CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-159

PHARMACOLOGY REVIEW

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

SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA number:	**22-159**
Drug Substance:	**Phentolamine mesylate (OraVerse®)**
PDUFA Goal Date:	9-Feb-2008
Sponsor:	**Novalar Pharmaceuticals, Inc.**
Reviewer name:	R. Daniel Mellon, Ph.D., Pharmacology Toxicology Supervisor
Division name:	Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #:	170
Review completion date:	13-Dec-2007
Recommendation:	**Approval.**

I have read Dr. Elizabeth Bolan's review of the nonclinical pharmacology and toxicology sections of NDA 22-159 and agree with her conclusion that the NDA may be approved. I also concur with her labeling recommendations.

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

/s/

R. Daniel Mellon
12/14/2007 05:37:38 PM
PHARMACOLOGIST

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:	**22-159**
SERIAL NUMBER:	**000 and 000-BP (11/16/07)**
DATE RECEIVED BY CENTER:	**April 9, 2007**
PRODUCT:	**OraVerse®; NV-101, phentolamine mesylate injection**
INTENDED CLINICAL POPULATION:	OraVerse® is indicated for the reversal of soft tissue anesthesia and the associated functional deficits resulting from an intraoral injection of a local anesthetic containing a vasoconstrictor.
SPONSOR:	**Novalar Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**All nonclinical information in the above submission**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Elizabeth A. Bolan, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Dominic Chiapperino, Ph.D.**

Date of review submission to Division File System (DFS): December 3, 2007

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This NDA can be approved from a nonclinical pharmacology/toxicology perspective.

B. Recommendation for nonclinical studies

There are no recommendations for nonclinical studies.

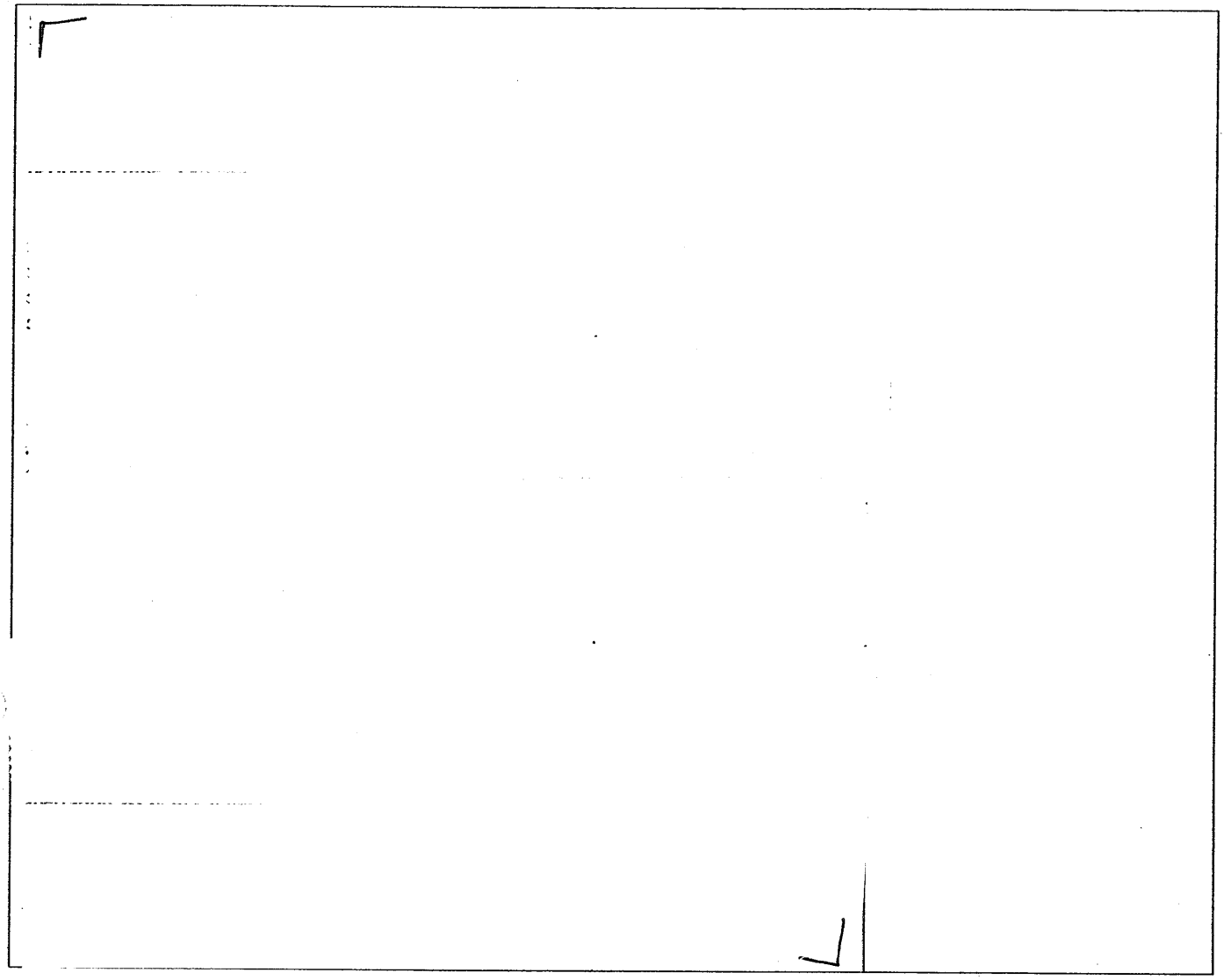
C. Recommendations on labeling

The table below contains the draft labeling submitted by the sponsor, the proposed changes and the rationale for the proposed changes.
Please note that there is one outstanding issue. We have requested that the sponsor provide updated exposure values for the Pregnancy section (5.2) of the label. The exposure values in the current draft of the label are the same values found in the original Regitine® label and do not seem to be updated for the OraVerse® product. An addendum will be submitted when the sponsor provides the updated values.

sponsor's labeling	*proposed changes*	*rationale*
[redacted]		

b(4)

b(5)



b(4)

b(5)

II. Summary of nonclinical findings

Brief overview of nonclinical findings

In support of the NDA for OraVerse® (phentolamine mesylate), the sponsor conducted a single-dose local tolerance study and a battery of genetic toxicology studies with phentolamine mesylate and — impurities/degradants found in the drug product, ——— b(4)
——— A Segment I male fertility study with oral administration of phentolamine mesylate was also included in the NDA. Repeat-dose toxicology, reproductive and developmental toxicology and carcinogenicity studies are not required for this 505(b)(2) application for the proposed indication.

Local toxicology: In the single-dose local toxicology study no test article-related changes were observed in any of the parameters examined with the exception of the histopathology

of local tissues. Minimal to mild inflammation was seen at the injection site of all groups. The 1X clinical formulation group showed muscle degeneration and fibrosis which was not seen at the higher doses. Minimal to moderate hemorrhaging in lymph nodes and minimal inflammation in lymph nodes and salivary glands was observed in both vehicle and treated animals. Several vehicle and 10X clinical formulation dogs showed minimal inflammation and degeneration in the trigeminal ganglia. Nerve fiber degeneration was observed in the superior alveolar nerve of one 1X clinical formulation dog but nerve fiber degeneration was not observed at higher doses. In the absence of intact (un-injected) control dogs, it is not possible to determine whether the pathologies observed in nerves, muscle and surrounding tissues seen in the vehicle group are due to pre-existing lesions or to needle placement. This drug product will be administered via a commonly used dental needle. Any potential pathology resulting from needle placement would be no greater than an injection of the dental anesthetic and is therefore of no toxicologic concern. It is concluded that phentolamine mesylate, [redacted] t doses up to ten times the intended human dose, did not cause considerably greater levels of toxicity than the vehicle injection in this study. b(4)

Genetic toxicology: The genotoxic potential of phentolamine mesylate was evaluated in the *in vitro* Bacterial Reverse Mutation Assay (Ames Test), the *in vitro* Chromosome Aberration Assay using CHO cells and the *in vivo* Mouse Micronucleus Assay. The sponsor submitted two separate studies for each test. The first Ames Test submitted did not utilize a high enough concentration of the test article and was concluded to be invalid. Phentolamine mesylate was negative in the second Ames Test. Phentolamine mesylate was negative in the first *in vitro* Chromosome Aberration Assay in both the presence and absence of metabolic activation and negative in the second assay in the presence of metabolic activation. In the second assay in the absence of metabolic activation, the high concentration showed an equivocal result. In both *in vivo* Micronucleus Assays, phentolamine mesylate was negative. The weight of evidence suggests that phentolamine mesylate is most likely not mutagenic or clastogenic.

The levels of [redacted] impurities [redacted] exceed ICH guidelines. The sponsor conducted the Bacterial Reverse Mutation Assay (Ames Test) and the *in vitro* Mammalian Chromosome Aberration Assay for both compounds. [redacted] was negative in both assays [redacted] was negative in the Ames Test and positive in the *in vitro* Mammalian Chromosome Aberration Assay. To further assess the clastogenic potential of [redacted] it was evaluated in the *in vivo* Micronucleus Test where it yielded a negative result. The weight of evidence suggests that [redacted] are most likely not mutagenic or clastogenic. b(4)

Male Fertility: The sponsor has submitted a male fertility study with phentolamine mesylate. At concentrations up to 143 times human therapeutic exposure levels, phentolamine mesylate was shown to have no adverse effects on male fertility in the rat.

A. Pharmacologic activity

OraVerse® (phentolamine mesylate) is being developed to accelerate recovery from soft tissue anesthesia induced by local dental anesthetics containing a vasoconstrictor such as

epinephrine. Phentolamine is a short-acting α1 and α2-adrenergic receptor antagonist that produces generalized vasodilation and smooth muscle relaxation. When stimulated by epinephrine, α-adrenergic receptors on small blood vessels cause smooth muscle contraction resulting in a constriction of blood flow. Local application of phentolamine can reverse the vasoconstrictive effects of epinephrine-containing dental anesthetics due to the ability of phentolamine to antagonize α-adrenergic receptors. The vasodilation results in increased clearance of the anesthetic from the anesthetized site bringing about a faster return to normal sensation.

