Males:

Mean body weight at the end of the administration period for the 300 mg/kg/day dose was 4% lower than that in the vehicle treated group. Mean body weight after the 1000 mg/kg/day dose was 6% lower than vehicle treated group (Table 2).

Females

Mean body weight in the 3000 mg/kg/day group was 8% lower than that in the vehicle treated group (Table 2).

Table 2. Summary of body weight

Sex	Dosage (mg/kg/day)	No. of animals	Mean body weight in Week 104 (g)
Male	0 ^{a)}	26	503 (+37%) ^{c)}
	100	36	504
	300	34	483 (-4%) ^{d)}
	1000	35	472* (-6%) ^{d)}
	0 ^{b)}	30	368
Female	0 ^{a)}	43	311 (+19%) ^{c)}
	300	38	313
	1000	37	302
	3000	39	285* (-8%) ^{d)}
	0 ^{b)}	45	262

a): Vehicle control group received corn oil, b): Negative control group received water for injection

c): Values in parentheses indicate percentage of change against the negative control mean (+: increase).

d): Values in parentheses indicate percentage of change against the vehicle control mean (-: decrease).

*: $p \leq 0.05$ (statistically significant difference from the vehicle control group)

Food Consumption

Males

No treatment-related changes were observed in the 100 or 300 mg/kg/day groups. In the 1000 mg/kg/day group, slightly higher values for food consumption were observed throughout the administration period with statistical significance at almost all time points.

Females

No treatment-related changes were observed in the 300 mg/kg/day group. In the 1000 and 3000 mg/kg/day groups, slightly higher values for food consumption were observed throughout the administration period with statistical significance at many time points at the 3000 mg/kg/day dose.

Hematology

1)Males:

No significant effects on hematological parameters were observed.

2)Females

A statistically significant increase in platelet counts and eosinophil ratio was observed at the 3000 mg/kg/day dose. A dose-dependent and statistically significant increase in lymphocyte and neutrophil ratios was observed at the 1000 and 3000 mg/kg/day doses.

Table 3. Summary of Hematology (Sponsor's table)

Sex	Male			Female		
	100	300	1000	300	1000	3000
Dosage (mg/kg/day)	100	300	1000	300	1000	3000
No. of animals used	50	50	50	50	50	50
No. of animals	36	34	35	38	37	39
Platelet	N	N	N	N	N	-13%*
Lymphocyte ratio	N	N	N	N	+12%*	+24%*
Neutrophil ratio	N	N	N	N	-9%*	-16%*
Eosinophil ratio	N	N	N	N	N	-30%*

Values in the table indicate percentage of change against the vehicle control mean (+: increase, -: decrease).

N: No remarkable changes, *: $p \leq 0.05$ (statistically significant difference from the vehicle control group)

Non-Neoplastic Changes

Treatment-related non-tumor lesions that showed an increase/decrease in incidence and/or severity were observed in the liver, eye, optic nerve, glandular stomach, testis, urinary bladder and kidney (Table 4). Many of these effects were decreases in incidence. In the cases where incidences increased, these increases were not dose-dependent or not different from control animals.

Table 4. Summary of Non-Tumor Lesions (Sponsor's table)

Sex	Male					Female				
	0 ^{a)}	100	300	1000	0 ^{b)}	0 ^{a)}	300	1000	3000	0 ^{b)}
Dosage (mg/kg/day)										
Effective No. of animals	50	50	50	50	50	50	50	50	50	50
Liver										
Vacuolation, hepatocyte, periportal										
(total, ± to ++)	28	27	17	15	5	27	26	19	5	7
Proliferation, bile ductal (total, ±/+)	18	20	13	13	50	27	27	25	13	17
Eye										
Atrophy, retinal										
(total)	44	50	44	48	49	48	45	50	49	50
(± to ++)	26	11	14	16	22	17	13	23	26	27
(+++)	18	39	30	32	27	31	32	27	23	23
Cataract										
(total)	36	46	40	46	46	47	44	46	45	48
(± to ++)	32	28	29	30	34	38	28	39	39	38
(+++)	4	18	11	16	12	9	16	7	6	10
Inflammation, anterior chamber										
(total, ± to ++)	8	20	18	23	16	10	15	6	1	9
Optic nerve										
Atrophy										
(total, ± to ++)	6	18	16	15	15	8	13	6	1	9
Stomach										
Dilatation, glandular, cystic (total, ±/+)	33	44	40	47	50	48	46	50	49	50
Testis										
Atrophy, seminiferous tubular										
(total, ± to +++)	37	43	42	47	50	NA	NA	NA	NA	NA
Urinary bladder										
Inflammation (total, ± to +++)	14	5	5	4	3	1	0	0	2	0
Kidney										
Mineralization, cortical (total, ±/+)	10	3	0	0	2	1	1	1	0	0

a): Vehicle control group received corn oil, b): Negative control group received water for injection

Number in the table indicates the number of animals with respective lesions.

±: Minimal, +: Mild, ++: Moderate, +++: Severe, NA: Not applicable

Neoplastic Changes

There was no treatment-related increase in either the number of tumors or tumor-bearing animals in either sex (Table 5).

Table 5. Number of tumors and Tumor Bearers (Sponsor's table)

Sex	Male					Female				
	0 ^{a)}	100	300	1000	0 ^{b)}	0 ^{a)}	300	1000	3000	0 ^{b)}
Dosage (mg/kg/day)										
No. of animals used	50	50	50	50	50	50	50	50	50	50
Total No. of tumors	132	172	155	161	191	77	81	88	68	77
No. of benign tumors	119	156	141	146	166	60	63	71	47	56
No. of malignant tumors	13	16	14	15	25	17	18	17	21	21
Total No. of tumor bearing animals	39	45	48	47	50	41	43	46	40	43
No. of benign tumor bearers	36	44	47	46	50	34	36	43	32	37
No. of malignant tumor bearers	12	13	12	14	23	14	16	16	19	18
No. of multiple tumor bearers	32	40	40	44	48	19	24	25	19	23

a): Vehicle control group received corn oil, b): Negative control group received water for injection

Number in the table indicates the number of tumors or animals

Tumors that demonstrated a change in incidence are summarized in Table 6.
Table 6. Incidence Summary of Major Tumors (Sponsor's table)

Sex	Male					Female				
	0 ^{a)}	100	300	1000	0 ^{b)}	0 ^{a)}	300	1000	3000	0 ^{b)}
Dosage (mg/kg/day)										
No. of animals used	50	50	50	50	50	50	50	50	50	50
Adrenal										
Pheochromocytoma	1	8	5	7	4	3	5	1	1	0
Thyroid										
Adenoma, C cell	2	9	6	4	4	9	6	8	4	5
Carcinoma, C cell	0	4	2	1	0	1	1	1	2	1
Adenoma + Carcinoma, C cell	2	13*	8	5	4	10	7	9	6	6
Pituitary										
Adenoma, anterior	14	15	17	16	23	13	21	18	12	21
Uterus										
Polyp, endometrial stromal	NA	NA	NA	NA	NA	13	14	24	13	16
Adenoma	NA	NA	NA	NA	NA	0	0	1	3	0
Adenocarcinoma	NA	NA	NA	NA	NA	1	0	1	1	1
Adenoma + Adenocarcinoma	NA	NA	NA	NA	NA	1	0	2	4	1
Hemolymphoreticular										
Leukemia, large granular lymphocytic	4	1	1	3	15	6	8	11	13	13

a): Vehicle control group received corn oil, b): Negative control group received water for injection

Number in the table indicates the number of animals with respective lesions.

*: p<0.01 (statistically significant difference from the vehicle control group, common tumor, Peto's test, pairwise comparison)

NA: Not applicable

In the adrenal, a slight increased incidence of pheochromocytoma was observed in males in all dose groups without statistical significance. In the thyroid, a slightly

increased incidence of C cell adenoma was observed in males in the 100 and 300 mg/kg/day groups and a statistically significant increase of C cell tumors (adenoma and carcinoma) was observed only in the 100 mg/kg/day group compared to vehicle controls. However, there was no dose-dependency for these tumors. In the pituitary gland, there was a slightly higher incidence of anterior adenoma observed in females at the 300 mg/kg/day group, but this effect did not reach statistical significance. In the uterus, a slightly higher incidence of endometrial stromal polyp was observed in the 1000 mg/kg/day group without statistical significance. A slightly higher incidence of adenoma and combined incidence of adenoma and adenocarcinoma were observed at the 3000 mg/kg/day group. These effects also did not reach statistical significance. In the hemolymphoreticular system, slightly higher incidence of large granular lymphocytic leukemia was observed in females in the 3000 mg/kg/day group without statistical significance.

Toxicokinetics

After the first dose, the C max and AUC 0-24hr values in males and females increased with increasing doses, although the extent of increase was less than the dosage ratio. The C max and AUC 0-24hr values at 300 and 1000 mg/kg/day in males after the first dose were slightly higher than those in females. Repeating the dose, the C max and AUC 0-24hr values in males decreased, except for the AUC 0-24hr value at 1000 mg/kg/day that was comparable. In females, the C max value at 1000 mg/kg/day increased, although the values at 300 and 3000 mg/kg/day decreased. The AUC 0-24hr value at 1000 mg/kg/day increased, but the values at 300 and 3000 mg/kg/day were comparable (Table 7).

Table 7. Toxicokinetics of TAK-536 M-II (Sponsor's table)

Sex	Male (n=3)			Female (n=3)		
	Dosage (mg/kg/day)	100	300	1000	300	1000
TAK-536 M-II						
T_{max} (h)						
Day 1 (1st dose)	1.0	2.0	1.0	1.0	1.0	1.0
Week 52 (363rd dose)	1.0	1.0	1.0	1.0	1.0	2.0
C_{max} (ng/mL)						
Day 1 (1st dose)	8135	18097	29984	15269	18544	22565
Week 52 (363rd dose)	6742	9409	12807	7643	24925	16571
AUC_{0-24h} (ng·h/mL)						
Day 1 (1st dose)	39323	103512	181161	58292	108943	180789
Week 52 (363rd dose)	27574	57521	178839	51674	213967	180850

Plasma drug concentrations were also measured for the vehicle control group (1 hour after dosing on Day 1 and in Week 52), and the concentrations of TAK-536 M-II were less than the quantification limit (5 ng/mL).

SUMMARY

Carcinogenicity of TAK-536 M-II was assessed in F344/Jcl rats. Doses administered by gavage were 0 (corn oil), 100 (male only), 300, 1000 and 3000 (female only) mg/kg/day.

Results:

- 1) There was no treatment-related decrease in survival rate in either sex.
- 2) No treatment-related clinical signs and palpable masses were observed in either sex.
- 3) Body weights in treated animals decreased in males at all doses and females in the 3000 mg/kg/day group. These effects were minimal.
- 4) Food consumption tended to increase in both sexes.
- 5) Hematological effects in treated animals were seen only in females. At the 3000 mg/kg/day doses, there was a decrease in platelet counts.
- 6) Histopathology: No statistically significant increase in the incidence treatment-related tumors or non-tumor lesions were observed in either sex.
- 7) Toxicokinetics: After the first dose, the C max and AUC 0-24hr values in males and females increased with increasing doses, although the extent of increase was less than the dosage ratio. The C max and AUC 0-24hr values at 300 and 1000 mg/kg/day in males after the first dose were slightly higher than those in females. Repeating the dose, the C max and AUC 0-24hr values in males decreased, except for the AUC 0-24hr value at 1000 mg/kg/day that was comparable. In females, the C max value at 1000 mg/kg/day increased, although the values at 300 and 3000 mg/kg/day decreased. The AUC 0-24hr value at 1000 mg/kg/day increased, but the values at 300 and 3000 mg/kg/day were comparable

In conclusion, after 24-months of administration, TAK-536 M-II did not cause a statistically significant increase in incidence of tumors in rats.

Twenty-six Week Dietary Carcinogenicity Study of TAK-536 M-II in Tg.rasH2 Mice

TAK-536 M-II did not produce any drug-related neoplasms in the 6-month transgenic ras mouse carcinogenicity study. Review dated 9/23/10 is attached below:

Study Title: Twenty-six-week Dietary Carcinogenicity Study of TAK-536 M-II in Tg.rasH2 Mice

Study Facility: (b) (4)

Study Number: 07-472

Study Dates: Feb. 8, 2008- July 7, 2009

GLP Compliance: Yes

QA Report: Yes

Methods

Dosing: Male and female Tg.rasH2 mice (25/sex/group) were given either normal diet or diet containing TAK-536 M-II. Mice received diet containing 1.25, 3.5 and 5% TAK-536 M-II. For treatment groups, 30 animals/sex (non-transgenic mice) were utilized for TK analysis.

Basis for Dose Selection: In a 4-week dietary range-finding toxicity study of TAK-536 M-II, dietary levels of 0 (normal diet), 1.25%, 2.5% or 5% of TAK-536-MII were studied in non-transgenic mice. The highest concentration resulted exposures 40X (males) and 60X (females) those exposures seen in humans. Weight loss of 7.2% was seen at the highest concentration in males. Thus, these concentrations were suggested by the ECAC, but the sponsor chose to use concentrations of 1.25, 3.5 and 5%.

Clinical Signs: Animals were examined 2X daily for clinical signs and once per week for any palpable masses.

Body Weights: Animals were weighed once per week in the first 13 weeks of treatment and then biweekly until study termination/completion.

Food Intake: Food intake was measured once per week in the treated animals only.

Water Intake: Not reported.

Hematology: Not reported.

Clinical Chemistry: Not reported.

Urinalysis: Not reported.

Organ Weights: The brain, heart, liver, kidneys, lung, spleen, thymus and testes or ovaries were weighed at terminal necropsy.

Histopathology: After 26 weeks, all remaining animals from all the groups were subjected to a necropsy. Microscopic examination was performed on the following tissues:

Adrenal Glands	Parathyroid Glands
Aorta	Pituitary Gland
Bone (femur and sternum)	Prostate Gland
Bone Marrow (femur and sternum)	Salivary Gland
Brain	Sciatic Nerve
Epididymides	Seminal Vesicles
Esophagus	Skeletal Muscle (thigh)
Eyes	Small Intestine (duodenum, jejunum, and ileum)
Gall Bladder	Spinal Cord (cervical, thoracic, and lumbar)
Gross Lesions	Spleen
Harderian Glands	Stomach
Heart	Testes
Kidneys	Thymus
Large Intestine (cecum, colon, rectum)	Thyroid Glands
Liver	Tongue
Lungs and Bronchi	Trachea
Lymph Nodes (mesenteric, mediastinal and mandibular)	Urinary Bladder
Mammary Gland with adjacent skin	Uterus
Nasal Cavity	Vagina
Ovaries	
Pancreas	

Toxicokinetics: Twelve TK animals per sex and concentration (3/sex/conc./timepoint) were bled at after continuous ad. lib. test article exposure treatment in the diet during the second (after 7 days of exposure) and twenty-sixth study week. Test-article treated animals were bled at 8AM, 2PM, 8PM and 8AM the next day. Diet control animals (3/sex) were bled once at 8AM during the second and twenty-sixth week of treatment.

Statistical Analysis: The following table lists the tests used to perform the statistical analysis of the data:

Table 1. Staistical Analyses and Mathematical Functions (from Sponsor)

Parameter	Statistical Test or Mathematical Function	Program
Mortality & Selected Clinical Observations	Fisher’s Exact Test Generalized Wilcoxon tests*	Microsoft® Excel 2003 ¹ SAS Version 8.2
Tumor Incidence	Fisher’s Exact Test Peto Analysis Test*	Microsoft® Excel 2003 ¹ SAS Version 8.2
Body Weights, Body Weight Gain and Weekly Food Consumption, Organ Weights (Absolute and Relative)	Means and Standard Deviations, ANOVA, Dunnett’s t-test	Provantis™ Version 6.5.0.1
Absolute (Total) Body Weight Gain, Total Food Consumption	ANOVA, Dunnett’s t-test	Minitab Version 15.0

Note: A probability level of p<0.05 was used to determine statistical significance.

¹ Excel worksheet templates are validated for functionality, accuracy and worksheet security according to (b) (4) SOPs ODQP2725 and OPIS2108.

* = Was performed by a designated subcontractor.

Results

Mortality

- 1) **Males** One male in the control group died before study end and two each in the middle and high concentration group. Fifteen urethane treated animals died during the study (Table 2).
- 2) **Females** One female animal in the mid-concentration group and two in the high concentration group died before the end of the study. Eleven urethane treated animals died during the study (Table 2).

Clinical Signs

- 1) **Males** There were no statistically significant differences comparing diet control to treated animals.
- 2) **Females** The only statistically significant difference between control diet and test-article treated diet animals was in increase in rapid and shallow breathing in the 1.25% concentration group (Table 2).

Body Weights

Males:

Group body weight for the 5% concentration group was significantly decreased compared to control from day 8 until terminal sacrifice (Table 2).

Females

Group body weight was significantly decreased in the 5% concentration group on day 183 when compared to the diet control group (Table 2).

Food and Compound Consumption

Males

A statistically significant decrease in food consumption was observed in males comparing the 5% diet group and control diet mice beginning in week 4 and lasting through week 23. Compound consumption was lower in males than females (Table 2).

Females

No statistically significant changes in food consumption were observed in females comparing treated and control mice for the whole study (Table 2).

Organ Weights

Males

Decreases in heart, liver, kidneys, brain thymus and testes weights were significant in the 5% treatment group most likely due to decrease in body weight (Table 2).

