CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

213072Orig1s000

NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 213072
Supporting document/s:	SDN 1
Applicant's letter date:	07/29/2019
CDER stamp date:	07/29/2019
Product:	Rosuvastatin and Ezetimibe Tablets
Indication:	Primary (b) (4) non-familial) hyperlipidemia (b) (4) and Homozygous familial hypercholesterolemia.
Applicant:	Althera Life Sciences, LLC.
Review Division:	Division of Diabetes, Lipid Disorders and Obesity
Reviewer:	Shaji Theodore, MVSc, PhD, DABT
Supervisor/Team Leader:	C. Lee Elmore, PhD
Division Director:	Lisa Yanoff, MD (Acting)
Project Manager:	Richard E. Whitehead

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 213072 are owned by Althera Life Sciences, LLC. or are data for which Althera Life Sciences, LLC. has obtained a written right of reference. Any information or data necessary for approval of NDA 213072 that Althera Life Sciences, LLC. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 213072.

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Althera Life Sciences submitted a 505(b)(2) marketing application for ROSZET® tablets, a new fixed-dose combination (FDC) of rosuvastatin (5, 10, 20 or 40 mg) and ezetimibe (10 mg), with reliance on FDA's previous findings of safety and effectiveness for rosuvastatin (CRESTOR®, NDA 021366) and ezetimibe (ZETIA®, NDA 021445). Rosuvastatin/ezetimibe FDC tablets are intended for the treatment of primary (b) (4) hyperlipidemia and homozygous familial hypercholesterolemia. The applicant's bridging strategy primarily relies upon human bioequivalence studies with rosuvastatin/ezetimibe FDC tablets compared to Crestor and Zetia alone. From a nonclinical perspective, reliance on the safety of the listed drugs, in the absence of a combination toxicity study, hinges on identification of any residual safety concerns for the combination based on all available sources of information. No nonclinical safety concerns were identified a priori for the combination. However, forced degradation studies of the rosuvastatin/ezetimibe FDC tablets identified (b) (4) as a major degradant impurity that exceeds the qualification threshold of 0.5% per ICH Q3B(R2). To qualify this impurity, the applicant conducted a 90-day comparative rodent oral repeat-dose toxicity study and two in vitro genotoxicity studies, in accordance with ICH Q3B(R2) qualification criteria. This review summarizes the established nonclinical safety information for rosuvastatin and ezetimibe as they relate to the current product, summarizes the findings of the three nonclinical studies conducted to support impurity safety, and provides a nonclinical recommendation regarding marketing of rosuvastatin/ezetimibe FDC tablets.

1.2 Brief Discussion of Nonclinical Findings

Rosuvastatin belongs to the statin class of drugs that reduce cholesterol synthesis by competitively inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in de novo cholesterol biosynthesis. Ezetimibe inhibits dietary cholesterol absorption by blocking the Niemann-Pick C1 like 1 (NPC1L1) protein, a cholesterol transporter localized in the brush border of small intestine. Both drugs lead to increased liver expression of LDL receptors and decreased plasma LDL cholesterol. Thus, rosuvastatin and ezetimibe represent non-overlapping and complementary strategies to reduce plasma LDL cholesterol.

Extensive nonclinical and clinical safety data are available for rosuvastatin and ezetimibe individually, and rosuvastatin and ezetimibe are commonly co-administered in clinical practice. The pharmacological and toxicological profiles of rosuvastatin and ezetimibe do not significantly overlap. The safety of combinations of ezetimibe with an array of statins (i.e., statins marketed at the time of ezetimibe approval) are described in ezetimibe product labeling; no significant toxicological interactions are reported. To establish that reliance on the safety of rosuvastatin and ezetimibe as individual components is justified, the applicant conducted three clinical bioequivalence bridging studies in healthy subjects that compared the highest and lowest doses of the new rosuvastatin/ezetimibe FDC against the concomitant administration of respective doses

of rosuvastatin and ezetimibe tablets individually. According to the clinical pharmacology review, bioequivalence was established. While controlled clinical safety data with rosuvastatin and ezetimibe are limited, per the clinical review, it is reassuring that no new adverse effects distinct from those seen with rosuvastatin or ezetimibe alone were observed in human subjects. Per the clinical pharmacology review, no clinically significant drug-drug interactions are expected for the combination. These data are consistent with the acceptable safety profiles of rosuvastatin and ezetimibe coadministration demonstrated in broader clinical practice, which supports the conclusion that additive therapeutic effects of rosuvastatin and ezetimibe may be anticipated without risk of synergistic toxicity[1][2][3].

