CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

200153Orig1s000

PHARMACOLOGY REVIEW(S)

Signed off in DARRTS on 2/2/2012

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	200153
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	4/28/2011
PRODUCT:	Atozet tablets (ezetimibe/atorvastatin fixed dose combination tablets), tablet strengths are 10/10, 10/20, 10/40, 10/80 mg).
INTENDED CLINICAL POPULATION:	Primary hypercholesterolemia, and homozygous familial hypercholesterolemia.
SPONSOR:	MSP Singapore Company, LLC (MSP). The application is submitted by Merck Sharp & Dhome Corp., North Wales, PA, on behalf of MSP
DOCUMENTS REVIEWED:	e-CTD submission.
REVIEW DIVISION:	Division of Metabolism and Endocrinology Products.
PHARM/TOX REVIEWER:	Indra Antonipillai
PHARM/TOX SUPERVISOR:	Karen Davis Bruno
DIVISION DIRECTOR:	Mary Parks
PROJECT MANAGER:	Kati Johnson
Date of review submission to DARRTS:	2/2/2012

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Executive Summary

1. Recommendations

A. **Recommendation on approvability**

Pharmacology recommends approval of this fixed dose combination product (Atozet) for the proposed indication.

B. **Recommendation for Nonclinical Studies**:

The previous studies conducted with ezetimibe and atorvastatin under NDA 21-445 (approved in 2002) are adequate to support the current fixed dose combination drug product (atozet). In addition, MSP has provided a 3-month toxicity/ toxicokinetics study of atorvastatin amorphous in dogs and 2 gene-toxicity studies with atorvastatin amorphous in the current NDA application to qualify the impurities excipients in their atorvastatin amorphous used in altozet. Previously crystalline form of atorvastatin has been used in the reference listed drug (Lipitor from Pfizer), and in NDA 21-445. The preclinical studies are adequate to support the recommended doses of up to 10/80 mg of zetia/atorvastatin (in atozet) per day.

C. **Recommendation on Labeling:** The pre-clinical labeling section for the fixed dose combination is in general similar to the approved zetia label and Lipitor label, however a change in the contraindications section is recommended, see page 61 of the review.

II. Summary of Nonclinical Findings:

A. Brief Review of Nonclinical studies

Both ezetimibe and atorvastatin are approved drug products for oral use in USA as zetia, (NDA 21-445) and Lipitor (NDA 20-702). Extensive nonclinical studies have been conducted with the approved Zetia and Lipitor (crystalline form of atorvastatin), as well as co-administration of both drugs under NDA 21-445.
In the current application, sponsor has conducted one 3-month toxicity study in dogs and two gene-toxicity studies with atorvastatin amorphous to qualify the impurities present in their amorphous atorvastatin used in the fixed dose combination of ezetimibe and atorvastatin (atozet).

B. Pharmacologic activity

Both are lipid lowering drugs. Atorvastatin is an HMG-CoA reductase inhibitor. Ezetimibe is a cholesterol absorption inhibitor. Combination of two drugs has been shown to have additive effects on lowering LDL-cholesterol in patients (NDA 21-445).

C. Nonclinical safety issues relevant to clinical use

No new nonclincal safety issues relevant to the clinical use have been identified with the fixed dose combination Atozet compared to zetia or atorvastatin alone.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

NDA number: NDA 200-153
Review Number: 1

Sequence number/date/type of submission: 4/28/2011 (original application), it is a 505(b)(2) application submitted in an electronic format (e-CTD). 4/29/2011 (draft labeling package insert). Initially, this application was submitted on 9/2/2009. However, sponsor's manufacturing facilities were not ready for GMP inspection, therefore this application was not fileable.

Information to sponsor: Yes ( ) No (X)

Sponsor: Merck Sharp & Dhome Corp., North Wales, PA has submitted this application on behalf of MSP Singapore Company, LLC (MSP), a joint venture between Merck & Co., Inc. and Schering Corporation

Manufacturer for drug substance: The manufacturer of ezetimibe drug substance will be MSP International GmbH (Singapore branch). The manufacturer of atorvastatin calcium (amorphous) drug substance is Dr. Reddy's Laboratories Ltd in Andhra Pradesh, India (Type II, DMF # 18468). The manufacturer of the fixed dose combination drug product Atozet (ezetimibe /atorvastatin tablet) is MSD International GmbH (Puerto Rico); this will be an immediate release-film coated (b) (4) tablet.

Reviewer name: Indra Antonipillai, Ph.D. Pharmacology Reviewer.
Division: Division of Metabolic and Endocrine Products,
Review completion date: 12/14/11

Drug:

Trade name: Atozet or MK-0653C tablets. The tablets are a fixed dose combination of ezetimibe and atorvastatin. The tablet strengths are 10/10, 10/20, 10/40, 10/80 mg.

Combination code name: MK-0653C (or SCH 900068). It is a combination of ezetimibe (SCH 58235 or L-000829161) and atorvastatin (SCH 412387 or L000776336, or MK-9396).

Generic name for two drugs (list alphabetically): Ezetimibe + atorvastatin.

Brand names for two drugs: Zetia (MSP) + Lipitor (Pfizer).

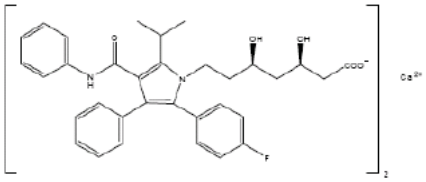
Chemical name: Ezetimibe's chemical name is 1-(4-fluorophenyl)-3®-(3-(4-fluorophenyl)-3(S)-hydroxypropyl)-4(S)-4-hydroxyphenyl)-2-azetidione.

Atorvastatin's chemical name is

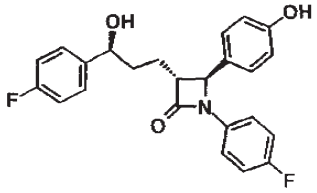
[R-(R*, R*)]-2-(4-fluorophenyl)-ß, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1)

Molecular formula/molecular weight of **ezetimibe**: $C_{24}H_{21}F_2NO_3$/409.4
Molecular formula/molecular weight of **atorvastatin**: $(C_{33}H_{34}FN_2O_5)_2Ca$/1155.36

Structure of atorvastatin



Structure of ezetimibe



SCH 58235

Drug class: Both are lipid lowering drugs. Ezetimibe is a cholesterol absorption inhibitor. Atorvastatin is an HMG-CoA reductase inhibitor. Combination of two drugs has already been shown to have additive effects on lowering LDL-cholesterol in patients.

Relevant INDs/NDAs/DMFs: IND 101,953 (fixed dose combination of ezetimibe and atorvastatin). Zetia (ezetimibe NDA 21-445), Lipitor (atorvastatin, NDA 20-702, Pfizer). DMF 18468 (for atorvastatin calcium amorphous drug substance from Dr. Reddy's Laboratories Ltd.).

Indication: Treatment of hypercholesterolemia and homozygous familial hypercholesterolemia (HoFH).

Clinical formulation: The combination drug MK-0653C (or fixed dose combination of ezetimibe/atorvastatin tablets) will be available with the fixed dose of ezetimibe (10 mg) and variable doses of atorvastatin (i.e. in 10/10, 10/20, 10/40, 10/80 mg of ezetimibe/ atorvastatin respectively). Ezetimibe is already marketed by MSP. Although atorvastatin is also a marketed drug, the current sponsor will use their own atorvastatin calcium amorphous in the fixed dose combination which is. manufactured by Dr. Reddy's Laboratories Ltd (see Type II DMF 18468). A copy of the authorization letter to reference DMF 18468 has been provided by the sponsor.

The composition of the fixed dose combination drug product is shown below. Sponsor states that qualitatively there were no new impurities/degradates present in the ezetimibe /atorvastatin combination tablet relative to the individually approved drug formulation. The excipients used in the film-coated (b) (4) tablet (b) (4) are compendial grade. Thus, no novel excipients are used in the formulation of ATOZET tablet. All excipients comply with corresponding USP and/or NF monographs.

Ezetimibe/Atorvastatin FDC Tablet – Market Composition

Components	Compendial Testing	Function	Unit Strength (Ezetimibe/Atorvastatin)			
			10/10 mg/mg	10/20 mg/mg	10/40 mg/mg	10/80 mg/mg
(b) (4)			mg/tablet			
Ezetimibe (SCH58235),	---	Active	10.00			
(b) (4)						
Atorvastatin Calcium[§] (amorphous) (equivalent free acid)[‖]	---	Active	10.34 (10.00)	20.68 (20.00)	41.36 (40.00)	82.73 (80.00)
(b) (4)						(b) (4)
Film-Coating:						
(b) (4						
Total Coated Tablet Wt.:			624.0 mg	364.1 mg	519.7 mg	830.3 mg
						(b) (4)

Route of administration: Oral

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

Studies reviewed in this submission: One three month toxicity study in dogs and 3 geno-toxicity studies (one non-GLP and two GLP studies) with atorvastatin calcium amorphous.

2.6.1. INTRODUCTION AND DRUG HISTORY

Both Zetia (ezetimibe) and Lipitor (atorvastatin, crystalline) are lipid lowering drugs. The safety profile of atorvastatin (HMG-CoA reductase inhibitor, NDA 20-702) has been well characterized, and that of ezetimibe (zetia, a cholesterol absorption inhibitor) is available from the approved NDA 21-445.

The Sponsor now wants to market a (b) (4) fixed-dose combination (FDC) tablets of ezetimibe with atorvastatin. This application was initially submitted on 9/2/2009, however, sponsor's manufacturing facilities were not ready for GMP inspection, therefore this application was not fileable**.** The combination of two drugs in a single formulation here will supposedly improve patient compliance compared to co-administration of two medications. Overall, the development program for the ezetimibe/ atorvastatin combination tablet is similar to that followed for the approved ezetimibe/simvastatin combination tablet registration (NDA 21687, Vytorin).

Sponsor states that co-administration of ezetimibe with atorvastatin 10 mg results in a similar mean % change in LDL-C (approximately -53%) as atorvastatin 80 mg alone (approximately -54%). Therefore, by combining the two different mechanisms of action of these agents (inhibition of endogenous cholesterol synthesis by atorvastatin and inhibition of cholesterol absorption across the intestinal wall by ezetimibe), the coadministration of ezetimibe 10 mg + atorvastatin (10, 20, 40, or 80 mg) in this study will result in more effective reduction of LDL-C than atorvastatin or ezetimibe alone.

Atorvastatin calcium (amorphous) drug substance will be manufactured at Dr. Reddy's Laboratory ltd., at Andhra Pradesh in India. Complete information on the drug substance is included in Type II Drug Master File 18468, and a letter of authorization has been provided.

This atorvastatin calcium amorphous drug substance contains the following amounts of impurities, metals, isomer and other organic solvents, etc. (see Tables Table 2.3.S.4 below):

Atorvastatin Calcium (amorphous) Specification

Test	Specification	Analytical Procedure
Description	White to off-white powder	Visual
Identification	(b) (4)	
IR-Spectrum		
Test for calcium		
Water by KF		
Heavy Metals		
Related Substances by HPLC‡		
(b) (4		
Assay by HPLC		

Atorvastatin Calcium Specifications

Test	Specification	Analytical Procedure
(b) (4)		(b) (4)
Particle Size§		
		(b) (4)

The solvents used in the manufacture of atorvastatin calcium amorphous are controlled to ensure conformance to ICH Q3C residual solvent requirements and are specified in the supplier's DMF.

2.6.2 PHARMACOLOGY

No new nonclinical pharmacology studies with the fixed dose combination (FDC) of ezetimibe and atorvastatin have been provided in the current submission. The pharmacology of ezetimibe was presented in the approved NDA 21-445, and of atorvastatin in the marketed NDA 20-702.

Most of the pharmacology and toxicity studies with the co-administration of ezetimibe and atorvastatin have been conducted previously under NDA 21-445.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The pharmacokinetics / toxicokinetics data are available from the co-administration toxicity studies of ezetimibe and atorvastatin in rats and dogs conducted under NDA 21-445.

2.6.6 Toxicology:

2.6.6.3 Repeat-dose toxicity

The toxicity of co-administration of ezetimibe and atorvastatin was also evaluated under NDA 21-445, these included 3-month toxicity studies in rats and dogs, embryo-fetal development studies in rats and rabbits and gene-toxicity studies.

New Pharmacology/toxicology studies submitted in the current application

Drug product excipients, impurities and degradants

Sponsor states that the batch of atorvastatin calcium crystalline (batch number 76590-003) that was used in above co-administration toxicity studies conducted under NDA 21-445, was not fully characterized' as those studies were not meant to be impurity/ degradate-qualifying studies.

The current NDA contains a tabular summary of the batches of atorvastatin calcium (amorphous; also referenced as L-000776336 and MK-9396) used in studies conducted by Merck to qualify degradants of atorvastatin. [Table 2.4: 1] below provides the nomenclature for the impurities used in the current NDA.

Table 2.4: 1 Atorvastatin Calcium (amorphous) Impurities and Alternate Nomenclature

Impurity Name	Alternate Impurity Nomenclature
(b) (4)	

The proposed specifications for impurities/degradants in the fixed-dose combination drug product of ezetimibe and atorvastatin calcium (amorphous) are presented below in [Table 2.4: 2]. Based on the levels of the impurities/degradation products in atorvastatin calcium (amorphous) batch L-000776336-004G002, which was used in the three-month dog study [Sec. 2.6.7.17A], Table 2.4: 2 presents the levels qualified, as well as the supporting genetic toxicology information for each impurity/degradation product.

Table 2.4: 2 Proposed Specifications for Impurities/Degradants in the Ezetimibe/ Atorvastatin Calcium (amorphous) Fixed Dose Drug Product are shown below (this Table is same as Table 2.6.6:4).

Impurities/Degradates	Specifications Proposed for Drug Product	Levels Qualified in Toxicology Study[a]	Qualified in Ames and In vitro Chromosomal Aberration (CA) Assays
Ezetimibe-Related Degradate			
(b) (4)			
Atorvastatin-Related Degradates			
(b) (4)			

[Sec. 2.6.7.17A; 2.6.7.17B, 2.6.7.17C, 2.6.7.17D]

Sponsor states that atorvastatin lactone is a major (inactive) metabolite of atorvastatin and undergoes interconversion with the parent (active) open-acid form in vivo. Humans are significantly exposed to atorvastatin lactone and data indicate that the lactone is formed in the acid environment of the stomach and is also seen in rat, dog and human liver microsomes. Based on this data, the atorvastatin lactone is a major metabolite of atorvastatin and as such has been qualified.

As stated earlier, sponsor has conducted one 3-month toxicity study in dogs, and 3 gene-toxicity studies in the current application to qualify the impurities/degradents in the atorvastatin amorphous in their drug product. Note that the 3-month toxicity study conducted here was with anther investigational cholesterol absorption inhibitor (MK-

6213), and not ezetimibe. However, this study included a group of animals administered atorvastatin calcium (amorphous) alone at 10 mg/kg/day.

Following is a New toxicity study conducted in the current application to support atorvastatin amorphous:

3-Month Oral Co-administration Toxicity Study of MK-6213 /L-000776336 in Dogs. (Note that MK-6213 is another cholesterol absorption inhibitor, however L000776336 is sponsor's own atorvastatin amorphous calcium from Dr. Reddy's Laboratory).

Therefore, note that the subject of this study is sponsor's atorvastatin calcium amorphous (and not another cholesterol absorption inhibitor MK-6213 here).

Study no: TT #07-6039
Volume #, and page #: e-CTD Submission.
Conducting laboratory and location: Merck facility at Riom, in France. However data were reported by (b) (4)

Date of study initiation: 5/22/07
GLP compliance: Yes.
QA report: yes (X) no ( ). In accordance with French Ministry of Health GLP regulation
Drug lot #, and % purity: These are shown below.

Test Article: Lot number of atorvastatin calcium amorphous (L-000776336) is 004G002

Lot number of MK-6213 is L-001662326-000Y024. Note that MK-6213 {also known as L-001662326} is another cholesterol absorption inhibitor like ezetimibe from Merck.

Factor Used to Calculate Doses as Base Compound: MK-6213: (b) (4) (base compound), L-000776336: (b) (4) (calcium salt)

Purity: Purity of atorvastatin amorphous was 99.4%. Purity of MK-6213 was 99%.

Formulation/vehicle: Imwitor 742/Polysorbate 80 (1:1 w/w).

Weight at Study Start: Females: 5.7 to 10.7 kg; Males: 5.7 to 9.9 kg

Doses in administered units:

Beagle dogs were assigned to 5 groups of 3 females and 3 males each that received 0/10, 10/10, 50/10, or 200/10 mg/kg/day of MK-6213/L-000776336 combination or vehicle only. MK-6213 and L-000776336 were administered as the base compound and the calcium salt, respectively, in Imwitor 742/Polysorbate 80 (1:1 w/w).