Females

A decrease in liver and brain weights seen in the 5% treatment group was also likely due to a decrease in body weight (Table 2).

Non-Neoplastic and Neoplastic Changes**Males**

Nasal hemangiosarcomas were observed in all treatment groups (2 in LC, 2 in the MC and 1 in the HC) but not in diet controls. In the historical control data base, only 1 nasal hemangiosarcoma was noted in control diet male mice. Statistically, there was no significant positive trend. There were no significant pre-neoplastic lesions in the nasal cavities of either sex (Table 2).

Females

Nasal hemangiosarcomas were observed in 3 animals consuming the highest concentration. Statistically, this was a significant positive trend (Table 2).

Toxicokinetics

TK analysis revealed that exposure of TAK-536 M-II in both sexes increased with increasing dose level. The AUC_{0-24hr} values were lower during week 26 than week 2 in both sexes. The AUC_{0-24hr} values were almost comparable between males and females except for the values at 3.5% in week 2 and 5% in week 26 which were higher in females than males (Table 2).

SUMMARY

Carcinogenicity of TAK-536 M-II was assessed in Tg.rasH2 mice. Drug given in the diet consisted of 1.25, 3.5 and 5% concentrations.

In conclusion, after 26-weeks of dietary intake, TAK-536 M-II did not cause a statistically significant increase in incidence of tumors in transgenic mice.

Table 2. Tabulated Summary of Twenty-six Week Dietary Carcinogenicity Study of TAK-536 M-II in Tg.rasH2 Mice

Animal	Main Study (Tg.rasH2) mice, approximately 9-10 weeks of age at study start				
Test article	Control ¹	Positive control (urethane) ²	TAK-536 M-II		
Dosage (%)	0	1000 mg/kg	1.25	3.5	5
Drug intake (mg/kg/day) (M:F)	NA	NA	1762:2407	5026:6951	8134:11182
No. of animals (M:F)	25:25	25:25 ³	25:25	25:25	25:25
Mortality (M:F)	1:0	15:11	2:0	2:1	2:2
Clinical signs	-	Rapid & shallow respiration, thin (M) alopecia (F), ruffled fur (F), hunched appearance (F)	-	-	-
Body weight	-	↓ Day 8-36, 64 (M) ↓ Day 8, 113, 127 (F)	-	-	↓ Day 8-183 (M) ↓ Day 183 (F)
Total Body weight gains	-	↓	-	-	↓
Total Food consumption	-	↓	-	-	-
Necropsy	-	Pulmonary and splenic lesions	-	-	-
Organ weights (Absolute)	-	NA	-	-	↓ Heart, ↓ Kidneys & ↓ Thymus (M) ↓ Liver
Organ weights (Relative)	-	NA	-	-	↑ Brain, ↑ Testes
Histopathology	Hs of the nasal cavity M0:F0 Hs in various organs M3:F5	Alveolar bronchiolar adenomas alveolar bronchiolar carcinomas and splenic hemangiosarcomas	Hemangiosarcomas in the nasal cavity ⁴		
			M2:F0	M2:F0	M1:F3
			Hemangiosarcoma in various organs		
			M3:F3	M8:F6	M1:F3
Animal	TK Study (CByB6F1) mice, approximately 9-10 weeks of age at study start				
Test article	Control	TAK-536 M-II			
Dosage (%)	0	1.25	3.5	5	
No. of animals (M:F)	10:10	30:30	30:30	30:30	
Mortality (M:F)	0:0	1:0	0:0	1:1	
Plasma concentration [M:F, Mean (n=3)]					
AUC _{0-24h} (ng·h/mL)	Week 2	NC:NC	50211:63885	181479:800988	987942:1255815
	Week 26	NC:NC	47292:43464	101364:105897	514422:925704
Conclusion: No treatment-related neoplastic or non-neoplastic lesions were observed in Tg.rasH2 mice treated with daily diet administration of TAK-536 M-II with 1.25%, 3.5% or 5% for 26-Weeks, although the PETO analysis showed a positive trend for nasal cavity hemangiosarcomas in female mice.					

¹: Basal diet, Harlan TEKLAD Global Diet.

²: Urethane in saline at dose of 1000 mg/kg in three intraperitoneal injections (one each on Day 1, 3, and 5).

³: Five additional female animals were added in Group 2 Positive Control due to unexpected early death.

⁴: Positive trend for females (PETO Analysis).

Hs: Hemangiosarcoma. M: Male, F: Female, -: No treatment-related changes, ↑: Increase, ↓: Decrease

NA: Not applicable, NC: Not calculated

Reproductive Toxicity Studies of TAK-536 M-II

Seg. I and Seg. II studies were performed. No noteworthy findings were observed in these studies.

Seg. I

Effects of TAK-536 M-II on Fertility and Early Embryonic Development to Implantation in Rats

Study Facility: [REDACTED] (b) (4)

Study Number: BA07245

Study Dates: 8/27/07 – 4/30/08

GLP Compliance: Statement indicates that this study was performed in compliance with GLP regulations.

Animals: CrI:CD(SD) rats (M=10 weeks old, F=9 weeks old at initiation of dosing)

Drug Administration: TAK-536 M-II was suspended in corn oil and administered to males by gavage 14 days before mating, through the mating period and until 1 day before necropsy (42-45 days total). The compound was administered to females from 14 days before mating, through the mating period and until day 6 of gestation. They were then necropsied on day 13 of gestation.

Dose Levels: 0 (vehicle), 120, 600 and 3000 mg/kg/day. There were 20 animals/group/sex for the toxicity studies and 4 animals/group/sex for the satellite (PK) studies.

Observations/Measurements: **Mating:** On day 14 of treatment, males and females were paired on a one-to-one basis in the same group in each male cage. Copulation was confirmed by the presence of a copulatory plug or the presence of sperm in a vaginal smear. The day copulation was confirmed was designated as day 0 of gestation. The mating index (# of mated females/# of females used X 100) and the fertility index (# of pregnant females/ # of mated females X 100) were calculated. Mating was not conducted in the satellite group. **Females:** All dams in the toxicity group were euthanized and necropsied on Day 13 of gestation. The numbers of corpora lutea and implantations were recorded. The number of live embryos was recorded and the viability of pre and post-implantation embryos was measured. The estrous cycle was analyzed. The ovaries, uterus and stomach were removed for macroscopic analysis. **Males:** On Day 42-45 of the study, all males in the toxicity group were weighed and euthanized. The testes, epididymes, prostate and seminal vesicles were removed and weighed.

Results: No animals died in any group. At the highest dose, grayish-white stools were observed in both sexes. In all groups, body weight, body weight gain and food consumption were not affected. No treatment-related changes were noted in gross pathological findings or organ weights in any treated group. There were no treatment-related changes noted in estrous cycles, mating index, fertility index, numbers of corpora lutea, implantations, live embryos or viability of pre- or post-implantation embryos in any treated group.

The C_{max} and AUC_{0-24h} values on Days 1 and 15 of dosing increased to a lesser extent than the increases in doses, with the exception that the C_{max} values in males on Day 15 were comparable between 600 and 3000 mg/kg/day. The C_{max} values on Day 15 were lower than those on day 1 in males and were comparable to those on Day1 in females. The AUC_{0-24h} values in both sexes on Day 15 were lower than those on day 1, with the exception that the AUC_{0-24h} value in females at 600 mg/kg/day on Day 15 was comparable to that on Day 1 of dosing. The T_{max} values on Days 1 and 15 were between 1.5 and 6 hr in males and between 0.7 and 3 hr in females.

Conclusion: The NOAELs are 3000 mg/kg/day or above for general toxicity and for reproductive function in parental animals and for early embryonic development.

Table 8.

Effects of TAK-536 M-II on Fertility and Early Embryonic Development to Implantation in Rats (Study No. BA07245)

Animals	CrI:CD(SD) rats, males and females, 10 and 9 weeks of age, respectively, at initiation of dosing				
Test article	Control	TAK-536 M-II			
Dosage level (mg/kg/day)	0	120	600	3000	
Dosage volume (mL/kg/day)	10	10	10	10	
No. of animals (M:F)	20+4 ^{a)} :20+4 ^{a)}	20+4 ^{a)} :20+4 ^{a)}	20+4 ^{a)} :20+4 ^{a)}	20+4 ^{a)} :20+4 ^{a)}	
Males					
Mortality	0	0	0	0	
Clinical signs	–	–	–	GWS	
Body weights	–	–	–	–	
Body weight gain	–	–	–	–	
Food consumption	–	–	↑ (D4, 8, 11, 14)	↓ (D1), ↑ (D4, 8, 11, 14)	
Gross pathology	–	–	–	–	
Organ weights ^{b)}	–	–	–	–	
Toxicokinetics for plasma levels of TAK-536 M-II (n=3)					
C _{max} (ng/mL)	D1	ND	15859 ± 2316	28092 ± 509	49703 ± 20999
	D15	ND	7025 ± 2689	17593 ± 8167	18163 ± 4994
AUC _{0-24h} (ng·h/mL)	D1	ND	86679 ± 16062	304311 ± 88403	568226 ± 69937
	D15	ND	58639 ± 27045	206405 ± 97494	247500 ± 65960
Females					
Mortality	0	0	0	0	
Clinical signs	–	–	–	GWS	
Body weights	–	–	–	–	
Body weight gain	–	–	–	–	
Food consumption	–	–	–	↑ (D4, 8, 11, 14, G3, 6)	
Gross pathology	–	–	–	–	

Control: Corn oil –: No treatment-related effects

GWS: Grayish white stools

↑: Increase ↓: Decrease D: Day of dosing

G: Gestation day

ND: Not detected

^{a)}: Satellite animals for toxicokinetics^{b)}: Testes, epididymides, prostate and seminal vesicles

(continued)

Animals	CrI:CD(SD) rats, males and females, 10 and 9 weeks of age, respectively, at initiation of dosing				
Test article	Control	TAK-536 M-II			
Dosage level (mg/kg/day)	0	120	600	3000	
Dosage volume (mL/kg/day)	10	10	10	10	
No. of animals (M:F)	20+4 ^{a)} :20+4 ^{a)}	20+4 ^{a)} :20+4 ^{a)}	20+4 ^{a)} :20+4 ^{a)}	20+4 ^{a)} :20+4 ^{a)}	
Females					
Estrous cycles	Length (days)	4.2 ± 0.6	4.5 ± 1.4	4.3 ± 0.4	4.3 ± 0.5
	No. of cycles ^{c)}	3.1 ± 0.5	3.0 ± 0.5	3.0 ± 0.3	2.9 ± 0.3
Mating index (%)	100	100	100	100	
Fertility index (%)	95.0	100	90	95.0	
No. of corpora lutea	16.6 ± 2.1	16.6 ± 2.1	16.6 ± 4.6	17.6 ± 3.6	
Toxicokinetics for plasma levels of TAK-536 M-II (n=3)					
C _{max} (ng/mL)	D1	ND	12865 ± 9561	17747 ± 2859	30997 ± 5458
	D15	ND	11741 ± 9776	19886 ± 4517	28324 ± 11350
AUC _{0-24h} (ng·h/mL)	D1	ND	83335 ± 16174	235364 ± 40694	404271 ± 98752
	D15	ND	56438 ± 20492	235532 ± 74669	294995 ± 116540
Embryos					
No. of pregnant animals	19	20	18	19	
No. of implantations	15.5 ± 2.1	15.3 ± 2.1	13.6 ± 5.8	14.4 ± 4.0	
Pre-implantation loss (%)	6.3 ± 6.4	7.7 ± 7.2	23.0 ± 29.8	18.7 ± 21.2 s	
Post-implantation loss (%)	2.0 ± 4.2	3.6 ± 4.9	5.6 ± 9.8	5.9 ± 6.4 s	
Earl (%)	0	0	0	0	
Resorb (%)	2.0 ± 4.2	3.6 ± 4.9	5.6 ± 9.8	5.9 ± 6.4 s	
Dead (%)	0	0	0	0	
No. of live embryos	15.3 ± 2.4	14.8 ± 2.1	12.7 ± 5.6	13.6 ± 4.0	
Conclusion	NOAEL for general toxicity in males: 3000 mg/kg/day or above NOAEL for reproductive function of males: 3000 mg/kg/day or above NOAEL for general toxicity in females: 3000 mg/kg/day or above NOAEL for reproductive function of females: 3000 mg/kg/day or above NOAEL for early embryonic development: 3000 mg/kg/day or above				

Control: Corn oil

^{a)}: Satellite animals for toxicokinetics^{c)}: (No. of transitions from diestrus to estrus + No. of transitions from estrus to diestrus)/2

D: Day of dosing

ND: Not detected

s: Significantly different from control at P ≤ 0.05 (Shirley-Williams' test)

Early: Implantation sites only without conceptuses such as embryos and placentas

Resorb: Unformed conceptuses

Dead: Formed embryos with placentas

NOAEL: No-observed-adverse-effect level

Seg. II Studies**Effects of TAK-536 M-II on Embryo-Fetal Development in Rats****Study Facility:** Takeda Pharmaceutical Company, Ltd., Osaka, Japan**Study Number:** BA07193**Study Dates:** 8/10/07 – 4/30/08**GLP Compliance:** Statement indicates that this study was performed in compliance with GLP regulations.**Animals:** CrI: CD(SD) pregnant female rats (10-11 weeks old at initiation of mating)

Drug Administration: TAK-536 M-II was suspended in corn oil and administered orally by gavage to pregnant female rats at dosage levels of 0, 120, 600 and 3000 mg/kg/day from Day 6 to Day 17 of gestation in order to assess adverse effects on dams and embryo-fetal development. Animals underwent Cesarean section and were necropsied on Day 20 of gestation.

Dose Levels: 0 (vehicle), 120, 600 and 3000 mg/kg/day. There were 20 dams/group.

Observations/Measurements: **Dams:** Body weight, body weight gain and food consumption were measured. Gross pathology of the dams was performed. The numbers of corpora lutea and implantations were counted. **Fetuses:** The number of live and dead fetuses was counted. Fetal body weight, placental weight, and the sex of the fetuses were measured. Any visceral or skeletal malformations were noted in the live fetuses.

Results: **Dams:** All dams survived the duration of the study. There were grayish-white stools seen at the highest dose. No gross lesions were seen in the dams at any dose. There were no effects on body weight, body weight gains and food consumption at any dose. No treatment-related effects were seen in the number of corpora lutea, placental weight, implantations, live fetuses, sex ratio and pre- or postimplantation loss. **Fetuses:** Fetal body weights was not affected. No external malformations were observed in any group. No treatment-related skeletal or visceral abnormalities were seen in any treated group.

Conclusion: The NOAEL for general toxicity in dams and for embryo-fetal development was considered to be 3000 mg/kg/day or above.

Table 9.

**Effects of TAK-536 M-II on Embryo-Fetal Development in Rats
(Study No. BA07193)**

Animals	CrI:CD(SD) rats, dams, 10-11 weeks of age			
Test article	Control	TAK-536 M-II		
Dosage level (mg/kg/day)	0	120	600	3000
Dosage volume (mL/kg/day)	10	10	10	10
No. of pregnant females	20	20	20	20
Dams				
Mortality	0	0	0	0
Clinical signs	–	–	–	GWS
Body weights	–	–	–	–
Body weight gain	–	–	–	–
Food consumption	–	–	–	↓ (G6), ↑ (G8, 12, 16, 18)
Gross pathology	–	–	–	–
No. of corpora lutea	17.0 ± 2.5	17.3 ± 2.4	16.7 ± 2.6	16.5 ± 3.1
No. of implantations	14.5 ± 1.6	15.4 ± 1.5	14.8 ± 1.4	14.3 ± 3.0
Pre-implantation loss (%)	13.7 ± 10.5	9.8 ± 9.8	10.3 ± 9.2	12.2 ± 17.6
Fetuses				
Post-implantation loss (%)	5.7 ± 14.9	3.8 ± 5.6	6.0 ± 6.1	6.6 ± 13.8
Early (%)	0	0	0	0
Resorb (%)	5.4 ± 15.0	3.8 ± 5.6	6.0 ± 6.1	6.6 ± 13.8
Dead (%)	0.3 ± 1.3	0	0	0
o. of live fetuses	13.8 ± 2.8	14.8 ± 1.6	13.9 ± 1.5	13.4 ± 3.6
Male proportion [M/(M+F)]	0.53 ± 0.12	0.49 ± 0.14	0.46 ± 0.14	0.51 ± 0.12
Fetal weights (g) Male	4.30 ± 0.31	4.22 ± 0.28	4.24 ± 0.31	4.30 ± 0.22
Female	4.04 ± 0.25	4.01 ± 0.22	4.02 ± 0.29	4.05 ± 0.24
Placental weights (g) Male	0.485 ± 0.084	0.492 ± 0.043	0.503 ± 0.073	0.481 ± 0.045
Female	0.455 ± 0.049	0.472 ± 0.036	0.484 ± 0.047	0.464 ± 0.044

Control: Corn oil

–: No treatment-related effects

GWS: Grayish white stools

↓: Decrease

↑: Increase

G: Gestation day

Early: Implantation sites only, early deaths after implantation

Resorb: Unformed fetuses

Dead: Formed fetuses with placentae or macerated fetuses

(continued)

Animals	CrI:CD(SD) rats, dams, 10-11 weeks of age			
Test article	Control	TAK-536 M-II		
Dosage level (mg/kg/day)	0	120	600	3000
Dosage volume (mL/kg/day)	10	10	10	10
No. of pregnant females	20	20	20	20
Fetuses				
External malformations (%)	0	0	0	0
Visceral malformations (%)	4.7 ± 7.3	2.1 ± 5.0	7.7 ± 11.8	2.8 ± 5.8
Major malformations (%)				
Membranous VSD	0	0	1.6 ± 4.8	0
Pe. left umbilical artery	1.4 ± 4.4	0	0	0
Thymic remnant in neck	3.9 ± 7.0	2.1 ± 5.0	7.0 ± 11.8	2.2 ± 5.4
Dilated renal pelvis	0	0	0	0.6 ± 2.8
Skeletal malformations (%)	0	0.8 ± 3.7	0.8 ± 3.7	0
Major malformations (%)				
Absent rib	0	0.8 ± 3.7	0	0
Short rib	0	0	0.8 ± 3.7	0
Skeletal variations (%)	20.7 ± 18.5	23.0 ± 25.5	23.1 ± 21.4	20.9 ± 18.5
Major variations (%)				
Bi. os. thoracic centrum	3.2 ± 7.0	1.9 ± 6.1	0.7 ± 3.2	0 s
Du. os. thoracic centrum	0.7 ± 3.2	1.9 ± 6.1	0	1.2 ± 3.6
Bi. os. lumbar centrum	0.6 ± 2.8	0	0	0
Su. lumbar vertebra	0	0.7 ± 3.2	0	0.7 ± 3.2
Lumbarization	1.4 ± 6.4	0	0.6 ± 2.8	0
Bi. os. sternebra	0.6 ± 2.8	0	1.5 ± 4.5	0.6 ± 2.8
Cervical rib	0	0	2.3 ± 5.7	0.6 ± 2.5
Full supernumerary rib	0.7 ± 3.2	0	0.6 ± 2.8	0.7 ± 3.2
Short supernumerary rib	16.3 ± 17.6	19.8 ± 26.4	18.6 ± 20.1	19.1 ± 18.6
o. of sac. and cau. vertebrae	8.6 ± 0.5	8.6 ± 0.5	8.5 ± 0.5	8.5 ± 0.5
Conclusion	NOAEL for dams: 3000 mg/kg/day or above NOAEL for embryo-fetal development: 3000 mg/kg/day or above TAK-536 M-II has no teratogenicity.			