The potential for product-specific safety issues was also evaluated by various physicochemical means. In general, these analyses did not demonstrate product specific safety issues. However, forced degradation studies of rosuvastatin/ezetimibe FDC tablets conducted by the applicant identified (b) (4) as a major degradant that exceeded the specification of 0.5% set per ICH Q3B(R2). The need for qualification of any impurities and degradants was considered. To address the issue, the applicant conducted a 90-day comparative rodent oral repeat-dose toxicity study and two in vitro genotoxicity studies in accordance with ICH Q3B(R2) qualification criteria. The 90-day study evaluated the toxicity of ezetimibe containing the impurity against ezetimibe alone. Ezetimibe or (b) (4) impurity at the high dose ((b) (4) mg/kg/day, (b) (4) fold greater than the human therapeutic dose (10 mg/day), based on AUC) did not cause any treatment-related adverse effects. The high dose ((b) (4) mg/kg/day) is thus considered the no observed adverse effect level (NOAEL). The two genotoxicity studies conducted were an in vitro bacterial reverse mutation assay (Ames assay) and an in vitro mammalian chromosomal aberration assay in human lymphocytes, which evaluated the mutagenic and clastogenic potential of (b) (4) . (b) (4) was not genotoxic in the two in vitro assays at any dose irrespective of the status of metabolic activation.

In conclusion, based on the above discussion, there are no nonclinical concerns for the marketing of the fixed-dose combination of rosuvastatin and ezetimibe that should preclude marketing approval of rosuvastatin/ezetimibe FDC tablets.

Table 1. Safety Margin for Ezetimibe and (b) (4) Impurity

Toxicity	Species	NOAEL (mg/kg/day)	Key Findings	Safety Margin AUC*
90-day repeat-dose oral toxicity in rodents	SD Rats	M, F- (b) (4) (high dose)	No adverse effects observed up to high dose	(b) (4) fold

*AUC in human: (b) (4) ng.hr/ml at 10 mg/day; AUC in rat: (b) (4) ng.hr/ml at (b) (4) mg/kg/day

[1] Ballantyne CM, et al., 2007, American Journal of Cardiology, 99(5): 673-80

[2] Bays HE, et al., 2011, American Journal of Cardiology, 108(5): 14

[3] Ballantyne CM, et al., 2014, Atherosclerosis, 232(1): 86-93

1.3 Recommendations

1.3.1 Approvability

From a Pharm-Tox point of view, the nonclinical studies submitted with NDA-213072 and previous FDA findings of safety and efficacy for Rosuvastatin (Crestor) and Zetia (Ezetimibe) are adequate to support approval of ROSZET® (Rosuvastatin and Ezetimibe, Fixed Dose Combination Tablets).

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

In accordance with the Pregnancy and Lactation Labeling (Drugs) Final Rule, the (b) (4) and the associated statement in parenthesis from Section 8.1 (Pregnancy) of the applicant's proposed label should be removed. No other changes or additions to the applicant's proposed label are recommended from a nonclinical perspective.

2 Drug Information

2.1 Drug

CAS Registry Number
Rosuvastatin Calcium USP: 147098-20-2
Ezetimibe USP: 163222-33-1

Generic Name
Rosuvastatin: Rosuvastatin Calcium
Ezetimibe: Ezetimibe

Code Name
AL-320

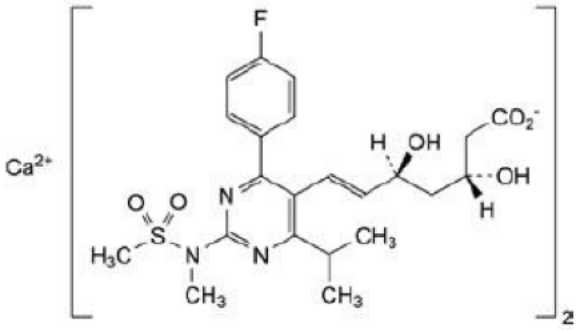
Chemical Name
Rosuvastatin Calcium: 6-Heptenoic acid, 7-[4-(4-fluorophenyl)-6-(1-methylethyl)-2 [methyl(methylsulfonyl)amino]- 5-pyrimidinyl]-3,5-dihydroxy-, calcium salt (2:1)