The dogs received the drug in a capsule once daily.

Oral by capsules (10-mL "Lock Ring" Porcine Gelatine Capsules, batch 1846. (b) (4)

4) **Dosing Volume**

1 mL/kg (distributed into 1 or 2 capsules depending upon individual body weights)

	Females	Males
Control (vehicle)	3	3
L-000776336		
10 mg/kg/day	3	3
MK-6213/L-000776336		
10/10 mg/kg/day	3	3
50/10 mg/kg/day	3	3
200/10 mg/kg/day	3	3

Dose levels were selected on the basis of results from a previous study with MK-6213/L-000776336 combination in Dogs (TT #07-6009).

The dosing formulations were collected for analysis, and all assay results were within the acceptable concentration range.

Parameters examined are stated below

Type and Frequency of Observations and Analyses

1) **Physical Examinations**

Daily observation for mortality and physical signs

2) **Body Weights**

Measured pretest and once a week thereafter

3) **Food Consumption**

Daily food consumption was estimated 5 times per week, except in Study Weeks 4, 8, 12, and 13 when food consumption was estimated only 4 times due to fasting for scheduled blood collections for hematology, serum biochemistry and urinalysis or due to holidays.

4) **Ophthalmic Examinations**

Ophthalmic examinations (indirect ophthalmoscopy and slit lamp biomicroscopy) were performed on all animals pretest and in Study Weeks 6 and 12. Examinations were facilitated by ocular instillation of 0.5% tropicamide (MYDRIATICUM® 0.5%, [redacted] (b) (4) [redacted] (b) (4)

5) **Electrocardiographic Examinations**

Electrocardiograms were recorded pretest and in Study Week 12 from all dogs in right lateral recumbency. Recordings were made from leads I, II, III, aVR, aVL, aVF, V1 (CV_5RL), and V2 (V_{10}). The heart rate, PR, QRS, and QT intervals were measured.

Hematological/ Clinical chemistry Examinations: Blood samples were collected from all dogs (fasted) pretest, and in study weeks 4, 8, and 12.

Urinalyses: Overnight urine collections were performed on all dogs (fasted) in study weeks 8 and 12.

Toxicokinetics: Blood samples were collected from all dogs (non-fasted) for TK at approximately 0.5, 1, 2, 4, 8, and 24 hours post-dose after the first dose and in Study Week 13.

Bile Biochemical Examination: Bile was collected from all fasted dogs at the scheduled final necropsy

Gross Pathology: This was conducted at sacrifice.
Organs weighed: Following organs were weighed as shown below.

adrenals	pituitary
brain	prostate
heart	spleen
ovaries	testes
kidneys	thyroids
liver	thymus

Histopathology: This was performed at sacrifice in all animals in organs listed below:

brain (including cerebral cortex, subcortical white matter, basal ganglia, thalamus, hippocampus, midbrain, cerebellum, and medulla oblongata)

spinal cord (cervical)

eye

optic nerve

peripheral nerve (sciatic)

pituitary

adrenals

thyroids

parathyroids

skin (from inguinal mammary region)

mammary gland

salivary gland (mandibular)

heart (right and left atrial and ventricular regions and interventricular septum)

aorta

skeletal muscle (rectus femoris and masseter)

stomach (fundic and pyloric regions)

small intestine (duodenum, jejunum, and ileum)

Peyer's patches

large intestine (colon and cecum)

esophagus (distal region)

urinary bladder

kidneys

gallbladder

liver

lung

trachea

spleen

lymph nodes (mesenteric and retropharyngeal)

pancreas

thymus

prostate

testes

epididymides

uterus

cervix

ovaries

vagina

bone (rib at costochondral junction)

bone marrow (in bone section and as May Grünwald Giemsa's stained smears)

Mammary glands in males were evaluated when present in section. Tissues with gross observations (at the discretion of the pathologist) and liver were also processed, stained, and examined microscopically in all animals on study.

Hall's stain for bilirubin was performed on liver sections of dog #07-0266 at 200/10 mg/kg/day.

Results: Note that since another ezetimibe-like drug product (MK-6213 here) is not the subject of concern for this NDA, the reviewer will mostly concentrate on the control and atorvastatin amorphous alone (i.e. 0/0 and 0/10 ezetimibe /atorvastatin respectively) in this 3-month study in dogs.

Mortality: No treatment related mortality was observed.

Clinical signs: No treatment related clinical signs were observed

Body weights: No effects on body weights were noted.

Food consumption: No treatment related effects on food consumption were observed.

Ophthalmology: No treatment related effects on ophthalmology were observed

Hematology: No drug related effects on hematological parameters were observed

Electrocardiographic Examinations: No drug related effects were observed on electrocardiographic exams.

Clinical chemistry:
L-000776336 (i.e atorvastatin amorphous) alone produced increases in alanine aminotransferase activity (ALT males 97 vs 45 IU/L in controls; females 63 vs 30 IU/L in controls), and bilirubin levels (0.2 vs 0.1 mg/dl in controls) in both sexes. In females, ALP levels (152 vs 98 IU/L in controls) and creatinine kinase levels (411 vs 239 IU/L in controls) were increased. Cholesterol (males 49 vs 113 mg/dl in controls; females 57 vs 149 mg/dl in controls), triglycerides (males 23 vs 34 mg/dl in controls; females 28 vs 48 mg/dl in controls), and total protein levels (males 5.4 vs 5.7 g/L in controls; females 5.4 vs 5.6 g/L in controls) were decreased in both sexes. See Table.1 below:

Table 1. Blood chemistry parameters in a 3-month toxicity study in dogs at 0/0. 0/10, 10/10, 50/10, 200/10 of MK-6213/L-000776336 mg/kg/day respectively.

	Males					Females				
Dose (ppm)	0/0	0/10	10/10	50/10	200/10	0/0	0/10	10/10	50/10	200/10
Chol (mg/dl)	113	49	17	14	11	149	57	11	21	19
triglyceride (mg/dl)	34	23	34	41	24	48	28	17	26	20
Creat kinas (IU/L)	395	313	321	434	320	239	411	495	411	321
Bilirubin (mg/dl)	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2
	Males					Females				
Total protein g/L	5.7	5.4	4.9	5.2	5.1	5.6	5.4	5.0	4.8	5.5
Albumin (g/dl)	3.4	3.2	2.8	3.1	2.8	3.3	3.4	3.1	2.9	3.3
AST (IU/L)	43	42	72	71	63	41	56	79	78	86
ALT (IU/L)	45	97	743	727	542	30	63	505	820	940
ALP (IU/L)	132	117	320	270	251	98	152	203	242	316

Urine analysis: No drug related effects on urine analysis were observed.

Gross findings: No treatment related gross findings were observed.

Organ Weights: Atorvastatin amorphous produced decreased liver weights in males (243 vs 277 g in controls, by 12%; relative liver to brain weights were also decreased by 11%,) and thymus weights (2.4 vs 4.0 g in controls, by 40%; relative by 35%). Thymus weights were also decreased in females (2.89 vs 3.79 g in controls, by 24%). However sponsor does not consider these significant.

Here is sponsor' description on organ weights:

The variations in organ weights were of the type seen in untreated dogs in this laboratory and were unrelated to treatment. Although statistically significant by trend assessment ($p \leq 0.05$) when expressed as absolute and/or relative to brain weight, the decreased mean liver weights were considered unrelated to treatment because of the low magnitude of the change and because most drug-treated animals but 1 male dog at 200/10 mg/kg/day had liver weights in the 95% range of liver weights from weight-matched historical control animals.

Table 2. Liver organ weight changes are shown below in male + female dogs.
MK-6213/L-000776336: Three-Month Combination Oral Toxicity Study in Dogs. TT #07-6039

Summary Organ Weight Statistics

Parameter	Stat	C1	T1	T2	T3	T4
Liver (g)	Mean	254.57	239.15	209.08	207.51	194.47
	S. E.	18.11	9.86	16.05	4.72	13.80
	N	6	6	6	6	6
	P-value			0.085	0.026	0.030
Liver % Body Weight	Mean	2.99	2.67	2.53	2.50	2.25
	S. E.	0.28	0.12	0.26	0.19	0.09
	N	6	6	6	6	6
	P-value					0.230
Liver % Brain Weight	Mean	359.0	318.3	288.1	286.4	269.7
	S. E.	21.1	11.2	24.2	9.7	11.7
	N	6	6	6	6	6
	P-value		0.164	0.035	0.015	0.022

C1 = Control T1 = 0/10 mg/kg/day T2 = 10/10 mg/kg/day T3 = 50/10 mg/kg/day T4 = 200/10 mg/kg/day
Analysis Type : Trend analysis with ordinal dose scale; multiplicity adjusted
N : Group Size
S. E. : Standard Error
P-value : Multiplicity Adjusted for # of Tests

Table 3. Changes in Thymus weights are shown below in male + female dogs vs controls. Note that thymus weights were lower with atorvastatin amorphous (2.82 vs 3.91 g in controls, absolute by 10%; relative by 35%)

Summary Organ Weight Statistics

Parameter	Stat	C1	T1	T2	T3	T4
Thymus (g)	Mean	3.91	2.63	3.27	3.04	2.82
	S. E.	0.70	0.48	0.44	0.44	0.45
	N	6	6	6	6	6
	P-value					0.991
Thymus % Body Weight	Mean	0.05	0.03	0.04	0.04	0.03
	S. E.	0.01	0.01	0.00	0.00	0.01
	N	6	6	6	6	6
	P-value					0.998
Thymus % Brain Weight	Mean	5.49	3.52	4.47	4.19	3.96
	S. E.	0.96	0.66	0.59	0.61	0.67
	N	6	6	6	6	6
	P-value					0.986

C1 = Control T1 = 0/10 mg/kg/day T2 = 10/10 mg/kg/day T3 = 50/10 mg/kg/day T4 = 200/10 mg/kg/day
Analysis Type : Trend analysis with ordinal dose scale; multiplicity adjusted
N : Group Size
S. E. : Standard Error
P-value : Multiplicity Adjusted for # of Tests

The absolute changes in the male and female liver and thymus weights are shown below. Please see the changes in controls vs 0/10 ezetimibe /atorvastatin only, because the other co-administrations are not applicable here.

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Table. Absolute liver weights in males

Table B-5. MK-6213/L-000776336: Three-Month Combination Oral Toxicity Study in Dogs. TT #07-6039

Male ABSOLUTE ORGAN WEIGHTS

TREATMENT GROUP & ANIMAL NUMBER	NO. OF DOSES	BODY WEIGHT kilograms	Brain grams	Spleen grams	Heart grams	Kidney grams	Liver grams
Control							
07-0262M	91	8.1	73.35	21.95	74.95	37.80	280.29
07-0264M	91	9.5	71.28	24.21	87.89	50.86	265.43
07-0270M	91	9.6	71.98	28.28	82.69	56.79	285.93
MEAN		9.1	72.20	24.81	81.84	48.48	277.22
STD DEV		0.8	1.05	3.21	6.51	9.72	10.59
0/10 mg/kg/day							
07-0174M	91	9.9	75.60	21.35	76.70	43.95	264.18
07-0254M	91	8.5	73.15	24.13	79.08	54.09	212.86
07-0258M	91	11.1	76.09	27.16	80.33	46.99	254.31
MEAN		9.8	74.95	24.21	78.70	48.34	243.78
STD DEV		1.3	1.58	2.91	1.84	5.20	27.23
10/10 mg/kg/day							
07-0106M	91	7.9	80.98	17.42	78.92	45.11	179.80
07-0162M	91	6.1	71.95	12.46	62.07	40.24	214.93
07-0252M	92	10.6	69.26	23.93	67.09	45.57	229.90
MEAN		8.2	74.06	17.94	69.36	43.64	208.21
STD DEV		2.3	6.14	5.75	8.65	2.95	25.72
50/10 mg/kg/day							
07-0256M	91	10.5	72.31	22.27	92.02	43.48	215.42
07-0260M	91	8.8	74.23	23.68	78.75	50.77	191.84
07-0268M	92	9.4	72.12	18.55	75.61	47.69	210.72
MEAN		9.6	72.89	21.50	82.13	47.31	205.99
STD DEV		0.9	1.17	2.65	8.71	3.66	12.48
200/10 mg/kg/day							
07-0132M	91	10.1	78.98	30.62	80.95	53.98	249.94
07-0206M	91	8.2	74.21	21.33	78.77	51.99	210.79
07-0266M	92	9.6	79.16	24.06	76.23	53.08	203.30
MEAN		9.3	77.45	25.34	78.65	53.02	221.34
STD DEV		1.0	2.81	4.77	2.36	1.00	25.05

Table. Absolute liver weights in females

Table B-4. MK-6213/L-000776336: Three-Month Combination Oral Toxicity Study in Dogs. TT #07-6039

Female ABSOLUTE ORGAN WEIGHTS

TREATMENT GROUP & ANIMAL NUMBER	NO. OF DOSES	BODY WEIGHT kilograms	Brain grams	Spleen grams	Heart grams	Kidney grams	Liver grams
Control							
07-0183F	92	7.5	71.09	20.95	65.59	37.28	249.40
07-0187F	92	9.7	64.82	21.02	57.42	33.87	168.06
07-0265F	93	7.6	70.93	21.51	66.79	33.34	278.30
MEAN		8.3	68.95	21.16	63.27	34.83	231.92
STD DEV		1.2	3.57	0.31	5.10	2.14	57.16
0/10 mg/kg/day							
07-0101F	92	7.8	73.84	21.63	84.16	37.62	243.44
07-0261F	92	10.0	77.83	31.90	84.58	47.29	254.41
07-0269F	93	7.1	73.81	24.73	61.32	33.54	205.69
MEAN		8.3	75.16	26.09	76.69	39.48	234.51
STD DEV		1.5	2.31	5.27	13.31	7.06	25.56
10/10 mg/kg/day							
07-0121F	92	12.0	78.81	29.31	84.77	43.86	240.05
07-0245F	92	7.0	68.36	21.06	54.70	37.32	144.68
07-0257F	93	7.8	68.19	18.74	76.95	45.84	245.12
MEAN		8.9	71.79	23.04	72.14	42.34	209.95
STD DEV		2.7	6.08	5.56	15.60	4.46	56.58
50/10 mg/kg/day							
07-0197F	92	7.7	75.89	24.60	81.04	41.18	193.92
07-0251F	92	8.1	69.47	22.79	70.13	44.07	217.45
07-0259F	93	6.5	71.52	16.36	60.87	36.37	215.71
MEAN		7.4	72.29	21.25	70.68	40.54	209.03
STD DEV		0.8	3.28	4.33	10.10	3.89	13.11
200/10 mg/kg/day							
07-0169F	92	7.8	62.88	24.58	58.12	37.06	172.68
07-0253F	93	8.4	68.70	30.29	60.78	33.38	171.80
07-0267F	93	7.7	67.00	16.82	58.42	32.83	158.33
MEAN		8.0	66.19	23.90	59.11	34.42	167.60
STD DEV		0.4	2.99	6.76	1.46	2.30	8.04

Table. Absolute thymus weights in males.