Control: Corn oil s: Significantly different from control at $P \leq 0.05$ (Shirley-Williams' test)
VSD: ventricular septum defect Pe.: Persistent Bi.: Bipartite
os.: ossification Du.: Dumbbell Su.: Supernumerary
sac.: sacral cau.: caudal
NOAEL: No-observed-adverse-effect level

Effects of TAK-536 M-II on Embryo-Fetal Development in Rabbits

Study Facility: (b) (4)

Study Number: BA07195

Study Dates: 11/13/07- 6/11/08

GLP Compliance: There was a statement indicating that this study was conducted under GLP conditions.

Animals: Kbl:JW rabbits (18 weeks old). Pregnant rabbits were dosed from Day 6 to Day 18 of gestation.

Drug Administration: TAK-536 M-II was dissolved in 0.5 w/v% methylcellulose and administered by oral gavage to pregnant rabbits at the following dose levels: 0 (vehicle control), 300 mg/kg/day, 1000 mg/kg/day and 3000 mg/kg/day. There were 20 females/group. Systemic exposure in dams was also investigated in 5 females/group.

Observations/Measurements: **Dams**: Mortality and clinical signs were monitored 2X a day (before dosing and up to 1 hr after dosing). Body weight was measured in the dams on Days 0, 2, 5, 6, 8, 10, 12, 14, 16, 18, 19, 22, 25 and 29 (the day of gross pathology examination. Food consumption was measured on Days 3, 6, 7, 9, 11, 13, 15, 17, 19, 20, 23, 26, and 29 of gestation. On Day 29 of gestation, dams were euthanized and necropsied. External appearance and internal organs and tissues were observed macroscopically. Evidence of implantations and number of corpora lutea were noted. Thoracoabdominal organs (including uterus and ovaries) were collected and stored in formalin.

Fetuses/placentae: The numbers of implantations and live and dead fetuses were noted; external morphology (including the oral cavity, amniotic fluid and placentae) of live fetuses were noted; body weights of live fetuses were recorded. All live fetuses were euthanized by inhalation of carbon dioxide. The sex of all live fetuses was determined by the gross pathological technique and the internal organs/tissues were examined macroscopically. The head was cut parallel to the coronal suture of the parietal region and the cut surface was examined. The heart was fixed in 10 vol% neutral buffered formalin, stained with saturated picric acid aqueous solution. All live fetuses were cleared and stained with alizarin red S and the specimens were examined for skeletal malformations and variations and for the number of ossified sacral and caudal vertebrae.

The following indices were calculated: Pre-implantation loss [(Number of corpora lutea – Number of implantations) / Number of corpora lutea × 100], post-implantation loss (Total number of dead fetuses / Number of implantations × 100), sex ratios of live fetuses, and incidences of external, visceral and skeletal malformations and skeletal variations of live fetuses.

Toxicokinetics: Plasma concentrations (T_{max} , C_{max} and AUC_{0-24h}) of the compound were measured in 5 dams/groups on 2 days: on Day 6 of gestation at 0.5, 1, 2, 6, and 24 hr. Measurements were also made on Day 18 of gestation before, and 0.5, 1, 2, 6, and 24 hr after dosing.

Results: **Dams**: One dam each in the 1000 and 3000 mg/kg groups died on Days 23 and 12 of gestation, respectively. One dam aborted on Day 20 of gestation and another dam prematurely delivered on Day 28 of gestation in the control group. In addition, 1 dam in the 1000 mg/kg group aborted on Day 25 of gestation. In the 3000 mg/kg group, scant or no feces and scant or no urine were noted for 14 of 20 dams on Day 7 or 8 of gestation, immediately after initiation of the dosing period. Thereafter, scant or no feces were noted for 17 dams in total, including 3 females that exhibited scant feces on Day 25, 26 or 29 of gestation.

In the 300 and 1000 mg/kg groups, scant or no feces and scant or no urine were noted at the comparable incidences to the control group. No statistically significant differences in body weights were noted in any treated group as compared to the control group. In the 3000 mg/kg group, statistically significant decreases in food consumption were noted on Days 6 and 8 of gestation as compared to the control group. No gross pathological lesions were evident in any dam that underwent the Cesarean section. There were no statistically significant differences between the control and any treated group in the number of corpora lutea, implantations or live fetuses, pre- or post-implantation loss, or sex ratio (number of males/total number of fetuses), body weights or placental weights of live fetuses.

Fetuses: There were no statistically significant differences in the incidence of external malformations between the control and any treated group. There were no statistically significant differences in the incidence of visceral malformations between the control and any treated group. There were no statistically significant differences in the incidence of skeletal malformations between the control and any treated group.

In the 3000 mg/kg group, although a statistically significant increase was noted in the total incidence of skeletal variations, no statistically significant differences were noted in the incidence of each skeletal variation as compared to the control group. There were no statistically significant differences in the incidence of skeletal variations between the control and 300 or 1000 mg/kg groups.

A statistically significant increase was noted in the number of sacro-caudal vertebrae in the 3000 mg/kg group, but no statistically significant differences were noted in the 300 or 1000 mg/kg group as compared to the control group.

Toxicokinetics

The C_{max} values after the first and last doses increased with increasing dosage levels, except for comparable values after the first dose at 300 and 1000 mg/kg/day. The AUC_{0-24h} values after the first and last doses increased with increasing dosage levels. Although the C_{max} values at 1000 and 3000 mg/kg/day increased by repeated dosing, the C_{max} values in the first and last doses were comparable at 300 mg/kg/day. The AUC_{0-24h} values at all dosage levels increased by repeated dosing. The T_{max} values after the first dose were between 1.0 and 1.3 hours, and the values after the last dose were between 1.3 and 1.7 hours.

In the control group (0 mg/kg/day), TAK-536 M-II were not detected at 1 hour after the first or last dose.

Conclusion:

The no observed adverse effect level of TAK-536 M-II was considered to be 1000 mg/kg/day and above for embryo-fetal development based on the statistically significant increase in the number of sacro-caudal vertebrae in the 3000 mg/kg/day group.

Table 10

**Effects of TAK-536 M-II on Embryo-Fetal Development in Rabbits
(Study No. BA07195)**

Animals	Rabbits, Kbl:JW, 18-week-old females			
Test article	Control	TAK-536 M-II		
Dosage level (mg/kg/day)	0	300	1000	3000
Dosage volume (mL/kg/day)	10	10	10	10
No. of dams	20	19	18	20
Dams				
Mortality	0	0	1 (G23)	1 # (G12)
No. of aborted dams ¹⁾	2	0	1	0
Clinical signs (No. of dams)				
Scant or no feces	6	2	4	17 #
Scant or no urine	4	1	3	8 #
Body weights	–	–	–	–
Body weight gain	–	–	–	↓ (G6-12) #
Food consumption	–	–	–	↓ (G6, 8) #
Gross pathology	–	–	–	–
No. of corpora lutea	10.8 ± 1.7	10.8 ± 2.2	11.0 ± 1.9	10.5 ± 1.6
No. of implantations	8.8 ± 3.1	9.1 ± 2.5	7.9 ± 2.5	8.3 ± 2.6
Pre-implantation loss (%)	19.9 ± 26.0	14.8 ± 20.5	26.9 ± 25.4	22.0 ± 20.7
Toxicokinetics				
TAK-536 M-II	LQL			
Tmax (h)				
G6		1.0 ± 0.0	1.3 ± 0.6	1.2 ± 0.8
G18		1.3 ± 0.6	1.7 ± 0.6	1.7 ± 0.6
Cmax (ng/mL)				
G6		10189 ± 3900	10912 ± 3099	23098 ± 5876
G18		10941 ± 2953	22168 ± 7691	34420 ± 2539
AUC _{0-24h} (ng·h/mL)				
G6		24401 ± 14673	49199 ± 15665	233303 ± 24706
G18		34927 ± 12728	188039 ± 65698	391175 ± 57473
Fetuses				
Post-implantation loss (%)	7.9 ± 17.8	4.6 ± 13.2	15.8 ± 24.6	10.9 ± 13.8
Early (%)	0	0	6.3 ± 25.0	0
Resorb (%)	6.8 ± 17.6	4.6 ± 13.2	5.7 ± 10.0	9.6 ± 12.9
Dead (%)	1.1 ± 4.7	0	3.9 ± 7.6	1.3 ± 3.9

Control: 0.5 w/v% methylcellulose #: Adverse effects 1): Including prematurely delivered dam
 –: No treatment-related effects ↓: Decrease G: Gestation day
 LQL: Less than the quantification limit (5 ng/mL)
 Early: Implantation sites only, early deaths after implantation Resorb: Unformed fetuses
 Dead: Formed fetuses with placentae or macerated fetuses

(continued)

Animals	Rabbits, Kbl:JW, 18-week-old females			
Test article	Control	TAK-536 M-II		
Dosage level (mg/kg/day)	0	300	1000	3000
Dosage volume (mL/kg/day)	10	10	10	10
No. of dams	20	19	18	20
Fetuses				
No. of live fetuses	8.1 ± 3.3	8.7 ± 2.7	7.1 ± 2.5	7.6 ± 2.9
Male proportion [M/(M+F)]	0.47 ± 0.25	0.53 ± 0.16	0.49 ± 0.18	0.50 ± 0.24
Fetal weights (g)				
Male	40.8 ± 6.0	45.3 ± 6.7	43.7 ± 5.9	44.8 ± 5.5
Female	42.3 ± 8.2	45.5 ± 6.2	43.9 ± 7.2	43.6 ± 6.0
Placental weights (g)				
Male	3.23 ± 0.61	3.54 ± 0.68	3.51 ± 0.59	3.49 ± 0.60
Female	3.33 ± 0.69	3.63 ± 0.84	3.36 ± 0.75	3.46 ± 0.48
External malformations (%)	0	2.4 ± 5.8	0	0
Major malformations (%)				
Hemorrhage	0	1.0 ± 4.2	0	0
Visceral malformations (%)	3.0 ± 8.7	1.5 ± 4.7	4.1 ± 12.0	0.8 ± 3.3
Major malformations (%)				
Thymic remnant in neck	0	0.7 ± 2.9	3.0 ± 11.5	0.8 ± 3.3
Skeletal malformations (%)	0.7 ± 2.9	0.5 ± 2.1	1.5 ± 5.7	0.8 ± 3.3
Major malformations (%)				
Fused sternebra	0	0.5 ± 2.1	0	0.8 ± 3.3
Skeletal variations (%)	21.0 ± 21.9	36.9 ± 26.4	26.6 ± 29.5	41.5 ± 26.7 s
Major variations (%)				
Su. lumbar vertebra	3.5 ± 7.0	7.8 ± 14.6	2.6 ± 7.2	7.4 ± 13.9
Lumbarization	0.6 ± 2.4	1.4 ± 4.5	0	1.0 ± 3.0
Bi. os. sternebra	0.5 ± 2.0	1.4 ± 3.4	0	1.5 ± 4.5
Cervical rib	2.4 ± 5.6	2.0 ± 4.9	4.0 ± 15.5	7.6 ± 12.2
Full supernumerary rib	6.1 ± 13.6	13.2 ± 17.7	6.0 ± 14.9	12.8 ± 23.3
Short supernumerary rib	12.6 ± 14.9	20.7 ± 22.3	17.7 ± 25.5	28.6 ± 27.7
No. of sac. and cau. vertebrae	19.1 ± 0.4	19.2 ± 0.4	19.2 ± 0.5	19.5 ± 0.3 w
Conclusion	NOAEL for dams: 1000 mg/kg/day NOAEL for embryo-fetal development: 3000 mg/kg/day and above TAK-536 M-II has no teratogenicity.			

Control: 0.5 w/v% methylcellulose

s: Significantly different from control at $P \leq 0.05$ (Shirley-Williams' test)w: Significantly different from control at $P \leq 0.05$ (Williams' test)

Su.: Supernumerary

Bi.: Bipartite

os.: ossification

sac.: sacral

cau.: caudal

NOAEL: No-observed-adverse-effect level

11 Integrated Summary and Safety Evaluation

Primary and Secondary Pharmacodynamic Activity

TAK-491, also known as azilsartan medoxomil, is a prodrug that is rapidly hydrolyzed to TAK-536, which is a potent, selective antagonist of angiotensin II type 1 (AT1) receptors. Typically, TAK-536 was used to conduct *in vitro* studies, and both TAK-491 and TAK-536 were used to conduct the *in vivo* studies. The clinically used AT1 receptor blockers (ARB) olmesartan, telmisartan, valsartan, irbesartan, losartan and candesartan were tested in some *in vitro* studies as comparators for TAK-536. In some *in vivo* experiments, TAK-491 was compared to olmesartan medoxomil (OLM or OM, the prodrug for olmesartan) and TAK-536 was compared to losartan.

TAK-536 was examined for All receptor binding affinity, dissociation from All receptors, and effects on All-induced vasoconstriction *in vitro*. The results of *in vitro* receptor binding studies indicate that TAK-536 is a potent, selective antagonist of human AT1 receptors, with 50% inhibitory concentration (IC₅₀) values of 0.62 to 2.6 nmol/L in cloned human AT1 receptors. The metabolites, TAK-536 M-I (primary metabolite in animals) and TAK-536 M-II (primary metabolite in human), demonstrated weak binding affinity for AT1 receptors; the respective IC₅₀ values were 2300 and 1100 nmol/L, which are approximately 1770- and 850-fold greater than that for TAK-536.

In screening studies, TAK-536 was >10,000-fold more selective for AT1 receptors compared with AT2 receptors based on differences in IC₅₀ values. When compared in the same experiment, the affinity of TAK-536 for AT1 receptors was either similar to or greater than those of olmesartan, telmisartan, valsartan, and irbesartan.

TAK-491, TAK-491F (K⁺ - free acid), TAK-536, and TAK-536 M-II all were examined for pharmacodynamic activity in broad receptor, ion channel, and enzyme screens. Pharmacodynamic profiling studies of TAK-491 or TAK-536 were conducted *in vitro* and *in vivo*. The dissociation of TAK-536 from human AT1 receptors was much slower compared with olmesartan, telmisartan, valsartan, and irbesartan.

TAK-536 selectively inhibited All-induced constriction of isolated rabbit aorta strips *in vitro*; the effect was similar to that of olmesartan and more potent than that of losartan and its active metabolite EXP3174.

TAK-491 and TAK-536 were studied for their effects on blood pressure in normotensive animals and in rat and dog models of hypertension *in vivo*. A single oral dose of TAK-491 inhibited an All-induced pressor response in conscious male rats for up to 24 hours and reduced blood pressure in spontaneously hypertensive rats (SHRs). Repeated daily dosing of TAK-491 to SHRs reduced blood pressure across a 2-week period without producing tachycardia; the animals did not experience rebound hypertension after termination of treatment. The *in vivo* antihypertensive effects of TAK-536 were tested

also in animal models similar to those used in the TAK-491 studies. Similar to TAK-491, TAK-536 had statistically significant and dose-dependent antihypertensive effects at doses of ≥ 0.1 mg/kg.