Ezetimibe: (3R,4S)-1-(4-Fluorophenyl)-3-[(S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-azetidin-2-one

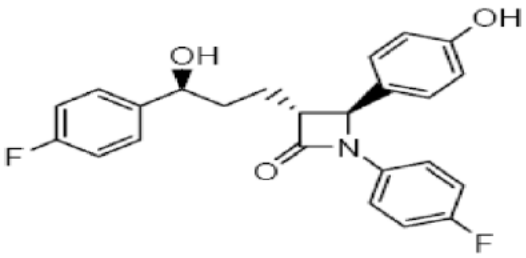
Molecular Formula/Molecular Weight
Rosuvastatin Calcium: $C_{44}H_{54}CaF_2N_6O_{12}S_2$ / 1001.4
Ezetimibe: $C_{24}H_{21}F_2NO_3$ / 409.43

Structure or Biochemical Description

Rosuvastatin Calcium



Ezetimibe



Pharmacologic Class

HMG CoA-Reductase Inhibitor / Dietary Cholesterol Absorption Inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND-136378
NDA-021366 (Crestor)
NDA-021445 (Zetia)
DMFs:
Rosuvastatin Calcium USP – [redacted]
Ezetimibe USP – DMF [redacted]; DMF [redacted]

2.3 Drug Formulation

ROSZET® is a fixed dose combination of the active pharmaceutical ingredients rosuvastatin calcium USP and ezetimibe USP as a tablet. ROSZET is a [redacted] tablet containing 5, 10, 20 or 40 mg Rosuvastatin calcium [redacted] and 10mg of Ezetimibe [redacted]. The excipients used in the formulation are compendial grade (per USP monograph) and are listed in the FDA inactive ingredient database and do not exceed maximum potency listed.

Name of Ingredient	Reference	Function	Quantity per tablet (mg) for respective dose			
			40mg/ 10mg	20mg/ 10mg	10mg/ 10mg	5mg/ 10mg
[redacted]						

(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients in the formulation.

2.5 Comments on Impurities/Degradants of Concern

No process related impurities of concern were identified in the rosuvastatin/ezetimibe FDC. Forced degradation studies of rosuvastatin/ezetimibe FDC identified [redacted] (b) (4) as a major degradant impurity which exceeded the specification of 0.5% set per ICH Q3B. This degradant was qualified in a 90-day rodent comparative toxicity study and two in vitro genotoxicity studies as per ICH Q3B. The studies did not identify any toxicological concern related to [redacted] (b) (4). A detailed review of the qualifying studies can be found under general and genetic toxicology sections.

2.6 Proposed Clinical Population and Dosing Regimen

Rosuvastatin/ezetimibe FDC is intended for adult patients with primary ([redacted] (b) (4) non-familial) hyperlipidemia [redacted] (b) (4) and homozygous familial hypercholesterolemia [redacted] (b) (4). Dose ranges from 5mg rosuvastatin/10 mg ezetimibe to 40mg rosuvastatin/10 mg ezetimibe taken once daily orally.

2.7 Regulatory Background

Althera Life Sciences, LLC., submitted a Type-B meeting (Pre-IND 136378) request to seek FDA guidance on the development of their new fixed-dose combination product ROSZET® (rosuvastatin/ezetimibe FDC) on August 3, 2017. Following a written and oral feedback received on November 2, 2017, the applicant submitted IND 136378 on October 17, 2018 for which the applicant received FDA feedback on a clinical protocol (December 4, 2018) and CMC information (February 12, 2019) and submitted revisions on April 30, 2019. As per FDA guidance, the applicant conducted studies to demonstrate bioequivalence of rosuvastatin/ezetimibe tablets to co-administration of Crestor® (rosuvastatin) and Zetia® (ezetimibe) as bridging studies for its New Drug Application (NDA#213072) under the 505(b)2 pathway.