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Continued
Table B-5. MK-6213/L-000776336: Three-Month Combination Oral Toxicity Study in Dogs. TT #07-6039

Male ABSOLUTE ORGAN WEIGHTS

TREATMENT GROUP & ANIMAL NUMBER	NO. OF DOSES	Adrenal grams	Pituitary grams	Thyroid grams	Testis grams	Prostate grams	Thymus grams
Control							
07-0262M	91	1.09	0.0705	0.62	14.75	1.08	2.88
07-0264M	91	0.94	0.0548	1.16	17.01	6.02	3.72
07-0270M	91	0.98	0.0583	0.73	15.71	7.23	5.48
MEAN		1.00	0.0612	0.84	15.82	4.78	4.03
STD DEV		0.08	0.0082	0.29	1.13	3.26	1.33
0/10 mg/kg/day							
07-0174M	91	1.00	0.0577	0.57	14.74	8.73	2.19
07-0254M	91	1.11	0.0742	0.49	15.69	5.15	1.74
07-0258M	91	0.68	0.0656	0.64	13.74	5.14	3.15
MEAN		0.93	0.0658	0.57	14.72	6.34	2.36
STD DEV		0.22	0.0083	0.08	0.98	2.07	0.72
10/10 mg/kg/day							
07-0106M	91	1.19	0.0575	0.40	18.07	1.68	3.84
07-0162M	91	0.92	0.0443	0.33	9.97	0.81	1.57
07-0252M	92	0.95	0.0562	0.54	13.65	3.18	4.13
MEAN		1.02	0.0527	0.42	13.90	1.89	3.18
STD DEV		0.15	0.0073	0.11	4.06	1.20	1.40
50/10 mg/kg/day							
07-0256M	91	1.00	0.0596	0.47	15.44	5.84	5.04
07-0260M	91	0.80	0.0674	0.53	12.96	4.54	2.10
07-0268M	92	1.21	0.0796	1.52	14.36	2.86	3.03
MEAN		1.00	0.0689	0.84	14.25	4.41	3.39
STD DEV		0.21	0.0101	0.59	1.24	1.49	1.50
200/10 mg/kg/day							
07-0132M	91	1.08	0.0677	0.66	23.02	8.59	1.63
07-0206M	91	0.94	0.0745	0.61	15.78	1.72	2.60
07-0266M	92	1.08	0.0732	0.46	16.41	2.74	3.26
MEAN		1.03	0.0718	0.58	18.40	4.35	2.50
STD DEV		0.08	0.0036	0.10	4.01	3.71	0.82

Table. Absolute thymus weights in females

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Continued
Table B-4. MK-6213/L-000776336: Three-Month Combination Oral Toxicity Study in Dogs. TT #07-6039

Female ABSOLUTE ORGAN WEIGHTS

TREATMENT GROUP & ANIMAL NUMBER	NO. OF DOSES	Adrenal grams	Pituitary grams	Thyroid grams	Ovary grams	Thymus grams
Control						
07-0183F	92	0.93	0.0542	0.55	0.64	4.41
07-0187F	92	0.99	0.0594	0.65	0.86	1.19
07-0265F	93	1.22	0.0532	0.69	0.68	5.77
MEAN		1.05	0.0556	0.63	0.73	3.79
STD DEV		0.15	0.0033	0.07	0.12	2.35
0/10 mg/kg/day						
07-0101F	92	1.40	0.0597	0.69	0.63	3.39
07-0261F	92	1.33	0.0633	0.75	0.87	1.04
07-0269F	93	1.16	0.0547	0.42	0.83	4.25
MEAN		1.30	0.0592	0.62	0.78	2.89
STD DEV		0.12	0.0043	0.18	0.13	1.66
10/10 mg/kg/day						
07-0121F	92	1.55	0.0686	0.73	1.52	4.14
07-0249F	92	0.83	0.0572	0.49	2.14	3.68
07-0257F	93	1.00	0.0702	0.42	2.76	2.25
MEAN		1.13	0.0653	0.55	2.14	3.36
STD DEV		0.38	0.0071	0.16	0.62	0.99
50/10 mg/kg/day						
07-0197F	92	1.29	0.0642	0.63	0.72	3.34
07-0251F	92	1.35	0.0581	0.82	4.33	2.33
07-0259F	93	0.91	0.0545	0.52	0.40	2.41
MEAN		1.18	0.0589	0.66	1.82	2.69
STD DEV		0.24	0.0049	0.15	2.18	0.56
200/10 mg/kg/day						
07-0169F	92	1.06	0.0690	0.56	1.51	1.04
07-0253F	93	0.99	0.0575	0.60	0.85	4.70
07-0267F	93	0.93	0.0621	0.49	0.55	2.89
MEAN		0.99	0.0631	0.55	0.97	3.14
STD DEV		0.07	0.0062	0.06	0.49	1.45

Histopathology: No significant changes in histopathology were observed with atorvastatin amorphous, except in the liver, where focal fibrosis was noted in 1/3 male dogs vs 0/3 controls, see below. Additionally, 1/1 female dogs had focal pneumonia in the lungs (not noted in controls).

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TABLE B-3. MK-6213/L-000776336: Three-Month Combination Oral Toxicity Study in Dogs. TT #07-6039
Summary Incidence of Histomorphology

Group Number	Female 1	2	3	4	5	Male 1	2	3	4	5
Liver (CONT.)										
Pigmentation	-	-	1	1	-	-	-	-	-	2
Centrilobular Region, Cellular infiltration, Mixed	-	-	-	-	-	-	-	-	1	-
Kupffer Cell, Hypertrophy	-	-	2	1	3	-	-	-	2	2
Bile Ductule, Hyperplasia	-	-	2	1	3	-	-	1	3	1
Liver										
NO. EXAMINED MICROSCOPICALLY	3	3	3	3	3	3	3	3	3	3
Not Remarkable	3	3	1	1	-	3	2	2	-	-
Fibrosis, Focal	-	-	-	-	-	-	1	-	-	-
Glycogen depletion	-	-	1	-	3	-	-	-	2	2
Lung										
NO. EXAMINED MICROSCOPICALLY	3	1	0	1	3	3	1	1	0	3
Not Remarkable	3	-		-	3	3	1	-		2
Bronchopneumonia, Focal	-	-		1	-	-	-	1		-
Pneumonia, Focal	-	1		-	-	-	-	-		1
Kidney										
NO. EXAMINED MICROSCOPICALLY	3	0	0	0	3	3	0	0	0	3
Not Remarkable	2				2	3				2
Cast	1				-	-				-
Cellular infiltration, Multifocal	-				1	-				-
Pyelonephritis, Chronic	-				-	-				1

KEY: GROUP 1 = Control
GROUP 2 = 0/10 mg/kg/day
GROUP 3 = 10/10 mg/kg/day
GROUP 4 = 50/10 mg/kg/day
GROUP 5 = 200/10 mg/kg/day
- = NOT PRESENT.

Sponsor description of histopathology changes are provided below. This description includes changes with combination of MK-6213, i.e. another ezetimibe-like drug and their own amorphous atorvastatin at 0/10, 50/10, 200/10 mg respectively of Mk06213/atorvastatin.

Very slight or slight bile ductule hyperplasia, characterized by the presence of prominent perilobular immature bile ductules, made up of flattened to cuboidal and slightly basophilic epithelial cells, was seen in all drug-combination groups from both genders. This change was very slight at 10/10 mg/kg/day, slight in females and very slight to slight in males at 50/10 mg/kg/day, and very slight to slight in females and slight in males at 200/10 mg/kg/day.

Very slight or slight Kupffer cell hypertrophy, characterized by prominent, plump, occasionally brownish pigment-laden Kupffer cells, was seen in all drug-combination groups but males at 10/10 mg/kg/day. This change was slight in females at 10/10 mg/kg/day, very slight in females and very slight to slight in males at 50/10 mg/kg/day, and very slight to slight in females and very slight in males at 200/10 mg/kg/day.

Pigmentation, characterized by foci of intra- or inter-hepatocellular greenish coarse granules, and identified as bile by Hall's stain for bilirubin (in 200/10-mg/kg/day animal #07-0266), was seen and graded very slight in 1 female at 10/10 mg/kg/day and 50/10 mg/kg/day each, and in 2 males, very slight or slight, at 200/10 mg/kg/day.

Glycogen depletion, characterized by dense, slightly basophilic hepato-cellular cytoplasm throughout the liver section, was observed in females at 10/10 mg/kg/day, in males at 50/10 mg/kg/day and in both genders at 200/10 mg/kg/day, and was always graded very slight.

Treatment-Related Histomorphologic Changes
(Incidence, n=3)

	MK-6213/L-000776336 (mg/kg/day)							
	Females				Males			
	0/10	10/10	50/10	200/10	0/10	10/10	50/10	200/10
Liver:								
Bile Ductule Hyperplasia	-	2[a]	1[a]	3[a]	-	1[a]	3[a]	1[a]
Kupffer Cell Hypertrophy	-	2[a]	1[a]	3[a]	-	-	2[a]	2[a]
Pigmentation	-	1[a]	1[a]	-	-	-	-	2[a]
Glycogen Depletion	-	1[a]	-	3[a]	-	-	2[a]	2[a]

[a] Treament-related change based on incidence and/or severity.
- = No treatment-related change.

3-Month Combination Oral Toxicity Study in Dogs.

Sponsor's conclusions are stated below:

Liver changes included bile ductule hyperplasia, Kupffer cell hypertrophy (not seen in males at 10/10 mg/kg/day), pigmentation (not seen in females at 200/10 mg/kg/day or in males at 10/10 mg/kg/day or 50/10 mg/kg/day), and glycogen depletion (not seen in females at 50/10 mg/kg/day or in males at 10/10 mg/kg/day). No treatment-related histomorphological liver changes were noted in the L-000776336-only treatment group.

Based on histomorphologic liver changes, there was no no-observed effect level (NOEL) for postmortem changes attributed to the MK-6213/L-000776336 combination.

Toxicokinetics:

L-000776336 or atorvastatin amorphous (10 mg/kg/day) in dogs accumulated over time and values were higher in week-13 (1010 nM.hr), compared to day-1 (465 nM.hr). Cmax values also increased over time (week-13 values were 316 nM, vs day-1 which were 145 nM). Thus exposures of atorvastatin were 2-3 fold higher in week-13 vs day-1. Note that para-hydroxy atorvastatin and ortho-hydroxy atorvastatin were not measured in this study (these both were measured in the co-administration studies of ezetimibe and atorvastatin crystalline, in a 3-month dog study under NDA 21-445). The TK parameters of L-000776336 (or atorvastatin amorphous) in dogs are sown below:

L-000776336

Absorption of L-000776336 was rapid in all dose groups including L-000776336-only groups with mean T_{max} occurring between 1.5 and 2.3 hours after dosing. Plasma drug elimination was rapid at all doses of MK-6213/L-000776336 with no measurable L-000776336 plasma concentrations (< lower limit of quantitation) 24 hours postdosing.

L-000776336

Taking into account the high inter-animal variability, there were no consistent and substantial (i.e., less than 2-fold) sex-related differences in systemic exposure ($AUC_{0\text{-}24\ hr}$) or C_{max} on Study Day 1 and in Study Week 13.

Absorption of L-000776336 was rapid in all dose groups including L-000776336-only groups with mean T_{max} occurring between 1.5 and 2.3 hours after dosing. Plasma drug elimination was rapid at all doses with no measurable L-000776336 plasma concentrations (< lower limit of quantitation) 24 hours postdosing. Taking into account the inter-animal variability, co-administration of MK-6213 did not change the systemic exposure and C_{max} of L-000776336 on Study Day 1 or in Study Week 13 at any of the combination dose levels.

Systemic exposure and C_{max} were approximately 2.4-fold and 2.9-fold greater in Study Week 13 than on Study Day 1, respectively.

Mean Plasma L-000776336 Toxicokinetic Parameters - Study Day 1

	MK-6213/L-000776336 (mg/kg/day)[a]			
	Sexes Combined			
	0/10	10/10	50/10	200/10
$AUC_{0\text{-}24\ hr}$ (nM•hr)	465 ± 102	697 ± 112	370 ± 110	438 ± 72.8
C_{max} (nM)	145 ± 37.0	168 ± 34.3	114 ± 34.3	101 ± 13.6
T_{max} (hr)	1.8 ± 0.17	2.3 ± 0.33	1.5 ± 0.22	2.3 ± 0.33

Values are the mean ± SEM.
[a] No drug levels detected above the lower limit of quantitation in control plasma samples.

3-Month Combination Oral Toxicity Study in Dogs.

Mean Plasma L-000776336 Toxicokinetic Parameters - Study Week 13

	MK-6213/L-000776336 (mg/kg/day)[a]			
	Sexes Combined			
	0/10	10/10	50/10	200/10
$AUC_{0\text{-}24\ hr}$ (nM•hr)	1010 ± 105	1630 ± 306	895 ± 192	1180 ± 224
C_{max} (nM)	316 ± 56.5	563 ± 119	271 ± 51.4	366 ± 80.4
T_{max} (hr)	2.0 ± 0	2.0 ± 0	1.7 ± 0.21	2.2 ± 0.40

Values are the mean ± SEM.
[a] No drug levels detected above the lower limit of quantitation in control plasma samples.

3-Month Combination Oral Toxicity Study in Dogs.

MK-6213 exposures are not relevant here, and are therefore not shown below.

Sponsor's conclusion of this study are stated below

Conclusions

In conclusion, MK-6213 was administered orally (by capsule) in combination with a fixed dose of L-000776336 to dogs once daily at doses of 10/10, 50/10, or 200/10 mg/kg/day for approximately 3 months. L-000776336 was also administered at 10 mg/kg/day to dogs orally once daily alone for reference purposes. Treatment-related very slight to slight histomorphologic liver changes were observed in all drug-treated combination groups without a dose-response related to MK-6213. Liver changes included bile ductule hyperplasia, Kupffer cell hypertrophy (not seen in males at 10/10 mg/kg/day), pigmentation (not seen in females at 200/10 mg/kg/day or in males at 10/10 mg/kg/day or 50/10 mg/kg/day), and glycogen depletion (not seen in females at 50/10 mg/kg/day or in males at 10/10 mg/kg/day). No treatment-related histomorphological liver changes were noted in the L-000776336-only treatment group.

Based on histomorphologic liver changes, there was no no-observed effect level (NOEL) for postmortem changes attributed to the MK-6213/L-000776336 combination.

In summary in a 3-month oral co-administration toxicity study of MK-6213/L000776336 in dogs (another cholesterol absorption inhibitor/atorvastatin amorphous), doses of 0/0, 0/10, 10/10, 50/10, 200/10 mg/kg/day of MK-6213/atorvastatin were administered to dogs orally in a capsule (in a vehicle imwitor 742: polysorbate 1:1 w/w). Atorvastatin AUC exposures in combined sexes were approximately 2-fold higher in week-13, (1010 nM/hr at 0/10 mg/kg/day of MK - 6213/atorvastatin) vs on day-1 (465 nM/hr), suggesting acumulation of atorvastatin over time. In both sexes, atorvastatin amorphous produced increases in plasma ALT (males 97 vs 45 IU/L in controls; females 63 vs 30 IU/L in controls), and bilirubin levels (0.2 vs 0.1 mg/dl in controls in both sexes). ALP levels (152 vs 98 IU/L in controls) and creatinine kinase levels (411 vs 239 IU/L in controls) were increased in females only, while cholesterol and triglycerides were decreased in both sexes. Atorvastatin amorphous decreased absolute liver weights by 6% (239 vs 254 g in controls, relative by 11%,) and thymus weights by 10% (2.82 vs 3.91 g in controls; relative by 35%) in combined sexes. However, no associated changes in histopathology were observed in the thymus. Target organ of toxicity in males may be liver, as 1/3 dogs had focal fibrosis in the liver(vs 0/3 in controls. Thus 10 mg/kg/day of atorvastatin calcium amorphous in dogs (or 200 mg/m^2/day) in the above 3-month study provides the safety margin of approximately 4-fold in human subjects (at the highest dose of 80 mg/day clinical dose or 49 mg/kg/m^2, assuming 60 kg weight), based on body surface area. Note that this study has a limited value, as it did not compare the marketed atorvastatin (crystalline form) with the current atorvastatin (amorphous form). However, no significant new toxicity was noted with atorvastatin amorphous in dogs.

Sponsor makes following statement for qualifying this study

"A three-month oral toxicity study was conducted in dogs administered atorvastatin calcium (amorphous) and an investigational cholesterol absorption inhibitor (MK-6213) (TT #07- 6039). This study included a group of animals administered atorvastatin calcium (amorphous) alone at 10 mg/kg/day. The MK-0653C drug product has the potential to form impurities/degradants of atorvastatin calcium (amorphous), of which the content in the drug product may exceed the qualification threshold of (b) (4) *per day total daily intake for the 80 mg dose strength of atorvastatin calcium (amorphous). No new toxicities were identified at 10 mg/kg/day atorvastatin calcium (amorphous) in this study. The data from this group were determined to be sufficient for the purposes of atorvastatin impurity/degradate qualification at the proposed drug product specification provided in [Table 2.4: 2]". See page 11 for Table 2.4.2.*

6.6.6.4 GENETIC TOXICOLOGY

Following new geno-toxicity studies have been conducted with atorvastatin impurities in the current NDA application of Atozet.

Sponsor has conducted the bacterial mutagenicity assays and in vitro chromosomal aberration studies to assess the potential genotoxicity of impurities /degradants found in atorvastatin calcium (amorphous).