B. Nonclinical safety issues relevant to clinical use

There are no nonclinical safety issues relevant to clinical use.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

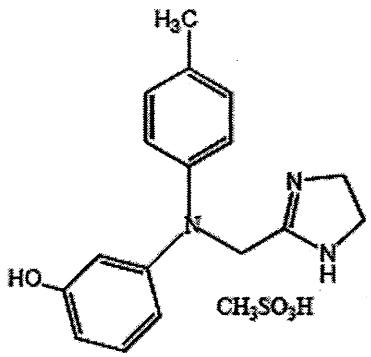
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-159
Review number: 1
Sequence number/date/type of submission: 000/April 9, 2007/original submission
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Novalar Pharmaceuticals, Inc., San Diego, CA
Manufacturer for drug substance: b(4)

Reviewer name: Elizabeth A. Bolan, Ph.D.
Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #: 170
Review completion date: November 21, 2007

Drug:

Trade name: OraVerse®
Generic name: phentolamine mesylate
Code name: NV-101
Chemical name: *m*-[*N*-(2-imidazolin-2-ylmethyl-*p*-toluidino) phenol monomethanesulfonate (salt)
CAS registry number: *[65-28-1]*
Molecular formula/molecular weight: $C_{17}H_{19}N_3O \cdot CH_4O_3S$ MW= 377.46
Structure:



Relevant INDs/NDAs/DMFs:

IND/NDA/DMF	*drug/compound*	*sponsor*	*division*	*status*
IND 65,095	phentolamine mesylate: NV-101	Novalar Pharma	DAARP	Active

NDA 8-278	phentolamine mesylate: Regitine® (RLD)	Novartis	DCRP	Approved, but not currently on market
			NA	reviewed: 10/29/07
			NA	reviewed: 9/6/07

b(4)

Drug class: α_1 and α_2 adrenergic receptor antagonist and peripheral vasodilator

Intended clinical population: OraVerse® is indicated for the reversal of soft tissue anesthesia and the associated functional deficits resulting from an intraoral injection of a local anesthetic containing a vasoconstrictor.

Clinical formulation: The OraVerse® drug product is an injection solution. The components of the OraVerse® drug product are listed in the table below. All excipients can be found in approved drug products at equal or greater levels and therefore do not pose any unique toxicological concerns.

Ingredient	Function	Unit Formulation (per mL)	Unit Formulation (per 1.7 mL cartridge)
Phentolamine mesylate, USP	Active pharmaceutical ingredient		0.40 mg
EDTA Na_2, USP			
D-Mannitol, USP			
Sodium acetate			
Acetic acid, USP	pH adjustment agent		
NaOH, NF	pH adjustment agent		

Abbreviations: EDTA, ethylenediaminetetraacetic acid; NF, National Formulary; q.s., quantity sufficient;

b(4)

Proposed commercial specifications of impurities in the OraVerse® drug product

Purity		

b(4)

At the proposed specifications, — impurities, ——— exceeded ICH Q3A(R2) and ICH Q3B(R2) threshold for safety qualification. The sponsor performed the appropriate genetic toxicology and local toxicity studies. There are no structural alerts for either compound. Neither ——— showed toxicity in the studies conducted and can be considered adequately qualified at concentrations up to ten times the maximal human dose. b(4)

——— has been shown to be extractable from the ——— rubber used for the cap and plunger in all three extraction conditions tested (M3.2.P.2.4.1 table 11). No leachable assessment was performed. Based on the extraction data, exposure levels for ——— could potentially reach ———. ——— does not contain a structural alert but no information on its potential for toxicity was provided. We asked the sponsor to provide a toxicological assessment of ——— based on what is known from the literature to determine the safe level of exposure of ——— via the intended route of administration for the product. The toxicological assessment of ——— submitted by the sponsor summarized the literature and has been found adequate. The Sponsor also noted that the DMF holder, ——— has stated that the ——— rubber used for the cap and plunger is used in FDA approved products. No further studies for ——— will be required. b(4)

The sponsor has performed analysis for the possible presence of ——— ——— in the drug substance using a validated gas chromatography-mass spectroscopy (GC-MS) procedure. A total of 6 batches of drug substance have been tested with a result of "none detected" in each of the 6 batches. b(4)

Route of administration: The route of administration is intraoral submucosal injection. The dosing regimen following an injection of a local anesthetic containing a vasoconstrictor is:

½ cartridge (0.2 mg) of OraVerse® when ½ cartridge of local anesthetic has been administered

1 cartridge (0.4 mg) of OraVerse® when 1 cartridge of local anesthetic has been administered

2 cartridges (0.8 mg) of OraVerse® when 2 cartridges of local anesthetic has been administered

If the weight of the patient is between 15-30 kg, the maximum recommended dose of OraVerse® is ½ cartridge (0.2 mg).

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

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of 22-159 are owned by Novalar or are data for which Novalar has obtained a written right of reference. Any information or data necessary for approval of 22-159 that Novalar 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 described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Novalar does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of 22-159.

Studies reviewed within this submission:

Study Title	*Study Number*
Pharmacology	
Effects of Intraoral Injections of 2% Lidocaine with 1:100,000 Epinephrine and Phentolamine Mesylate on Local Blood Flow in the Dog	044-002
General Side Effect Profile Screening Program (Receptor Binding Screen)	05-1585
Toxicology	
Local Tolerance at the Intraoral Injection Site and Systemic Toxicity Study of Phentolamine Mesylate,	044-001
A Rat Male Fertility Study with Phentolamine Mesylate via Oral Gavage Administration	96-4097
Genetic Toxicology	
Salmonella/Mammalian Microsome Preincubation Mutagenicity Assay (Ames Test) with Phentolamine Mesylate	T5796-502
Bacterial Reverse Mutation Assay (Ames Test) with Phentolamine Mesylate	
Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells with Phentolamine Mesylate	T8199.337
In Vitro Mammalian Chromosome Aberration Assay with Phentolamine Mesylate	
In Vivo Mouse Bone Marrow Micronucleus Test with Phentolamine Mesylate	96067
Mammalian Erythrocyte Micronucleus Test with Phentolamine Mesylate	
Bacterial Reverse Mutation Assay (Ames Test) with	
Bacterial Reverse Mutation Assay (Ames Test) with	
Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells with	
Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells with	
Mammalian Erythrocyte Micronucleus Test with	

b(4)

Studies <u>not</u> reviewed within this submission:
The rat male fertility study (study # 96-4097) and several genetic toxicology studies with phentolamine (study #s T5796-502, T8199.337, 96067) have been previously reviewed by Dr. Jeri El-Hage from the Division of Metabolic and Endocrine Products. Results have been summarized under the appropriate headings in this NDA review.

2.6.2 PHARMACOLOGY

The reference listed drug, Regitine® (phentolamine mesylate), was approved in 1952 for the indication of prevention or control of hypertensive episodes that may occur in pheochromocytoma as a result of stress or manipulation during preoperative preparation and surgical excision. It is also indicated for the prevention of dermal necrosis and

sloughing following intravenous administration or extravasation of norepinephrine. Regitine® is also indicated for diagnosis of pheochromocytoma by the Regitine® blocking test (Novartis, 1998). Although Regitine® is no longer marketed, a generic phentolamine mesylate is available. The NDA for Regitine® has not been withdrawn.

2.6.2.1 Brief summary

Phentolamine is a short-acting α1 and α2 adrenergic receptor antagonist that produces generalized vasodilation and smooth muscle relaxation. Pharmacologic actions on cardiac and smooth muscle include cardiac stimulation and peripheral vasodilation. Phentolamine has a wide range of pharmacological actions in addition to α-adrenergic blockade including sympathomimetic, antihypertensive, antihistaminic, and cholinomimetic activity. Increased GI motility is observed secondary to the inhibition of α2-mediated vagal tone. Phentolamine also blocks 5-HT receptors, releasing histamine from mast cells and increases gastric acid and pepsin secretion.

2.6.2.2 Primary pharmacodynamics

With regard to the α-adrenergic blocking actions, phentolamine is frequently used for management of catecholamine-induced hypertensive crises (*i.e.* pheochromocytoma; Varon and Marik, 2000;Perret and Enrico, 2003). Intercavernosal injections of phentolamine have also been used in clinical trials for treatment of erectile dysfunction, although there are no products approved by the FDA for this indication (Montorsi, et al., 2003).

Mechanism of action: Phentolamine reverses the vasoconstrictive effects of epinephrine-containing dental anesthetics via blockade of the α-adrenergic receptor.

Drug activity related to proposed indication: OraVerse® (phentolamine mesylate) is being developed to accelerate recovery from soft tissue anesthesia induced by local dental anesthetics containing a vasoconstrictor such as epinephrine. Alpha-adrenergic receptors on small blood vessels cause smooth muscle contraction resulting in a constriction of blood flow when stimulated by epinephrine from a local anesthetic product. Local application of phentolamine to the gingival submucosa can reverse the vasoconstrictive effects of epinephrine-containing anesthetics due to the ability of phentolamine to antagonize α-adrenergic receptors. The vasodilation results in increased clearance of the anesthetic from the anesthetized site bringing about a faster return to normal sensation.

2.6.2.3 Secondary pharmacodynamics

Phentolamine has a wide range of pharmacological actions in addition to α-adrenergic blockade. Phentolamine has been shown to inhibit *in vitro* human platelet aggregation induced by catecholamines, adenosine diphosphate, platelet-activating factor, collagen, thrombin and the calcium ionophore A23187 (Anfossi, et al., 1990). Phentolamine also inhibits K_{ATP} and delayed rectifier K_V potassium channels in vascular smooth muscle cells, cardiac ventricular cells, insulin-producing pancreatic β-cells, and cavernosal smooth muscle (Ibbotson, et al., 1993;Proks and Ashcroft, 1997;McPherson, 1993).

Study title: Effects of Intraoral Injections of 2% Lidocaine with 1:100,000 Epinephrine and Phentolamine Mesylate on Local Blood Flow in the Dog

Key study findings: This non-GLP pharmacology study demonstrates that mucosal blood flow, as measured by laser doppler flowmetry, was locally increased by an intraoral injection of phentolamine mesylate when given after an injection of a vasoconstrictor-containing anesthetic in beagle dogs. Phentolamine mesylate did not affect blood flow in the contralateral side and did not affect systemic blood pressure.