3 Studies Submitted

3.1 Studies Reviewed

- U-16379: 90-Day Repeat Dose Oral Gavage Comparative Toxicity Study of Ezetimibe API vs. Ezetimibe API with [redacted] (b) (4) Impurity in Sprague Dawley Rats
- U-17071: Bacterial Reverse Mutation Test for [redacted] (b) (4) Impurity
- U-16393: In Vitro Mammalian Chromosome Aberration Test for [redacted] (b) (4) Impurity Using Human Peripheral Blood Lymphocytes

4 Pharmacology

ROSZET® tablet is a fixed-dose combination of rosuvastatin (5, 10, 20 or 40 mg) and ezetimibe (10 mg) taken once daily for the treatment of primary ((b) (4) non-familial) hyperlipidemia (b) (4) and homozygous familial hypercholesterolemia. No new pharmacology studies were submitted by the applicant with NDA 213072 for rosuvastatin/ezetimibe FDC. The applicant refers to the label for CRESTOR® (NDA 021366) and ZETIA® (NDA 021445) for rosuvastatin and ezetimibe respectively, and published literature for pharmacological information.

Mechanism of action related to proposed indication
Rosuvastatin (from the label for CRESTOR®)

Rosuvastatin is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme that converts HMG-CoA to mevalonate, a precursor of cholesterol. In vivo studies in animals, and in vitro studies in cultured animal and human cells have shown rosuvastatin to have a high uptake into, and selectivity for, action in the liver, the target organ for cholesterol lowering. In in vivo and in vitro studies rosuvastatin produces its lipid-modifying effects in two ways. First, it increases the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL. Second, rosuvastatin inhibits hepatic synthesis of VLDL, which reduces the total number of VLDL and LDL particles.

Ezetimibe (from the label for ZETIA®)

Ezetimibe reduces blood cholesterol by inhibiting the absorption of cholesterol by the small intestine. Ezetimibe has a mechanism of action that differs from those of other classes of cholesterol-reducing compounds (statins, bile acid sequestrants [resins], fibric acid derivatives, and plant stanols). The molecular target of ezetimibe has been shown to be the sterol transporter, Niemann-Pick C1-Like 1 (NPC1L1), which is involved in the intestinal uptake of cholesterol and phytosterols.

Ezetimibe does not inhibit cholesterol synthesis in the liver or increase bile acid excretion. Instead, ezetimibe localizes at the brush border of the small intestine and inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. This causes a reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood; this distinct mechanism is complementary to that of statins and of fenofibrate

5 Pharmacokinetics/ADME/Toxicokinetics

The nonclinical pharmacokinetic/toxicokinetic parameters of rosuvastatin and ezetimibe were characterized individually previously under NDA 021366 and NDA 021445 respectively. The clinical pharmacokinetics for rosuvastatin and ezetimibe is described in the labels for CRESTOR® (NDA 021366) and ZETIA® (NDA 021445) respectively.

6 General Toxicology

With NDA 213072, the applicant submitted three toxicology studies to qualify a degradation impurity ([redacted]) in accordance with ICH Q3(B) qualification criteria. Those three studies, a 90-day comparative rodent oral repeat-dose toxicity study and two in vitro genotoxicity studies are reviewed below.

6.1 Repeat-Dose Toxicity

Study title: 90-Day Repeat Dose Oral Gavage Comparative Toxicity Study of Ezetimibe API vs. Ezetimibe API with [redacted] Impurity in Sprague Dawley Rats

Study no.:	U-16379
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	February 28, 2017
GLP compliance:	Yes, OECD Principles of Good Laboratory Practice
QA statement:	Yes
Drug, lot #, and % purity:	Ezetimibe/#614400515/100% [redacted]/090217A/99.94%

Key Study Findings

Methods

Doses:	Ezetimibe, 0, [redacted], and [redacted] mg/kg/day [redacted], 0, [redacted] mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	5 ml/kg
Formulation/Vehicle:	0.4% methyl cellulose in purified water
Species/Strain:	Rats/Sprague Dawley
Number/Sex/Group:	12 rats/sex/group
Age:	8 weeks
Weight:	Males, ~262g; Females, ~207g
Satellite groups:	10 males + 10 females
Justification of dose selection:	The high and low doses for the study are [redacted] and [redacted] fold greater than human therapeutic dose based on AUC. The HD is limited by saturation of absorption.

Key Findings

- There were no ezetimibe- or [redacted]-related adverse effects at any dose administered.

- The NOAEL for this study is the high dose of (b) (4) mg/kg/day based on lack of adverse effects at this dose. The high dose is (b) (4) fold greater than the human therapeutic dose (10 mg/day) based on AUC.