Sponsor states *that an exploratory bacterial mutagenicity study was conducted (using doses of 8 to 1500 mcg/plate) to assess the genotoxic potential of commonly identified impurities/ degradants found in atorvastatin calcium (amorphous). The impurities /degradants tested were* (b) (4) *and a mixture (5:1) of* (b) (4) *An initial trial was conducted with a top dose of 250 mcg/plate of* (b) (4) *and 500 mcg/plate of (5:1) mixture of* (b) (4) *with and without activation. Trial 2 with a (5:1) mixture of* (b) (4) *was conducted at 1500 mcg/plate in order to test both the impurities/ degradants present in the mixture up to at least 250 mcg/plate. The* (b) (4) *and* (b) (4) *present in a milligram of the mixture, when tested at a top dose of 1500 mcg/plate would be equivalent to* (b) (4) *of the* (b) (4) *and* (b) (4) *) per plate of the* (b) (4) *respectively. The impurities/degradants (* (b) (4) *or 5:1 mixture of* (b) (4) *) were found to be negative in the bacterial mutagenicity assay.*

Additional bacterial mutagenicity [Sec. 2.6.7.17C] and in vitro chromosomal aberration assays [Sec. 2.6.7.17D] were conducted using atorvastatin calcium (amorphous) batch L-000776336-004G027 [Sec. 2.6.7.4B] containing the following impurity/degradant levels: (b) (4) *Both the bacterial mutagenicity assay and in vitro chromosomal aberration assay were negative and therefore provide further support for the proposed specifications for the impurity/degradation products of atorvastatin calcium (amorphous).*

These new gene-toxicity studies are reviewed below:

1. **Ames assay with atorvastatin amorphous (study # SN 08348)**:

EXPLORATORY BACTERIAL MUTAGENICITY STUDY OF ATORVASTATIN (SCH 412387) IMPURITIES

This exploratory non-GLP bacterial mutagenicity study was conducted to assess the genotoxic potential of commonly identified impurities/degradants found in atorvastatin calcium (amorphous) [Sec. 2.6.7.17B]. The impurities/degradants tested were (b) (4) and a mixture (5:1) of (b) (4) Each (b) (4) was tested at levels up to 250 µg/plate. Sponsor states that All of the impurities/degradants (b) (4) were found to be negative for genotoxicity.

This non-GLP study that was conducted by Schering-Plough Research Institute, at Summit, NJ. The study was initiated on 9/16/2008.

Methods:
The potential mutagenicity of SCH 412387 impurities, [redacted], were assessed in the *Salmonella*/mammalian microsome (Ames test) and *Escherichia*/mammalian microsome plate incorporation reverse mutation assays [redacted] with and without metabolic activation.

The batch number of impurity A [redacted]) was 20080500360 and of B was 20080500361 [redacted] The concentrations of impurity A and B tested were 0, 4, 8, 16, 32, 63, 125, 250 µg/plate; additionally impurity B was tested up to 500 ug/plate. Thus the highest doses chosen for this assay were 250 to 500 µg/plate. In the microbial mutagenesis assays, atorvastatin at doses up to 500 µg/plate did not induce increased numbers of revertants in any of the *Salmonella typhimurium* or *Escherichia coli* test strains with or without metabolic (S-9) activation.

Sponsor's methods, study design and results are provided below:

Bacterial Mutagenicity Study of SCH 412387 impurities A&B (SN 08348): **Materials/Methods**	
Objective:	The objective of this study was to evaluate the mutagenicity of SCH 412387 impurities A&B, in the Ames *Salmonella*/mammalian microsome and *Escherichia*/mammalian microsome reverse mutation assays.
Test Article:	SCH 412387 impurities A&B in dimethylsulfoxide prepared on the day of dosing
	Batch No.: For (A) 20080500360 – [redacted] For (B) 20080500361- [redacted].
	Storage Conditions: Refrigerated, 2-8°C
Solvent Control:	Dimethylsulfoxide (DMSO)
Positive Controls:	9-Aminoacridine 2-Aminoanthracene Cumene hydroperoxide 1-Methyl-3-nitro-1-nitrosoguanidine 2-Nitrofluorene Sodium azide
Metabolic Activation System:	Aroclor 1254-induced rat liver S9
	Lot No.: 1921
	Supplier: [redacted]
Method of Exposure:	Plate incorporation method
Test System (Bacterial Tester Strains):	Histidine auxotrophs: *Salmonella typhimurium* tester strains TA1535, TA97a, TA98, TA100 and TA102
	Tryptophan auxotroph: *Escherichia coli* tester strain WP2*uvr*A [redacted]
	Reference bacterial stocks: Stored below -70°C and periodically regenerated and confirmed for retention of strain-specific phenotypes.
	Plate Identification: Labeled with the study number, treatment date and the treatment group number (color-coded for each bacterial strain).

STUDY DESIGN

Bacterial Mutagenicity Study of SCH 412387 impurities (A)		
Bacterial Strain	SCH 412387 impurity (A) (μg/plate)	
	Trial 1 Nonactivation[a]	Trial 1 Activation[b]
TA1535, TA97a, TA98, TA100, TA102 and WP2*uvr*A	0,[c] 3.9,7.8,15.6,31.3,62.5,125,250	0,[c] 3.9,7.8,15.6,31.3,62.5,125,250
a: 0.5 mL sodium phosphate buffer (pH 7.4) will be added to each culture tube prior to plating.		
b: 0.5 mL S9 mix will be added to each culture tube prior to plating.		
c: Solvent control plates will be dosed with 100 μL/plate DMSO		

STUDY DESIGN

Bacterial Mutagenicity Study of SCH 412387 impurities (B)		
Bacterial Strain	SCH 412387 impurity (B) (μg/plate)	
	Trial 1 Nonactivation[a]	Trial 1 Activation[b]
TA1535, TA97a, TA98, TA100, TA102 and WP2*uvr*A	0,[c] 7.8,15.6,31.3,62.5,125,250,500	0,[c] 7.8,15.6,31.3,62.5,125,250,500
Bacterial Strain	Trial 2 Nonactivation[a]	Trial 2 Activation[b]
TA1535, TA97a, TA98, TA100, TA102 and WP2*uvr*A	0,[c] 750, 1000, 1250, 1500	0,[c] 750, 1000, 1250, 1500
a: 0.5 mL sodium phosphate buffer (pH 7.4) will be added to each culture tube prior to plating.		
b: 0.5 mL S9 mix will be added to each culture tube prior to plating.		
c: Solvent control plates will be dosed with 100 μL/plate DMSO		

Bacterial Mutagenicity Study of SCH 412387 impurities (A&B) (SN 08348): **Study Design - Positive Controls**				
Bacterial Strain[a]	Nonactivation[b]		Activation[c]	
	Positive Control	Dose (μg/plate)[d]	Positive Control	Dose (μg/plate)[d]
TA1535	Sodium Azide	5	2-Aminoanthracene	2.5
TA97a	9-Aminoacridine	75	2-Aminoanthracene	2.5
TA98	2-Nitrofluorene	5	2-Aminoanthracene	2.5
TA100	Sodium Azide	5	2-Aminoanthracene	2.5
TA102	Cumene hydroperoxide	100	2-Aminoanthracene	5
WP2*uvr*A	MNNG[e]	4	2-Aminoanthracene	20
a: Dosing procedures and incubation conditions for the positive controls were the same as those for the test article				
b: 0.5 mL of 0.1M sodium phosphate buffer (pH 7.4) was added to each culture tube prior to plating				
c: 0.5 mL S9 mix was added to each culture tube prior to plating				
d: The positive controls will be dosed with 100 μL/plate				
e: MNNG = 1-Methyl-3-nitro-1-nitrosoguanidine				

Bacterial Mutagenicity Study of SCH SCH 412387 Impurities (A&B) (SN 08348): **Observations and Measurements**	
Optical Density:	Determined for overnight bacterial cultures at 650 nm using a spectrophotometer
Colony Counting and Evaluation of Background Lawns:	Revertant colonies were scored with an automatic colony counter Background lawns were scored visually.
Cytotoxicity:	Determined by decreases in revertant colony counts and inhibition of background bacterial lawn growth. Cytotoxicity to revertant colonies was determined by an approximate 30% reduction in revertant colony counts below the concurrent solvent control, as well as additional factors based on sound scientific judgment.

Results:

An initial trial was conducted with a top dose of 25 µg/plate of (A) and 500 µg/plate of (B) with and without metabolic activation system **(Tables 1-4)**.

Trial 2 was conducted with (B) with and without metabolic activation at 1500 µg/plate top dose in order to test both the impurities present in the mixture up to at least 250 µg/plate. (b) (4)

(b) (4)

In the absence of activation:

SCH 412387 (A) or (B) were not cytotoxic in any of the strains tested in the absence of metabolic activation system **(Table 1, 3 and 5)**.

SCH 412387 (A or B) did not induce an increase in revertant colony counts at any dose in any strain tested relative to the solvent controls in the absence of metabolic activation system. Mean revertant counts are included in **(Tables 1, 3 and 5)**

In the presence of activation:

SCH 412387 (A) was not cytotoxic in any of the strains tested except in TA98 in the presence of metabolic activation system at a concentration of 250 µg/plate **(Table 2)**.

SCH 412387 (B) was not cytotoxic in any of the strains tested except in TA1535 in the presence of metabolic activation at a concentration of 1500 µg/plate **(Table 6)**.

SCH 412387 (A or B) did not induce an increase in revertant colony counts at any dose in any strain tested relative to the solvent controls in the presence of metabolic activation system. Mean revertant counts are included in **(Tables 2, 4 and 6)**.

See Tables 1, 3, and 5 below in the absence of metabolic activation

Note that the Assay A (trial 1) was conducted with 250 mcg/plate of impurity A.

Table 1 Mutagenicity Assay (A) Trial 1– Nonactivation Phase Summary of Results

Summary Of Results: **Non-Activation**
Mean (n=3) Revertants Per Plate (Cytotoxicity)

Dose (µg/Plate)	Bacterial Strains					
	TA1535	TA97a	TA98	TA100	TA102	WP2*uvr*A
0 (Solvent Control: DMSO)	10(0)	83(0)	13(0)	114(0)	211(0)	56(0)
3.9 (SCH 412387 Impurity A)	9(0)	70(0)	17(0)	112(0)	187(0)	51(0)
7.8 (SCH 412387 Impurity A)	10(0)	77(0)	18(0)	124(0)	147(0)	57(0)
15.6 (SCH 412387 Impurity A)	12(0)	74(0)	15(0)	113(0)	180(0)	54(0)
31.3 (SCH 412387 Impurity A)	5[a](0)	86(0)	22(0)	108(0)	207(0)	52(0)
62.5 (SCH 412387 Impurity A)	9(0)	88(0)	14(0)	113(0)	219(0)	49(0)
125 (SCH 412387 Impurity A)	11(0)	83(0)	14(0)	121(0)	223(0)	52(0)
250 (SCH 412387 Impurity A)	11(0)	85(0)	19(0)	132(0)	211(0)	54(0)
5 (Sodium Azide)	1180*(0)	---	---	1203*(0)	---	---
5 (2-Nitrofluorene)	---	---	493*(0)	---	---	---
75 (9-Aminoacridine)	---	1561*(0)	---	---	---	--
4 MNNG	---	---	---	---	---	667*(0)
200 (Cumene Hydroperoxide)	---	---	--	---	176**(0)	---

* = ≥ two-fold increase (three-fold for TA1535) in revertant colonies over the corresponding solvent control indicates a positive response

** Positive control did not attain required value

Cytotoxicity to Background Lawn: (0) = none

a: Slight decrease in mean revertant colony counts was considered incidental and not cytotoxicity because of the absence of a dose-response.

--- Not applicable

Table 3. Two Assays with the impurity B were conducted using up to 500 and 1500 mcg/plate respectively (Trial 1 and Trial 2). Table 3 is with 500 mcg/plate, and Table 5 is 1500 mcg/plate

Table 3 Mutagenicity Assay (B) Trial 1 – Nonactivation Phase Summary of Results

Summary Of Results: **Non-Activation**
Mean (n=3) Revertants Per Plate (Cytotoxicity)

Dose (µg/Plate)	Bacterial Strains					
	TA1535	TA97a	TA98	TA100	TA102	WP2*uvr*A
0 (Solvent Control: DMSO)	11 (0)	70 (0)	11(0)	106(0)	141(0)	23 (0)
7.8 (SCH 412387 Impurity B)	13 (0)	77 (0)	10 (0)	104 (0)	121 (0)	22 (0)
15.6 (SCH 412387 Impurity B)	12 (0)	65 (0)	11(0)	107(0)	114(0)	25(0)
31.3 (SCH 412387 Impurity B)	14 (0)	82 (0)	9 (0)	124 (0)	131 (0)	25 (0)
62.5 (SCH 412387 Impurity B)	10(0)	65 (0)	14 (0)	106 (0)pt	128 (0)	43 (0)
125 (SCH 412387 Impurity B)	14(0)pt	85 (0)pt	15 (0)pt	98 (0)pt	153 (0)pt	28 (0)
250 (SCH 412387 Impurity B)	9(0)pt	64 (0)pt	13 (0)pt	80 (0)pt	107 (0)pt	53 (0)
500 (SCH 412387 Impurity B)	10 (0)pt	72 (0)pt	12(0)pt	93 (0)pt	149 (0)pt	50 (0)
5 (Sodium Azide)	1221*(0)	---	---	1398*(0)	---	---
5 (2-Nitrofluorene)	---	---	559*(0)	---	---	---
75 (9-Aminoacridine)	---	1495*(0)	---	---	---	--
4 MNNG	---	---	---	---	---	700*(0)
200 (Cumene Hydroperoxide)	---	---	--	---	1048*(0)	---

* = ≥ two-fold increase (three-fold for TA1535) in revertant colonies over the corresponding solvent control indicates a positive response

Cytotoxicity to Background Lawn: (0) = none

pt = precipitate

--- Not applicable

Table 5. Second assay of impurity B with 1500 mcg/plate (Trial 2).

Table 5 Mutagenicity Assay (B) Trial 2– Nonactivation Phase Summary of Results

Summary Of Results: **Non-Activation** Mean (n=3) Revertants Per Plate (Cytotoxicity)						
	Bacterial Strains					
Dose (µg/Plate)	TA1535	TA97a	TA98	TA100	TA102	WP2*uvr*A
0 (Solvent Control: DMSO)	14(0)	97(0)	14(0)	117(0)	282(0)	58(0)
750 (SCH 412387 Impurity B)	13(0)pt	90(0)pt	14(0)pt	108(0)pt	290(0)pt	50(0)pt
1000 (SCH 412387 Impurity B)	14(0)pt	78(0)pt	16(0)pt	92(0)pt	299(0)pt	56(0)pt
1250 (SCH 412387 Impurity B)	13(0)pt	86(0)pt	14(0)pt	114(0)pt	316(0)pt	62(0)pt
1500 (SCH 412387 Impurity B)	13(0)pt	68(0)pt	17(0)pt	104(0)pt	310(0)pt	51(0)pt
5 (Sodium Azide)	1132*(0)	---	---	1487*(0)	---	---
5 (2-Nitrofluorene)	---	---	582*(0)	---	---	---
75 (9-Aminoacridine)	---	1672*(0)	---	---	---	--
4 MNNG	---	---	---	---	---	727*(0)
200 (Cumene Hydroperoxide)	---	---	--	---	1633*(0)	---
* = ≥ two-fold increase (three-fold for TA1535) in revertant colonies over the corresponding solvent control indicates a positive response Cytotoxicity to Background Lawn: (0) = none pt = precipitate --- Not applicable						

See Tables 2, 4, and 6 below in the presence of metabolic activation

Note that the Assay A (trial 1) was conducted with 250 mcg/plate of impurity A.

Table 2 Mutagenicity Assay (A) Trial 1 – Activation Phase Summary of Results

Summary Of Results: **Activation**
Mean (n=3) Revertants Per Plate (Cytotoxicity)

Dose (µg/Plate)	Bacterial Strains					
	TA1535	TA97a	TA98	TA100	TA102	WP2*uvr*A
0 (Solvent Control: DMSO)	10(0)	128(0)	28(0)	128(0)	276(0)	66(0)
3.9 (SCH 412387 IMPURITY A)	30(0)	134(0)	18[a](0)	127(0)	275(0)	62(0)
7.8 (SCH 412387 IMPURITY A)	11(0)	144(0)	23(0)	131(0)	273(0)	62(0)
15.6 (SCH 412387 IMPURITY A)	7[a](0)	134(0)	17[a](0)	137(0)	264(0)	62(0)
31.3 (SCH 412387 IMPURITY A)	9(0)	127(0)	28(0)	130(0)	271(0)	57(0)
62.5 (SCH 412387 IMPURITY A)	8(0)	123(0)	23(0)	122(0)	280(0)	66(0)
125 (SCH 412387 IMPURITY A)	10(0)	129(0)	20(0)	118(0)	288(0)	57(0)
250 (SCH 412387 IMPURITY A)	11(0)	104(0)	18[b](0)	124(0)	283(0)	65(0)
2.5 (2-Aminoanthracene)	184*(0)	971*(0)	825*(0)	1087*(0)	---	---
5 (2-Aminoanthracene)	---	---	---	---	1142*(0)	---
20 (2-Aminoanthracene)	---	---	---	---	---	502*(0)

* = ≥ two-fold increase (three-fold for TA1535) in revertant colonies over the corresponding solvent control indicates a positive response

Cytotoxicity to Background Lawn: (0) = none

a: Slight decrease in mean revertant colony counts was considered incidental and not cytotoxicity because of the absence of a dose-response.

b: Cytotoxicity to revertant colonies (approximately ≥30% decrease in revertant colonies compared to concurrent solvent control)

--- Not applicable

Table 4. First assay of impurity B with 500 mcg/plate (Trial 2).