Oral or intravenous (IV) dosing of TAK-536 to conscious rats and dogs dose-dependently inhibited the pressor response caused by an IV infusion of All. In rats, doses producing a 50% response inhibition (ID_{50}) after oral and IV dosing were 0.06 mg/kg and 0.03 mg/kg, respectively. In dogs, the respective ID_{50} values were 0.34 mg/kg and 0.06 mg/kg. The metabolite TAK-536 M-I failed to inhibit an All-induced pressor response *in vivo* at a dose of 1.0 mg/kg, administered intraperitoneally, whereas TAK-536 inhibited the pressor response dose-dependently at doses of 0.01, 0.1, and 1 mg/kg. These results indicate that the TAK-536 M-I metabolite is unlikely to have antihypertensive activity *in vivo*.

In dogs with renal hypertension produced by unilateral constriction of the renal artery, a single oral dose of TAK-491 reduced SBP for up to 24 hours without producing tachycardia. In all these studies, TAK-491 produced dose-related antihypertensive effects which achieved statistical significance at doses ≥ 0.1 mg/kg. On a mg/kg basis, TAK-491 had consistently greater and longer-lasting antihypertensive effects compared with olmesartan.

In normotensive rats, oral dosing of TAK-536 at 0.1 and 1 mg/kg increased plasma renin concentrations, but had no significant effect on blood pressure or plasma aldosterone levels although these same doses of TAK-536 had produced significant reductions in blood pressure in SHRs. A single, oral dose of TAK-536 at 0.1 to 1 mg/kg to SHRs produced average reductions in blood pressure of 25 to 45 mmHg. With multiple oral dose administration across 14 days, TAK-536 at $\square 0.1$ mg/kg/day reduced blood pressure in SHRs by 20 to 50 mmHg across a 24 hour period. There was no reflex tachycardia during treatment and no rebound hypertension after cessation of dosing.

TAK-536 also was tested in the deoxycorticosterone acetate (DOCA) salt-dependent model of hypertension, in which rats with unilateral nephrectomy are given a subcutaneous implant of the synthetic mineralocorticoid, DOCA. These animals develop profound hypertension if they are maintained on sodium-enriched drinking water and the model is considered a salt sensitive, low renin, form of hypertension, with volume overload. TAK-536 had no effect on blood pressure in this rat model even at the high oral dose of 10 mg/kg. This result was not unexpected since other ARBs, such as losartan and olmesartan medoxomil, also are ineffective in reducing blood pressure in this low-renin model. The lack of efficacy for TAK-536 in this model supports the pharmacologic specificity of this compound at AT1 receptors; neither ARBs nor ACE inhibitors affect DOCA-induced hypertension, although endothelin antagonists, diuretics, such as hydrochlorothiazide (HCTZ), and beta blockers, such as propranolol, attenuate it.

Oral dosing of TAK-536 reduced blood pressure in renal hypertensive rat models (1 or 2 kidneys, 1 clip on renal artery) during the acute (hyper-reninemia) and chronic (normo-reninemia) postoperative periods. TAK-536 at 1.0 mg/kg, oral, had no effect on blood pressure in normotensive rats, although blood pressures in SHR were reduced up to 45 mmHg at this dose. In normotensive rats, oral dosing of TAK-536 increased plasma renin concentrations at 0.1 and 1 mg/kg, but not at 0.01 mg/kg, a dose sub-threshold for reducing blood pressure in SHR. TAK-536 prevented the increase in plasma aldosterone levels and blood pressure produced by IV infusions of All in rats; the minimally effective doses were 0.1 and 1.0 mg/kg for inhibition of All effects on aldosterone secretion and blood pressure, respectively, although these doses of TAK-536 had no statistically significant effect on blood pressure or aldosterone secretion in normotensive rats in the absence of an All infusion. These results indicate that TAK-536 is more potent in reducing the blood pressure of hypertensive rats than normotensive rats.

In order to detect any potential off-target activities associated with exposure to TAK-491, TAK-491F, TAK-536, or TAK-536 M-II, each compound was screened for its potential binding to a broad range of receptors and ion channels and for effects on enzyme activities using standard *in vitro* assay protocols. Aside from effects at AT1 receptors, the most potent effect of TAK-491F was inhibition of PDE4 activity, with an IC_{50} value of 0.835 $\mu\text{mol/L}$. TAK-491 also demonstrated inhibition of PDE4 activity. However, since TAK-491 is not detectable in human plasma after oral administration of TAK-491 at doses up to 320 mg, this nonspecific activity of TAK-491 is considered not to be of clinical relevance. The results from these screening experiments confirmed that TAK-536 does not inhibit ACE and indicated that other off-target activities seen with TAK-491 and related compounds occurred only at concentrations that are at least 10 times higher than those likely to be attained after an 80 mg oral dose of TAK-491, the highest proposed clinical dose.

Safety Pharmacology

The effects of TAK-491 or TAK-536 on the central nervous, respiratory, and cardiovascular systems were evaluated in GLP-compliant safety pharmacology studies. These studies were conducted *in vitro* (hERG channel assay, isolated guinea pig papillary muscles) or *in vivo* in telemeterized dogs and in rats (general effects on the central nervous system and respiration).

There were no effects of treatment on neurobehavioral or respiratory parameters in rats. Neither TAK-491 nor TAK-536 affected heart rate or any electrocardiogram parameter in telemeterized dogs or during the course of subchronic dog toxicity studies; an expected decrease in blood pressure was seen. TAK-536 did not inhibit hERG channel currents when assayed. The primary human metabolite (TAK-536 M-II) was also separately tested in the hERG assay and was found to be negative.

In summary, the results of pharmacodynamic studies indicate that TAK-536 is a potent antagonist at AT1 receptors *in vitro* and *in vivo* across a number of species and that dosing of TAK-491 or TAK-536 acts to reduce blood pressure in animal models of normo- and supra-renin hypertension. The metabolites TAK-536 M-I and TAK-536 M-II have little activity on AT1 receptors or on blood pressure.

Pharmacokinetics/ADME

TAK-491 (azilsartan medoxomil) is a prodrug of TAK-536. Based on molecular weights, a dose of 1.33 mg/kg TAK-491 is equivalent to 1 mg/kg TAK-536. TAK-491 is also a potassium salt, therefore, plasma concentrations of TAK-491 are expressed as TAK-491F (TAK-491 salt free form).

Nonclinical pharmacokinetic studies were conducted in mice, rats, rabbits, dogs, and monkeys. Extensive pharmacokinetic and metabolism studies were conducted in rats and dogs since those 2 species were the major species used in the toxicology program. The oral formulation of TAK-491 used in the rat and dog studies was 0.5% aqueous solution of methylcellulose suspension containing 0.05% (w/v) citric acid to prevent the conversion of TAK-491 to TAK-536. The oral formulation of TAK-536 used in the rat and dog studies was 0.5% (w/v) gum arabic. Saline solution containing a small amount of sodium hydroxide (1.08 mmol/L) was used as the intravenous (IV) formulation for TAK-536. The formulation used in the nonclinical pharmacokinetic and metabolism studies was similar to those used in the toxicology studies.

The absorption, distribution, metabolism, and excretion of TAK-536 were studied in rats, dogs, and monkeys following administration of [¹⁴C]TAK-536. TAK-491 was rapidly hydrolyzed, by gut and plasma aryl esterase to form the active moiety TAK-536, *in vivo* after oral administration of [¹⁴C]TAK-491. The systemic bioavailability of TAK-536, after oral dosing of TAK-491, was estimated to be 12.4% in rats and 53.9% in dogs. The respective bioavailability of oral TAK-536 in fed rats and dogs was 14.6% and 24.3% and was increased to 40.9% (rats) and 39.2% (dogs) when animals were fasted. In fasted animals, drug half-lives were approximately 5.2 hours in rats, 2.8 hours in dogs, and 1.4 hours in monkeys. C_{max} was achieved at 0.7 hours in rats and dogs and 1.7 hours in monkeys after oral dosing under fasted conditions.

The linearity in the pharmacokinetics of TAK-536 was investigated in rats and dogs after single oral administration of TAK-491. The increases in the maximum observed plasma concentration (C_{max}) and area under the plasma concentration-time curve (AUC) values for TAK-536 were greater than dose-proportional between 0.3 to 3 mg/kg expressed as TAK-536.

Caco-2 studies indicate that TAK-491 was not a P-glycoprotein (P-gp) substrate and both TAK-491 and TAK-536 were low permeability drugs. TAK-491 had some inhibitory effect on the P-gp-mediated efflux activity while TAK-536 did not show any P-gp inhibitory effect.

Following oral administration of [¹⁴C]TAK-536 to rats, total radioactivity was distributed widely to tissues, with relatively high concentrations in the liver.

[¹⁴C]TAK-491 and its related compounds were highly bound to plasma protein (>99.9% in rats and >97.1% in dogs) and poorly distributed into blood cells (<2% in rats and <3% in dogs) after single oral administration of [¹⁴C]TAK-491 to rats and dogs. [¹⁴C]TAK-536 exhibited high *in vitro* plasma/serum protein binding in plasma of mice (99.8%), rats (≥99.8%), dogs (≥98.8%), and humans (99.5%), and protein binding was broadly concentration-independent in all species.

After a single oral administration of [¹⁴C]TAK-491 to pregnant rats, radioactivity was gradually transferred to the fetuses via the placenta. Following administration of [¹⁴C]TAK-491 to lactating rats, plasma radioactivity was distributed into milk. The majority of the radioactivity in the plasma and milk was from TAK-536.

The biotransformation of [¹⁴C]TAK-491 was investigated *in vitro* in rats, dogs, and humans and *in vivo* in rats and dogs. Most of the radioactivity in plasma was as the active moiety TAK-536. *In vitro*, TAK-491 was rapidly hydrolyzed to TAK-536 in the plasma from rats, dogs, monkeys, and humans and in the incubation mixture with human hepatic and intestinal S9 fractions. TAK-536 was either further decarboxylated to form the pharmacologically inactive metabolite, TAK-536 M-I, primarily by cytochrome P-450 (CYP) 2C8 (abbreviated as CYP2C8), or was O-dealkylated to form another pharmacologically inactive metabolite, TAK-536 M-II, primarily by CYP2C9. Incubation of [¹⁴C]TAK-536 with microsomes expressing human CYP isoforms indicated that CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 all were capable of metabolizing TAK-536. All human Phase I metabolites of TAK-536 were present in rat and dog excreta.

Both TAK-491 and TAK-536 have low potency to induce CYP3A activity *in vitro*. TAK-491 inhibited some CYP isoforms *in vitro*, but because it is converted rapidly to TAK-536 *in vivo* during the absorption phase, this activity is unlikely to be clinically significant. TAK-536 itself had no inhibitory activity (50% inhibitory concentration [IC₅₀] values >100 μmol/L) against human hepatic CYP isoforms. Based on *in vitro* studies, TAK-491 and TAK-536 are unlikely to have clinically significant potential for drug-drug interactions at anticipated clinical exposures.

The biotransformation of TAK-536 was investigated *in vitro* in mice, rats, dogs, monkeys, and humans and both TAK-536 M-I and TAK-536 M-II was formed. All metabolites formed by human hepatic microsomes were also formed by hepatic microsomes from animal species.

Following oral administration of [¹⁴C]TAK-491 to rats and dogs, TAK-536 was the main component in plasma (93.7% and 90.0%, respectively). TAK-536 was metabolized and then excreted mainly into feces. In a 0-120 hour postdose period, 95.0% and 96.8% of the dosed radioactivity were recovered in rat and dog feces, respectively. Only 2% and 5.4% of the dosed radioactivity were excreted in rat and dog urine, respectively in the

same time collection period. More than 80% of the radioactivity in rat and dog feces was TAK-536 M-I. TAK-536 M-II, a major metabolite in humans, was a minor metabolite in animals.

Following oral administration of [¹⁴C]TAK-536 to rats, dogs, and monkeys only a small amount of unchanged TAK-536 was detected in urine and feces indicating that TAK-536 is almost completely metabolized before being eliminated. In biliary-cannulated rats, 22.2% and 32.4% of total ¹⁴C in bile was composed of unchanged TAK-536 and TAK-536 M-I, respectively.

Toxicology

The toxicology program for TAK-491 included assessment of this compound and/or the active moiety, TAK-536, in preliminary and definitive single (rat, ≤ 2000 mg/kg; dog ≤ 30 mg/kg) and repeat-dose toxicity studies in rats (up to 26 weeks, ≤ 2000 mg/kg) and dogs (up to 26 weeks with TAK-491 (≤ 60 mg/kg) and up to 52 weeks with TAK-536 (≤ 300 mg/kg), rodent carcinogenicity studies, genotoxicity studies, and reproduction and developmental toxicity studies. Recovery from changes occurring after 4 weeks of dosing TAK-491 was examined in dogs. The major human metabolite, TAK-536 M-II, was examined in rat and dog repeat-dose toxicity studies (up to 13 weeks in duration), in 6-month transgenic mouse and 2-year rat carcinogenicity assays, and in genotoxicity and reproduction/developmental studies. A full listing of all Toxicology studies conducted is presented in the Appendix. NOAELs, AUCs and human exposure ratios for pivotal repeat-dose toxicity studies are presented in 2 Tables (based on AUC for TAK-536 and for TAK-536 M-II) at the end of this summary.

Acute Toxicity

Single or escalating dose oral toxicity studies performed with azilsartan medoxomil in rats and dogs up to 2000 mg/kg. However, death occurred at an IV dose of 40 mg/kg in male rats and 200 mg/kg in female rats.

Chronic Toxicity

In oral repeat dose toxicity studies, TAK-491-related deaths occurred in mice at doses of 200 mg/kg/day, in dogs at 200 mg/kg/day in males and 300 mg/kg/day in females. No deaths occurred in rats at doses up to 2000 mg/kg/day.

Mice

In a 13-week toxicity study in mice with TAK-491, deaths occurred at ≥200 mg/kg/day; the no-observed-adverse-effect level (NOAEL) in this study was <20 mg/kg/day.

Rats

In repeat-dose toxicity studies in rats with TAK-491, increases in white blood cells (WBC) and adrenal weights were observed in males at ≥ 600 mg/kg/day in the 13-week study, and in males and females at 2000 mg/kg/day in the 13- and/or 26-week studies.

Toxicologic findings of dark red foci with corresponding observation of erosion in the glandular stomach were noted in the 26-week rat study with TAK-491 in males at ≥ 200 mg/kg/day and in females at 2000 mg/kg/day. Minimal to moderate erosion in the glandular stomach was observed in 3/15 males in the 200 mg/kg group and 14/15 males and 10/15 females in the 2000 mg/kg group. Minimal erosion was also observed in a few females in the control, 2 and 20 mg/kg groups. Mild ulcer in the glandular stomach was observed in 1/15 males in the 2000 mg/kg group. The NOAEL's for TAK-491 in the 26-week rat study were 20 mg/kg/day for males and 200 mg/kg/day for females. Olmesartan, the other ARB that has a medoxomil side chain, produced ulceration in the stomach of dogs in a 3-month repeat dose study.

Kidney histopathological effects were also observed in this 26-week study in rats. Minimal or mild hypertrophy of the juxtaglomerular cells was observed in 5/15 males and 6/15 females in the 2 mg/kg group, 14/15 males and 15/15 females in the 20 mg/kg group and 15/15 animals in both sexes in the 200 and 2000 mg/kg groups. Minimal to moderate intimal proliferation of the interlobular artery, was observed in 14/15 males and 10/15 females in the 20 mg/kg group, 15/15 males and 10/15 females in the 200 mg/kg group and 15/15 males and 12/15 females in the 2000 mg/kg/day group. These effects appear to be class effects of ARB's.

Finally, histopathological effects were observed in the adrenal gland in this 26-week study. Minimal or mild atrophy of the zona glomerulosa was observed in 7/15 females in the 2 mg/kg group, 14/15 males and 15/15 females in the 20 mg/kg group and 15/15 animals in both sexes in the 200 and 2000 mg/kg groups. Again, these effects appear in many toxicology studies using ARB's.

Other effects seen in this 26-week study: There was a dose-dependent decrease in body weight (from 5 to 17%); food consumption decreased significantly at 200 and 2000 mg/kg/day in males and only at 2000 mg/kg/day in females; In males, there was a dose-dependent decrease in urine osmotic pressure (10-32%) and a decrease in plasma sodium concentrations from 20 to 2000 mg/kg/day (not dose dependent (-20%)); hematological effects: in males, there was a dose-dependent decrease in RBC number (3-7%); in both sexes there were decreases in hemoglobin concentrations and hematocrit that were not dose-dependent; other hematological effects were seen mainly at the highest dose given to both sexes. These include an increase in the reticulocyte ratio in males, platelets in both sexes, WBC in both sexes; blood chemistry: dose-dependent decreases in AST, ALT, and LDH in both sexes (21-73%); dose-dependent increase in triglycerides in males (27-39%); decrease in phospholipids in males (8-16%); dose-dependent increase in BUN in males (14-43%); dose-dependent increase in creatinine in males (19-26%); dose-dependent increase in plasma potassium in males

(7-12%); dose-dependent decreases in TP and albumin in both sexes (3-13%). Organ weights: dose-dependent decrease in body weight at necropsy in males (6-19%); dose-dependent decrease in thymus weight in males (11-34%); dose-dependent decrease in absolute heart weight (15-29%) in both sexes although this effect was more pronounced in males; dose-dependent decrease in splenic weight (8-22%) in males; dose-dependent increase in relative adrenal weight (14-50%) in males.