Study no.: 044-002
Volume #, and page #: eCTD 0000 4.2.1.1.1
Conducting laboratory and location: b(4)
Date of study initiation: June 8, 2006
Drug, lot #, and % purity: Lot # M51228, 99.9%

Methods
This study uses the drug product formulation and the vehicle is the formulation minus the active ingredient. The formulation is detailed in the Clinical Formulation section above.

group	*pretreatment*	*followed by*	*n=*
1	2% lidocaine with 1:100,000 epinephrine	--	4
2		235 mcg/mL phentolamine mesylate	4
3		vehicle	4

b(4)

Species/strain: beagle dog (
Number/sex/group: 4 M/group
Route, formulation, volume, and infusion rate: intraoral injection in one maxillary quadrant in a volume of 51 µL/kg at a rate of 0.6 mL per 20 seconds.
Age: 14-20 months
Weight: 10.7-15.2 kg

The phentolamine mesylate dose (per injection) administered to dogs in this study was the dog equivalent dose of the single injection dose given to dental patients in the clinical trials. The single injection clinical dose of 0.4 mg for adults was adjusted to dogs by converting the human dose to mg/kg assuming a human body weight of 60 kg, and multiplying that dose by the dog scaling factor of 1.8. This is calculated as 0.4 mg divided by 60 kg = 0.00666 mg/kg times 1.8 = 0.012 mg/kg = dog equivalent dose. The dose volume for a 10 kg dog dosed with 0.235 mg/mL solution of phentolamine mesylate was 0.51 mL per quadrant. The 0.235 mg/mL concentration used in the dog is the same concentration used in the clinical trials.
Each dog was sedated with an intravenous injection of 16 mg/kg sodium thiopental and placed in a stereotaxic headholder. The dogs were masked to a surgical plane of anesthesia, intubated and maintained on ~1 L/min of oxygen and 2.0% isoflurane. The femoral artery was catheterized to monitor systemic blood pressure during the procedure. Arterial blood

pressure was monitored and recorded every two minutes throughout the procedure. Two probes were positioned in identical positions over the mucogingival junction at the midline of the first premolar tooth on each side of the maxilla. The probes were adjusted to obtain maximal blood perfusion measurements by laser doppler flowmetry. Blood flow on side of the injection and contralateral side was measured. Baseline blood flow measurements were taken for all animals over a period of ten minutes. After baseline recordings, each dog was given an intraoral injection of 0.3 mL of 2% lidocaine with 1:100,000 epinephrine (referred to as "lidocaine") in the maxilla. The animals in Group 1 were observed for 30 minutes after the lidocaine injection. Groups 2 and 3 were observed for approximately 10 minutes after the lidocaine injection after which an injection of either phentolamine mesylate (Group 2) or vehicle (Group 3) was made in the same location as the lidocaine injection. The volume of phentolamine mesylate or vehicle administered was 0.051 mL/kg and the injection was given over a period of 20 seconds. The animals in Groups 2 and 3 were monitored for at least 30 minutes following the treatment injection and blood flow was recorded with laser doppler flowmetry.

Blood flow was sampled 20 times per minute throughout the study period. For each dog, a mean value was calculated for each one minute time period by averaging the 20 samples collected during each one minute period. This sampling rate and averaging interval minimized noise inherent in the assay. The ten one-minute periods of the baseline were averaged to form a single mean baseline value. The average blood flow measure from each one-minute period following lidocaine injection and following phentolamine mesylate injection or vehicle, were recorded as percent change from the mean baseline value. Statistics were performed on each one-minute interval for 29 minutes after the lidocaine injection. The ten one-minute periods between the lidocaine injection and the treatment injection were also averaged to form a single mean lidocaine baseline value. The average blood flow measurement from each one-minute period following the treatment injection were recorded as percent change from the mean lidocaine baseline value. Statistics were performed on each one-minute interval for 29 minutes after the treatment injection.

Reviewer's note:
The names of the statistical tests used to evaluate the data are not given.

Results
Within five minutes of the lidocaine injection, blood flow decreased in the area surrounding the injection site in all groups. The mean decrease in blood flow was consistent throughout all groups with a mean percent change in blood perfusion units ranging from -40% (Group 1) to -58% (Group 3). Decreased blood flow continued in Group 1 throughout the remainder of the 30-minute observation period. In Group 2 the post treatment injection mean percent change from the lidocaine injection ranged from 170% to 778%. Thirty minutes after the treatment injection blood perfusion for three of the four dogs in Group 2 had returned to baseline levels. The fourth dog was monitored for an additional 12 minutes with no decrease observed. In Group 3, for the 29 minutes after the treatment injection, the mean percent change from the lidocaine injection ranged from -57% to 82%. With the exception of the first two minutes (82% and 14%, respectively) after the lidocaine injection, the mean percent change was below -6% for the monitoring period. The increase in mean

percent change from the lidocaine injection during the first 2 minutes is in large part due to a single dog.

Statistical analysis of the groups for mean percent change from the baseline value showed a significant ($p \leq 0.05$) change for Group 2 at 13, 19-24, 26, and 29 minutes post lidocaine dose with a highly significant ($p \leq 0.01$) change present at 25, 27, and 28 minutes post lidocaine dose. Mean percent change of blood flow in Group 2 was significantly greater than in Group 3 at 7-23 minutes ($p \leq 0.05$) with highly significant ($p \leq 0.01$) changes occurring at 9, 10, and 14-16 minutes post treatment injection. There were no significant changes in blood flow on the contralateral side during the procedure and there were no noteworthy changes in blood pressure as a result of the administration of the anesthetic or the treatment.

Discussion and Conclusions

Reviewer's Comment:
This study demonstrates that mucosal blood flow, as measured by laser doppler flowmetry, was locally increased by an intraoral injection of phentolamine mesylate when given after an injection of a vasoconstrictor-containing anesthetic. Phentolamine mesylate did not affect blood flow in the contralateral side and did not affect systemic blood pressure.

Study title: General Side Effect Profile Screening Program

Key study findings: The binding of phentolamine mesylate was shown to be selective for α-adrenergic receptors in comparison to the battery of receptors tested in the assay. The absence of significant dose dependent binding of ——— suggest that they are inactive at the receptor types tested. b(4)

Study no.: 05-1585
Volume #, and page #: eCTD 0000 4.2.1.2.1
Conducting laboratory and location: ——— b(4)
Date of study initiation: April 9, 2007

Methods
Observations and times
The objective of this *in vitro* study was to individually assess the binding affinities of phentolamine and the — mpurities found in the drug product, ——— ——— at 63 cloned human receptor sites. The — test articles were assayed for receptor binding at concentrations of 1 and 10 µM against a standard battery of neurotransmitter, peptide, steroid and growth factor receptors, transporters, ion channels, and enzymes. b(4)

——— has specified a set of criteria to interpret the results of their assays. The baseline range is between -20% to 20% inhibition. A compound would be considered to be a negative inhibitor if it shows inhibition greater than -20%. Compounds that show

inhibition in the range of 20% to 49% are considered marginally active. An active compound is one that shows inhibition greater than 50% inhibition and displays a dose-dependent relationship.

Results

At 1 and 10 μM, phentolamine mesylate maximally inhibited binding in both the α1 and α2 adrenergic nonselective (NS) assays (α1: 102.5% and 104.1%; α2: 100.2% and 104.6%, respectively; Table 1). At the 10 μM high concentration, phentolamine mesylate inhibited binding of opioid NS (55.5%), SERT (75.8%), 5-HT NS (79.9%), and sodium ion channel site 2 (63.1%) at levels considered to qualify the compound as active but the magnitude of the inhibition was not dose dependent (Table 1). ———— b(4)

———

Reviewer's note:

Phentolamine has been shown to bind to the K^+_{ATP} channel at the Kir 6.2 site (Proks and Ashcroft, 1997). This specific subunit of the K^+ channel was not assayed in the ——— study. As expected, phentolamine did not displace any of the three K^+ channel ligands utilized in the ——— say: glibenclamide, apamin and astemizole, which bind to the SUR1 subunit of the K^+_{ATP} channel, Ca^{2+}-activated and voltage-gated K^+ channels, respectively. Although the list of receptors tested in this assay is not exhaustive, it is standard in the field and it provides an adequate overview of the selectivity of the compounds tested. b(4)

Discussions and Conclusions

The objective of this *in vitro* study was to individually assess the binding affinities of phentolamine mesylate, ———— at 63 cloned human receptor sites. Although all — ompounds did show displacement of several radioligands in the screen, with the exception of the α-adrenergic receptors for phentolamine mesylate, the criteria for an active compound are not satisfied in any of the cases. The sponsor states that mean peak plasma concentration of phentolamine observed after a dose of 0.8 mg OraVerse® in healthy human subjects was 10 nM (Study # NOVA 04-PK, Section 2.7.2.2.1). The sponsor suggests that these concentrations, it would be unlikely that phentolamine would interact with the receptors where it was considered marginally active or active at the highest concentration tested. This reviewer agrees with the sponsor and concludes that this study has demonstrated that phentolamine mesylate is selective for α-adrenergic receptors as compared to the other receptor sites tested. The absence of significant dose-dependent binding of ———— suggests that — compounds are inactive at the receptor types tested in this screen. b(4)

Table 1

	Results		
	receptor in assay (ligand)	***% Inhibition at 1 μM***	***% Inhibition at 10 μM***
phentolamine mesylate	α1 adrenergic, non-selective [^{3}H]-7-MeO-Prazosin	**102.5**	**104.1**
	α2 adrenergic, non-selective [^{3}H]-RX 821002	**100.2**	**104.6**
	opioid, non-selective [^{3}H]-Naloxone	21.6	**55.5**
	SERT [^{3}H]-Citalopram	22.0	**75.8**
	5-HT, non-selective [^{3}H]-LSD	33.6	**79.9**
	Sodium ion channel, site 2 [^{3}H]-Batrachotoxin	11.4	**63.1**
	[		]

b(4)

note: bolded numbers indicate greater than 50% inhibition

2.6.2.4 Safety pharmacology

Novalar has not conducted any nonclinical safety studies for phentolamine. Phentolamine has been previously approved by the FDA and has been on the market for 55 years. The long history of use precludes the need for such studies.

The sponsor states that no formal safety pharmacology studies of phentolamine were identified in electronic literature searches. However, three nonclinical pharmacology studies were summarized by the sponsor which addressed the cardiac effects of phentolamine administration in cats, dogs and monkeys. The studies demonstrated several known pharmacological effects of α-adrenergic antagonists and did not provide any new information.

The following safety pharmacology overview has been taken from the publicly available text *Goodman and Gilman's The Pharmacological Basis of Therapeutics 11th edition* (2006), unless otherwise noted.

Reviewer's note:
Taking into consideration the proposed dose and local route of administration for this drug product, the potential for many of the adverse effects noted below are not necessarily relevant.

Neurological effects: No neurological effects of phentolamine have been identified from the literature.

Cardiovascular effects: Hypotension is the major adverse effect of phentolamine. In addition, reflex cardiac stimulation may cause alarming tachycardia, cardiac arrhythmias, and ischemic cardiac events, including myocardial infarction.

Pulmonary effects: The *AHFS Drug Information* database states that phentolamine dilates bronchial smooth muscle, although presumably via β-adrenergic receptors at very high doses where selectivity for α-adrenergic receptors is lost.

Renal effects: No renal effects of phentolamine have been identified from the literature.

Gastrointestinal effects: Phentolamine has been shown to stimulate GI smooth muscle and may result in abdominal pain, nausea, and exacerbation of peptic ulcer. Phentolamine also enhances gastric acid and pepsin secretion.

Abuse liability: The sponsor has not conducted specific studies to evaluate abuse liability. A review of the literature and assessment of the pharmacology of phentolamine indicate that phentolamine would not demonstrate any abuse liability concerns.

2.6.2.5 Pharmacodynamic drug interactions
According to the sponsor's NDA submission, no articles on pharmacodynamic drug interaction studies in animals were available from electronic searches of the literature.

No documented human interactions for phentolamine were found in the *Drug Interaction Facts* database.

Phentolamine has the potential to antagonize drugs that stimulate the α-adrenergic receptor. However, taking into consideration the local route of administration and the low systemic exposure, drug interactions should not be a concern.