Table 4 Mutagenicity Assay (B)Trial 1– Activation Phase Summary of Results

Summary Of Results: **Activation**
Mean (n=3) Revertants Per Plate (Cytotoxicity)

Dose (µg/Plate)	Bacterial Strains					
	TA1535	TA97a	TA98	TA100	TA102	WP2*uvr*A
0 (Solvent Control: DMSO)	10(0)	93 (0)	30(0)	114(0)	206(0)	47(0)
7.8 (SCH 412387 Impurity B)	11(0)	94(0)	24(0)	105(0)	173 (0)	55(0)
15.6 (SCH 412387 Impurity B)	9 (0)	90 (0)	30(0)	130(0)	142 (0)	46(0)
31.3 (SCH 412387 Impurity B)	12 (0)	87 (0)	30(0)	103(0)	205 (0)	54(0)
62.5 (SCH 412387 Impurity B)	8 (0)	98 (0)	32(0)	107(0)	232 (0)	52(0)
125 (SCH 412387 Impurity B)	10 (0)pt	89 (0)	29 (0)	92 (0)	171 (0)	58(0)
250 (SCH 412387 Impurity B)	8 (0)pt	85 (0)pt	14(0)pt	88(0)pt	188 (0)pt	59(0)
500 (SCH 412387 Impurity B)	8 (0)pt	79 (0)pt	13(0)pt	91 (0)pt	124(0)pt	53(0)
2.5 (2-Aminoanthracene)	208*(0)pt	887*(0)	493*(0)	666*(0)	---	---
5 (2-Aminoanthracene)	---	---	---	---	463*(0)	---
20 (2-Aminoanthracene)	---	---	---	---	---	755*(0)

* = ≥ two-fold increase (three-fold for TA1535) in revertant colonies over the corresponding solvent control indicates a positive response

Cytotoxicity to Background Lawn: (0) = none

pt = precipitate

--- Not applicable

a: Cytotoxicity to revertant colonies(approximately ≥30% decrease in revertant colonies compared to concurrent solvent control.

Table 6. Second assay of impurity B with 1500 mcg/plate (Trial 2).

Table 6 Mutagenicity Assay (B) Trial 2– Activation Phase Summary of Results

Summary Of Results: **Activation**
Mean (n=3) Revertants Per Plate (Cytotoxicity)

Dose (µg/Plate)	Bacterial Strains					
	TA1535	TA97a	TA98	TA100	TA102	WP2*uvr*A
0 (Solvent Control: DMSO)	11(0)	94(0)	22(0)	114(0)	404(0)	60(0)
750 (SCH 412387 IMPURITY B)	9(0)pt	95(0)pt	20(0)pt	109(0)pt	394(0)pt	57(0)pt
1000 (SCH 412387 IMPURITY B)	10(0)pt	90(0)pt	22(0)pt	91(0)pt	395(0)pt	65(0)pt
1250 (SCH 412387 IMPURITY B)	10(0)pt	87(0)pt	21(0)pt	104(0)pt	424(0)pt	55(0)pt
1500 (SCH 412387 IMPURITY B)	7[a](0)pt	82(0)pt	25(0)pt	110(0)pt	407(0)pt	63(0)pt
2.5 (2-Aminoanthracene)	206*(0)	958*(0)	830*(0)	1265*(0)	---	---
5 (2-Aminoanthracene)	---	---	---	---	995*(0)	---
20 (2-Aminoanthracene)	---	---	---	---	---	779*(0)

* = ≥ two-fold increase (three-fold for TA1535) in revertant colonies over the corresponding solvent control indicates a positive response
Cytotoxicity to Background Lawn: (0) = none
pt = precipitate
--- Not applicable
a: Cytotoxicity to revertant colonies(approximately ≥30% decrease in revertant colonies compared to concurrent solvent control.

In conclusion, in this non-GLP Ames assay, atorvastatin at doses up to 500 µg/plate did not induce increased numbers of revertants in any of the *Salmonella typhimurium* or *Escherichia coli* test strains with or without metabolic (S-9) activation.

Sponsor's conclusions are stated below:

Conclusion

SCH 412387 impurities (A&B) did not induce an increase in revertant colony counts in any strain tested and were, therefore, not mutagenic in the bacterial mutagenicity assay, with or without metabolic activation, under the conditions of this study.

Note that additional bacterial mutagenicity assays and chromosomal aberration assay were conducted using atorvastatin calcium (amorphous) batch L-000776336-004G027 containing the following impurity/degradant levels: (b) (4) [redacted]

2. **Ames assay with atorvastatin amorphous (study # TT-098047)**:
This was GLP/QA study, conducted at Merck Research Laboratories West point, PA. The drug lot number for MK 9396 was L-000776336-004G027, it was 99.08% pure. The study was initiated on 5/13/2009. Note that MK 9396 is atorvastatin amorphous; it also has code names SCH 58235 -412387 or L000776336.

The objective of this study was to determine whether MK-9396 (atorvastatin amorphous) and its degradate induced mutations. The concentrations tested were 30, 100, 300, 1000, 3000, and 5000 µg/plate. The highest dose chosen for this assay was 5000 µg/plate.

Sponsor's methods and results are provided below.

Methods

MK-9396 and its degradate was evaluated for mutagenic potential in a microbial mutagenesis test system using mutant strains of *Salmonella typhimurium* (TA1535, TA97a, TA98, and TA100) and *Escherichia coli* (WP2 uvrA pKM101). In this test system, mutation was measured as reversion to histidine prototrophy of *Salmonella* test strains which are histidine auxotrophs and as reversion to tryptophan prototrophy of an *E. coli* test strain which is a tryptophan auxotroph. The test article was tested with and without a liver microsomal enzyme activation system (S-9) prepared from rats treated with phenobarbital and beta-naphthoflavone. The test article was tested using triplicate plates with and/or without metabolic activation for each strain tested. This study was conducted at Merck Research Laboratories, West Point, Pennsylvania, U.S.A., from 14-May to 18-May-2009, in accordance with Standard Operating Procedures.

MK-9396 (Atorvastatin) was used as a solution in Dimethyl Sulfoxide (DMSO). An active factor of 1.10 was used for calculating drug weight for the calcium salt. The final concentrations tested were 30, 100, 300, 1000, 3000, and 5000 µg/plate with and without S-9 metabolic activation.

a. Identification of Test Article

MK-9396

Lot number: L-000776336-004G027

Therapeutic Class: Atorvastatin

Purity: 99.08 area percent by LCAP

Active Factor: 1.10

Results

The results of the Microbial Mutagenesis Assay (TT #09-8047) indicated that MK-9396 did not produce any 2-fold or greater increases in revertants relative to control. The positive control and diagnostic mutagens showed appropriate S-9- and strain-dependent increases in revertants. Precipitate was seen on the plates at test concentrations of 1000 µg/plate and greater, but did not interfere with scoring of the plates. No inhibition of bacterial lawn growth was noted at any concentration tested. Inhibition of revertant growth was noted at 3000 µg/plate and greater in TA97a with metabolic activation only.

MK-9396 and its degradate did not induce 2-fold or greater dose-related increases in revertants relative to the solvent control in any of the test strains and thus is not detectably mutagenic in the Microbial Mutagenesis Assay.

Table 1. Ames assay with atorvastatin in the absence and presence of metabolic activation system (study # TT 09-8047)

2.6.7.17C Other Toxicity Studies (Genotoxicity: In Vitro) **Study No.:** [Ref. 4.2.3.7.6: TT098047] **Test Article**: MK-9396

TT #09-8047	TA100 18-May-2009				TA1535 18-May-2009				TA97a 18-May-2009			
	Without S-9		With S-9		Without S-9		With S-9		Without S-9		With S-9	
Conc. µg/Plate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 µg	162.1	18.9	181.6	14.2	24.3	6.7	22.1	4.7	204.3	20.1	252.8	17.9
30 µg	166.3	9.7	186.0	24.6	30.3	5.0	23.0	4.6	187.7	17.2	267.0	31.5
100 µg	152.7	19.5	180.0	28.8	21.7	5.7	24.7	3.5	201.0	6.1	263.0	6.2
300 µg	161.0	19.9	183.7	6.5	18.0	5.0	28.0	3.6	198.3	19.7	242.7	11.9
1000 µg	150.3	11.9	178.3	15.3	25.0*	11.4	27.3	4.0	202.0	11.8	251.3	8.5
3000 µg	171.3*	4.9	180.7	17.9	15.7*	3.5	20.0*	7.0	177.7*	18.0	208.3	5.9
5000 µg	180.7*	11.2	181.0*	10.6	17.3*	1.5	28.7*	1.5	170.0*	2.6	186.3*	3.2

Salmonella his$^+$ or *Escherichia coli* trp$^+$ Revertants per Plate

	TA98 18-MAY-2009				WP2 uvrA pKM101 18-MAY-2009							
	Without S-9		With S-9		Without S-9		With S-9		Without S-9		With S-9	
Conc./Plate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 µg	41.7	7.3	47.0	8.0	152.8	16.1	158.3	17.0				
30 µg	44.3	2.5	45.3	3.1	138.3	23.8	160.7	10.0				
100 µg	43.3	14.3	54.3	10.0	142.7	7.5	178.7	13.4				
300 µg	37.7	3.2	47.3	6.7	133.0	12.8	159.3	18.0				
1000 µg	40.0	5.3	47.0	3.6	151.0	11.1	168.0	2.6				
3000 µg	45.7*	8.4	48.3	15.3	141.3*	21.1	156.7	25.7				
5000 µg	41.0*	8.7	59.0*	4.0	175.0*	19.0	165.3*	17.7				

Salmonella typhimurium/E. coli genotypes:
TA100 his G46 (base substitution) ΔuvrB rfa pKM101 (R factor).
TA1535 his G46 (base substitution) ΔuvrB rfa.
TA97a his D6610 (frameshift) ΔuvrB rfa pKM101 (R factor).
TA98 his D3052 (frameshift) ΔuvrB rfa pKM101 (R factor).
WP2 uvrA pKM101 (trp-) ΔuvrA pKM101 (R factor).

S-9 = Metabolic activation.
SD = Standard deviation.
* = Precipitate; did not interfere with scoring.

Table. Results with the positive controls are shown below in the absence of metabolic activation:

Results with Positive Control Mutagens
Salmonella his$^+$ or *Escherichia coli* trp$^+$ Revertants per Plate

Mutagen†	S-9 +/-	Conc./ Plate	*Salmonella typhimurium* strains							
			TA1535 Fold Inc		TA97a Fold Inc		TA98 Fold Inc		TA100 Fold Inc	
Sodium azide	-	0.75 µg	24.4		1.1		1.2		3.8	
ICR-191	-	1.5 µg	1.1		3.6		1.0		1.1	
2-Nitrofluorene	-	1.0 µg	1.0		1.7		7.9		3.2	
4NQO	-	1.0 µg	3.9		4.0		13.7		11.8	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
DMSO	-	100 µL	24.3	6.7	204.3	20.1	41.7	7.3	162.1	18.9
2AA	-	1.0 µg	32.0	4.4	214.7	18.6	46.3	0.6	175.3	9.0
2AA	-	2.0 µg	35.0	5.2	222.0	11.8	57.7	11.6	171.3	14.7
DMSO	+	100 µL	22.1	4.7	252.8	17.9	47.0	8.0	181.6	14.2
2AA	+	1.0 µg	131.7	16.3	658.7	21.2	438.7	2.1	806.0	72.8
2AA	+	2.0 µg	241.0	25.4	1230.0	30.6	999.0	161.0	1591.3	89.3
Distilled Water	-	100 µL	21.7	1.2	203.3	19.0	36.7	5.7	173.3	20.8

Table 1 continued. The positive controls are shown below in the absence and presence of metabolic activation:

Results with Positive Control Mutagens
Salmonella his$^+$ or *Escherichia coli* trp$^+$ Revertants per Plate

Mutagen†	S-9 +/-	Conc./ Plate	*Escherichia coli* strains	
			WP2 uvrA pKM101 Fold Inc	
Sodium azide	-	0.75 µg	1.1	
ICR-191	-	1.5 µg	1.1	
2-Nitrofluorene	-	1.0 µg	1.0	
4NQO	-	1.0 µg	5.9	
			Mean	SD
DMSO	-	100 µL	152.8	16.1
2AA	-	2.0 µg	149.3	23.5
2AA	-	5.0 µg	153.7	15.9
DMSO	+	100 µL	158.3	17.0
2AA	+	2.0 µg	428.7	52.0
2AA	+	5.0 µg	1101.7	68.1
Distilled Water	-	100 µL	164.3	31.0

† = Sodium azide was dissolved in water; all other compounds, in DMSO.
SD = Standard Deviation.
S-9 = Metabolic activation.

2AA = 2-Aminoanthracene DMSO = Dimethyl sulfoxide 4NQO = 4-Nitroquinoline-N-oxide

In conclusion in the microbial mutagenesis assays, atorvastatin amorphous (MK 9396) at doses up to 1000 µg/plate did not induce increased numbers of revertants in any of the *Salmonella typhimurium* or *Escherichia coli* test strains with or without metabolic (S-9) activation.

3. **Chomosomal aberrations in vitro in Chinese hamster Ovary (CHO) cells**

The study was conducted at Merck Research Laboratories West point, PA. The drug lot number for MK 9396 was: L-000776336-004G027 (calcium salt), it was 99.08% pure. Dose ranges used in the study are stated below.

Note that two study numbers are provided below. The first study (TT #09-8642) is a range-finding study and was not a GLP study. The second study is the actual chromosomal aberration study (TT #09-8643), and was a GLP study. The study details and methods are described below:

Study	Study #	Initiation Date	Start Date	Termination Date
Range-Finding Cytotoxicity	TT #09-8642	13-May-2009	19-May-2009	20-May-2009
Chromosomal Aberrations	TT #09-8643	21-May-2009	28-May-2009	04-Jun-2009

a) Dose Ranges Used in Assays

Range-Finding Assay:	TT #09-8642
Doses Tested	with and without S-9 (3-hour treatments): 1, 10, 25, 50, 100, 250, 500, 1000 and 1400 µM without S-9 (20-hour treatment): 0.5, 1, 10, 25, 50, 100, 250, 500, 1000 and 1400 µM
Aberration Assay:	TT #09-8643
Doses Tested	with S-9 (3-hour treatment): 100, 150, 200, 225, 250, 300, and 400 µM without S-9 (3-hour treatment): 100, 150, 200, 225, 250, 300, 350, and 400 µM without S-9 (20-hour treatment): 50, 100, 150, 175, 200, 225, 250, and 300 µM
Doses Scored for Aberrations	with S-9 (3-hour treatment): 150, 225, and 250 µM without S-9 (3-hour treatment): 100, 150, and 225 µM without S-9 (20-hour treatment): 50 µM and 100 µM

The study details are described below.

2.6.7.17D Other Toxicity Studies (Genotoxicity: In Vitro)	**Report Title:** Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells	**Test Article:** MK-9396
Test for Induction of: Chromosome Aberrations **Strains:** Chinese Hamster Ovary cells, subclone WBL **Metabolizing System:** Beta-naphthoflavone and phenobarbital-induced rat liver S-9, 1.5% in cultures **Vehicle for Test Article:** Dimethyl sulfoxide [DMSO], 1% in cultures **Treatment:** Three separate treatments – 3-hour treatments with and without S-9 and continuous treatment for 20 hr without S-9. Harvested 20 hr after beginning of treatment.	**No. of Independent Assays:** 1 **No. of Replicate Cultures:** 1 **No. of Cells Analyzed/Culture:** 200 except positive controls where aberration frequencies were high. **Vehicle for Positive Controls:** Distilled water	**Study No.:** [Ref. 4.2.3.7.6: TT098642], (TT #09-8642; TT #09-8643) **Location in CTD:** Vol. Section **GLP Compliance:** Yes, except TT #09-8642 was a range-finding (RF) study. **Date of Treatment**: 19-May-2009 (TT#09-8642), 28-May-2009 (TT#09-8643)
Cytotoxic Effects: TT #09-8642 (RF); Marked cytotoxicity. Based on cell counts at 24 hours, there were marked reductions in cell number after treatments with and without S-9. The estimated dose to reduce growth by about 50% was above 250 µM with S-9 and at 250 µM (3-hour treatment) or between 100 µM and 250 µM (20-hour treatment) without S-9. **TT #09-8643 (Aberrations);** Marked cytotoxicity. At the top doses scored, 250 µM with S-9 and 225 µM (3-hour treatment) or 100 µM (20-hour treatment) without S-9, cell growth at 20 hours was reduced to 58, 52, and 69% of concurrent solvent controls, respectively. Slightly higher doses were excessively toxic. The two lower doses scored for the 3-hour treatments with and without S-9 activation were selected to span a range of cytotoxicities. For the 20-hour treatment without S-9, there were only two doses scored, because higher doses were excessively toxic. Typically three dose levels are scored to help define a dose response and to cover a range of toxicities. Two doses are considered adequate here, because an appropriate toxicity range was achieved, the dose levels were closely spaced, and there was no evidence for an increase in aberrations. **MK-9396 = atorvastatin calcium (amorphous); batch number L-000776336-004G027**		

Criteria for a positive assay are described below:

Criteria for a Positive Result

The tests with and without S-9 activation and different treatment durations are considered separately. A positive point is a statistically significant increase in the percentage of cells with aberrations, which is also outside the overall historical control range. An assay is generally considered positive if there are two positive points within a series, without greatly exceeding a 50% reduction in growth.