In repeat-dose toxicity studies in rats with TAK-536, the active moiety, toxicologic findings of dark red foci with corresponding observation of erosion in the glandular stomach were noted at oral doses up to 3000 mg/kg/day in the 4- and 13-week studies. In the 26-week rat study with TAK-536, toxicologic, but probably pharmacologically-mediated, finding of basophilic renal tubules occurred at ≥ 10 mg/kg/day. The NOAEL for TAK-536 in the 26-week rat study was 1 mg/kg/day.

Dogs

In the 26-week dog study with TAK-491, toxicologic, but probably pharmacologically-mediated, findings of renal tubular dilatation and basophilia were observed in females at 60 mg/kg/day. The NOAELs in this 26-week dog study were 60 mg/kg/day for males and 12 mg/kg/day for females.

In the 26- and 52-week dog study with TAK-536, deaths occurred in 1 female at 100 mg/kg/day and in 1 male and 2 females at 300 mg/kg/day. These dogs showed severe clinical signs, gross and microscopic changes indicative of uremia. Toxicologic but probably pharmacologically-mediated findings of renal tubular dilatation and regeneration were observed in the surviving dogs at ≥ 100 mg/kg/day. The NOAEL for TAK-536 in these 26- and 52-week dog studies was 30 mg/kg/day.

General Toxicology Summary

The renal and adrenal effects in rats, and renal effects in dogs, occur at AUC levels equal to or lower than human exposure at the MRHD of 80 mg, whereas the GI effects occur at approximately 20-fold higher AUC in rats and 4 to 5-fold higher AUC in dogs.

In 13-week repeat-dose toxicity studies in rats, using the metabolite TAK-536 M-II, the toxicity of the kidney, adrenal gland and stomach were not observed. NOAELs of 300 mg/kg/day (male) and 3000 mg/kg/day (female) were reported. Similarly, GI and renal effects (observed in 52-week dog studies with TAK-536) were also not observed in 13-week studies of this metabolite in dogs, where NOAELs of 2000 mg/kg/day were reported. These findings again suggest that the majority of the observed toxicities with TAK-491 and TAK-536 may be pharmacologically mediated as TAK-536 M-II is relatively devoid of pharmacological activity.

Genotoxicity

TAK-491, TAK-491F, and TAK-536 were examined for genotoxicity using standard assays. In reverse mutation assays, TAK-491 was not mutagenic, although TAK-491F induced gene mutations to levels approximately 2.1-fold higher than those of the negative control group in the presence of S9 activation. Neither TAK-536 nor TAK-536 M-II was mutagenic in these assays.

TAK-491 induced structural chromosomal aberrations in Chinese hamster lung (CHL) cells in the absence (but not presence) of S9 activation at cytotoxic concentrations. TAK-536 also induced chromosomal aberrations in CHL cells at cytotoxic concentrations in the absence and presence of S9 activation. TAK-491 and TAK-536 did not induce unscheduled DNA synthesis in rats, and did not induce micronucleated cells in mouse and/or rat bone marrow *in vivo*. The dose of TAK-491 used in mice also was sufficient to qualify the effects of TAK-536 M-II in this assay. TAK-536 did not induce chromosomal aberrations in mouse lymphoma cells.

Carcinogenicity

Both TAK-491 and TAK-536 M-II were separately evaluated for carcinogenicity in 26-week Tg.rasH2 mouse studies or in 24-month rat studies. TAK-536 was evaluated in 24-month studies in both rat and mouse. All dose selections were evaluated and approved by the Executive CAC, and no evidence of carcinogenicity was apparent in any of the studies.

TAK-491 was not carcinogenic at the highest oral gavage dose of 450 mg/kg/day (based on MTD) in a 26-week Tg.rasH2 mouse study or at the highest oral gavage dose of 600 mg/kg/day (MTD basis) in a 24-month rat study. Exposure ratios, as measured by TAK-536, were 7X and 17X human exposure, in male and female mice, respectively. In rats, human exposure ratios were 25X and 28X, in male and female rats, respectively. These exposure margins are based on a MRHD of 80 mg/day of TAK-491.

Similarly, dosing with azilsartan (TAK-536) was also not carcinogenic at the highest dietary dose of 100 mg/kg/day (MTD basis) in a 24-month mouse study or at the highest dietary dose of 300 mg/kg/day (MTD basis) in a 24-month rat study. Exposure margins were 13X and 25X for male and female mice, and 15X and 13X for male and female rats, respectively. These margins are based on a daily MRHD of 5mg TAK-536, thus these studies were considered adequate, at that time, because TAK-536 was the proposed drug product. These ratios are irrelevant now that TAK-491 is the actual drug product and the MHRD for TAK-491 is 80 mg/day.

Finally, TAK-536 M-II (the major metabolite in human) was not carcinogenic at the highest dietary concentration of 5% (MFD basis) in a 26-week Tg.rasH2 mouse study or at the highest oral gavage doses of 1000 mg/kg/day (male) or 3000 mg/kg/day (female) in a 24-month rat study. Exposure margins were 21X and 38X for male and female

mice, and 7.4X for both male and female rats, respectively, relative to human AUC for TAK-536 M-II at the MRHD of 80 mg/day TAK-491.

Reprotoxicity

Administration of TAK-491 up to 1000 mg/kg/day to male and female rats (beginning 14 days before mating, through the mating period and until the day before necropsy for males; 14 days before mating, through the mating period and until day 6 of gestation for females) had no effect on mating or fertility indices or on reproductive performance of pregnant F₀ females. However, there was a dose-dependent decrease in body weight at necropsy (from 5 to 7%) and a dose-dependent decrease in body weight gain (22-32%) over the whole dose range (10 to 1000 mg/kg/day) in both males and females (during gestation). There also was a decrease in food consumption that was not dose-dependent (10% at all doses) in both males and females. Significant decreases in all these effects were observed at the lowest dose of 10 mg/kg/day in both males and females. At this dose, the exposure in males was 164 µg.hr/ml (exposure margin of 4.2X) and in females 188 µg.hr/ml (exposure margin of 4.8X). There was no effect on the F₁ fetuses as assessed by body weight, external, skeletal and visceral examinations. Thus, the study suggests that TAK-491 does not have adverse effects on the F₁ generation when given to the F₀ generation before conception and during the early stages of pregnancy.

TAK-491 (10, 100 and 1000 mg/kg/day) was administered to pregnant rats during the period of organogenesis from Day 6 to Day 17 of gestation. In the dams, statistically significant decreases in body weight gain and food consumption were observed at all doses. Body weight gain was decreased to the same extent at all doses (5%). Food consumption was decreased also by the same amount at all doses (7%).

In the fetuses, there was a statistically significant increase in the frequency of short supernumerary rib at the highest dose only. No effects were observed at the other doses. There was a dose-dependent increase in the frequency of dilated renal pelvis: 3.7% at 100 mg/kg/day and 6.84% at 1000 mg/kg/day. The frequency of this variation seen in control animals was 1.43%. This finding may represent a pseudohydronephrosis. The cranial-facial defects reminiscent of that seen in humans on ACE-inhibitors, and expected of any important blockade of the renin/angiotensin/aldosterone system (RAAS) during organogenesis, was not observed, consistent with insensitivity of animal models to detect this teratogenic effect of RAAS blockade. Accordingly, labeling should still carry a warning not to use this during pregnancy.

No toxicokinetic data was provided in this study.

Thus, in this study, there was general toxicity seen in the dams, no reproductive toxicity in the dams, and an increase in skeletal and visceral variations in the fetuses which reached statistical significance at 1000 mg/kg/day (166 mg/kg/day when converted to the human dose based on surface area). The human dose is 80 mg/day.

TAK-491 (10, 30 and 50 mg/kg/day) was administered to pregnant rabbits during the period of organogenesis from Day 6 to Day 18 of gestation. There was a dose-dependent increase in the number of deaths: 2 at 10 and 7 at 50 mg/kg/day. Body weight also decreased dose-dependently: 5% at mid-dose and 8.7% at high dose. Food consumption decreased 29% at lowest dose and 66% at the highest dose. There was an increase in plasma sodium: 0.7% at the lowest dose and 2% at the highest dose. A decrease in plasma calcium: 3.6% at the lowest dose and 17% at the highest dose. An increase in BUN: 350% at mid-dose and 622% at the highest dose. An increase in creatinine: 10% at the lowest dose and 1279% at the highest dose. An increase in inorganic phosphorus: 67% at the mid-dose and 115% at the highest dose. An increase in total cholesterol: 36% at the mid-dose and 50% at the highest dose. An increase in glucose: 4% at the lowest dose and 17% at the highest dose. An increase in potassium: 14% at the mid-dose and 22% at the highest dose. A decrease in chloride: 2% at the mid-dose and 5% at the highest dose.

In reproduction: Post-implantation loss was seen only at the highest dose: a 179% increase. Embryo-fetal deaths increased: 300% at the mid-dose and 536% at the highest dose. Finally, there was a decrease in the number of live fetuses: 19% only seen at the highest dose. The exposure to TAK-536 in this study was 368 µg·hr/ml at the mid-dose of 30 mg/kg/day, the NOAEL for embryo-fetal development. This is an exposure margin of 9.2X the human exposure at 80 mg/day.

A perinatal and postnatal toxicity study was performed with TAK-491 (0.1, 1 and 10 mg/kg/day) in rats. Dams were administered the test article from Day 6 of gestation to Day 21 after delivery. In the dams, there was dose-dependent decrease in body weight gain: 2% at the mid-dose and 5% at the high dose (during lactation). Additionally, there was a non-dose dependent decrease in food consumption: 14% at both the mid-dose and high dose (during gestation).

In F1 pups, a decrease in live births was observed: 3% at the lowest dose and 12% at the highest dose. A decrease in viability index: 3% only observed at the highest dose. A dose-dependent decrease in body weight during lactation: 5% at the mid-dose (day 7) and 15% at the high dose (day 22). Developmentally delayed incisor eruption was observed: 31% at the mid-dose and 34% at the high dose. Finally, renal pelvis dilatation was observed: increase of 1.32 % at all doses. The F2 pups were normal in all areas. No TK analysis was performed in these animals.

Since the major metabolite of azilsartan medoxomil, TAK-536 M-II, is found in only small amounts all species used in toxicology testing, including rats and rabbits, reproductive toxicology studies were performed with this compound.

TAK-536 M-II (120, 600 and 3000 mg/kg/day) was administered to male rats 14 days before mating through the mating period and until 1 day before necropsy (42-45 days total) and to female rats 14 days before mating, through the mating period and until Day 6 of gestation to study whether this metabolite had any effects on fertility and early embryonic development. All animals survived the duration of the study. Grayish-white

stools were observed in all animals at the highest dose administered (3000 mg/kg/day). There was a transient decrease in food consumption in males, but no other effects were seen in males, females or offspring. This study demonstrates that TAK-536 M-II did not affect the F₁ generation after administration of the metabolite to the F₀ generation before conception and during the early stages of pregnancy.

TAK-536 M-II (120, 600 and 3000 mg/kg/day) was administered to pregnant rats from gestation day 6 to gestation day 17 to assess the effect of the metabolite on organogenesis. All dams survived the duration of the study. Grayish-white stools were observed in all dams at the highest dose (3000 mg/kg/day). There was a transient but significant decrease in food consumption in dams at 3000 mg/kg/day. No other effects were seen in dams or offspring. Thus, the metabolite appeared to have no adverse effects on dams or teratogenic effects on the offspring.

TAK-536 M-II (300, 1000 and 3000 mg/kg/day) was administered to pregnant rabbits from gestation day 6 to gestation day 18 to assess the effect of the metabolite on organogenesis in this species. Toxicological effects were observed only in does at the highest dose tested. There was a decrease in food consumption and suppressed body weight gain at 3000 mg/kg/day. One doe died in this group. One died in the 1000 mg/kg/day group. The death of this latter animal was attributed to dosing error. The exposure to this metabolite on gestation day 6 at the 1000 mg/kg/day dose (49 µg.hr/ml) is very close to (2x) that seen in human after 80 mg (24 µg.hr/ml). There were no effects on the embryo-fetal development at any dose. (AUC 233 ug.hr/ml at gestation day 6 with the highest dose). The exposure margin relative to human was approximately 10X.

Tabulated findings relating dose, AUC and human exposure multiples.

NOAELs, AUCs and human exposure ratios for pivotal repeat-dose toxicity studies are presented in the following 2 Tables (based on AUC for TAK-536 and for TAK-536 M-II).

Exposure Margins (as TAK-536) at the Proposed Clinical Dose of 80 mg/day TAK-491

Endpoint From Nonclinical Toxicity Studies with TAK-491 or TAK-536	Species	Dose Duration	NOAEL (mg/kg/day)	TAK-536 AUC(0-24) (µg-hr/mL) (a)	Exposure Margin (b)	Source
NOAEL/repeat-dose	Rat (M)	26 weeks	20	237	5.9	491-00167
NOAEL/repeat-dose	Rat (F)	26 weeks	200	931	23.4	491-00167
NOAEL/repeat-dose	Dog (M)	26 weeks	60	173	4.3	491-00166
NOAEL/repeat-dose	Dog (F)	26 weeks	12	45.9	1.2	491-00166
NOAEL/repeat-dose (c)	Dog (M)	26/52 weeks	30	78.0	2.0	536-C-46-00267/536-C-46-00263
NOAEL/repeat-dose (c)	Dog (F)	26/52 weeks	30	81.7	2.1	536-C-46-00267/536-C-46-00263
NOAEL/carcinogenicity	Mouse (M)	26 weeks	450	292	7.3	491-10809-001A
NOAEL/carcinogenicity	Mouse (F)	26 weeks	450	693	17.4	491-10809-001A
NOAEL/carcinogenicity	Rat (M)	104 weeks	600	1013	25.4	491-10808
NOAEL/carcinogenicity	Rat (F)	104 weeks	600	1128	28.3	491-10808
NOAEL/embryo-fetal development	Rabbit (F)	2 weeks	30	368	9.2	491-00247

F=female, M=male.

(a) Data at last collection interval.

(b) Nonclinical AUC(0-24)/Clinical AUC(0-tlqc). Human plasma AUC(0-tlqc) values were based on data obtained in healthy subjects dosed with TAK-491 80 mg [491-017]; human AUC(0-tlqc) of 39.84 µg-hr/mL (geometric mean).

(c) Dogs in this study were dosed with TAK-536 for 52 weeks with a 26-week interim sacrifice. AUC(0-24) values provided here were obtained in Week 52.

TAK-536 M-II Exposure Margins at the Proposed Clinical Dose of 80 mg/day TAK-491

Endpoint from Nonclinical Toxicity Studies (a)	Species	Dose Duration	TAK-536 M-II AUC(0-24) (µg-hr/mL) (b)	Exposure Margin (c)	Source
NOAEL/repeat-dose	Rat (M)	13 weeks	153	6.3	536-C-46-00454
NOAEL/repeat-dose	Rat (F)	13 weeks	143	5.9	536-C-46-00454
NOAEL/repeat-dose	Dog (M)	13 weeks	151	6.2	536-C-46-00458
NOAEL/repeat-dose	Dog (F)	13 weeks	185	7.6	536-C-46-00458
NOAEL/carcinogenicity	Mouse (M)	26 weeks	514	21.2	536-10010
NOAEL/carcinogenicity	Mouse (F)	26 weeks	926	38.1	536-10010
NOAEL/carcinogenicity	Rat (M)	104 weeks	179	7.4	536-10008
NOAEL/carcinogenicity	Rat (F)	104 weeks	181	7.4	536-10008
NOAEL/embryo-fetal development	Rabbit (F)	2 weeks	391	16.1	536-C-46-00464

F=female, M=male.

(a) NOAEL based on studies using TAK-536 M-II.

(b) Values at last collection interval.

(c) Nonclinical AUC(0-24)/Clinical AUC(0-tlqc). Human plasma AUC(0-tlqc) values were based on data obtained in healthy subjects dosed with TAK-491 80 mg [491-017]; human AUC(0-tlqc) of 24.29 µg-hr/mL (geometric mean).