The following italicized text regarding drug interactions with phentolamine was excerpted verbatim from the *Clinical Pharmacology* online database.

Since phentolamine is an antagonist at alpha-receptors, it can antagonize drugs that stimulate this receptor. One of the main clinical uses of phentolamine is to prevent tissue

damage from vasopressors if they extravasate during IV infusion (Bedford Laboratories, 1999;Bey, et al., 1998). Phentolamine inhibits the alpha-mediated effects of adrenergic agonists such as methoxamine, norepinephrine (levarterenol), and phenylephrine. Phentolamine decreases, but does not reverse, the pressor response to metaraminol. Regarding epinephrine, phentolamine can antagonize its alpha-receptor-mediated actions and exaggerate its beta-adrenergic responses (e.g., hypotension, vasodilation, and tachycardia; (American Reagent, 2003). In high doses, dopamine stimulates alpha-receptors; phentolamine can be used to prevent tissue damage from dopamine and may possibly antagonize dopamine's vasopressor activity (DuPont Pharmaceuticals, 1991;Bey, et al., 1998).

In general, the administration of an alpha-blocker such as phentolamine with ephedra, ma huang alkaloids (Haller and Benowitz, 2000) can reduce the vasopressor activity of the ephedra alkaloid (Haller and Benowitz, 2000). In addition, other sympathomimetics can antagonize the effects of antihypertensives such as alpha-blockers when administered concomitantly (Chua and Benrimoj, 1988;Bey, et al., 1998;Haller and Benowitz, 2000;Kernan, et al., 2000;American Reagent, 2003;Bradley, 1991).

Ethanol ingestion can have an additive vasodilatory effect (Johnson, et al., 1986) in patients concurrently taking phentolamine, which can lead to postural hypotension and tachycardia.

Although the duration of action of phentolamine is short, its onset is very rapid. The hypotensive effects of phentolamine can be additive with that of other antihypertensive agents (Bedford Laboratories, 1999).

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Table 1: Nonclinical Pharmacology Studies of Phentolamine Sponsored by Novalar

Section	Study Title (Study No.)	Species	Route	Duration	Dose/ Concentration
2.6.2.2.2.1	Effects of Intraoral Injections of 2% Lidocaine with 1:100,000 Epinephrine and Phentolamine Mesylate on Local Blood Flow in the Dog (Study No. 044-002)	Dog	Intraoral submucosal injection	Single dose	0, 0.012 mg/kg phentolamine
					1×10^{-6}, 1×10^{-5} M phentolamine
2.6.2.3.1	General Side Effect Profile Screening Program (Study No. 05-1585)	*In Vitro*	Not applicable	Not applicable	[redacted] b(4)

Abbreviation: [redacted]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Formal studies were not submitted with the NDA.

2.6.4.1 Brief summary

As stated in the publicly available text *Goodman and Gilman's The Pharmacological Basis of Therapeutics 11th edition* (2006), the pharmacokinetic properties of phentolamine are not known, although the drug is extensively metabolized.

2.6.4.2 Methods of Analysis

No studies were submitted with the NDA.

2.6.4.3 Absorption

Formal studies were not submitted with the NDA.
Phentolamine is only about 20% as active after oral administration as after parenteral administration. About 10% of a parenteral dose can be recovered in the urine as active drug; the fate of the remainder is unknown. It is not known whether phentolamine crosses the placenta or appears in milk (*AHFS Drug Information* database).

2.6.4.4 Distribution

Formal studies were not submitted with the NDA.
see Absorption

2.6.4.5 Metabolism

Formal studies were not submitted with the NDA.
see Absorption

2.6.4.6 Excretion

Formal studies were not submitted with the NDA.
see Absorption

2.6.4.7 Pharmacokinetic drug interactions

No pharmacokinetic drug interactions have been identified.

2.6.4.8 Other Pharmacokinetic Studies

No pharmacokinetic studies were submitted with the NDA.

2.6.4.9 Discussion and Conclusions

The pharmacokinetic properties of phentolamine are not known, although the drug is extensively metabolized. About 10% of a parenteral dose can be recovered in the urine as active drug; the fate of the remainder is unknown.

2.6.4.10 Tables and figures to include comparative TK summary

Toxicokinetics summary tables were not submitted with the NDA.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

A pharmacokinetics tabulated summary was not submitted with the NDA.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: A single dose local toxicology study was performed with the clinical formulation of OraVerse® and the — impurities, ——————— at doses up to ten times the to-be-marketed formulation. No test article-related changes were observed in any of the parameters examined with the exception of the histopathology of local tissues. Minimal to mild inflammation was seen in the injection site of all groups. The 1X clinical formulation group showed muscle degeneration and fibrosis which was not seen at the higher doses. Minimal to moderate hemorrhaging in lymph nodes and minimal inflammation in lymph nodes and salivary glands was observed in both vehicle and treated animals. Several vehicle and 10X clinical formulation group dogs showed minimal inflammation and degeneration in the trigeminal ganglia. Nerve fiber degeneration was observed in the superior alveolar nerve of one 1X clinical formulation group dog but nerve fiber degeneration was not observed at higher doses. b(4)

In the absence of intact (un-injected) control dogs, it is not possible to determine whether the pathologies observed in nerves, muscle and surrounding tissues seen in the vehicle group are due to pre-existing lesions or to needle placement. This drug product will be administered via a commonly used dental needle. Any potential pathology resulting from needle placement would be no greater than an injection of the dental anesthetic and is therefore of no toxicologic concern. It is concluded that phentolamine mesylate, ——— ——————— at doses up to ten times the intended human dose, did not cause considerably greater levels of toxicity than the vehicle injection in this study. b(

Genetic toxicology: The genotoxic potential of phentolamine mesylate was evaluated in the *in vitro* Bacterial Reverse Mutation Assay (Ames Test), the *in vitro* Chromosome Aberration Assay using CHO cells and the *in vivo* Mouse Micronucleus Assay. The sponsor submitted two separate studies for each test. The first Ames Test submitted did not utilize a high enough concentration of the test article and was concluded to be invalid. Phentolamine mesylate was negative in the second Ames Test. Phentolamine mesylate was negative in the first *in vitro* Chromosome Aberration Assay in both the presence and absence of metabolic activation and negative in the second assay in the presence of metabolic activation. In the second assay in the absence of metabolic activation, the high concentration showed an equivocal result. In both *in vivo* Micronucleus Assays, phentolamine mesylate was negative. The weight of evidence suggests phentolamine mesylate is most likely not mutagenic or clastogenic.

The levels of — impurities, ——————— , exceed ICH guidelines. The sponsor conducted the Bacterial Reverse Mutation Assay (Ames Test) and the *in vitro* Mammalian Chromosome Aberration Assay for — compounds ——— as negative in both assays and ——— was negative in the Ames Test and positive in the *in vitro* b(4)

Mammalian Chromosome Aberration Assay. However, in IT submission 073, the sponsor concluded that ——— was *negative* in the *in vitro* Mammalian Chromosome Aberration Assay. We consulted Dr. David Jacobson-Kram and he concurred that the result was positive. To further assess the clastogenic potential of ——— it was evaluated in the *in vivo* Micronucleus Test where it yielded a negative result. ——— are concluded to be genotoxic. b(4)

Carcinogenicity: The sponsor did not conduct a carcinogenicity assessment for phentolamine mesylate. The ICH M3 Guideline indicates that carcinogenicity assessment would not be required for a drug that would not be used either continuously for ≥ 6 months or intermittently over a lifetime such that the total exposure would add up to approximately six months time. At this time, carcinogenicity assessment is not required for the drug product. There is one report in the literature by Poulet *et al.* describing a 24-month carcinogenicity study which shows development of hibernomas in rats dosed with phentolamine mesylate (Poulet, et al., 2004). Due to the acute indication sought in this NDA, the results from the Poulet *et al.* study will not be considered clinically relevant.

Reproductive toxicology: The sponsor has submitted a male fertility study with phentolamine mesylate. At concentrations up to 143 times human therapeutic exposures level, phentolamine mesylate was shown to have no adverse effects on male fertility in the rat.

2.6.6.2 Single-dose toxicity

Study title: Local tolerance at the intraoral injection site and systemic toxicity study of phentolamine mesylate, ——— in the dog

Key study findings: It is concluded that phentolamine mesylate, ——— ——— at doses up to ten times the intended human dose, did not cause considerably greater levels of toxicity than the vehicle injection in this study. b(4)

Study no.: 044-001
Volume #, and page #: eCTD 0000 M4.2.3.1 Study-044-001
Conducting laboratory and location: ———
Date of study initiation: May 3, 2006
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:

compound	*lot #*	*purity, method*
phentolamine mesylate	M51228	99.9%, *
———		

b(4)

* method not stated

Methods

Doses:

drug	*µg/kg*	*human dose (dog equivalent)*
phentolamine mesylate	12	1x
	120	10x
[redacted]	0.27	2.7%
	2.7	27%
[redacted]	0.2	2%
	2.0	2 %

b(4)

Formulation of Vehicle

Ingredient	Quantity (per mL)
EDTA Na_2, USP	[redacted]
D-Mannitol, USP	
Sodium acetate trihydrate, USP	
Acetic acid, USP	[redacted]
Sodium hydroxide, NF	
[redacted]	

b(4)

NF = National Formulary; q.s. = quantity sufficient; USP = United States Pharmacopeia

Species/strain: beagle dog

Number/sex/group or time point (main study): 7 M/group (see table below for study design)

Route, formulation, volume, and infusion rate: single intraoral injection in one maxillary quadrant and one mandibular quadrant on the right side of the mouth in a volume of 51 µL/kg at a rate of 0.6 mL per 20 seconds.

Satellite groups used for toxicokinetics or recovery: none

Age: 16-18 months

Weight: 13.9-22.4 kg

Sampling times: necropsy on day 2, 4 or 15

Unique study design or methodology (if any): none

Prior to administration of the test article each dog received a s.c. injection of atropine sulfate (0.04 mg/kg) and approximately 15 minutes later an i.m. injection of ketamine HCl (13.6 mg/kg) and xylazine (1.36 mg/kg) for sedation.

Study Number: 044-001

Table 2: Study Design, Dose Levels, Dosing Solution Concentrations and Dosing Volumes

Group	Total n	Treatment	Dose (µg/kg)	Dosing Solution Concentration (µg/mL)	Free Base Dose (µg/kg)	Free Base Dosing Solution Concentration (µg/mL)	Percent of Phentolamine Free Base	Volume of Dosing Solution per kg of Body Weight (µL)	Day of Necropsy		
									2	4	15
1	7	Control (Vehicle)	0	0	0	0	na	51	3	2	2
2	7	Phentolamine mesylate	12	235	8.9	175	na	51	3	2	2
3	7	Phentolamine mesylate	120	2350	89.5	1752	na	51	3	2	2
4	7	Phentolamine mesylate	120	2350	89.5	1752	na	51	3	2	2
5	7	Phentolamine mesylate	0	0	0.0	0	na	51	3	2	2

b(4)

	treatment (fold human dose)			
group number	***phentolamine mesylate***			***description***
1	-	-	-	vehicle
2	1X	1X	1X	clinical formulation*
3	10X	10X	10X	10X clinical formulation
4	10X	-	-	10X active ingredient only
5	-	10X	10X	10X impurities only

b(4)

*note that the clinical formulation contains
respectively, which slightly exceed the specifications of th mpurities.