A single positive point is considered equivocal until repeated in another assay.

If any of the tests with S-9 activation or without S-9 is positive, the overall conclusion will be positive.

The assay is considered positive if positive results are obtained in any of the series, with or without metabolic activation.

Results
In the range-finding assay (TT #09-8642, non-GLP), a dose range for the chromosomal aberration assay was selected. For the actual aberration assay (TT #09-8643, GLP) dose range selected was up to 400 µM in the 3-hour treatments (with and without S-9), and up to 300 µM in the 20-hour treatment (without S-9). These doses were selected based on cyto-toxicity, i.e. a dose that would produce growth reduction not greatly exceeding 50% of concurrent solvent controls.

1). Preliminary Solubility and Range-Finding Cytotoxicity Assay (TT #09-8642)
The results are summarized in Table 1. In the solubility test (in study TT09-8642), MK-9396 was soluble in DMSO at about 696 mM after warming at 37ºC. For the range-finding cytotoxicity test, the top concentration selected was 1400 µM (to ensure testing at the limits of solubility in cultures). Precipitate was noted in cultures at ≥ 250 µM (after the 3-hour treatment with S-9), and at ≥ 500 µM (after the 3- and 20-hour treatments without S-9). In cultures, cytotoxicity was noted at the end of treatments with and without S-9, as rounded and dead cells were observed at ≥ 500 µM after 3-hour treatments, and at ≥ 250 µM after 20 hour treatment (see Table 1 below). Therefore, high dose cultures with and without S-9 were discarded due to excessive toxicity or precipitate.

Table 1. Dose-range assay from non-GLP study# TT 09-8642.

Table 1. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

TT #09-8642: Range-Finding Assay

Summary of Cytotoxicity Results at 20 Hours

Treatment	Cell Growth (PD, % Control)	Treatment	Cell Growth (PD, % Control)
With S-9 (3 hours)		Without S-9 (20 hours)	
DMSO 1%	100	DMSO 1%	100
DMSO 1%	100	DMSO 1%	100
CP 40 µM	36	MK-9396 (µM)	
MK-9396 (µM)		0.5	101
1	ND	1	99
10	ND	10	88
25	ND	25	86
50	105	50	81
100	104	100	78
250[a]	81	250	23
500[b]	Discarded	500[a]	Dead, Discarded
1000[b]	Discarded	1000[b]	Dead, Discarded
1400[b]	Dead, Discarded	1400[b]	Dead, Discarded
Without S-9 (3 hours)		PD = Population doubling. DMSO = Dimethyl sulfoxide. ND = Not done. CP (Cyclophosphamide) is the positive control. [a] Precipitate evident at end of treatment. [b] Precipitate evident at beginning and end of treatment.	
DMSO 1%	100		
DMSO 1%	100		
MK-9396 (µM)			
1	ND		
10	ND		
25	ND		
50	95		
100	93		
250	51		
500[a]	Discarded		
1000[b]	Discarded		
1400[b]	Dead, Discarded		

2) Chromosomal Aberration assay (TT #09-8643, see Tables 2 to 4)

a) Cytotoxicity (Table 2)

As stated earlier, for the actual aberration assay (TT #09-8643, GLP) dose range selected was up to 400 µM in the 3-hour treatments (with and without S-9), and up to 300 µM in the 20-hour treatment (without S-9), see Table 2. There was precipitate and marked cytotoxicity noted in this assay. Precipitate was observed in cultures at 250 µM at the end of the 3-hour treatments with S-9, and the high doses (above 100 uM) were excessively toxic at the end of the 20-hour treatment without S-9.

b) Chromosomal Aberrations (Tables 2 to 4)

The top dose levels scored for chromosome aberrations were limited by cytotoxicity. The top doses scored were 250 µM with S-9 and 225 µM without S-9 (3-hour treatment) or 100 µM without S-9 (20-hour treatment), cell growth was reduced at these doses to 58%, 52%, and 69% of concurrent solvent controls, respectively. Doses only slightly higher were excessively toxic. Test article precipitate was noted at 250 µM with S-9. Therefore, two lower doses were scored for the 3-hour treatments with and without S-9 activation to span a range of cytotoxicities.

Sponsor states that for the 20-hour treatment without S-9, there were only two doses scored, because higher doses were excessively toxic. Typically three dose levels are scored to help define a dose response and to cover a range of toxicities. Two doses are considered adequate here, because an appropriate toxicity range was achieved, the dose levels were closely spaced, and there was no evidence for an increase in aberrations.

The overall results are summarized in Table 2, and details are provided in Table 3.

Sponsor states that the concurrent solvent control values are in the expected range based on our historical controls (Table 4), and the high-dose positive controls with and without S-9 activation induced significant increases in aberrations over the concurrent solvent controls, indicating that the assay was working as expected.

After the 3-hour treatment with S-9, there was an increase in endo-reduplication, a form of polyploidy. The highest level seen 4.0% endoreduplicated cells at 225 µM and 250 µM, was within our historical control range of 0.0 to 9.0% for polyploidy (Table 4). Endoreduplication in cultured cells occurs spontaneously and as a result of treatment with various agents. Possible mechanisms for endoreduplication include inhibition of normal DNA synthesis and/or temporary cell cycle block in the G2 phase.

Sponsor states that the assay for MK-9396 and its degradate was negative. There were no positive points in cultures treated with MK-9396, i.e., statistically significant increases in the percentages of cells with aberrations over concurrent solvent controls that are also outside the typical historical control range. The maximum level of aberrations observed was 4.0% at 100 µM after a 20-hour treatment without S-9, and is within the typical historical control range of 0.0 to 5.0% (Table 4).

However, note that these historical controls are taken from the current study.

Table 2. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

TT #09-8643: Assay for Chromosomal Aberrations

Summary of Cytotoxicity and Aberrations at 20 Hours

Treatment	Cell Growth (PD, % Control)	% Aberrant Cells	Frequency Abs per 100 Cells[a]	% Endos[b]
With S-9 (3-Hour Treatment)				
DMSO (1%)	100	1.00	1.00	0.2
DMSO (1%)	100	1.00	1.00	0.0
Cyclophosphamide (5 µM)[c]	71	28.00	34.00	NS
Cyclophosphamide (10 µM)[d]	44	80.00**	160.00	NS
MK-9396				
100 µM	105	NS		
150 µM	94	2.50	4.00	0.6
200 µM	86	NS		
225 µM	70	1.00	2.00	4.0
250 µM[e]	58	3.00	3.00	4.0
300 µM[e]	28	NS		
400 µM[e]	Dead, Discarded			
Without S-9 (3-Hour Treatment)				
DMSO (1%)	100	1.00	1.00	NS
DMSO (1%)	100	1.00	3.00	NS
Mitomycin C (0.5 µM)[c]	75	26.00	36.00	NS
Mitomycin C (1.5 µM)[d]	56	64.00**	160.00	NS
MK-9396				
100 µM	87	1.50	1.50	NS
150 µM	87	1.50	1.50	NS
200 µM	58	NS		
225 µM	52	1.50	1.50	NS
250 µM	28	NS		
300 µM[e]	17	NS		
350 µM[e]	0	NS		
400 µM[e]	Dead, Discarded			

Continued

Table 2. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

TT #09-8643: Assay for Chromosomal Aberrations

Summary of Cytotoxicity and Aberrations at 20 Hours

Treatment	Cell Growth (PD, % Control)	% Aberrant Cells	Frequency Abs per 100 Cells[a]	% Endos[b]
Without S-9 (20-Hour Treatment)				
DMSO (1%)	100	2.50	2.50	NS
DMSO (1%)	100	1.50	1.50	NS
MK-9396				
50 µM	79	0.50	0.50	NS
100 µM	69	4.00	8.00	NS
150 µM	34	NS		
175 µM	29	NS		
200 µM	27	NS		
225 µM	17	NS		
250 µM[e]	14	NS		
300 µM[e]	6	NS		

PD = Population doubling.
NS = Not scored.
DMSO = Dimethyl sulfoxide.
Cyclophosphamide (CP) and Mitomycin C (MMC) are positive controls.
[a] The total number of aberrations per 100 cells, since a cell may have more than one aberration.
[b] The number of endoreduplicated metaphases based on 500 cells.
200 cells scored for aberrations per point except where noted.
[c] 50 cells scored for aberrations.
[d] 25 cells scored for aberrations.
[e] Precipitate observed during treatment.
** A positive point that is statistically significant $p \leq 0.01$ compared to the relevant control group using a one-sided Fisher's exact test and is also outside the historical solvent control range.

Table 3a. Chromosomal aberration assay with atorvastatin amorphous (with S-9) at 3-hour treatment period.

Table 3. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

TT #09-8643: Assay for Chromosomal Aberrations

Summary of Aberration Types at 20 Hours

Treatment	Cells Scored	% Cells With Abs	Frequency of Aberrations (Per 100 Cells)							
			Total Abs	Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	Severely Damaged	Pulverized Chromosome	Gaps
With S-9 (3-Hour Treatment)										
DMSO 1%	200	1.00	1.00	0.50	0.00	0.50	0.00	0.00	0.00	2.00
DMSO 1%	200	1.00	1.00	0.50	0.00	0.50	0.00	0.00	0.00	0.50
Cyclophosphamide 5 µM	50	28.00	34.00	6.00	18.00	10.00	0.00	0.00	0.00	2.00
Cyclophosphamide 10 µM	25	80.00	160.00	16.00	92.00	12.00	0.00	4.00	0.00	24.00
MK-9396										
150 µM	200	2.50	4.00	1.00	2.00	1.00	0.00	0.00	0.00	1.50
225 µM	200	1.00	2.00	0.00	0.50	1.50	0.00	0.00	0.00	0.00
250 µM	200	3.00	3.00	1.00	0.00	1.00	1.00	0.00	0.00	0.50

Table 3b. Chromosomal aberration assay with atorvastatin amorphous (without S-9) at 3-hour treatment period.

Table 3. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

TT #09-8643: Assay for Chromosomal Aberrations

Summary of Aberration Types at 20 Hours

Treatment	Cells Scored	% Cells With Abs	Frequency of Aberrations (Per 100 Cells)							
			Total Abs	Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	Severely Damaged	Pulverized Chromosome	Gaps
Continued Without S-9 (3-Hour Treatment)										
DMSO 1%	200	1.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.50
DMSO 1%	200	1.00	3.00	0.50	0.50	2.00	0.00	0.00	0.00	1.50
Mitomycin C 0.5 µM	50	26.00	36.00	8.00	18.00	10.00	0.00	0.00	0.00	6.00
Mitomycin C 1.5 µM	25	64.00	160.00	56.00	64.00	40.00	0.00	0.00	0.00	8.00
MK-9396										
100 µM	200	1.50	1.50	0.50	0.00	0.50	0.50	0.00	0.00	1.00
150 µM	200	1.50	1.50	0.00	0.00	1.50	0.00	0.00	0.00	2.50
225 µM	200	1.50	1.50	0.50	0.00	0.50	0.50	0.00	0.00	1.50

Table 3c. Chromosomal aberration assay with atorvastatin amorphous (without S-9) at 20-hour treatment period. Note that in this assay at 100 uM, the chromosome deletions were higher (6.5 vs 2.0 in controls), the total aberrations were higher (8 vs 2.5 in controls), and % of cells with aberration were higher (4 vs 2.5% in controls), however these were within the historical control range (0-5%).

Table 3. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

TT #09-8643: Assay for Chromosomal Aberrations

Summary of Aberration Types at 20 Hours

Treatment	Cells Scored	% Cells With Abs	Frequency of Aberrations (Per 100 Cells)							
			Total Abs	Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	Severely Damaged	Pulverized Chromosome	Gaps
Continued										
Without S-9 (20-Hour Treatment)										
DMSO 1%	200	2.50	2.50	0.50	0.00	2.00	0.00	0.00	0.00	0.50
DMSO 1%	200	1.50	1.50	0.50	0.00	0.50	0.50	0.00	0.00	3.00
MK-9396										
50 µM	200	0.50	0.50	0.00	0.00	0.50	0.00	0.00	0.00	1.50
100 µM	200	4.00	8.00	0.50	0.00	6.50	1.00	0.00	0.00	2.00

Abs = Aberrations (Excluding Gaps, Pulverized Chromosome, Translocations and Abnormal Monocentrics).
Severely damaged (SD) cell counted as 10 aberrations.

The historical controls are provided below in Table 4.

Table 4(a) below shows the structural chromosomal aberrations (without S-9) of 0 to 5%, which is actually from the current study (study # 09-8643). Note that the time frame of these historical control data has not been provided (i.e. if incubation time was 3 hours or 20 hours).

In the current aberration assay with atorvastatin amorphous at 20 hour treatment period, without S-9 at doses of 100 uM, not only the % of cells with aberrations were higher (4% vs 1.5-2.5%), but also the chromosome deletions were higher (6.5 vs 2.0 in controls), and the total aberrations were higher (8 vs 2.5 in controls)

Table 4. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

Historical Controls For Chinese Hamster Ovary Cells

A. Negative and Solvent Controls: Percentages of Cells With Structural Chromosome Aberrations

1. Studies From TT #99-8655 to TT #09-8643

	With S-9	Without S-9
Combined mean percent ± standard deviation:	1.57 ± 0.98	1.45 ± 0.91
Number of cultures scored[a]:	550	1055
Range for individual cultures:	0.00 to 5.50	0.00 to 5.00
	With and Without S-9	
Overall mean percent ± standard deviation:	1.49 ± 0.93	
Total cells scored:	308377	
Cells with more than one aberration:	0.18	

[a] 200 cells are typically scored per culture.
Includes concurrent control data.
The typical control range is 0.0% to 5.0%. In TT #07-8627, 5.5% was obtained for 1 solvent control culture.

Table 4(b) below shows the endo-reduplication (with S-9); this Table shows the endo-reduplication frequency range of 0-3.4% (with S-9); again current study (study # 09-8643) is included in the historical controls. Also note that the time frame of these historical control data has not been provided (i.e. if incubation time was 3 or 20 hours)

In the current assay with atorvastatin amorphous, the frequency of cells with endo-reduplication was increased (4% vs 0.2%).

Continued.

Table 4. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

Historical Controls For Chinese Hamster Ovary Cells

C. Negative and Solvent Controls: Frequency of Endoreduplication (Only Studies With Endoreduplication Included)

1. Studies from TT #87-8614 to TT #03-8658

	With S-9	Without S-9
Combined mean percent ± standard deviation:	0.31 ± 0.56	0.06 ± 0.15
Number of cultures scored[a]:	171	144
Range for individual cultures:	0.00 to 3.40	0.00 to 1.20
	With and Without S-9	
Overall mean percent ± standard deviation:	0.20 ± 0.44	
Total cells scored:	157500	
Total endoreduplicated metaphases:	310	
Overall range for individual cultures:	0.00 to 3.40	
[a] 500 cells are typically scored per culture.		

2. Studies From TT #03-8687 to TT #09-8643

	With S-9	Without S-9
Combined mean percent ± standard deviation:	0.20 ± 0.26	0.09 ± 0.18
Number of cultures scored[a]:	181	160
Range for individual cultures:	0.00 to 1.60	0.00 to 1.20
	With and Without S-9	
Overall mean percent ± standard deviation:	0.15 ± 0.23	
Total cells scored:	171096	
Total endoreduplicated metaphases:	256	
Overall range for individual cultures:	0.00 to 1.60	
[a] 500 cells are typically scored per culture. Includes concurrent control data.		

Table 4(c) below shows the frequency of polyploidy range (with S-9) which is taken from one study (# 03-8684). In the current aberration assay with atorvastatin amorphous at 20 hour treatment period, in the absence of metabolic activation at doses of 100 uM, the % of cells with aberrations were higher (4% vs 1.5-2.5%). Note again that the time frame of these historical control data has not been provided (i.e. if incubation time was 3 or 20 hours).

Continued.

Table 4. MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

Historical Controls For Chinese Hamster Ovary Cells

D. Negative and Solvent Controls: Frequency of Polyploidy (Only Studies With Polyploidy Included)

1. Studies from TT #00-8657 to TT #03-8682

	With S-9	Without S-9
Combined mean percent ± standard deviation:	1.65 ± 1.19	1.91 ± 1.22
Number of cultures scored[a]:	46	111
Range for individual cultures:	0.20 to 4.60	0.00 to 6.00
	With and Without S-9	
Overall mean percent ± standard deviation:	1.83 ± 1.22	
Total cells scored:	79000	
Total polyploid metaphases:	1457	
Overall range for individual cultures:	0.00 to 6.00	
[a] 500 cells are typically scored per culture.		