12 Appendix/Attachments

1. Pharmacology Overview – Comprehensive listing of all submitted pharmacology studies conducted, including Safety Pharmacology
2. Pharmacokinetics Overview - Comprehensive listing of all submitted pharmacokinetic studies
3. Toxicology Overview - Comprehensive listing of all submitted toxicology studies , including General Tox, Genetic Tox, Reproductive Tox and Carcinogenicity studies

TAK-491

2.6.3.1 Pharmacology Overview

2.6.3.1 Pharmacology Overview

Type of Study (Test Article)	Test System	Method of Administration	Testing Facility	TPC Report Number
Primary Pharmacodynamics				
In Vitro Studies				
Receptor binding (TAK-536)	hAT1 receptors coated on membranes	In vitro	Takeda Pharmaceutical Co., Ltd.	TAK-491-00053-001R
Receptor binding (TAK-536)	Rabbit or bovine AT1 or AT2 receptors	In vitro	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
Receptor binding dissociation (TAK-536)	hAT1 receptors	In vitro	Takeda Pharmaceutical Co., Ltd.	TAK-536-10013
Angiotensin II-induced artery contraction (TAK-536)	Rabbit aorta	In vitro	Takeda Pharmaceutical Co., Ltd.	TAK-491-00054-001R
Angiotensin II-induced artery contraction (TAK-536)	Rabbit aorta	In vitro	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
Receptor binding (TAK-536, TAK-536 M-I, TAK-536 M-II)	hAT1 receptors	In vitro	Takeda Pharmaceutical Co., Ltd.	TAK-491-00138
Receptor binding (TAK-536, TAK-536 M-I)	Bovine AT1 receptors	In vitro	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00171
In Vivo Studies				
Angiotensin II-induced pressor response (TAK-491)	Rat/Sprague-Dawley	Oral, gavage	Takeda Pharmaceutical Co., Ltd.	TAK-491-00055-001R
Hypertension and HR (TAK-491)	SHR	Oral, gavage	Takeda Pharmaceutical Co., Ltd.	TAK-491-00056-001R
Hypertension and HR (TAK-491)	SHR	Oral, gavage	Takeda Pharmaceutical Co., Ltd.	TAK-491-00057-001R
Hypertension and HR (TAK-491)	Dog/beagle with 2 kidney, 1 clip renal hypertension	Oral, gavage	Takeda Pharmaceutical Co., Ltd.	TAK-491-00249
Hypertension and HR (TAK-491)	Dog/beagle with 2 kidney, 1 clip renal hypertension	Oral, gavage	Takeda Pharmaceutical Co., Ltd.	TAK-491-00058-001R
Angiotensin II-induced pressor response (TAK-536)	Rat/strain not indicated	Oral, gavage or IV	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A

TAK-491

2.6.3.1 Pharmacology Overview

2.6.3.1 Pharmacology Overview (continued)

Type of Study (Test Article)	Test System	Method of Administration	Testing Facility	TPC Report Number
Primary Pharmacodynamics (continued)				
In Vivo Studies (continued)				
All-induced pressor response (TAK-536)	Dog/beagle	Oral, gavage or IV	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057.002A
Hypertension and HR (TAK-536)	SHR	Oral, gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
Hypertension and HR (TAK-536)	Rat/Wistar with 2 kidney, 1 clip renal hypertension	Oral, gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
Hypertension and HR (TAK-536)	Rat/Wistar with 1 kidney, 1 clip renal hypertension	Oral, gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
Hypertension and HR (TAK-536)	Dog/beagle with 2 kidney, 1 clip renal hypertension	Oral, gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
Hypertension (TAK-536)	Rat/Wistar with DOCA/salt induced hypertension	Oral, gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
BP and HR (TAK-536)	Normotensive Rat/Wistar	Oral, gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
Renin-Angiotensin-Aldosterone System (TAK-536)	Rat/normotensive, strain not indicated	Oral, gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00057-002A
All-induced pressor response (TAK-536, TAK-536 M-I)	Rat/strain not indicated	IP	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00171
Secondary Pharmacodynamics (Test Article)				
In Vitro Studies				
Receptor, ion channel binding, and enzyme activity (TAK-491F)	Various receptors and enzymes	In vitro	(b) (4)	TAK-491-00185
Receptor, ion channel binding, and enzyme activity (TAK-491)	Various receptors and enzymes	In vitro	(b) (4)	TAK-491-00200
In vitro assays, receptor, ion channel binding, and enzyme activity (TAK-536)	Various receptors and enzymes	In vitro	(b) (4)	TAK-536-C-46-00434
Receptor, ion channel binding, and enzyme activity (TAK-536)	Various receptors and enzymes	In vitro	(b) (4)	TAK-536-C-46-00440

TAK-491

2.6.3.1 Pharmacology Overview

2.6.3.1 Pharmacology Overview (continued)

Type of Study (Test Article)	Test System	Method of Administration	Testing Facility	TPC Report Number
Secondary Pharmacodynamics (continued)				
In Vitro Studies (continued)				
Receptor, ion channel binding, and enzyme activity (TAK-536 M-II)	Various receptors and enzymes	In vitro	(b) (4)	TAK-536-C-46-00435-001A
Receptor, ion channel binding, and enzyme activity (TAK-536 M-II)	Various receptors and enzymes	In vitro	(b) (4)	TAK-536-C-46-00441
IRS-1 tyrosine phosphorylation (an insulin sensitivity marker) (TAK-536)	Rat primary skeletal muscle cells	In vitro	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00371
In Vivo Studies				
Effects on proteinuria (TAK-491)	Rat/Wistar fatty (diabetic) and Wister lean (non-diabetic control)	Oral, gavage	Takeda Pharmaceutical Co., Ltd.	TAK-491-00060-001R
Insulin sensitivity measured by hyperinsulinemic-euglycemic clamp technique (TAK-491)	SHR	Oral, gavage	Takeda Pharmaceutical Co., Ltd.	TAK-491-00059-001R
Insulin sensitivity measured by hyperinsulinemic-euglycemic clamp technique (TAK-536)	SHR	Oral, gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00359
Glucose intolerance and adipocyte differentiation (TAK-536)	Mouse/KK-Ay (diabetic) and C57BL/6 (non-diabetic control)	Oral, dietary admixture	(b) (4)	Publication, (AJH, 2007, 20(5):579-86)
Various in vivo screening for CNS, cardiovascular, renal gastrointestinal, immune, and metabolic effects (TAK-536)	Various	Oral by gavage, IP, and ICVT	(b) (4)	TAK-536-C-46-00434
Safety Pharmacology				
Effects on the central nervous system Behavior observation (Irwin's test) (TAK-491)	Rat/Sprague-Dawley	Oral, gavage	(b) (4)	TAK-491-00031-001A
Effects on the cardiovascular systems hERG current (whole cell clamp method) (TAK-536)s	HEK293 cells expressing hERG channels	In vitro	(b) (4)	TAK-491-00035

TAK-491

2.6.3.1 Pharmacology Overview

2.6.3.1 Pharmacology Overview (continued)

Type of Study (Test Article)	Test System	Method of Administration	Testing Facility	TPC Report Number
Safety Pharmacology (continued)				
Effects on the cardiovascular systems (continued)				
hERG current (whole cell clamp method) (TAK-536 M-II)	HEK293 cells expressing hERG channels	In vitro	(b) (4)	TAK-536-C-46-00375
Action potential parameters (TAK-536)	Guinea pig/ Hartley, isolated papillary muscle	In vitro		TAK-491-00033-001A
BP, HR, and ECG (TAK-491)	Dog/beagle (telemetry)	Oral, gavage		TAK-491-00040-001A
Effects on the respiratory systems				
Respiratory rate, tidal volume, minute volume, and Penh (TAK-491)	Rat/Sprague-Dawley	Oral, gavage		TAK-491-00032-001A
General Pharmacology of TAK-536				
Various studies in central nervous, autonomic nervous, cardiovascular, renal, gastrointestinal, immune and reproductive systems (TAK-536)	Mouse/ICR, rat/Sprague-Dawley, guinea pig/Hartley, rabbit/New Zealand white, cat/mongrel, dog/beagle	In vitro, oral by gavage, and intraduodenal	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00058
Pharmacodynamic Drug Interactions				
Plasma diabetes parameters and body weight (TAK-536)	Rat/Wistar fatty (diabetic) and Wistar lean (non-diabetic control)	Oral, gavage	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00360-002R

All=angiotensin II, AT1=angiotensin II type 1 receptor, AT2=angiotensin II type 2 receptor, BP=blood pressure, CNS=central nervous system, DOCA=deoxycorticosterone-acetate, ECG=electrocardiogram, hAT1=human AT1 receptors, HEK=human embryonic kidney, hERG=human *ether-à-go-go*-related gene, HR=heart rate, ICVT=intracerebroventricular, IP=intraperitoneal, IRS-1=tyrosine phosphorylation of insulin receptor substrate-1, IV=intravenous, OM=olmesartan medoxomil, Penh=enhanced pause, SHR=spontaneously hypertensive rat, TAK-491F=TAK-491 salt free form, TPC=Takeda Pharmaceutical Co., Ltd.

TAK-491

2.6.5.1 Pharmacokinetics Overview

2.6.5.1 Pharmacokinetics Overview

Type of Study	Test System	Method of Administration	Testing Facility	TPC Report Number	
Absorption					
Plasma TAK-536 concentration (1250 and 5000 mg/kg/day for 2 days)	Mice	Oral gavage	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00070	
Absorption (TAK-491F and metabolites)	Rats	Oral gavage, IV	(b) (4)	TAK-491-00079	
Absorption (radioactivity)	Rats	Oral gavage, IV		TAK-491-00043	
Linearity in plasma concentration of TAK-491F and metabolites	Rats	Oral gavage		TAK-491-10039	
Absorption (radioactivity) after repeated doses	Rats	Oral gavage		TAK-491-10034	
Absorption site of [¹⁴ C]TAK-491	Rats	Intra-gastric		TAK-491-10035	
Radioactivity concentration in portal plasma	Rats	Intra-jejunal		TAK-491-10036	
Lymphatic absorption of radioactivity	Rats	Oral gavage		TAK-491-10037	
Enterohepatic circulation	Rats (bile duct-cannulated)	Intraduodenal		TAK-491-00075	
Absorption of TAK-536	Rats, dogs	Oral gavage		Takeda Chemical Industries, Ltd.	TAK-536-C-46-00017
Absorption and excretion of [¹⁴ C]TAK-536	Rats, dogs	Oral gavage, IV		Takeda Chemical Industries, Ltd.	TAK-536-C-46-00122
Pharmacokinetics of [¹⁴ C]TAK-536	Rats, dogs	Oral gavage, IV	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00292	
Absorption	Dogs	Oral gavage, IV	(b) (4)	TAK-491-00051	
Absorption (TAK-491F, metabolites)	Dogs	Oral gavage, IV		TAK-491-00080	
Linearity in plasma concentration of TAK-491F and metabolites	Dogs	Oral gavage		TAK-491-10040	
Absorption of [¹⁴ C]TAK-536	Monkeys	Oral gavage		TAK-536-C-46-00413	
Absorption of TAK-536	Monkeys	Oral gavage, IV	TAK-536-C-46-00414		
Permeability study of TAK-491	Caco-2 cells	In vitro	TAK-491-00214		
Inhibitory effect of TAK-491 on [³ H]Digoxin transport	Caco-2 cells	In vitro	TAK-491-00215		
Permeability study of TAK-536	Caco-2 cells	In vitro	TAK-536-C-46-00445		
Inhibitory effect of TAK-536 on [³ H]Digoxin transport across Caco-2 cells	Caco-2 cells	In vitro	TAK-536-C-46-00446		

TAK-491

2.6.5.1 Pharmacokinetics Overview

2.6.5.1 Pharmacokinetics Overview (continued)

Type of Study	Test System	Method of Administration	Testing Facility	TPC Report Number
Distribution				
Distribution in bone marrow	Mice, rats	Oral gavage	Takeda Chemical Industries, Ltd. (b) (4)	TAK-536-C-46-00170
Tissue distribution	Rats (albino & pigmented)	Oral gavage		TAK-491-00052-001A
Whole body autoradiography	Rats	Oral gavage		TAK-491-10041
Plasma protein binding and blood cell distribution of radioactivity	Rats	Oral gavage		TAK-491-10044
Placental transfer of [¹⁴ C]TAK-491	Rats	Oral gavage		TAK-491-10046
Plasma protein binding and blood cell distribution of radioactivity	Dogs	Oral gavage		TAK-491-10045
Plasma protein binding of [¹⁴ C]TAK-536	Mice, rats, dogs, and humans	In vitro		TAK-491-10042
Distribution of [¹⁴ C]TAK-536 in blood cells	Rats, dogs, and humans	In vitro		TAK-491-10043
Metabolism				
Metabolite characterization	Rats and human hepatic microsomes	Oral gavage, in vitro	Takeda Pharmaceutical Co. Ltd. (b) (4)	TAK-491-00087
Metabolites in urine, feces, and bile	Rats	Oral gavage, ID		TAK-491-00076
Metabolite profiles of portal plasma and GI	Rats	Intra-jejunal		TAK-491-10048
Metabolite profiles in the maternal and fetal plasma	Rats (pregnant)	Oral gavage		TAK-491-10047
Metabolite profiles in the plasma and milk	Rats (pregnant)	Oral gavage		TAK-491-10050
Metabolite M-I identification	Rats	Oral gavage		TAK-536-C-46-00199
Metabolites in urine and feces	Dogs	Oral gavage		TAK-491-00077
Plasma metabolite profiling	Monkeys	Oral gavage		TAK-536-C-46-00416
Urine and fecal metabolite profiling	Monkeys	Oral gavage		TAK-536-C-46-00417
Metabolism of [¹⁴ C]TAK-536 by hepatic microsomes	Human and animal hepatic microsomes	In vitro		TAK-491-00048
Identification of CYP involved in the metabolism of [¹⁴ C]TAK-536	Human hepatic and CYP-expressing microsomes	In vitro		TAK-491-00049
Hydrolysis of [¹⁴ C]TAK-491 to [¹⁴ C]TAK-536	Rat, dog, human hepatic and intestinal S9 fractions	In vitro		TAK-491-00047

TAK-491

2.6.5.1 Pharmacokinetics Overview

2.6.5.1 Pharmacokinetics Overview (continued)

Type of Study	Test System	Method of Administration	Testing Facility	TPC Report Number
Metabolism (continued)				
Effect of diisopropyl fluorophosphate on hydrolysis of [¹⁴ C]TAK-491	Human plasma	In vitro	(b) (4)	TAK-491-00120
Inhibition of cytochrome P450 isoforms	Microsomes expressing human CYP isoforms	In vitro	Takeda Analytical Laboratories, Ltd.	TAK-491-00050
Hydrolysis of [¹⁴ C]TAK-491 to [¹⁴ C]TAK-536	Human serum albumin	In vitro	(b) (4)	TAK-491-00126
Stability of [¹⁴ C]TAK-491 in plasma	Rat, dog, and human plasma	In vitro	(b) (4)	TAK-491-00078
Hydrolysis pathway of [¹⁴ C]TAK-491	Rat, and human hepatic and intestinal S9 fractions	In vitro	(b) (4)	TAK-491-00238
CYP3A induction by TAK-536	Human hepatocytes	In vitro	Takeda Pharmaceutical Co. Ltd.	TAK-491-00086
Inhibitory effect of TAK-491 on CYP activity	Human hepatic microsomes	In vitro	(b) (4)	TAK-491-10051
Inhibitory effect of TAK-536 on CYP activity	Human hepatic microsomes	In vitro	(b) (4)	TAK-491-10052
CYP3A induction by TAK-491	Human hepatocytes	In vitro	(b) (4)	TAK-491-10053
CYP3A induction by TAK-536	Human hepatocytes	In vitro	(b) (4)	TAK-491-10054
Excretion				
Excretion	Rats	Oral gavage	(b) (4)	TAK-491-00044
Excretion (biliary)	Rats (bile duct-cannulated)	Intraduodenal	(b) (4)	TAK-491-00045
Lacteal secretion	Rats	Oral gavage	(b) (4)	TAK-491-10049
Excretion of [¹⁴ C]TAK-536	Monkeys	Oral gavage	(b) (4)	TAK-536-C-46-00413
Pharmacokinetic Drug-Drug Interactions				
Pioglitazone inhibited absorption and elimination of TAK-536	Monkeys	Oral gavage, IV	(b) (4)	TAK-536-C-46-00415

TAK-491

2.6.5.1 Pharmacokinetics Overview

Page 4 of 4
07 January 2010**2.6.5.1 Pharmacokinetics Overview (continued)**

Type of Study	Test System	Method of Administration	Testing Facility	TPC Report Number
Others				
Inhibition of CYP by Chlorthalidone	Human hepatic microsomes and microsomes expressing human CYP	In vitro	Takeda Analytical Laboratories Ltd.	TAK-491CLD-00068
Inhibition of CYP by Chlorthalidone	Microsomes expressing human CYP	In vitro	(b) (4)	TAK-491CLD-00069
Permeability study of Chlorthalidone	Caco-2 cells	In vitro	(b) (4)	TAK-491-00272

Additional Information:

CYP=cytochrome P450, GI=gastrointestinal, IV=intravenous administration, TAK-536=active moiety of TAK-491, TPC=Takeda Pharmaceutical Co., Ltd.