Observations and times:

Results

Mortality: Morbidity and mortality observations were done twice daily throughout the experiment. No abnormal observations were noted.

Clinical signs: The injection sites and oral cavity were visually inspected once pretreatment and at 10 min, 1, 3, and 24 hr post dosing and on days 3, 8, and 15 in all surviving dogs. The injection sites were evaluated for soft tissue appearance, color and

presence of bleeding. One dog in Group 3 and one dog in Group 5 had hematomas noted at 10 min, 1 hr and 3 hr time points only. One dog in Group 3 showed erythroplakia at 24 hr time point only. Several of the dogs in all groups, including vehicle, displayed pigmented (melanin present) mucosa predose and at early time points (up to day 4) which was not noted at later time points. Hyperemia was observed at 10 min and 1 hr in all groups, including vehicle, and in one dog at 3 hr in Group 5. Minimal bleeding in one or two dogs was seen at 10 min in Groups 3, 4 and 5. One incidence of moderate bleeding was seen in Group 2 at the 10 min time point only. None of these local oral cavity observations were considered by the sponsor to be related to the test article.

The clinical signs observed in this study were emesis, decreased feces and diarrhea. Emesis was observed across all groups, including vehicle. With the exception of two dogs in Group 4, emesis was observed in all dogs on days 1 and 2 and was attributed by the sponsor to the anesthesia. All groups, including control, showed a decrease in feces on days 1 and 2 which is most likely related to fasting prior to dosing. Diarrhea was observed in one dog in a test article group prior to dosing. None of the clinical signs observed were considered by the sponsor to be related to the test article.

Body weights: Body weights were recorded on all dogs at randomization, dosing and in surviving dogs on days 2, 4, 8, and 15. The test article did not have a marked effect on body weight in this study.

Food consumption: Food consumption was determined daily throughout the treatment period. Food was withheld at least 12 hours prior to dosing, 6 hours prior to clinical pathology evaluations, during urine collection and at least 6 hours prior to necropsy. There were no effects of the test article on food consumption.

Ophthalmoscopy: Ophthalmology examinations were performed predose and prior to necropsy. The eyes were dilated with 1% tropicamide ophthalmic solution and examined with an indirect ophthalmoscope. No test article related changes were observed.

EKG: EKG was not evaluated.

Hematology: Blood samples for hematology were collected predose and from surviving dogs on day 2, 3, and 15. The following hematology parameters were measured:

Erythrocyte Count	Differential WBC Count:
Reticulocyte Count	lymphocyte
Hemoglobin	neutrophil
Hematocrit	eosinophil
Mean Cell Volume	monocyte
Mean Cell Hemoglobin	basophil
Mean Cell Hemoglobin Concentration	Coagulation factors:
Leukocyte Count	Activated Partial Thromboplastin Time
Platelet Count	Prothrombin Time

There were no test article-related changes in hematological parameters.

Clinical chemistry: Blood samples for clinical chemistry were collected predose and from surviving dogs on day 2, 3, and 15. The following clinical chemistry parameters were measured:

Urea Nitrogen	Albumin
Glucose	Globulin
Aspartate Aminotransferase	Albumin/Globulin Ratio
Alanine Aminotransferase	Cholesterol
Alkaline Phosphatase	Creatinine
Lactate Dehydrogenase	Calcium
Sodium	Phosphate
Potassium	Total Bilirubin
Chloride	Triglycerides
Total Serum Protein	Gamma Glutamyl Transferase

No test article-related changes in clinical chemistry parameters were observed.

Urinalysis: Urine Samples were collected 0-6 and 18-24 hour post dosing. The following parameters were measured:

Specific Gravity	Urobilinogen
Volume	Bilirubin
pH	Blood
Protein	Gross Appearance
Glucose	Nitrite
Ketones	Leukocytes

No test-article changes in urinalysis were observed.

Gross pathology: All dogs were subjected to a full necropsy following treatment. Dogs were sedated with 16 mg/kg sodium thiopental IV, given a bolus of sodium heparin (200 IU/kg) and maintained on a halothane/O_2 mixture. The dogs were perfused via the left cardiac ventricle. Petechia and/or ecchymosis were observed at the mandibular or maxillary injection sites in all groups. The findings of petechia and ecchymosis are considered by the sponsor not to be directly related to the test article because they were observed in all groups at one or more of the necropsy intervals and most likely related to the injection itself.

Organ weights: Organ weights were obtained at necropsy and absolute and relative (to body weight) values were recorded. Paired organs were weighed together. There were no test article-related changes in absolute or relative organ weights.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

The sponsor evaluated all tissues in the vehicle group (Group 1) and high dose group (Group 3) with the exception of the inferior alveolar nerve (mandibular) and alveolar branches of the superior alveolar nerve (maxillary) in which all groups were evaluated.

Minimal to mild inflammation was seen in the injection site (lip) of all groups (1/1 for each group) except Group 2 where muscle degeneration and fibrosis occurred (2/2). Minimal to moderate hemorrhaging in the mediastinal lymph nodes was seen in both vehicle (5/6) and Group 3 (5/7). Moderate, diffuse inflammation in the mediastinal lymph nodes was seen in the vehicle group in one dog. Minimal multifocal hemorrhage was observed in Group 3 in the mesenteric (2/7) and submandibular (3/7) lymph nodes and not in vehicle dogs. Minimal focal inflammation was observed in the submaxillary salivary gland in one dog each in the vehicle (1/7) and Group 3 (1/7). Minimal to mild multifocal inflammation was seen in Group 3 (4/7) in the submaxillary salivary gland. Several vehicle (3/7) and Group 3 (4/7) dogs showed minimal inflammation and degeneration in the trigeminal ganglia. In Group 3, minimal axonopathy (1/7) and mineralization (1/7) was also observed. The table below is excerpted from the pathology report and details the histologic comparisons between all groups for the lip (injection site) and between the vehicle and Group 3 for the mediastinal, mesenteric and submandibular lymph nodes, the submaxillary salivary gland and the trigeminal ganglia.

b(4)

Pathology - Intergroup Comparison of Histologic Pathology Observations

96-88-018 - Local Tolerance at the Intraoral Injection Site and Systemic Toxicity Study of Phentolamine Mesylate in the Dog

Observations: Neo-Plastic and Non Neo-Plastic
Removal Reasons: All of those SELECTED

	MALES				
	Group 1 Control	Group 2 Trtmt 2	Group 3 Trtmt 3	Group 4 Trtmt 4	Group 5 Trtmt 5
Number of Animals on Study :	7	7	7	7	7
Number of Animals Completed:	(7)	(7)	(7)	(7)	(7)
LIP(INJECTION SITE):					
Examined	(1)	(2)	(1)	(1)	(1)
Within Normal Limits	0	0	0	0	0
Inflammation; Lymphohistiocytic; Focal	(1)	(0)	(0)	(1)	(0)
Minimal	1	0	0	1	0
Inflammation; Lymphohistiocytic; Multifocal	(0)	(0)	(1)	(0)	(1)
Minimal	0	0	1	0	0
Mild	0	0	0	0	1
Degeneration; Muscle; Multifocal	(0)	(2)	(0)	(0)	(0)
Mild	0	2	0	0	0
Fibrosis; Muscle; Multifocal	(0)	(2)	(0)	(0)	(0)
Mild	0	2	0	0	0
LYMPH NODE, MEDIASTINAL:					
Examined	(6)	(0)	(7)	(0)	(0)
Within Normal Limits	1	0	2	0	0
Not Examined: NOT PRESENT	1	0	0	0	0
Hemorrhage; Diffuse	(2)	(0)	(2)	(0)	(0)
Minimal	0	0	1	0	0
Mild	2	0	1	0	0
Hemorrhage; Multifocal	(5)	(0)	(5)	(0)	(0)
Minimal	1	0	2	0	0
Mild	1	0	1	0	0
Moderate	1	0	0	0	0
Inflammation; Suppurative; Diffuse	(1)	(0)	(0)	(0)	(0)
Moderate	1	0	0	0	0
LYMPH NODE, MESENTERIC:					
Examined	(7)	(0)	(7)	(0)	(0)
Within Normal Limits	7	0	5	0	0
Hemorrhage; Multifocal	(0)	(0)	(2)	(0)	(0)
Minimal	0	0	2	0	0
LYMPH NODE, SUBMANDIBULAR:					
Examined	(7)	(0)	(7)	(0)	(0)
Within Normal Limits	7	0	4	0	0
Hemorrhage; Multifocal	(0)	(0)	(3)	(0)	(0)
Minimal	0	0	3	0	0

SUBMAXILLARY SALIVARY GLAND:					
Examined	(7)	(0)	(7)	(0)	(0)
Within Normal Limits	6	0	3	0	0
Inflammation; Lymphohistiocytic; Focal	(1)	(0)	(1)	(0)	(0)
Minimal	1	0	1	0	0
Inflammation; Lymphohistiocytic; Multifocal	(0)	(0)	(3)	(0)	(0)
Minimal	0	0	2	0	0
Mild	0	0	1	0	0
TRIGEMINAL GANGLIA:					
Examined	(7)	(0)	(7)	(0)	(0)
Within Normal Limits	4	0	3	0	0
Inflammation; Lymphohistiocytic; Focal	(1)	(0)	(1)	(0)	(0)
Minimal	1	0	1	0	0
Inflammation; Lymphohistiocytic; Multifocal	(1)	(0)	(2)	(0)	(0)
Minimal	1	0	2	0	0
Degeneration; Neuron; Focal	(1)	(0)	(0)	(0)	(0)
Minimal	1	0	0	0	0
Axonopathy; Focal	(0)	(0)	(1)	(0)	(0)
Minimal	0	0	1	0	0
Mineralization; Focal	(0)	(0)	(1)	(0)	(0)
Minimal	0	0	1	0	0

In addition to the standard battery of tissues, the inferior alveolar nerve (mandibular) and alveolar branches of the superior alveolar nerve (maxillary) were evaluated for all groups. Nerve fiber degeneration was observed in the superior alveolar nerve of one Group 2 dog (1/7) sacrificed 24 hours after test article administration. The nerve fiber changes were characterized by axonal degeneration and fragmentation with myelin swelling. No other degenerative or inflammatory changes in tissue surrounding the affected nerve were observed. The inferior alveolar nerve was found to be within normal limits in this dog. Mild and moderate inflammation of the inferior alveolar nerve were observed in all groups including vehicle. Minimal degeneration was observed in one vehicle dog (1/7) in the superior alveolar nerve at 72 hours. The sponsor states that the cause of the moderate degeneration of the superior alveolar nerve in Group 2 is most likely not due to the test article because similar damage was not seen at the higher dose condition and degeneration of a lesser degree was seen in one vehicle dog. The sponsor also asserts that the pathology is most likely due to needle placement. The table below is reproduced from the pathology report and details the histologic comparisons of all groups for the inferior and superior alveolar nerves.