2. Studies From TT #03-8684 to TT #09-8635

	With S-9	Without S-9
Combined mean percent ± standard deviation:	2.83 ± 1.91	2.24 ± 1.42
Number of cultures scored[a]:	112	161
Range for individual cultures:	0.00 to 9.00	0.00 to 7.00
	With and Without S-9	
Overall mean percent ± standard deviation:	2.48 ± 1.66	
Total cells scored:	136000	
Total polyploid metaphases:	3375	
Overall range for individual cultures:	0.00 to 9.00	
[a] 500 cells are typically scored per culture. Includes concurrent control data.		

In conclusion, in the chromosomal aberration assay in CHO cells, atorvastatin amorphous was negative at 3 hour treatment period in the absence of metabolic activation at doses up to 225 uM (cell growth was reduced to 52%). However, at 3 hour treatment period in the presence of metabolic activation at doses of 225 & 250 uM (cell growth reduced to 70% and 58% respectively), frequency of cells with endo-reduplication was increased (4% vs 0.2%, see Table 2). Similarly, at 20 hour treatment period, in the absence of metabolic activation at doses of 100 uM (cell growth was reduced to 69%), the % of cells with aberrations were higher (4% vs 1.5-2.5%). However, sponsor concludes that this is within the range of historical control range in their studies. In conclusion the test was considered negative. Sponsor's conclusions are stated below.

Sponsor's summary of this study is provided below

MK-9396: Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells. TT #09-8642 and TT #09-8643

MK-9396 and its degradate was evaluated for its potential to cause chromosomal aberrations in Chinese hamster ovary (CHO) cells (subclone WBL).

MK-9396, also known as atorvastatin (calcium salt), was prepared as a solution in Dimethyl Sulfoxide (DMSO) and diluted 100-fold into medium. A formula weight of 577.688 was used for calculating molarity. The amount of the compound weighed was adjusted by a potency factor of 1.06. MK-9396 was tested with and without a metabolic activation system (S-9) prepared from the livers of rats treated with beta-naphthoflavone and phenobarbital. A range-finding assay was done to select a dose range for the chromosome aberration assay. Cytotoxicity was assessed as reductions in cell growth or monolayer confluence. The range-finding and aberration assays involved 2 treatment durations; 3-hour treatment with or without S-9 and a continuous treatment without S-9 (about 20 hours). The concurrent solvent control cultures were treated with 1% DMSO. Positive controls (cyclophosphamide with S-9 activation or mitomycin C without S-9) were included. The cells were fixed for analysis of chromosome aberrations about 20 hours from the beginning of treatment (about 1.5 normal cell cycle lengths).

Precipitate and marked suppression of growth were found in the range-finding assay, TT #09-8642, and the top doses for the chromosomal aberration assay were based both on cytotoxicity and on the apparent limit of solubility of MK-9396 in tissue culture medium.

In the chromosomal aberrations assay, TT #09-8643, the top doses of MK-9396 scored for aberrations were selected such that reductions in cell growth did not greatly exceed about 50% of concurrent controls. The treatment levels of MK-9396 scored for aberrations were 150, 225, and 250 µM with S-9 (3-hour treatment), and 100, 150, and 225 µM (3-hour treatment) or 50 µM and 100 µM (20-hour treatment) without S-9. At the top doses scored, cell growth at 20 hours was reduced to 58, 52, and 69% of controls respectively, and precipitate of test compound was evident at the high dose with S-9. Slightly higher doses were excessively toxic. The high-dose positive controls induced significant increases in aberrations over the concurrent solvent controls. The chromosomal aberration assay of MK-9396 and its degradate was negative.

Sponsor's Conclusions

MK-9396 and its degradate was negative in the in vitro assay for chromosomal aberrations in CHO cells. The highest concentrations scored were 250 µM with S-9 and 225 µM without S-9 (3-hour treatment), or 100 µM (20-hour treatment) without S-9.

Sponsor states that *in exploratory assay, the impurities/degradants tested were* (b) (4) *Each* (b) (4) *was tested at levels up to 250 µg/plate. All of the impurities/degradants* (b) (4) *were found to be negative for genotoxicity. In additional assays (Ames, and chromosomal aberration assay, using atorvastatin calcium (amorphous) batch L-000776336-004G027 containing the following impurity/degradant levels:* (b) (4) *showed that both the bacterial mutagenicity assay and in vitro chromosomal aberration assay were negative and therefore provide further support for the proposed specifications for the impurity/degradation products of atorvastatin calcium (amorphous).*

In summary, the 3-month oral toxicity study in dogs, and the bacterial mutagenicity and in vitro chromosomal aberration assays support the proposed specifications for the atorvastatin calcium (amorphous) impurity/degradation products provided in [Table 2.6.6: 4].

2.6.6.6 Reproductive and developmental toxicology

Following reproductive toxicity studies have been reviewed under NDA 21-445 with the co-administration of ezetimibe + atorvastatin.

In a segment II teratology study with ezetimibe + atorvastatin **in rats (Study # SN-99506)**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + atorvastatin (25, 50, 100 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or atorvastatin alone (100 mg/kg/day). Maternal exposures of the total drug on GD 17 were 8.6, 21.3, 66.2 µg.h/ml at 1000/25, 1000/50, 1000/100 mg/kg/day respectively. In a previous rat study of SCH 58235 with 1000 mg/kg/day on GD 15, exposures were 4.9 µg.h/ml (study #96383). Thus exposure to total drug increased by 2-10 fold when SCH 58235 was co-administered with atorvastatin. Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235. Exposures to atorvastatin free acid, para-hydroxy atorvastatin, and ortho hydroxy atorvastatin were all slightly decreased when high dose of atorvastatin (100 mg/kg/day) was co-administered with SCH 58235. The HD combination significantly decreased the gestation body weight gain (by 10%, 62* g vs 69 g in the control), food consumption (by 8%, 22 vs 24 g/rat/day in controls) in rats, decreased the mean body weights of fetuses, and produced increased incidences of skeletal variations (reduced ossification of sternebrae which sponsor attributes to decreased fetal body weight). Maternal/Developmental NOAEL was 1000 mg/kg SCH 58235 + 50 mg/kg atorvastatin, based on decreased maternal/fetal body weights, maternal FC and increased incidence of reduced ossification of sternebrae.

In a segment II teratology study with ezetimibe + atorvastatin **in rabbits (study # 99507)**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + atorvastatin (5, 25, 50 mg/k/day) from day 7-19 of gestation. Control animals received the vehicle or atorvastatin alone (50 mg/kg/day). Maternal exposures of the total drug on GD 19 were 124, 132, 149 µg.h/ml at 1000/5, 1000/25, 1000/50 mg/kg/day

respectively. In a previous rabbit study of SCH 58235 with 1000 mg/kg/day on GD 19, exposures to total drug were 113 μg.h/ml (study #96385). Thus exposure to the total drug did not significantly increase when SCH 58235 was co-administered with atorvastatin (124-149 in the present study vs 113 μg.h/ml with the drug alone in a previous study). Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235. Coadministration of SCH 58235 with atorvastatin 50 mg/kg increased the exposure to atorvastatin free acid by 1.5 fold (323 vs 214 ng.h/ml) and to ortho-hydroxy atorvastatin by 2.5 fold (1441 vs 584 ng.h/ml), but had no effect on the mean systemic exposure to para-hydroxy atorvastatin (167 vs 152 ng.h/ml). All combination doses produced increased clinical signs (a reduced number and small fecal pellets in 6-8/20 rabbits vs 0-1/20 in vehicle or atorvastatin controls), decreased food consumption in rabbits (by 30, 33 & 53% respectively), and HD combo produced decreased BW gains by 38%. All combination doses produced skeletal malformations (fused caudal vertebra and sternebrae), MD & HD combination (25 and 50 mg/kg atorvastatin + 1000 mg/kg SCH 58235) produced visceral malformations (gallbladder absent, ectopic/misshapen kidneys), and HD combination produced external malformations of kinked tail (1.3% in fetuses and 5.9% in litters) and increased skeletal variations (sternebrae assymetrical). NOAEL could not be established for the maternal toxicity as all combination doses decreased food consumption and produced fecal changes, and HD combo decreased BW gain. Maternal NOAEL and developmental NOAEL were both 1000 mg/kg SCH 58235 + < 5 mg/kg/day atorvastatin. Developmental NOAEL was based on increased skeletal malformations (fused caudal vertebra and sternabra) with all doses of combination, MD and/or HD combo produced external (kinked tail) and visceral malformations (gallbladder absent). Sponsor's no effect level (NOEL) for both maternal and in utero effects was also < 5 mg/kg/day atorvastatin + 1000 mg/kg SCH 58235.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The drug atorvastatin is a cholesterol-lowering drug; it is an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, an enzyme that catalyzes the rate-limiting step in cholesterol synthesis. Atorvastatin has been marketed extensively by Pfizer for many years (NDA 20-702) for oral administration under the trade name Lipitor.

Ezetimibe is a lipid lowering drug that selectively inhibits the intestinal absorption of cholesterol. Ezetimibe has been marketed since 2002 (NDA 21-445) for oral administration under the trade name Zetia.

In the current proposal, the fixed dose combination FDC) of two drugs is proposed for patients with heterozygous and homozygous hypercholesterolemia to provide a more convenient single tablet. This application is a resubmission after a refusal to file letter was sent to the sponsor, due to several CMC deficiencies in 2009. Sponsor is proposing to market 10/10, 10/20, 10/40, and 10/80 mg of ezetimibe /atorvastatin tablets in the current application. The sponsor has provided the comparative bioavailability after administration of a combination tablet vs the co-administration of individual tablets. However, the atorvastatin component of the 10/20 mg and 10/40 mg ezetimibe /atorvastatin FDC tablets are not bioequivalent to the co-administration of corresponding individual ezetimibe + atorvastatin tablets. The atorvastatin AUC0-∞ and Cmax of 10/20

mg FDC tablet is [redacted] (b) (4) than those of the co-administration of individual 10 mg ezetimibe plus 20 mg atorvastatin tablets. Similarly, the atorvastatin AUC0-∞ and Cmax of 10/40 mg FDC tablet is [redacted] (b) (4) than those of the co-administration of individual 10 mg ezetimibe plus 40 mg atorvastatin tablets.

The ezetimibe pharmacology and toxicology has been reviewed under NDA 21-445 (see review in DARRTS)

Ezetimibe + atorvastatin toxicology

The non-clinical toxicology studies in rats and dogs including the 3-month co-administration studies of ezetimibe + atorvastatin, as well as repro-toxicity and geno-toxicity studies have been reviewed under NDA 21-445.

Following is a brief summary of co-administration studies with ezetimibe (SCH-58238) + atorvastatin in rats and dogs from NDA 21-445.

In a 3-month toxicity study in rats with ezetimibe (or SCH 58235, study # SN 99500, doses of ezetimibe used were males 15, 15, 250, 250 mg/kg/day, females 15, 15, 50, 50 mg/kg/day) + atorvastatin (10, 30, 30, 100 mg/kg/day), the increases in AUC values of the total ezetimibe drug levels (SCH 58235) were not dose proportional and values were generally higher in week 5 (males 2.0, 2.1, 11.0, 19.6 μg.h/ml, females 2.9, 2.3, 12.8, 16.6 μg.h/ml respectively) vs on day 0 (males 1.9, 1.7, 10.8, 14.2 μg.h/ml, females 1.9, 2.0, 4.0, 6.3 μg.h/ml respectively), suggesting accumulation of ezetimibe over time. The combination increased the total and conjugated ezetimibe exposures. However, there were no consistent increases in atorvastatin (or metabolite) exposures with the combination, and in HD combination atorvastatin exposures in week 5 were lower in males (males/females 1.4/2.9 μg.h/ml, vs 2.4/1.6 μg.h/ml with atorvastatin alone, on day 0 these values were 2.92/2.97 μg.h/ml vs 3.0/6.9 μg.h/ml with atorvastatin alone), suggesting that the combination may decrease atorvastatin exposures in males, but increase these in females in week 5. The MD-HD combination doses produced decreases in mean BW (by 9-14%) and weight gains (by 14-24%) in males, and similar decreases in weights (4-7%) and weight gains (9-15%) in females. Mid and high dose combinations not only produced increases in plasma AST/ AP levels (by up to 2-3 fold), and sorbitol dehydrogenase or SDH levels (by up to 7 fold), but also produced toxicity in the liver. Liver weights were increased in females at all doses, and produced histopath findings in both sexes (biliary hyperplasia, hepatocellular hypertrophy and single cell necrosis with increased severity). Since all doses produced increases in liver weights in females and toxicity in the spleen ((pigment accumulation, hemosiderin, minimal in 0/10, 0/10, 2/10, 2/10, 2/10, 1/10 rats respectively), NOAEL could not be identified in females and was <15 mg/kg/day of SCH 58235 + <10 mg/kg/day of atorvastatin. In males, NOAEL was 15 mg/kg/day of SCH 58235 + 30 mg/kg/day of atorvastatin, higher doses not only produced toxicity in the liver but also in the heart, testes and prostate.

In a 3-month toxicity study in dogs with ezetimibe (or SCH 58235, study # SN 99501, doses=0.3, 3, 3, 30 mg/kg/day) + atorvastatin (1, 1, 10, 10 mg/kg/day), AUC exposures were slightly higher at two HD combinations, suggesting some accumulation of the total drug (SCH 58235) in week 5 (0.12, 0.8, 0.89, 3.9 μg.h/ml at 0.3/1, 3/1, 3/10, 30/10 mg/kg/day of SCH 58235/atorvastatin respectively) vs on day 0 (0.12, 0.66, 0.52, 3.4 μg.h/ml respectively). However, presence of atorvastatin in the combination did not

significantly effect the total (or conjugated and free) ezetimibe exposures. The combination also did not significantly increase the atorvastatin (or metabolites such as ortho-hydroxy atorvastatin /parahydroxy-atorvastatin) expsoures and values were not significantly different in week 5 (23, 21, 260, 329 ng.h/ml vs atorvastatin 473 ng.h/ml) than on day 0 (27, 34, 215, 293 ng.h/ml vs atorvastatin alone 302 ng.h/ml). In both sexes, all combination doses produced increases in plasma ALT (by 2-40 fold vs atorvastatin control). At two HD combinations, AST (by 1.5-2 fold vs atorvastatin control) & AP levels (by 3 fold vs atorvastatin control) were increased, while total protein and albumin levels were decreased in dogs (see Table). All combination doses produced significant decreases in cholesterol and TG levels. Two HD combinations decreased absolute liver weights in males by 21-26%. At mid-high doses (3/10, 30/10 mg/kg/day of SCH 58235/atorvastatin), toxicity was observed in the liver (bile duct hyperplasia, kuffer cell hypertrophy, increased eosinophilia). HD combination produced toxicity in the heart (hemorrhage acute focal) and lungs (fibrosis or hemorrhage). No NOAEL in this 3-month dog study could be established for the combination and was < 0.3/1 mg/kg/day of SCH 58235/atorvastatin, as all doses increased liver enzyme ALT in dogs, and produced liver toxicity. We concur with the sponsor that NOAEL in dogs was < 0.3 mg/kg/day of SCH 58235 + <1 mg/kg/day of atorvastatin.

Following is a summary of the new 3-month toxicity study with atorvastatin amorphous, with MK-6213 (which is another ezetimibe-like drug) in dogs. Note that this study has a group of animals administered with atorvastatin calcium amorphous alone at 10 mg/kg/day, which is the subject of this study.

In a 3-month oral co-administration toxicity study of MK- 6213/L000776336 in dogs (another cholesterol absorption inhibitor/atorvastatin amorphous), doses of 0/0, 0/10, 10/10, 50/10, 200/10 mg/kg/day of MK-6213/atorvastatin were administered to dogs orally in a capsule (in a vehicle imwitor 742: polysorbate 1:1 w/w). Atorvastatin AUC exposures in combined sexes in dogs were approximately 2-fold higher in week-13 (1010 nM/hr) vs on day-1 (465 nM/hr), suggesting accumulation of atorvastatin over time. In both sexes, atorvastatin amorphous produced increases in plasma ALT levels (males 97 vs 45 IU/L in controls; females 63 vs 30 IU/L in controls), and bilirubin levels (0.2 vs 0.1 mg/dl in controls in both sexes). In females, ALP levels (152 vs 98 IU/L in controls) and creatinine kinase levels (411 vs 239 IU/L in controls) were increased, while cholesterol and triglycerides levels were decreased in both sexes. In combined sexes, atorvastatin amorphous decreased absolute liver weights by 6% (239 vs 254 g in controls, relative by 11%) and thymus weights by 10% (2.82 vs 3.91 g in controls; relative by 35%). Histopathology showed that 1/3 dogs had focal fibrosis in the liver (vs 0/3 in controls), no other associated histopathology changes were noted in thymus or any other organ. Note that this toxicity study has a limited value, as it did not compare the marketed atorvastatin (crystalline form) with the current atorvastatin (amorphous form). However, no significant new toxicity was noted with atorvastatin amorphous in dogs. The 10 mg/kg/day of atorvastatin calcium amorphous in dogs (or 200 mg/m^2/day) in the above 3-month study provides the safety margin of approximately 4-fold in humans (at the highest clinical dose of 80 mg/day, or 49 mg/kg/m^2, assuming 60 kg average weight), based on body surface area.