Observations and Results

There was no ezetimibe- or (b) (4) -related mortality or adverse clinical signs at the two doses tested ((b) (4) mg/kg/day and (b) (4) mg/kg/day). No treatment related adverse effects on body weight, food consumption, ophthalmoscopy, and clinical chemistry were observed in animals administered ezetimibe or (b) (4) impurity. No adverse effects on organ weight, or adverse macroscopic or microscopic findings were noticed after administration of ezetimibe or (b) (4) impurity.

7 Genetic Toxicology

The genotoxicity of rosuvastatin and ezetimibe was individually characterized previously for CRESTOR® (NDA 021366) and ZETIA® (NDA 021445) respectively. Summaries of the genotoxicity studies are provided in the labels for CRESTOR® and ZETIA®.

Rosuvastatin (CRESTOR®, NDA 021366)

Rosuvastatin was not mutagenic or clastogenic with or without metabolic activation in the Ames test with Salmonella typhimurium and Escherichia coli, the mouse lymphoma assay, and the chromosomal aberration assay in Chinese hamster lung cells. Rosuvastatin was negative in the in vivo mouse micronucleus test.

Ezetimibe (ZETIA®, NDA 021445)

No evidence of mutagenicity was observed in vitro in a microbial mutagenicity (Ames) test with Salmonella typhimurium and Escherichia coli with or without metabolic activation. No evidence of clastogenicity was observed in vitro in a chromosomal aberration assay in human peripheral blood lymphocytes with or without metabolic activation. In addition, there was no evidence of genotoxicity in the in vivo mouse micronucleus test.

Rosuvastatin/Ezetimibe FDC (NDA213072)

Forced degradation studies of rosuvastatin/ezetimibe FDC identified a degradant (b) (4) , which was evaluated in two in vitro genotoxicity studies described in detail below. The (b) (4) impurity was not mutagenic or clastogenic in the genotoxicity studies reviewed below.

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

Study title: U-17071: Bacterial Reverse Mutation Test for [redacted] Impurity

Study no.:	U-17071
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	June 23, 2017
GLP compliance:	Yes, OECD Principles of Good Laboratory Practice
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] / 090217A/99.94%

Methods

Strains	Salmonella typhimurium TA 100, TA 1535, TA 98, TA 102, TA 1537
Concentration of test article in definitive study	Plate incorporation method: [redacted] and [redacted] mg/ml Pre-incubation method: [redacted], and [redacted] mg/ml
Dose /plate for each strain in the main study	Plate incorporation method: [redacted] and [redacted] µg/plate Pre-incubation method: [redacted] and [redacted] µg/plate
Basis of dose selection	Highest concentration was selected based on no observable cytotoxicity at the highest concentration tested ([redacted] µg/plate) and solubility up to [redacted] mg/ml in vehicle. Four lower concentrations were selected according to the OECD Guideline #471.
Negative control	Dimethyl sulfoxide (DMSO)
Formulation/Vehicle	DMSO
Incubation & sampling time	The five standard tester strains were incubated with the different concentrations of test article or vehicle in the presence or absence of S9 mix. The S9 mix was derived from Aroclor 1254 induced rat liver S9 homogenate. Plate incorporation method: 49 hours Pre-incubation method: 20 minutes pre-incubation followed by 66 hrs incubation in plate.
Comment on Study Validity	Studies were valid based on the following criteria: • The integrity of the test strains conformed to the test no 471 OECD guideline • Test strain titers were equal to 1 – 2 x 10^9 cells/ml in line with OECD test 471

Study evaluation criteria	Test item was considered mutagenic if • There was a concentration dependent increase in revertant count per plate over a minimum of two increasing concentrations in at least one strain with or without metabolic activation

Positive Control

Test strain	Activation	Chemicals	CAS number	Concentration (µg/ plate)
TA 98	Presence	2-aminoanthracene	613-13-8	4
	Absence	2-nitrofluorene	607-57-8	2
TA 100	Presence	2-aminoanthracene	613-13-8	4
	Absence	Sodium azide	26628-22-8	1
TA 1535	Presence	2-aminoanthracene	613-13-8	4
	Absence	Sodium azide	26628-22-8	1
TA 1537	Presence	2-aminoanthracene	613-13-8	4
	Absence	9-Aminoacridine	90-45-9	50
TA 102	Presence	2-aminoanthracene	613-13-8	30
	Absence	Mitomycin C	50-07-7	0.5