Pathology - Intergroup Comparison of Histologic Pathology Observations

06-RG-013 - Local Tolerance at the Intraoral Injection Site and Systemic Toxicity Study of Phentolamine Mesylate, b(4) in the Dog

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reasons: All of those SELECTED	Group 1 Control	Group 2 Trtmt 2	Group 3 Trtmt 3	Group 4 Trtmt 4	Group 5 Trtmt 5
Number of Animals on Study :	7	7	7	7	7
Number of Animals Completed:	(7)	(7)	(7)	(7)	(7)
MANDIBULAR (INFERIOR ALVEOLAR NERVE) INJECTION SITE:					
Examined	(7)	(7)	(7)	(7)	(7)
Within Normal Limits	6	5	7	6	6
Inflammation; Lymphohistiocytic; Focal	(0)	(0)	(0)	(0)	(1)
Minimal	0	0	0	0	1
Inflammation; Lymphohistiocytic; Perineural tissue; Focal	(1)	(2)	(0)	(1)	(0)
Minimal	1	2	0	0	0
Mild	0	0	0	1	0
MAXILLARY (SUPERIOR ALVEOLAR NERVE BRANCHES)/INJECTION SITE:					
Examined	(7)	(7)	(7)	(7)	(7)
Within Normal Limits	6	6	7	7	7
Degeneration; Nerve fiber; Focal	(1)	(0)	(0)	(0)	(0)
Minimal	1	0	0	0	0
Degeneration; Nerve fiber; Multifocal	(0)	(1)	(0)	(0)	(0)
Moderate	0	1	0	0	0

Toxicokinetics: Toxicokinetics were not assessed in this study.

Conclusions

No test article-related changes were observed in any of the parameters examined with the exception of the histopathology of local tissues. Minimal to mild inflammation was seen at the injection site of all groups. The 1X clinical formulation group (Group 2) showed muscle degeneration and fibrosis which was not seen at the higher doses. Minimal to moderate hemorrhaging in lymph nodes and minimal inflammation in lymph nodes and salivary glands was observed in both vehicle and treated animals. Several vehicle and 10X (Group 3) dogs showed minimal inflammation and degeneration in the trigeminal ganglia. Nerve fiber degeneration was observed in the superior alveolar nerve of one 1X clinical formulation dog but nerve fiber degeneration was not observed at higher doses.

In the absence of intact (un-injected) control dogs, it is not possible to determine whether the pathologies observed in nerves, muscle and surrounding tissues seen in the vehicle group are due to pre-existing lesions or to needle placement. This drug product will be administered via a commonly used dental needle. Any potential pathology resulting from needle placement would be no greater than an injection of the dental anesthetic and is therefore of no toxicologic concern. The sponsor concludes that phentolamine mesylate, _____ , at doses up to ten times the intended human dose, did not cause considerably greater levels of toxicity than the vehicle injection in this study. This reviewer concurs. b(4)

Histopathology inventory (optional)

Study	044-001
Species	dog
Adrenals	X*
Aorta	X
Bone Marrow (rib) smear	X*
Bone (femur) with joint	X*
Brain	X*
Cecum	X
Cervix	
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gingival mucosal tissue	X
Gross lesions	
Harderian gland	
Heart	X*
Ileum	X
Inferior alveolar nerve	X
Injection site	X
Jejunum	X

Kidneys	X*
Lachrymal gland	
Larynx	
Liver	X*
Lungs	X
Lymph nodes, mediastinal	X
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	
Nasal cavity	
Optic nerves	X
Ovaries	
Pancreas	X
Parathyroid	X*
Peripheral nerve	X
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	
Skeletal muscle	X
Skin	
Spinal cord	X
Spleen	X*
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X*
Tongue	X
Trachea	X
Trigeminal ganglia	X
Ureter	X
Urethra	X
Urinary bladder	X
Uterus	
Vagina	
Zymbal gland	

X, histopathology performed
*, organ weight obtained

2.6.6.3 Repeat-dose toxicity

No repeat-dose toxicity studies were submitted with the NDA. They were not required for this indication.

2.6.6.4 Genetic toxicology

b(4)

However, this reviewer has determined that the maximal concentration of phentolamine mesylate utilized in this assay is inadequate by current standards and therefore the study is deemed incomplete. The study will not be formally reviewed for the current NDA. The sponsor has submitted a second Ames Test (Study # ————) which will be reviewed. b(4)

Key findings: The study is invalid.

Study title: Bacterial Reverse Mutation Assay with Phentolamine Mesylate (Ames Test)

Key findings: Under the conditions of this study, phentolamine mesylate was not mutagenic in the Bacterial Reverse Mutation Assay.

Study no.: ————

Volume #, and page #: eCTD 000 M4.2.3.3.1
Conducting laboratory and location: ———— b(4)
Date of study initiation: January 5, 2007
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: phentolamine mesylate; lot # M51228; 99.9% per COA

Methods
Six to eight doses of test article as well as water vehicle and positive controls were plated in triplicate with overnight cultures of TA98, TA100, TA1535, TA1537 (Ames, et al., 1975) and WP2 *uvr*A (Green and Muriel, 1976) on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9 using the plate incorporation method (Maron and Ames, 1983). Positive controls were appropriate for each tester strain and metabolic activation condition. Five-hundred µL of S9 or sham mix, 100 µL of tester strain and 100 µL of vehicle, test article dilution or positive control were added to 0.8% melted selective top agar, vortexed and overlaid onto the surface of 25 mL Vogel-Bonner minimal medium E bottom agar (Vogel and Bonner, 1956). After solidification of the overlay, plates were inverted and incubated for approximately 48-72 hours at 37 ± 2°C. Plates that could not be counted immediately after incubation were stored at 2-8°C until colony counting could be conducted. The condition of the bacterial background lawn was evaluated for evidence of toxicity using a dissecting microscope. Precipitate was evaluated by the naked eye.

Revertant colonies for a given tester strain and activation condition, with the exception of positive controls, were counted either entirely by automated colony counter (Minicount Colony Counter, Imaging Products International) or entirely by hand.

In order to be considered a valid assay all *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion of the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion of the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls (TA98: 10-50; TA100: 80-240; TA1535: 5-45; TA1537: 3-21; WP2 *uvr*A: 10-60). Test strain culture titers must be $\geq 0.3 \times 10^9$ cells/mL in order to ensure that appropriate numbers of bacteria are plated on each plate. The mean of each positive control must exhibit at least a 3-fold increase in the number of revertants as compared to vehicle. A minimum of three non-toxic dose levels is also required to evaluate the assay data. Toxicity is described as a $\geq$ 50% reduction in the mean number of revertants per plate as compared to vehicle accompanied by an abrupt dose-dependent drop in the revertant count and/or a moderate reduction in the background lawn. A positive response would be indicated by a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Mean number of revertants per plate for TA1535 and TA1537 must be $\geq$ 3-fold the mean of the vehicle and TA98, TA100 and WP2 *uvr*A must be $\geq$ 2-fold the mean of the vehicle to be considered a positive response.

Strains/species/cell line: *Salmonella typhimurium* histidine auxotrophs utilized included: TA98, TA100, TA1535 and TA1537. *Escherichia coli* tryptophan auxotroph utilized: WP2 *uvr*A.

Doses used in definitive study: The doses used were: vehicle (water), 15, 50, 150, 500, 1500 and 5000 µg/plate in the presence and absence of metabolic activation by S9.

Basis of dose selection: The initial toxicity-mutation assay tested 1.5, 5.0, 15, 50, 150, 1500, 5000 µg/plate of the test article in water vehicle ± SP in the TA100 and WP2 *uvr*A strains and did not demonstrate precipitation or positive mutagenic response. Toxicity, as evidenced by bacterial lawn clearing, was observed at 5000 µg/plate with most test conditions. Based on the initial toxicity assay, the maximum dose utilized in the definitive study was 5000 µg/plate.

Negative controls: The vehicle, water, was used as the negative control.

Positive controls: The positive controls utilized for the respective strains are indicated in the sponsor's table (Table 1) below.

Table 1

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	Rat	2-aminoanthracene (b(4)) Lot No. 15216JA Exp. Date 10-Mar-2007 CAS No. 613-13-8 Purity 97.3%	1.0
WP2 *uvrA*			10
TA98	None	2-nitrofluorene Lot No. 03926DC Exp. Date 18-Aug-2010 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		sodium azide Lot No. 07383JH Exp. Date 31-Jul-2008 CAS No. 26628-22-8 Purity 99.9%	1.0
TA1537		9-aminoacridine Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. 90-45-9 Purity >97%	75
WP2 *uvrA*		methyl methanesulfonate Lot No. 05713JD Exp. Date 06-Nov-2008 CAS No. 66-27-3 Purity 99.9%	1,000

Incubation and sampling times: Plates were incubated 48 to 72 hours at 37±2°C.

Results

Study validity: The study is valid. The definitive and confirmatory studies utilized suitable numbers of replicate plates and appropriate counting methods. The positive controls demonstrated clear increases in tester strain revertants while the negative control (vehicle) was within historical range for the tester strains with this vehicle.

Study outcome: Both the definitive and confirmatory studies with phentolamine mesylate were negative. The sponsor summarized the results of the confirmative assay in Table 2 below. The data in the table indicate negative mutagenic responses in the presence and absence of exogenous metabolic activation with S9. No precipitate or toxicity was observed at concentrations up to 5000 µg per plate.