TAK-491
2.6.7.1 Toxicology Overview

Page 1 of 19
08 March 2010

2.6.7.1 Toxicology Overview

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Single-dose toxicity	Mouse/ICR	Oral, gavage/ 5% (w/v) gum arabic	Single dose	TAK-536: 0, 750, 1500, and 3000	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00077
	Rat/F344	Oral, gavage/ MC+CA	Single dose	TAK-491: 0, 500, and 2000	Yes	Takeda Pharmaceutical Co., Ltd.	TAK-491-00036
	Rat/F344	Oral, gavage/ MC+CA	Single dose	TAK-491: 600, 1000, and 2000	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00002
	Rat/Wistar	Oral, gavage/ 5% (w/v) gum arabic	Single dose	TAK-536: 0, 750, 1500, and 3000	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00078
	Rat/F344	Oral, gavage/ 5% (w/v) gum arabic	Single dose	TAK-536: 300	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00361
	Rat/F344	IV injection/ DMAc	Single dose	TAK-491: 0 (saline), 0 (vehicle), 12.5, 50, and 200	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00125
	Rat/F344	IV injection/ DMAc	Single dose	TAK-491: 0 (saline), 0 (vehicle), 8, 40, and 200	Yes	Takeda Pharmaceutical Co., Ltd.	TAK-491-00088-001A
	Rat/F344	IV injection/ 2% (w/v) propylene glycol + megl	Single dose	TAK-536: 0, 8, 40, 200, and 1000	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00364
	Rat/F344	IV injection/ 2% (w/v) propylene glycol + megl	Single dose	TAK-536: 0 (saline), 0 (vehicle), and 10	Yes	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00372-001A

TAK-491

Page 2 of 19

2.6.7.1 Toxicology Overview

08 March 2010

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Single-dose toxicity (continued)							
	Dog/beagle	Oral, gavage/ MC+CA	Escalating dose	TAK-491: 300 → 3 → 10 → 30 → 100	No	Takeda Chemical Industries, Ltd.	TAK-491-11321
	Dog/beagle	Oral, gavage/ MC+CA	Escalating dose	TAK-491: 0 → 0 → 0 → 0 → 0, 30 → 100 → 300 → 1000 → 2000	Yes	Takeda Pharmaceutical Co., Ltd.	TAK-491-00029
	Dog/beagle	Oral, gavage/ MC+CA	Escalating dose	TAK-491: 0 → 3 → 10 → 30	Yes	Takeda Pharmaceutical Co., Ltd.	TAK-491-00030-001A
	Dog/beagle	Oral, gavage/ 5% (w/v) gum arabic	Escalating dose	TAK-536: 0 → 0 → 0 → 0 → 0 → 0; 1 → 10 → 100 → 300 → 1000 → 2000	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00060
	Monkey/ cynomolgus	Oral, gavage/ MC	Escalating dose	TAK-536: 20 → 200 → 2000	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00363
Repeat-dose toxicity							
	Mouse/B6C3F1	Oral, gavage/ MC+CA	4 weeks	TAK-491: 0, 20, 200, and 2000	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00136
	Mouse/ CByB6F1 hybrid	Oral, gavage/ MC+CA	4 weeks	TAK-491: 0, 200, 600, 2000, and 3000	Yes	(b) (4)	TAK-491-00274-001A
	Mouse/B6C3F1	Oral, gavage/ MC+CA	13 weeks	TAK-491: 0, 20, 200, and 2000	Yes	(b) (4)	TAK-491-00163
	Mouse/B6C3F1	Oral, dietary admixture/ basal diet	13 weeks	TAK-536: 0, 3, 30, 300, and 3000	Yes	Takeda Chemical Industries, Ltd.	TAK-536- C-46-00243-002

TAK-491

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Repeat-dose toxicity (continued)							
	Rat/F344	Oral, gavage/ MC+CA	4 weeks	TAK-491: 0, 2, 20, <u>200</u> , and 2000	Yes	(b) (4)	TAK-491-00127
	Rat/F344	Oral, gavage/ 5% (w/v) gum arabic	4 weeks	TAK-536: 0, 3, 30, <u>300</u> , and 3000	Yes	(b) (4)	TAK-536-C-46-00061
	Rat/F344	Oral, gavage/ MC+CA	13 weeks	TAK-491: 0, <u>200</u> , 600, and 2000	Yes	(b) (4)	TAK-491-00143-001A
	Rat/F344	Oral, gavage/ 5% (w/v) gum arabic	13 weeks	TAK-536: 0, 3, 30, <u>300</u> , and 3000	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00207-002
	Rat/F344	Oral, dietary admixture/ basal diet	13 weeks	TAK-536: 0, 300, 1000, and 3000	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00251
	Rat/F344	Oral, gavage/ MC+CA	26 weeks	TAK-491: 0, 2, <u>20</u> , 200, and 2000	Yes	(b) (4)	TAK-491-00167
	Rat/F344	Oral, gavage/ 5% (w/v) gum arabic	26 weeks	TAK-536: 0, <u>1</u> , 10, 100, and 1000	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00266
	Dog/beagle	Oral, gavage/ MC+CA	2 weeks	TAK-491: 0, 10, <u>30</u> , and 100	No	Takeda Chemical Industries, Ltd.	TAK-491-11327-001A
	Dog/beagle	Oral, gavage/ MC+CA	4 weeks	TAK-491: 0, 3, <u>12</u> , 60, and 300	Yes	(b) (4)	TAK-491-00123
	Dog/beagle	Oral, gavage/ MC+CA	4 weeks with a 4 week recovery	TAK-491: 0 and 200	Yes	(b) (4)	TAK-491-00251
	Dog/beagle	Oral, gavage/ 5% (w/v) gum arabic	4 weeks	TAK-536: 0, 30, <u>100</u> , 300, and 1000	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00062-updated

TAK-491

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Repeat-dose toxicity (continued)							(b) (4)
	Dog/beagle	Oral, gavage/ 5% (w/v) gum arabic	13 weeks	TAK-536: 0, 10, 30, <u>100</u> , and 500	Yes		TAK-536-C-46-00208-002
	Dog/beagle	Oral, gavage/ MC+CA	26 weeks	TAK-491: 0, 3, <u>12</u> , and 60	Yes		TAK-491-00166
	Dog/beagle	Oral, gavage/ 5% (w/v) gum arabic	26 weeks (interim sacrifice) and 52 weeks	TAK-536: 0, 10, <u>30</u> , 100, and 300	Yes		TAK-536-C-46-00267, TAK-536-C-46-00263 (TK report)
Genotoxicity							
Mutagenic and carcinogenic potential	Computer models: mutagenicity/ <i>Salmonella</i> , carcinogenicity/rodents		Not applicable	In silico	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00168
Bacterial reversion assay	<i>S typhimurium</i> <i>E coli</i>	In vitro/DMSO	48 hours	TAK-491 ±S9: 1.5 to 5000 µg/plate	Yes	(b) (4)	TAK-491-00037-001A
	<i>S typhimurium</i> <i>E coli</i>	In vitro/DMSO	48 hours	T-1302593 (TAK-491F) ±S9: 1.22 to 5000 µg/plate	Yes		TAK-491-00231
	<i>S typhimurium</i> <i>E coli</i>	In vitro/DMSO	72 hours	TAK-536 ±S9: 156 to 5000 µg/plate	Yes	Takeda Analytical Research Laboratories, Ltd.	TAK-536-C-46-00063
Bacterial reversion assay, supplemental	<i>S typhimurium</i> <i>E coli</i>	In vitro/DMSO	48 hours	TAK-536 ±S9: 15.0 to 5000 µg/plate	Yes	(b) (4)	TAK-536-C-46-00460
Forward mutation assay	CHO cells (CHO/HGPRT)	In vitro/saline and NaOH	4 hours	TAK-536: -S9: 250 to 5000 µg/mL +S9: 250 to 3000 µg/mL	Yes		TAK-536-C-46-00135

TAK-491

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Genotoxicity (continued)							
Forward mutation assay	Mouse lymphoma cells (L5178Y/tk)	In vitro/saline and NaOH	3 hours	TAK-536: -S9: 312.5 to 2500 µg/mL +S9: 78.125 to 1250 µg/mL	Yes	(b) (4)	TAK-536-C-46-00241
Cytogenetic assay	CHL/IU cells	In vitro/DMSO	6 and 24 hours	TAK-491: 6 hours, -S9: 550 to 1000 µg/mL 6 hours, +S9: 700 to 900 µg/mL 24 hours, -S9: 200 to 400 µg/mL	Yes		TAK-491-00041
	CHL cells	In vitro/saline and NaOH	6, 24, and 48 hours	TAK-536: 6 hours, ±S9: 2.5 to 10 mmol/L 24 hours, -S9: 0.625 to 2.5 mmol/L 48 hours, -S9: 0.313 to 1.25 mmol/L	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00137
In vivo/in vitro UDS assay	Rat/F344 hepatocytes	Oral, gavage/ MC+CA	Single dose	TAK-491: 0, 1000, and 2000	Yes	(b) (4)	TAK-491-00042
	Rat/F344 hepatocytes	Oral, gavage/ 5% (w/v) gum arabic	Single dose	TAK-536: 0, 100, 300, 1000, and 3000	Yes		TAK-536-C-46-00134
Micronucleus assay	Mouse/CD-1, male	Oral, gavage/ MC+CA	2 doses	TAK-491: 0, 500, 1000, and 2000	Yes	(b) (4)	TAK-491-00038
Plasma concentrations to support TAK-491-00038	Mouse/CD-1, male	Oral, gavage/ MC+CA	2 doses	TAK-491: 2000	Yes		TAK-491-00164-001A
Micronucleus assay	Mouse/(C3H x SWV)F1, male	Oral, gavage/ 5% (w/v) gum arabic	2 doses	TAK-536: 0, 1250, 2500, and 5000	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00064

TAK-491

Page 6 of 19
08 March 2010

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Genotoxicity (continued)							
Plasma concentrations to support TAK-536-C-46-00064	Mouse/(C3H x SWV)F1	Oral, gavage/ 5% (w/v) gum arabic	2 doses	TAK-536: 1250 and 5000	No	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00070
Micronucleus assay	Rat/F344, male	Oral, gavage/ MC+CA	2 doses	TAK-491: 0, 500, 1000, and 2000	Yes	(b) (4)	TAK-491-00039
In vivo cytogenetic	Rat/F344	Oral, gavage/ 5% (w/v) gum arabic	Single dose	TAK-536: 0, 750, 1500, and 3000	Yes	(b) (4)	TAK-536-C-46-00162
Carcinogenicity							
	Mouse/ Tg.rasH2	Oral, gavage/ MC+CA	26 weeks	TAK-491: 0, 50, 150, and 450	Yes	(b) (4)	TAK-491-10809-001A
	Mouse/B6C3F1	Oral, dietary admixture/ basal diet	24 months	TAK-536: 0, 10, 30, and 100	Yes	(b) (4)	TAK-536-C-46-00297
	Rat/F344	Oral, gavage/ MC+CA	24 months	TAK-491: 0, 0, 60, 200, and 600	Yes	(b) (4)	TAK-491-10808
	Rat/F344	Oral, dietary admixture/ basal diet	24 months	TAK-536: 0, 10, 30, 100, and 300	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00326
Reproductive and developmental toxicity							
Fertility and early embryonic development to implantation	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	M: 14 days prior to mating through 1000 day prior to necropsy F: 14 days prior to mating through GD 6	TAK-491: 0, 10, 100, and 1000	Yes	(b) (4)	TAK-491-00248

TAK-491

Page 7 of 19

2.6.7.1 Toxicology Overview

08 March 2010

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Reproductive and developmental toxicity (continued)							
Fertility and early embryonic development to implantation	Rat/Wistar, male	Oral, gavage/ 5% (w/v) gum arabic	M: 9 weeks prior to mating through day prior to necropsy	TAK-536: 0, 10, 30, and 100	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00065-001
Plasma concentrations to support fertility and early embryonic development	Rat/Wistar, male	Oral, gavage/ 5% (w/v) gum arabic	M: 13 weeks	TAK-536: 10, 30, and 100	No	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00099
Fertility and early embryonic development to implantation	Rat/Sprague-Dawley	Oral, gavage/ 5% (w/v) gum arabic	M: 9 weeks prior to mating through day prior to necropsy F: 2 weeks prior to mating through day prior to necropsy	TAK-536: 0, 1, 3, and 10	Yes	(b) (4)	TAK-536-C-46-00257
Embryo-fetal development-range-finding	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	GD 6-GD 17	TAK-491: 0, 10, 100, and 1000	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00028
Embryo-fetal development	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	GD 6-GD 17	TAK-491: 0, 10, 100, and 1000	Yes	(b) (4)	TAK-491-00246
Embryo-fetal development-range-finding	Rat/Sprague-Dawley	Oral, gavage/ 5% (w/v) gum arabic	GD 6-GD 17	TAK-536: 0, 30, 300, and 3000	No	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00146
Embryo-fetal development	Rat/Sprague-Dawley	Oral, gavage/ 5% (w/v) gum arabic	GD 6-GD 17	TAK-536: 0, 3, 10, 30, and 100	Yes	(b) (4)	TAK-536-C-46-00209-updated
Embryo-fetal development-range-finding	Rabbit/Kbl:JW	Oral, gavage/ MC+CA	GD 6-GD 18	TAK-491: 0, 10, 30, and 100	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00046

TAK-491

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Reproductive and developmental toxicity (continued)							
Single-dose toxicokinetic study	Rabbit/Kbl:JW, non-pregnant	Oral, gavage/ MC+CA	Single dose	TAK-491: 10, 30, and 100	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00216
Embryo-fetal development	Rabbit/Kbl:JW	Oral, gavage/ MC+CA	GD 6-GD 18	TAK-491: 0, 10, 30, and 50	Yes	(b) (4)	TAK-491-00247
Embryo-fetal development-range-finding	Rabbit/Kbl:JW	Oral, gavage/ 5% (w/v) gum arabic	GD 6-GD 18	TAK-536: 0, 1, 3, 10, 30, 100, 300, and 1000	No	Takeda Chemical Industries, Ltd.	TAK-536-1616te_1635te
Embryo-fetal development	Rabbit/Kbl:JW	Oral, gavage/ 5% (w/v) gum arabic	GD 6-GD 18	TAK-536: 0, 20, 100, and 500	Yes	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00196
Pre- and postnatal development, including maternal function-range-finding	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	GD 6-LD 21	TAK-491: 0, 0.1, 1, and 10	No	(b) (4)	TAK-491-00250
Pre- and postnatal development, including maternal function	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	GD 6-LD 21	TAK-491: 0, 0.1, 1, and 10	Yes	(b) (4)	TAK-491-00253
Pre- and postnatal development, including maternal function-range-finding	Rat/Sprague-Dawley	Oral, gavage/ 5% (w/v) gum arabic	GD 15-LD 21	TAK-536: 0, 3, 30, and 300	No	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00180
Pre- and postnatal development, including maternal function	Rat/Sprague-Dawley	Oral, gavage/ 5% (w/v) gum arabic	GD 15-LD 21	TAK-536: 0, 1, 3, 10, and 30	Yes	(b) (4)	TAK-536-C-46-00258
Pre- and postnatal development, including maternal function (supplemental study)	Rat/Sprague-Dawley	Oral, gavage/ 5% (w/v) gum arabic	GD 15-LD 21	TAK-536: 0, 0.03, 0.1, and 0.3	Yes	(b) (4)	TAK-536-C-46-00466

TAK-491

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Juvenile Toxicity							
0- and 7-day-old neonates	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	Single dose	TAK-491: 0 days old: 20 and 100; 7 days old: 100 and 500	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00273
0- and 7-day old neonates, heart development, range-finding	Rat/Sprague-Dawley	Oral/gavage, MC+CA	7 days	TAK-491: 0, 200, and 1000 Enalapril: 100	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-10810
0- to 6-day old neonates, heart development, range-finding (supplemental study)	Rat/Sprague-Dawley	Oral/gavage, MC+CA	7 days	TAK-491: 0, 20, and 100	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-10811
7-day old neonates, repeat-dose combination toxicity study with TAK-491 and TAK-536 M-II	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	5 weeks	TAK-491 + TAK-536 M-II: 0, 20/2000, 100/2000, and 500/2000	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00276
Local Tolerance							
	Whole blood and plasma from healthy male humans	In vitro/distilled water containing propylene glycol and megl	Not applicable	TAK-536: 0 (saline), 0 (vehicle), and 0.5 mg/mL	Yes	(b) (4)	TAK-536-C-46-00368
	Rabbit/Kbl:JW	Intravenous injection/ distilled water containing propylene glycol and megl	Single dose	TAK-536: 0 (saline), 0 (vehicle), and 0.5 mg/mL	Yes		TAK-536-C-46-00370
	Rabbit/Kbl:JW	Paravenous/ injection/ distilled water containing propylene glycol and megl	Single dose	TAK-536: 0 (saline), 0 (vehicle), and 0.5 mg/mL	Yes		TAK-536-C-46-00369

TAK-491
2.6.7.1 Toxicology Overview

Page 10 of 19
08 March 2010

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Other Toxicity							
Mechanistic Studies							
Mechanism for increased blood urea nitrogen	Rat/F344, males	Oral, gavage/ 5% (w/v) gum arabic	2 weeks	TAK-536: 0, 1, 10, and 100	No	Takeda Chemical Industries, Ltd.	TAK-536-C-46-00075
Saline supplementation combination toxicity study with pioglitazone and TAK-536	Rat/Sprague-Dawley	Oral, gavage/ MC	4 weeks	Controls: 0 (tap water) and 0 (saline); TAK-536: 300 (tap water) and 300 (saline); Pioglitazone/TAK-536: 4/300 (tap water) and 4/300 (saline)	No	Takeda Pharmaceutical Co., Ltd.	AD-4833-536-00062
Metabolite Studies (TAK-536 M-II)							
Single-Dose Toxicity							
	Rat/F344	Oral, gavage/ corn oil or MC	Single dose	TAK-536 M-II: Corn oil: 1500 and 3000; MC: 10,000 (5000 BID)	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00451
	Rat/F344	Oral, gavage/ MC or subcutaneous/ saline containing NaOH	Single dose	TAK-536 M-II: Oral: 10 and 30; Subcutaneous: 0.6 and 2	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00365
	Dog/beagle	Oral, gavage/ MC	Escalating dose	TAK-536 M-II: 200→ 600→ 2000	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00465