Results

- The mean revertant colonies/plate in vehicle control were within the range of in-house historic spontaneous revertant counts in both pre-incubation and plate incorporation methods.
- The mean number of revertant colonies in all tested strains at the different concentrations tested, both in the presence and absence of metabolic activation, was comparable to that of the vehicle control in both pre-incubation and plate incorporation methods.
- Positive control induced 5- to 39-fold increase in the number of revertant colonies compared to vehicle control both in the presence and absence of metabolic activation in the pre-incubation and plate incorporation methods.
- The mean number of revertant colonies/plate in the positive control groups was within the range of in-house historic positive control values.

Therefore, under the test conditions, the test substance ((b) (4)) was not mutagenic in the tested species in the bacterial reverse mutation test in the presence or absence of metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: U-16393: In Vitro Mammalian Chromosome Aberration Test for [redacted] Impurity Using Human Peripheral Blood Lymphocytes

Study no.:	U-16393
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	April 12, 2017
GLP compliance:	Yes, as per OECD principles of GLP
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] / 090217A/99.94%

Methods

Cell line	Proliferating human peripheral blood lymphocytes
Concentrations in definitive study	[redacted], and [redacted] mg/mL
Basis of concentration selection	Based on preliminary cytotoxicity tests, the highest concentration of [redacted] mg/mL was selected to not exceed 45±5% cytotoxicity as per OECD test# 473. The two lower concentrations of [redacted] and [redacted] mg/mL were separated by a factor of √10
Negative control	Dimethyl sulfoxide (DMSO)
Positive control	Ethyl methane sulfonate in the absence of metabolic activation and cyclophosphamide (CPA) in the presence of metabolic activation
Formulation/Vehicle	DMSO
Incubation and sampling time	For short-term treatment, the cells were treated for 4hrs±30 min at 37°C both in the presence and the absence of metabolic activation using the S9 mix. The S9 mix was derived from Aroclor 1254 induced rat liver S9 homogenate. After 4hrs treatment cells were washed and reincubated for 19 hrs. For prolonged treatment the cells were treated up to 19hrs without metabolic activation at 37°C. After 20-22hrs, of incubation with colcemid, the cells were analyzed microscopically for chromosomal aberrations.

Study Validity

The study was considered valid due to reasons below:

- The concurrent vehicle control data was within the limits of the historical control data for the lab.
- The positive control induced a statistically significant increase in chromosomal aberration when compared to control. The positive control data are within the in-house historic range of values for the positive control.
- Cell proliferation in the vehicle control treatment did not indicate cytotoxicity.
- All three experimental conditions were tested and slides from all the groups were analyzed for adequate number of cells.
- The highest concentration tested was selected based on the allowable cytotoxicity limit set by OECD guideline #473.

Evaluation Criteria

- The test item was considered positive for chromosome aberration if there was a concentration-dependent increase or a reproducible increase in the number of cells with chromosome aberrations.
- Biological relevance of results was considered prior to statistical significance as per OECD 473 guideline. A statistically significant ($p \leq 0.05$) in the number of aberrations in one or more concentrations was considered positive.
- An increase in the number of polyploid cells indicate the potential of the test substance to inhibit mitosis and to induce chromosome aberrations.
- An increase in the number of cells with endoreduplicated chromosomes was considered to indicate potential of test substance to inhibit cell cycle progression.

Results

- A slight precipitation of test substance was observed at the highest concentration ((b) (4) mg/ml). The pH of the treatment media was comparable to that of the vehicle.
- Cytotoxicity in the treated group ranged from 3% to 43% and was within the limits of OECD 473 guideline.
- There was no increase (biologically or statistically) in chromosome aberrations at any concentration tested with (b) (4) impurity.
- The positive control induced a statistically significant increase in chromosomal aberration when compared to control.

Therefore, (b) (4) was non-clastogenic in the in vitro mammalian chromosome aberration test using human peripheral blood lymphocyte at the tested concentrations with and without exogenous metabolic activation under the short and prolonged duration treatment conditions employed.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

SHAJI THEODORE
05/04/2020 11:49:23 AM

CALVIN L ELMORE
05/04/2020 12:02:27 PM
I concur.