Table 2

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay

Test Article Id : Phentolamine mesylate
Study Number : — Experiment No : B2 b(4)

Average Revertants Per Plate ± Standard Deviation
Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	18 ± 3	161 ± 16	22 ± 2	6 ± 2	25 ± 6
50	14 ± 2	192 ± 14	21 ± 3	7 ± 2	27 ± 5
150	19 ± 11	130 ± 3	20 ± 2	4 ± 1	26 ± 6
500	21 ± 6	117 ± 6	18 ± 5	6 ± 1	30 ± 3
1500	23 ± 3	138 ± 14	27 ± 6	8 ± 2	24 ± 4
5000	14 ± 7	102 ± 15	19 ± 8	11 ± 1	17 ± 5
Positive	133 ± 29	515 ± 23	358 ± 41	232 ± 118	164 ± 21

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	30 ± 3	172 ± 22	19 ± 7	8 ± 2	34 ± 7
50	27 ± 9	159 ± 13	18 ± 4	5 ± 3	33 ± 1
150	36 ± 9	183 ± 46	20 ± 7	7 ± 3	32 ± 4
500	34 ± 5	164 ± 23	14 ± 4	9 ± 5	30 ± 8
1500	40 ± 6	164 ± 29	16 ± 2	11 ± 4	31 ± 3
5000	35 ± 9	163 ± 1	9 ± 3	8 ± 4	17 ± 3
Positive	181 ± 87	592 ± 77	100 ± 22	41 ± 4	326 ± 230

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

Study title: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells with Phentolamine Mesylate (Study # T8199.337)

b(4)

Note: Study # T8199.337 was previously reviewed by Jeri El-Hage, Ph.D. for — (submission date —) in the Division of Reproductive and Urologic Products. Dr. El-Hage found this study to be valid and concluded that phentolamine mesylate (maximal concentration: 200 µg/mL 2 hr –S9; 600 µg/mL 6 hr +S9) yielded a negative result in the Chromosome Aberrations Assay in CHO cells. The study will not be re-reviewed for the current NDA. The sponsor submitted a second Chromosome Aberrations Assay in CHO cells (Study —, which will be formally reviewed.

Key findings: Phentolamine mesylate was not clastogenic in the Chromosome Aberrations Assay in CHO cells.

Study title: *In vitro* Mammalian Chromosome Aberration Test with Phentolamine Mesylate

Key findings: Under the conditions of this study, phentolamine mesylate was concluded to be negative for the induction of structural and numerical aberrations in CHO cells in the S9-activated system. In the non-activated system, phentolamine mesylate was concluded to be negative for the induction of structural aberrations and equivocal for the induction of numerical aberrations in CHO cells. The sponsor has submitted two *in vivo* mammalian micronucleus tests (Studies # 96067 and [redacted] in the current NDA in order to more fully evaluate the clastogenic potential of phentolamine mesylate. b(4)

Study no.:

Volume #, and page #: eCTD 000 M4.2.3.3.1
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: January 8, 2007
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: phentolamine mesylate; lot # M51228; 99.9% per COA

Methods
Strains/species/cell line: Chinese hamster ovary (CHO) cells (Preston, et al., 1981).

Doses used in definitive study:
-S9 4-hr/16 hr: water vehicle; phentolamine mesylate 12.5, 25, 50, 100, 150, 200, 250, 300 µg/mL; or positive control Mitomycin C 0.2 µg/mL
+S9 4-hr/16 hr: water vehicle; phentolamine mesylate 12.5, 25, 50, 100, 150, 200, 250, 300 µg/mL; or positive control Cyclophosphamide 10 µg/mL
-S9 20-hr: water vehicle; phentolamine mesylate 1.25, 2.5, 5, 10, 15, 20, 25, 35 µg/mL; or positive control Mitomycin C 0.1 µg/mL

Basis of dose selection: Dose levels for the chromosome aberration assay were selected based on the results from a preliminary toxicity assay which examined a reduction of cell growth with exposure to the test article relative to vehicle. CHO cells were exposed to vehicle or the test article (0.377-3770 µg/mL) in the presence or absence of S9-induced metabolic activation for 4 hours with a 16 hour recovery time before harvesting and counting. Cells were also exposed to vehicle or the same range of doses of test article continuously for 20 hours in the absence of S9. The osmolality and pH of the highest concentration of test article were equivalent to vehicle. Substantial toxicity, as defined as at least 50% cell growth inhibition, was observed at dose levels $\geq$ 377 µg/mL in both the –S9 and +S9 4-hour exposure groups and at dose levels $\geq$ 11.31 µg/mL in the non-activated 20-hour continuous exposure group.

Negative controls: The vehicle, water, was used as the negative control.

Positive controls: Mitomycin C (MMC) was used as the positive control at 0.2 μg/mL for the 4-hour and at 0.1 μg/mL for the 20-hour non-activated groups. Cyclophosphamide (CP) was used as the positive control at 10 μg/mL and 20 μg/mL for the 4-hour S9-activated group.

Incubation and sampling times: CHO cells were seeded at ~5 x 10^5 cells per flask incubated at 37 ± 1°C in a humidified 5 ± 1% CO_2 in air for 16-24 hours. Duplicate cultures of CHO cells were exposed to vehicle or test article in the presence or absence of S9 metabolic activation for 4 hours with a 16-hour recovery time or in the absence of S9 activation for 20 hours continuous exposure before harvesting (Evans, 1976;Swierenga, et al., 1991). Two hours prior to harvesting, the metaphase arrestor Colcemid® was added to all conditions at a final concentration of 0.1 μg/mL and the cells were returned to the incubator. At the completion of the incubation time cells were harvested and an aliquot was removed from each culture for a concurrent toxicity test. The presence of test article precipitate was determined using the unaided eye and the cells were counted using a Coulter counter and viability was assessed by trypan blue exclusion. The remainder of the cells was centrifuged and the pellet was resuspended in 2-4 mL 0.075 M KCl and allowed to stand at room temperature for 4-8 minutes. Cells were collected by centrifugation, supernatant was aspirated and the cells were fixed with two washes with ~2 mL Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v) and stored overnight at 2-8°C. To prepare the slides, an aliquot of cells was dropped onto a slide and allowed to air dry. The slides were then stained with 5% Giemsa, air dried and permanently mounted.

The selection of dose levels for analysis of chromosome aberrations was based on the results of the concurrent toxicity test. The highest dose level selected for evaluation was the dose which induced ≥ 50% toxicity, as measured by cell growth inhibition, and included a sufficient number of scorable metaphase cells. Two additional lower doses were included in the analysis. The mitotic index was determined for each treatment group (percentage cells in mitosis per 500 cells scored). Metaphase cells with 20 ± 2 centromeres were examined under oil immersion. A minimum of 200 metaphase spreads were examined and scored for chromatid-type (including chromatid and isochromatid breaks and exchange figures such as quadriradials, triradials and complex rearrangements) and chromosome-type (chromosome breaks and exchange figures such as dicentrics and rings) aberrations. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome) and were considered part of an incomplete exchange if they were observed with an exchange figure. Severely damaged cells (≥ 10 aberrations) and pulverized chromosomes or cells were also recorded. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test and the Cochran-Armitage test was used to measure dose-responsiveness.

To be considered valid, the frequency of cells with structural chromosomal aberrations in the vehicle group must be within the range of the historical vehicle control. The percentage of cells with chromosome aberrations in the positive control also must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to vehicle. The test article would be considered to have a positive response when the percentage of cells with aberrations is

increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). A reproducible and significant increase at the high concentration only with no dose-response or a reproducible significant increase at one dose level other than the high dose with no dose-response would be considered positive. Test articles not demonstrating a statistically significant increase in aberrations would be considered negative.

Results

Study validity: This study is valid. It utilizes appropriate replicates and cell counting/viability methodology. The vehicles and positive controls for the activated and non-activated groups with 4 hr incubation are within the range of the historical data set and the positive controls are significantly higher than vehicle controls. No historical data is provided by the sponsor for the 20 hr incubation group. However, for these data the vehicle and all doses of the test article are within the range of the 4 hr incubation historical dataset. The positive control is significantly higher than the vehicle control.

Study outcome: The highest concentration used in each condition reached a level which produced adequate evidence of cytotoxicity characterized by cell counts and trypan blue exclusion. The highest concentrations evaluated were 150 µg/mL for 4 hr –S9, 150 µg/mL for 4 hr +S9 and 10 µg/mL 20 hr –S9 and produced adequate levels of toxicity: 54%, 55% and 54%, respectively (Table 1). The dose levels in the 4-hr non-activated condition selected for microscopic analysis were 25, 50 and 150 µg/mL. The percentage of cells that were positive for structural aberrations was not significantly increased above the vehicle at all concentrations (Tables 2 and 3). The 150 µg/mL concentration was significantly increased (8.5%) above vehicle (3.5%) for percentage of cells with numerical aberrations ($p \leq 0.05$, Fisher's Exact test; Tables 2 and 3). The two lower concentrations were not significantly increased above vehicle. The Cochran-Armitage test was negative for a dose response. The historical control values for the non-activated system for percent of combined numerical aberrations above vehicle show a mean of $1.5 \pm 1.4\%$ and a range of 0-6.5% (Table 6). Because of the lack of dose-dependence, the increase in numerical aberrations at the 150 µg/mL dose of phentolamine mesylate in the absence of metabolic activation is concluded to be equivocal.

There were no significant increases for either numerical or structural aberrations in the 20 hour non-activated system (Tables 2 and 5).

The dose levels in the 4 hr activated condition selected for microscopic analysis were 25, 50 and 150 µg/mL. The percentage of cells that were positive for numerical aberrations was not significantly increased above the vehicle at all concentrations. The 150 µg/mL concentration was significantly increased (4.5%) above vehicle (0%) for percentage of cells with structural aberrations ($p \leq 0.01$, Fisher's Exact test; Tables 2 and 4). The Cochran-Armitage test was also positive for a dose response ($p \leq 0.05$). The two lower concentrations were not significantly increased above vehicle. The historical control values for the activated system for percent of structural aberrations above vehicle show a mean of $0.7 \pm 1.0\%$ and a range of 0-5.0% (Table 7). The significant increase of 4.5% for the 150 µg/mL dose is concluded to not be biologically relevant because it falls within the historical

control range. Therefore, phentolamine mesylate in the presence of metabolic activation is concluded to be negative for clastogenicity. The tables provided by the sponsor are reproduced below.

Table 1

Treatment Time	Recovery Time	Harvest Time	S9	Toxicity* at highest dose scored (µg/mL)	Mitotic Index Reduction **	LED[1] for Structural Aberrations µg/mL	LED[1] for Numerical Aberrations µg/mL
4 hr	16 hr	20 hr	-	54% at 150	0%	None	†
20 hr	0 hr	20 hr	-	54% at 10	4%	None	None
4 hr	16 hr	20 hr	+	55% at 150	None	None	†

* cell growth inhibition

** relative to solvent control at high dose evaluated for chromosome aberrations

[1] LED = lowest effective dose

† Test article was considered to be equivocal for the induction of numerical aberrations in the non-activated system

Table 2

SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Water	-S9	4	8.0	200	200	0.000	±0.000	3.5	0.0
Phentolamine mesylate									
25	-S9	4	8.2	200	200	0.000	±0.000	4.0	0.0
50	-S9	4	8.4	200	200	0.010	±0.100	5.0	1.0
150	-S9	4	8.0	200	200	0.015	±0.122	8.5*	1.5
MMC, 0.2	-S9	4	7.5	200	50	0.220	±0.465	3.0	20.0**
Water	+S9	4	8.5	200	200	0.000	±0.000	1.5	0.0
Phentolamine mesylate									
25	+S9	4	7.7	200	200	0.015	±0.122	1.5	1.5
50	+S9	4	7.5	200	200	0.010	±0.100	3.0	1.0
150	+S9	4	8.6	200	200	0.050	±0.240	2.5	4.5**
CP, 10	+S9	4	7.2	200	100	0.300	±0.628	2.0	22.0**
Water	-S9	20	8.2	200	200	0.000	±0.000	2.0	0.0
Phentolamine mesylate									
2.5	-S9	20	8.4	200	200	0.000	±0.000	2.0	0.0
5	-S9	20	7.3	200	200	0.000	±0.000	2.0	0.0
10	-S9	20	7.9	200	200	0.000	±0.000	3.5	0.0
MMC, 0.1	-S9	20	6.9	200	50	0.360	±0.631	1.0	28.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's Exact test.