TAK-491

Page 11 of 19

2.6.7.1 Toxicology Overview

08 March 2010

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Metabolite Studies (TAK-536 M-II) (continued)							
Single-Dose Toxicity							
	Dog/beagle	Oral, gavage/ MC or subcutaneous/ saline containing NaOH	Singe dose	TAK-536 M-II: 30 (oral)→ 100 (oral)→ 0.8 (subcutaneous)	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00373
Repeat-Dose Toxicity							
	Mouse/CB6F1	Oral, gavage/ MC or corn oil or dietary admixture/basal diet	8 days	TAK-536 M-II: gavage/MC: 0, 2000, and 5000; gavage/corn oil: 0, 1000, and 3000; dietary admixture: 1.5% (M: 2819/F: 2828) and 5% (M:9048/F:10,215)	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00455
	Mouse/ C57BL/6J	Oral, gavage/ MC or subcutaneous/ saline containing NaOH	8 days	TAK-536 M-II: Controls: no treatment; Oral: 200 and <u>2000</u> ; Subcutaneous: 20 and <u>60</u>	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00452
	Mouse/ CBYB6F1 hybrid	Dietary admixture/basal diet	4 weeks	TAK-536 M-II: 0, 1.25% (1840/2455), 2.5% (3788/5144), and <u>5%</u> (<u>7712/9491</u>)	Yes	(b) (4)	TAK-536-C-46-00459
	Rat/F344	Oral, gavage/ MC	8 days	TAK-536 M-II: 0, 200, and 2000	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00449

TAK-491

Page 12 of 19

2.6.7.1 Toxicology Overview

08 March 2010

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Metabolite Studies (TAK-536 M-II) (continued)							
Repeat-Dose Toxicity (continued)							
	Rat/F344	Oral, gavage/ MC or dietary admixture/ basal diet	8 days	TAK-536 M-II: gavage: 0, 2000, and 5000; dietary admixture: 1.5% (M: 1589.6/F: 1681.2) and 5% (M: 5856.3/F: 5645.8)	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00450
	Rat/F344	Subcutaneous/ saline containing NaOH	7 days	TAK-536 M-II: 0, 20, and <u>60</u>	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00376
	Rat/F344, females	Oral, gavage/ corn oil	4 weeks	TAK-536 M-II: 0, 3000 (1500 BID), and 6000 (3000 BID)	No	(b) (4)	TAK-536-C-46-00456
	Rat/F344	Subcutaneous/ saline containing NaOH	4 weeks	TAK-536 M-II: 0, 6, 20, and <u>60</u>	Yes		TAK-536-C-46-00404-002A
	Rat/F344	Oral, gavage/ corn oil	13 weeks	TAK-536 M-II: 0, <u>300</u> , 1000, and 3000	Yes		TAK-536-C-46-00454
	Dog/beagle	Subcutaneous/ saline containing NaOH	7 days	TAK-536 M-II: 0, 10, and <u>30</u>	No		TAK-536-C-46-00374
	Dog/beagle	Subcutaneous/ saline containing NaOH	4 weeks	TAK-536 M-II: 0, 3, 10, and <u>30</u>	Yes		TAK-536-C-46-00410
	Dog/beagle	Oral, gavage/ MC	13 weeks	TAK-536 M-II: 0, 200, 600, and <u>2000</u>	Yes		TAK-536-C-46-00458

TAK-491
2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Metabolite Studies (TAK-536 M-II) (continued)							
Genotoxicity							
Mutagenic and carcinogenic potential	Computer models:		Not applicable	In silico	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00433
Bacterial reversion assay	<i>S typhimurium</i>	In vitro/DMSO	48 hours	TAK-536 M-II ±S9: 1.5 to 5000 µg/plate	Yes	(b) (4)	TAK-536-C-46-00378
Cytogenetic assay	CHL/IU cells	In vitro/DMSO	6 and 24 hours	TAK-536 M-II: 6 hours, ±S9: 25 to 100 µg/mL 24 hours, -S9: 25 to 100 µg/mL	Yes		TAK-536-C-46-00377
Cytogenetic assay-supplemental	CHL/IU cells	In vitro/DMSO	6 and 24 hours	TAK-536 M-II: 6 hours, -S9: 2688 to 3763 µg/mL 6 hours, +S9: 1613 to 2688 µg/mL 24 hours, -S9: 1613 to 2688 µg/mL	Yes		TAK-536-C-46-00447
Carcinogenicity							
	Mouse/ Tg.rasH2 hemizygous	Oral, dietary admixture/basal diet	26 weeks	0, 1.25% (M: 1762/F: 2407), 3.5% (M: 5026/F: 6951), and 5% (M: 8134/F: 11,182)	Yes		TAK-536-10010
	Rat/F344	Oral, gavage/ corn oil	24 months	TAK-536 M-II: 0 (negative control), 0 (vehicle control), 100 (M only), 300, 1000, 3000 (F only)	Yes		TAK-536-10008
Carcinogenicity (supplemental)	Rat/F344, females	Oral, gavage/ corn oil	24 months (e)	TAK-536 M-II: 0, (0 BID), 6000 (3000 BID)	Yes		TAK-536-10009

TAK-491

Page 14 of 19
08 March 2010

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Metabolite Studies (TAK-536 M-II) (continued)							(b) (4)
Reproductive Toxicity							
Fertility and early embryonic development to implantation	Rat/Sprague-Dawley	Oral, gavage/ corn oil	M: 14 days prior to mating through day prior to necropsy F: 14 days prior to mating through GD 6	TAK-536 M-II: 0, 120, 600, and 3000	Yes		TAK-536-C-46-00461
Embryo-fetal development-range-finding	Rat/Sprague-Dawley	Oral, gavage/ corn oil	GD 6-GD 17	TAK-536 M-II: 0, 300, 1000, and 3000	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00453
Embryo-fetal development	Rat/Sprague-Dawley	Oral, gavage/ corn oil	GD 6-GD 17	TAK-536 M-II: 0, 120, 600, and 3000	Yes	Ina Research Inc.	TAK-536-C-46-00462
Toxicokinetic study	Rabbit/Kbl:JW, non-pregnant	Oral, gavage/ MC	7 days	TAK-536 M-II: 1000, 3000, and 5000	No	Takeda Pharmaceutical Co., Ltd.	TAK-536-C-46-00463
Embryo-fetal development-range-finding	Rabbit/Kbl:JW	Oral, gavage/ MC	GD 6-GD 18	TAK-536 M-II: 0, 1000, 3000, and 5000	No		(b) (4) TAK-536-C-46-00457
Embryo-fetal development	Rabbit/Kbl:JW	Oral, gavage/ MC	GD 6-GD 18	TAK-536 M-II: 0, 300, 1000, and 3000	Yes		TAK-536-C-46-00464
Impurity Studies (b) (4)							
Bacterial reversion assay	<i>S typhimurium</i> <i>E coli</i>	In vitro/DMSO	48 hours	(b) (4) 15 to	Yes		TAK-491-00229-001A
Effects of ADH with NADPH on the bacterial reversion assay	<i>E coli</i>	In vitro/DMSO	48 hours	(b) (4) 5000 µg/plate	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00232

TAK-491
2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain (b)(4)	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Impurity Studies							
(continued)							
Effects of ADH and/or NADPH on the bacterial reversion assay-supplemental	<i>E. coli</i>	In vitro/DMSO	48 hours	(b)(4) 5000 µg/plate	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00252
Concentrations of diacetyl in the reaction mixture for the bacterial reversion assay	Reaction mixture	In vitro/DMSO	20 minutes	(b)(4) 7.14 mg/mL (5000 µg/plate)	No	Takeda Pharmaceutical Co., Ltd.	TAK-491-00275-001A
Cytogenetic assay	CHL/IU cells	In vitro/DMSO	6 and 24 hours	(b)(4) 6 hours, -S9: 400 to 1000 µg/mL 6 hours, +S9: 1100 to 1300 µg/mL 24 hours, -S9: 100 to 500 µg/mL	Yes	(b)(4)	TAK-491-00228-001A
Repeat-dose toxicity study	Dog/beagle	Oral, gavage/ MC+CA	4 weeks	TAK-491: 0 and 60 (b)(4) 00/0.6	Yes	(b)(4)	TAK-491-00230
Combination Toxicity Studies							
TAK-491 + TAK-536 M-II + CLD							
Single-dose double combination toxicokinetic study with TAK-491 plus CLD or TAK-536 M-II plus CLD	Rat/F344	Oral, gavage/ MC+CA	Single dose	TAK-491: 1000; TAK-536 M-II: 1000 and 5000; CLD: 15, 50, and 150; TAK-491/CLD: 1000/50, TAK-536 M-II/CLD: 1000/50 and 5000/50	No	Takeda Pharmaceutical Co., Ltd.	TAK-491CLD-11097

TAK-491

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Combination Toxicity Studies (continued)							
TAK-491 + TAK-536 M-II + CLD (continued)							
Single-dose double combination toxicokinetic study with TAK-491 plus CLD or TAK-536 M-II plus CLD - supplemental study	Rat/F344	Oral, gavage/ MC+CA	Single dose	TAK-536 M-II: 2000; CLD: 150, 300, and 600; TAK-491/CLD: 1000/600, TAK-536 M-II/CLD: 2000/600	No	Takeda Pharmaceutical Co., Ltd.	TAK-491CLD-11098
Preliminary double combination toxicity study with TAK-491 plus CLD or TAK-536 M-II plus CLD	Rat/F344	Oral, gavage/ MC+CA	2 weeks	Control: 0; TAK-491: 1000; TAK-536 M-II: 2000; CLD: 100 and 300; TAK-491/CLD: 100/100, 100/300, 1000/100, and 1000/300; TAK-536 M-II/CLD: 2000/100 and 2000/300	No	Takeda Pharmaceutical Co., Ltd.	TAK-491CLD-10170
Triple combination toxicokinetic study with TAK-491, TAK-536 M-II, and CLD	Rat/F344	Oral, gavage/ MC+CA	2 weeks	Control: 0; TAK-491/TAK-536 M-II/ CLD: 1000/2000/300	No	Takeda Pharmaceutical Co., Ltd.	TAK-491CLD-11099
Triple combination toxicity study with TAK-491, TAK-536 M-II, and/or CLD	Rat/F344	Oral, gavage/ MC+CA	13 weeks	Control: 0; CLD: 300; TAK-491/TAK-536 M-II: 100/2000 and 1000/2000; TAK-491/TAK-536 M-II/ CLD: 100/2000/100, 100/2000/300, and 1000/2000/300	Yes	(b) (4)	TAK-491CLD-00071

TAK-491

2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Combination Toxicity Studies (continued)							
TAK-491 + TAK-536 M-II + CLD (continued)							
Range-finding embryo-fetal development triple combination toxicity study with TAK-491, TAK-536 M-II, and CLD	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	GD 6-GD 17	Control: 0; CLD: 300; TAK-491/TAK-536 M-II: 1000/2000; TAK-491/TAK-536 M-II/ CLD: 100/2000/100 and 1000/2000/300	No	Takeda Pharmaceutical Co., Ltd.	TAK-491CLD-00067
Embryo-fetal development triple combination toxicity study with TAK-491, TAK-536 M-II, and/or CLD	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	GD 6-GD 17	Control: 0; CLD: 300; TAK-491/TAK-536 M-II: 1000/2000; TAK-491/TAK-536 M-II/ CLD: 1000/2000/300	Yes	(b) (4)	TAK-491CLD-00070-001A
TAK-491 + TAK-536 M-II + AML							
Preliminary triple combination toxicity study with TAK-491, TAK-536 M-II, and/or AML	Rat/F344	Oral, gavage/ MC+CA	2 weeks	Control: 0; AML: 20; TAK-491/TAK-536 M-II: 160/2000; TAK-491/TAK-536 M-II/ AML: 160/2000/20	No	Takeda Pharmaceutical Co., Ltd.	TAK-491CCB-00057-001A
Triple combination toxicity study with TAK-491, TAK-536 M-II, and/or AML	Rat/F344	Oral, gavage/ MC+CA	13 weeks	Control: 0; AML: 20; TAK-491/TAK-536 M-II: 160/2000; TAK-491/TAK-536 M-II/ AML: 40/2000/5, 80/2000/10, 160/2000/20	Yes	(b) (4)	TAK-491CCB-10001

TAK-491
2.6.7.1 Toxicology Overview

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Combination Toxicity Studies (continued)							
TAK-491 + TAK-536 M-II + AML (continued)							
Range-finding embryo-fetal development triple combination toxicity study with TAK-491, TAK-536 M-II, and AML	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	GD 6-GD 17	Control: 0; AML: 20; TAK-491/TAK-536 M-II: 160/2000; TAK-491/TAK-536 M-II/AML: 160/2000/20	No	Takeda Pharmaceutical Co., Ltd.	TAK-491CCB-00058
Embryo-fetal development triple combination study with TAK-491, TAK-536 M-II, and/or AML	Rat/Sprague-Dawley	Oral, gavage/ MC+CA	GD 6-GD 17	Control: 0; AML: 10; TAK-491/TAK-536 M-II: 80/2000; TAK-491/TAK-536 M-II/AML: 80/2000/10	Yes	Takeda Pharmaceutical Co., Ltd.	TAK-491CCB-10002
TAK-536 + Pioglitazone (f)							
Single-dose combination toxicokinetics study with pioglitazone and TAK-536	Monkey/ cynomolgus	Oral, gavage/ MC	Single dose	Pioglitazone/TAK-536: 0 (MC)/25→ 0 (citric acid granules)/25→ 36/25→ 36/50→ 36/50 (TAK-536 administered 30 minutes post pioglitazone dosing)	No	Takeda Pharmaceutical Co., Ltd.	AD-4833-536-00028-001A
Preliminary combination toxicity study with pioglitazone and TAK-536	Rat/Sprague-Dawley	Oral, gavage/ MC	4 weeks	Control: 0 (MC); Pioglitazone: 4 and 63; TAK-536: 30 and 300; Pioglitazone/TAK-536: 4/30, 4/300, 63/30, and 63/300	No	Takeda Pharmaceutical Co., Ltd.	AD-4833-536-00025

TAK-491
2.6.7.1 Toxicology Overview

Page 19 of 19
 08 March 2010

2.6.7.1 Toxicology Overview (continued)

Type of Study	Species/Strain	Method of Administration/ Vehicle	Duration of Dosing	Test Article(s): Daily Dose (mg/kg) (a) (b)	GLP Compliance (c)	Testing Facility (d)	TPC Report Number
Combination Toxicity Studies (continued)							
TAK-536 + Pioglitazone (f) (continued)							
Combination toxicity study with pioglitazone and TAK-536	Rat/Sprague-Dawley	Oral, gavage/ MC	13 weeks	Control: 0 (MC); Pioglitazone: 16; TAK-536: 3 and 30; Pioglitazone/TAK-536: 16/3 and 16/30	Yes		(b) (4) AD-4833-536-00037-001A
Preliminary combination toxicity study with pioglitazone and TAK-536	Monkey/ cynomolgus	Oral, gavage/ MC	4 weeks	Control: 0; Pioglitazone: 36; TAK-536: 200 and 600; Pioglitazone/TAK-536: 9/600, 36/200, and 36/600	No	Takeda Pharmaceutical Co., Ltd.	AD-4833-536-00026-001A
Preliminary combination toxicity study with pioglitazone and TAK-536, supplemental	Monkey/ cynomolgus, males	Oral, gavage/ MC	4 weeks	Control: 0; Pioglitazone/TAK-536: 9/50, 36/50, and 36/100	No	Takeda Pharmaceutical Co., Ltd.	AD-4833-536-00027

ADH=alcohol dehydrogenase, AML=amlodipine besylate, BID=twice daily, CA=0.05%-1.0% (w/v) citric acid, CHL=Chinese hamster lung, CHO=Chinese hamster ovary, CLD=chlorthalidone, DMAc=5% (w/v) *N,N*-dimethylacetamide, 80%-85% (w/v) polyethylene glycol 400, and saline solution (pH ≤5.5), *E coli*=*Escherichia coli*, F=female, GD=Gestation Day, HCl=hydrochloride, HGPRT=hypoxanthine-guanine phosphoribosyl transferase, IV=intravenous, JW=Japanese white, LD=Lactation Day, M=male, MC=0.5% (w/v) methylcellulose, megl=0.14%-17.40% (w/v) or 1.5 mg/mL meglumine, NADPH=nicotinamide adenine dinucleotide phosphate, NaOH=sodium hydroxide, S9=metabolic activator, *S typhimurium*=*Salmonella typhimurium*, tk=thymidine kinase, TPC=Takeda Pharmaceutical Co., Ltd., UDS=unscheduled DNA (deoxyribonucleic acid) synthesis.

(a) Unless otherwise specified. For repeat-dose toxicity, the lowest NOAEL (no-observed-adverse-effect level) for males or females is underlined.

(b) TAK-491, TAK-491 (b) (4) and AML doses were expressed as the salt free form, unless specified otherwise.

(c) US FDA Good Laboratory Practice Regulations (21 CFR Part 58), the Good Laboratory Practice Regulations promulgated by the Ministry of Health and Welfare of Japan (Ordinance No. 21, 26 March 1997), and the OECD Principles of Good Laboratory Practice (C(97)186/Final).

(d) Takeda Chemical Industries, Ltd. and Takeda Pharmaceutical Co., Ltd. are the same company.

(e) Duration of administration was planned to be 24 months (104 weeks); however, actual duration of administration was 42 weeks (299 days) for the control group and was 73 weeks for the 6000 mg/kg/day group, due to decreased survival. All animals (controls and treated) were sacrificed Week 73 (Day 512).

(f) Pioglitazone doses were expressed as pioglitazone (HCl).

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/s/

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