Genetic toxicology conclusions:

1. *Following genotoxicity studies with ezetimibe (*SCH 58235) *and atorvastatin (crystalline)* have been reviewed under NDA 21-445.

Co-administration of ezetimibe + atorvastatin was negative 1) in AMES test in all tester strains (study # SN 99502), 2) in the chromosome aberration assay in cultured whole blood human lymphocytes (study # SN 99503), and 3) it was not cytogenic at doses of 250-300 mg/kg/day in an in vivo micronucleus test in mice (study # SN 99508).

2. *Genotoxicity studies with atorvastatin amorphous* reviewed in the current application (NDA 200153).

The atorvastatin calcium amorphous was negative in AMES test in all tester strains (study # TT-098047). Atorvastatin amorphous was also tested for clastogenic activity in the Chinese hamster ovary cell (CHO cell) chromosome aberration assay. The results showed that atorvastatin amorphous was negative at 3 hour treatment period in the absence of metabolic activation at doses up to 225 uM (cell growth was reduced to 52% of controls). However in the presence of metabolic activation, at 3 hour treatment period, concentrations of 225-250 uM (cell growth reduced to 70% and 58% respectively) produced increased frequency of cells with endo-reduplication (4% vs 0.2%). Similarly, at 20 hour treatment period, in the absence of metabolic activation, 100 uM concentrations increased the % of cells with aberrations to 4% vs 1.5-2.5% (cell growth was reduced to 69% at 100 uM). However, these findings were within the range of historical control range in their studies, in conclusion, the test was considered negative.

Safety Evaluation

As indicated earlier, atorvastatin (NDA 20-702, crystalline form) & ezetimibe (NDA 21-445) are both approved drugs, atorvastatin has been in the market for many years and is approved at doses up to 80 mg/day. There is clinical experience with this drug in adults, major toxicities are known, and are associated with myopathy and liver dysfunction. Similarly ezetimibe is an approved drug (NDA 21-445) at doses of 10 mg/day, and its toxicities are well characterized.

Extensive non-clinical studies have been conducted with the co-administration of ezetimibe + atorvastatin in an approved NDA 21-445 (zetia). However in these non-clinical studies atorvastatin crystalline form of the drug was used. In the current application, sponsor will use their own atorvastatin calcium amorphous from Dr. Reddy's Laboratory and to qualify this product, sponsor has conducted three non-clinical studies. These studies include a 3-month toxicity study in dogs with another ezetimibe like drug + atorvastatin amorphous, but this study has a group of animals who received atorvastatin amorphous alone. The sponsor has also conducted geno-toxicity studies (GLP, bacterial mutagenicity and chromosomal aberration assay) with their atorvastatin amorphous to support impurity/degradant qualification found in their fixed dose combination (FDC) product.

Supportive information for new excipients used has been provided in the fixed dose combination tablets. The excipients used in the film-coated (b) (4) tablet (b) (4) are compendial grade, and no novel

excipients are used in the formulation of ATOZET tablet. Thus, all excipients comply with corresponding USP and/or NF monographs.

The sponsor is proposing 10 mg/day of ezetimibe and up to 80 mg/day of atorvastatin. Currently the recommended doses of ezetimibe are 10 mg/day (NDA 21-445) and of atorvastatin are up to 80 mg/day in the label (NDA 20-702). Thus, doses that will be used here have already been approved before. From the pharmacology /toxicology point of view this application is approvable.

Labeling Review: The labeling in general is acceptable. The preclinical sections of the label for the fixed dose combination are in general similar to the approved zetia label and Lipitor label. However, following changes in the contraindication section of the label are recommended.

1. Following is sponsor's suggested label:

4 CONTRAINDICATIONS

(b) (4)

[redacted]

Reviewer's recommended changes are in bold letters:

Nursing mothers. **It is not known whether atorvastatin is excreted into human milk; however a small amount of another drug in this class does pass into breast milk**. Because statins have the potential for serious adverse reactions in nursing infants, women who require ATOZET treatment should not breast-feed their infants. *[see Use in Specific Populations (8.3)].***[16[**

Justification for the changes:

Nursing mothers: The above sentence is added, because it is in the RLD (Lipitor) and zetia labels. Sponsor states that this is from FDA-approved circulars for LIPITOR and ZETIA, (b) (4) [redacted] Zetia label also states in the contraindication section that statins may pass into breast milk.

Recommendation: From the preclinical standpoint, approval of this application is recommended.

A. Reviewer signature: Indra Antonipillai

B. Supervisor signature Concurrence:----------

Non-concurrence: ----------
(see memo attached)

cc: IND Arch
HFD-510
HFD-510/davisbruno/antonipillai/chowdhury/coleman/johnson
Review code: AP
File name: nda200153 (atozet or ezetimibe +atorvastatin comb tablets)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

INDRA ANTONIPILLAI
02/02/2012
From the pharmacology/toxicology point of view, this application is approvable.

KAREN L DAVIS BRUNO
02/02/2012
concur with AP recommendation

Signed off in DARRTS on 6/26/11

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

NDA 200-153: This NDA is a 505(b)(2) application.
Submission date: 4/26/2011
Sponsor: MSP Singapore Company, LLC, Singapore, the application is submitted by Merck, Sharp & Dhome Corp., North Wales, PA. It is an eCTD submission
Drug: Atozet tablets (ezetimibe/atorvastatin combination), with code name MK-0653C (also referred as L-000829161 + L000776336). Tablet strengths are 10/10, 10/20, 10/40, 10/80 mg ezetimibe/atorvastatin respectively.

Introduction: This tablet is a combination of two approved drug products, ezetimibe (a selective inhibitor of intestinal cholesterol/phytosterol absorption) and atorvastatin (an HMG-CoA reductase inhibitor). This application was initially submitted on 9/2/2009 (we signed it off in DARRTS on 10/8/2009 as fileable). However, sponsor's manufacturing facilities were not ready for GMP inspections, therefore this application was not fileable. The sponsor has resubmitted this application on 4/26/11. Ezetimibe (NDA 21-445 MSP Singapore) and crystalline atorvastatin (NDA 20-702 Pfizer) are both marketed drugs. Sponsor is also referring to IND 101,953 for MK-0653C (ezetimibe/ atorvastatin). However, the current sponsor will use their own amorphous atorvastatin calcium in the fixed dose combination of Atozet, which is manufactured by Dr. Reddy's laboratories Ltd. in India; they have a letter of authorization from Dr. Reddy's laboratories (Type II DMF 18468). The fixed dose combination (FDC) product in the current NDA (a film coated, (b) (4) tablet) is proposed for patients with primary hypercholesterolemia and homozygous familial hypercholesterolemia.

The current sponsor has conducted a 3-month toxicity study in dogs with their amorphous atorvastatin calcium + MK-6213; note that MK-6213 is another investigational cholesterol absorption inhibitor and not ezetimibe. However, this study has a group of animals who received atorvastatin amorphous alone (at a dose of 10 mg/kg/day in dogs, study # TT #07-6039). They have also conducted geno-toxicity studies (bacterial mutagenicity and chromosomal aberration assay) with their atorvastatin amorphous drug product to support impurity/degradant qualification found in their FDC product.

ITEM: NDA 200-153	**YES**	**NO**	**COMMENT**
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	Yes		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	Yes		

3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	Yes		The sponsor had previously conducted 3-month rat and dog toxicity studies with ezetimibe + atorvastatin co-administered in animals under NDA 21-445. In the current NDA submission, sponsor has provided a 3-month toxicity/ toxicokinetics study of MK-6213 + atorvastatin amorphous in dogs and 3 gene-toxicity studies. MK-6213 is another cholesterol absorption inhibitor (b) (4) like ezetimibe. Sponsor explains that that they have used this study to qualify the atorvastatin impurities/degradents, as this study has an extra group of animals who were administered atorvastatin calcium amorphous alone. All other studies with the combination have already been conducted under NDA 21-445, in which ezetimibe was approved for monotherapy and for co-administration therapy with statins (simvastatin, atorvastatin, pravastatin and lovastatin).
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications /discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)	Yes		Have electronic files of the carcinogenicity studies been submitted for statistical review? N/A On 6/30/2009 we communicated to the sponsor that various different sources of atorvastatin, including atorvastatin used in the co-administration toxicity studies (NDA 21-445), the atorvastatin to be used in their fixed dose combination (FDC) product, and the atorvastatin (Lipitor) that is already marketed be thoroughly compared. If the data show that there are significant differences in the impurity profiles between these three versions of atorvastatin, a bridging toxicity in a single species may be required for marketing of their drug product. In the current submission, sponsor has conducted one three-month toxicity study in dogs with MK-6213 (another ezetimibe like drug + L000776336 (atorvastatin amorphous) and three gene-toxicity studies with their atorvastatin amorphous product to qualify the impurities/ degradants present in the amorphous atorvastatin All other non-clinical studies have already been conducted with the approved ezetimibe (NDA 21-445) and approved atorvastatin (NDA 20-702). No carcinogenicity studies were requested with the current combination formulation, as both drugs are approved drug products with carcinogenicity evaluations.

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (i.e., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?			Yes. As indicated earlier, all non-clinical studies with ezetimibe and atorvastatin have already been conducted under the approved NDA 21-445, and NDA 20-702 individually and these studies were adequately designed.
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (i.e., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	Yes		Sponsor has used their own amorphous atorvastatin calcium in the current fixed dose combination product. A three-month dog toxicity study has been conducted with the combination MK-6213/L000776336 to characterize the impurities in the new formulation. Note that although MK-6213 is another cholesterol absorption inhibitor (and not ezetimibe), this study has used L000776336 alone in dogs, which is their own atorvastatin calcium amorphous. This study is designed to qualify the impurities/ degradants present in the amorphous atorvastatin. The additional 3 gene-toxicity studies including bacterial mutagenicity and in vitro chromosomal aberration assays conducted with their atorvastatin amorphous to support impurity/degradant qualification of their drug product appear to be adequate for review.
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	yes		The route of administration in a 3-month toxicity study conducted in dogs was oral, which is the intended route in humans

8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	Yes		Yes, the draft labeling submitted in general is similar to the approved ezetimibe label or atorvastatin label, and data express human dose multiples in mg/m^2 or AUC levels.

ITEM	**YES**	**NO**	**COMMENT**
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	Yes		
10) Reasons for refusal to file: N/A The application is filable.			

Reviewing Pharmacologist: Indra Antonipillai, HFD-510

Supervisory Pharmacologist: Karen Davis-Bruno
File name: 200153-2011 filing

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

INDRA ANTONIPILLAI
06/27/2011
From the pharm/tox point of view, this application is fileable.

KAREN L DAVIS BRUNO
06/27/2011

Signed off in DARRTS on 10/8/2009

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

NDA 200-153: This NDA is a 505(b)(2) application.
Submission date: 9/2/2009
Sponsor: MSP Singapore Company, LLC, Singapore, the application is submitted by Merck and Co. Inc. It is an eCTD submission
Drug: (b) (4) tablets (ezetimibe/atorvastatin combination), with code name MK-0653C (also referred as L-000829161 + L000776336). Tablet strengths are 10/10, 10/20, 10/40, 10/80 mg ezetimibe/atorvastatin respectively.

Introduction: This tablet is a combination of two approved drug products, ezetimibe (a selective inhibitor of intestinal cholesterol/phytosterol absorption) and atorvastatin (an HMG-CoA reductase inhibitor). Ezetimibe (NDA 21-445 MSP Singapore) and crystalline atorvastatin (NDA 20-702 Pfizer) are both marketed drugs. However, the current sponsor will use their own amorphous atorvastatin calcium in the fixed dose combination of (b) (4) which is manufactured by Dr. Reddy's laboratories Ltd. in India. The combination product in the current NDA is proposed for patients with primary hypercholesterolemia and homozygous familial hypercholesterolemia.

The current sponsor has conducted a 3-month toxicity study in dogs with MK-6213/L000776336 (another cholesterol absorption inhibitor + atorvastatin) and three geno-toxicity studies with their atorvastatin drug product to support impurity/degradant qualification found in their FDC product.

ITEM: NDA 200-153	**YES**	**NO**	**COMMENT**
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	Yes		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	Yes		

3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	Yes		The sponsor had previously conducted 3-month rat and dog toxicity studies with ezetimibe + atorvastatin co-administered in animals under NDA 21-445. In the current NDA submission, sponsor has provided a 3-month toxicity/toxicokinetics study of MK-6213 + atorvastatin amorphous in dogs and 3 gene-toxicity studies. MK-6213 is another cholesterol absorption inhibitor (b) (4) like ezetimibe. It is not clear to this reviewer, as to why they did not use ezetimibe in the above combination toxicity study (and used another cholesterol absorption inhibitor); sponsor provides no justification for this study. All other studies with the combination have already been conducted under NDA 21-445, in which ezetimibe was approved for monotherapy and for co-administration therapy with statins (simvastatin, atorvastatin, pravastatin and lovastatin).

4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)	Yes		Have electronic files of the carcinogenicity studies been submitted for statistical review? N/A On 6/30/2009 we communicated to the sponsor that various different sources of atorvastatin, including atorvastatin used in the co-administration toxicity studies (NDA 21-445), the atorvastatin to be used in their fixed dose combination (FDC) product, and the atorvastatin (Lipitor) that is already marketed be thoroughly compared. If the data show that there are significant differences in the impurity profiles between these three versions of atorvastatin, a bridging toxicity in a single species may be required for marketing of their drug product. In the current submission, sponsor has conducted one three-month toxicity study in dogs with MK-6213 (another ezetimibe like drug + L000776336 (atorvastatin amorphous) and three gene-toxicity studies with their atorvastatin amorphous product to qualify the impurities/ degradants present in the amorphous atorvastatin All other non-clinical studies have already been conducted with the approved ezetimibe (NDA 21-445) and approved atorvastatin (NDA 20-702. No carcinogenicity studies were requested with the current combination formulation, as both drugs are approved drug products with carcinogenicity evaluations.

ITEM	**YES**	**NO**	**COMMENT**
5) Were the studies adequately designed (i.e., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?			Yes. As indicated earlier, all non-clinical studies with ezetimibe and atorvastatin have already been conducted under the approved NDA 21-445, and NDA 20-702 individually and these studies were adequately designed. Four 4 new toxicity studies been provided in the current submission to qualify their atorvastatin amorphous in the fixed dose combination.

6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (i.e., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	Yes		Sponsor has used their own amorphous atorvastatin calcium in the current fixed dose combination product. A three-month dog toxicity study has been conducted with the combination MK-6213/L000776336 to characterize the impurities in the new formulation. Note that MK-6213 is another cholesterol absorption inhibitor (and not ezetimibe). This study is designed to qualify the impurities/ degradants present in the amorphous atorvastatin. The additional 3 gene-toxicity studies including bacterial mutagenicity and in vitro chromosomal aberration assays conducted with their atorvastatin amorphous to support impurity/degradant qualification of their drug product appear to be adequate for review.
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	yes		The route of administration in a 3-month toxicity study conducted in dogs was oral, which is the intended route in humans
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	Yes		Yes, the draft labeling submitted in general is similar to the approved ezetimibe label or atorvastatin label, and data express human dose multiples in mg/m^2 or AUC levels.

ITEM	**YES**	**NO**	**COMMENT**

9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	Yes		
10) Reasons for refusal to file: N/A The application is filable. However, following non-filing deficiency needs to be communicated to the sponsor: Please provide your justification for providing a combination toxicology study with atorvastatin and MK-6213 (a cholesterol absorption inhibitor which is not ezetimibe) in a 3-month toxicity study in dogs (with MK-6213/L000776336, study TT #07-6039)			

Reviewing Pharmacologist: Indra Antonipillai, HFD-510

Supervisory Pharmacologist: Karen Davis-Bruno
File name: 200-153-filing

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

INDRA ANTONIPILLAI

10/08/2009

From the pharm/tox point of view this application is filable. Please see the non-filing deficiency that needs to be communicated to the sponsor.

KAREN L DAVIS BRUNO

10/08/2009