Table 3

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Phentolamine mesylate
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell
							Gaps	Chromatid		Chromosome				
			Numerical	Structural	Numerical	Structural		Br	Ex	Br	Dic	Ring		
Water	A	8.2	100	100	4	0	0	0	0	0	0	0	0	0.000
	B	7.8	100	100	3	0	0	0	0	0	0	0	0	0.000
Phentolamine mesylate														
25	A	8.0	100	100	4	0	0	0	0	0	0	0	0	0.000
	B	8.4	100	100	4	0	0	0	0	0	0	0	0	0.000
50	A	8.8	100	100	5	1	0	1	0	0	0	0	0	0.010
	B	8.0	100	100	5	1	0	1	0	0	0	0	0	0.010
150	A	7.8	100	100	8	2	0	1	1	0	0	0	0	0.020
	B	8.2	100	100	9	1	0	1	0	0	0	0	0	0.010
MMC, 0.3	A	7.4	100	25	3	20	0	3	3	0	0	0	0	0.240
	B	7.6	100	25	3	20	0	1	4	0	0	0	0	0.200

Treatment: CHO cells were treated for 4 hours at 37±1°C in the absence of an exogenous source of metabolic activation. Additional dose levels of 12.5 and 100 µg/mL were tested as a safeguard against excessive toxicity at higher dose levels but were not required for microscopic examination. Dose levels 200, 250 and 300 µg/mL were not analyzed due to excessive toxicity (cell growth inhibition).

Mitotic index = number mitotic figures x 100/500 cells counted.

%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

Table 4

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Phentolamine mesylate
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell
							Gaps	Chromatid		Chromosome				
			Numerical	Structural	Numerical	Structural		Br	Ex	Br	Dic	Ring		
Water	A	8.2	100	100	1	0	0	0	0	0	0	0	0	0.000
	B	8.6	100	100	2	0	0	0	0	0	0	0	0	0.000
Phentolamine mesylate														
25	A	8.0	100	100	1	2	1	0	1	0	1	0	0	0.020
	B	7.4	100	100	2	1	0	0	0	1	0	0	0	0.010
50	A	7.8	100	100	3	2	3	1	0	1	0	0	0	0.030
	B	7.2	100	100	3	0	1	0	0	0	0	0	0	0.000
150	A	8.4	100	100	2	7	1	2	4	0	1	1	0	0.080
	B	8.8	100	100	3	3	1	2	0	0	0	0	0	0.030
CP, 10	A	6.8	100	50	3	16	3	5	3	2	0	0	0	0.200
	B	7.6	100	50	2	28	1	11	4	5	0	0	0	0.400

Treatment: CHO cells were treated for 4 hours at 37±1°C in the presence of an exogenous source of metabolic activation. Additional dose levels of 12.5 and 100 µg/mL were tested as a safeguard against excessive toxicity at higher dose levels but were not required for microscopic examination. Dose levels 200, 250 and 300 µg/mL were not analyzed due to excessive toxicity (cell growth inhibition).

Mitotic index = number mitotic figures x 100/500 cells counted.

%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

Table 5

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Phentolamine mesylate
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

20-HOUR CONTINUOUS TREATMENT

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell
							Gaps	Chromatid		Chromosome				
			Numerical	Structural	Numerical	Structural		Br	Ex	Br	Dic	Ring		
Water	A	7.8	100	100	2	0	0	0	0	0	0	0	0	0.000
	B	8.6	100	100	2	0	0	0	0	0	0	0	0	0.000
Phentolamine mesylate														
2.5	A	8.8	100	100	2	0	0	0	0	0	0	0	0	0.000
	B	8.0	100	100	2	0	0	0	0	0	0	0	0	0.000
5	A	7.2	100	100	2	0	0	0	0	0	0	0	0	0.000
	B	7.4	100	100	2	0	0	0	0	0	0	0	0	0.000
10	A	8.2	100	100	3	0	0	0	0	0	0	0	0	0.000
	B	7.6	100	100	4	0	0	0	0	0	0	0	0	0.000
MMC,	A	6.6	100	25	1	32	3	8	3	0	0	0	0	0.440
0.1	B	7.2	100	25	1	24	1	4	3	0	0	0	0	0.280

Treatment: CHO cells were treated for 20 hours at 37±1°C in the absence of an exogenous source of metabolic activation. An additional dose level of 1.25 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination. Dose levels 15, 20, 25 and 35 µg/mL were not analyzed due to excessive toxicity (cell growth inhibition).

Mitotic index = number mitotic figures x 100/500 cells counted.

%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

Table 6

IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES
COMBINED NUMERICAL ABERRATIONS
(POLYPLOID AND ENDOREDUPLICATED CELLS)
2003-2005

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[2] (%)
Mean	1.5	1.6
±SD[1]	1.4	1.4
Range	0.0-6.5	0.0-6.5

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	2.1	1.9
±SD[1]	1.8	1.7
Range	0.0-10.0	0.0-6.0

1 SD = standard deviation.
2 Positive control for non-activated studies, Mitomycin C (MMC, 0.1-0.2 µg/mL).
3 Positive control for S9-activated studies, cyclophosphamide (CP, 10-20 µg/mL).

Table 7

IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES
STRUCTURAL ABERRATIONS
2003-2005

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[2] (%)
Mean	0.6	19.6
±SD[1]	0.9	6.5
Range	0.0-5.5	8.0-56.0

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	0.7	20.9
±SD[1]	1.0	9.3
Range	0.0-5.0	8.0-84.3

1 SD = standard deviation.
2 Positive control for non-activated studies, Mitomycin C (MMC, 0.1-0.2 µg/mL).
3 Positive control for S9-activated studies, cyclophosphamide (CP, 10-20 µg/mL).

Study title: *In vivo* Mouse Bone Marrow Micronucleus Test with Phentolamine Mesylate (Study # 96067)

Note: Study # 96067 has been previously reviewed by Jeri El-Hage, Ph.D. in the Division of Reproductive and Urologic Products. In this study, Dr. El-Hage concluded that phentolamine mesylate (maximal dose: 62.5 mg/kg) yielded a negative result in the *in vivo* Micronucleus Assay. The study will not be re-reviewed for the current NDA. The sponsor has submitted a second *in vivo* Micronucleus Assay (Study # ———————— .), which utilizes a maximal concentration of 100 mg/kg phentolamine mesylate, which will be formally reviewed. b(4)

Key findings: Phentolamine mesylate at doses up to 62.5 mg/kg did not cause chromosomal damage in bone marrow cells and is therefore concluded to be negative in the *in vivo* Micronucleus Assay.

Study title: Mammalian Erythrocyte Micronucleus Test with Phentolamine Mesylate

Key findings: Under the conditions of this study, phentolamine mesylate did not cause chromosomal damage in bone marrow cells and is therefore concluded to be negative in the *in vivo* Micronucleus Assay.

Study no.: [redacted]

Volume #, and page #: eCTD 000 M4.2.3.3.1
Conducting laboratory and location: [redacted]
Date of study initiation: January 5, 2007
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: phentolamine mesylate; lot # M51228; 99.9% per COA

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Methods
Strains/species/cell line: 6-8 week old ICR mice; Male weight range: 25.5-37.0 g; Female weight range: 19.5-31.0 g; [redacted]

Doses used in definitive study: vehicle (0.9% saline), 25, 50, 100 mg/kg

Basis of dose selection: A toxicity study was conducted using a single i.p. injection of phentolamine mesylate at 50, 100, 200 or 300 mg/kg. Mortality was observed at doses of 200 (3/6) and 300 mg/kg (6/6). Lethargy and palpebral closure of both eyes was seen in all mice at 50, 100 and 200 mg/kg. Piloerection was observed at 100 mg/kg (6/6) and 200 mg/kg (3/6). No mortality or life threatening clinical signs at doses up to and including 100 mg/kg were observed, therefore 100 mg/kg was considered the maximum tolerated dose and was selected as the high dose in the definitive study.

Negative controls: The vehicle, 0.9% NaCl (saline), was used as the negative control.

Positive controls: Cyclophosphamide (CP), which induces micronucleus formation, was used at 50 mg/kg body weight. Saline was used to dissolve the CP.

Incubation and sampling times: A solubility test was performed to determine the solubility of the test article and it was determined by the sponsor to use saline as the vehicle and diluent. The highest test article dosing solution was prepared at 30 mg/mL. The test article, vehicle and positive control were administered intraperitoneally at 10 mL/kg body weight.

The definitive micronucleus study was conducted using established and validated procedures (Heddle, 1973;Hayashi, et al., 1994;Mavournin, et al., 1990). The mice were randomized and placed into seven treatment groups of five/sex. An additional group of five mice per sex were administered the high dose to be used as a replacement group in the event of mortality. The study design is outlined in Table 1 which was provided by the sponsor.

Table 1

Treatment (10 mL/kg)	Number of Mice/Sex Dosed	Number of Mice/Sex Used for Bone Marrow Collection at	
		24 hrs post-dose	48 hrs post-dose
Vehicle Control: 0.9% sodium chloride	10	5	5
Test Article: Phentolamine mesylate			
Low dose (25 mg/kg)	5	5	0
Mid dose (50 mg/kg)	5	5	0
High dose (100 mg/kg)	15*	5	5
Positive Control: CP (50 mg/kg)	5	5	0

*Including 5 replacement mice/sex to ensure the availability of five mice for micronucleus analysis.

The mice were euthanized by CO_2 asphyxiation approximately 24 or 48 hours after drug administration. Five male and five female mice were treated with the positive control and euthanized at the 24 hour time point. After each mouse was euthanized, the groin area was cleansed with 70% ethanol and the femurs were exposed, separated just above the kneecaps and the heads of the femur were removed with a scissors. The bone marrow was flushed from the femurs using a syringe into a culture tube containing fetal bovine serum. The tubes were centrifuged at approximately 100 x g for 5 minutes and the supernatant was aspirated leaving approximately 0.1 mL of serum. The cells were resuspended and a small drop was placed on a microscope slide and spread along the length of the slide. The slides were fixed with methanol and stained with May-Gruenwald-Giemsa stain, permanently mounted and scored in a blind fashion. The number of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) among 200 erythrocytes (PCE + NCE) per mouse were determined. The number of micronucleated polychromatic erythrocytes (MPCE) were then determined for 2000 PCE per mouse. The dose group means were calculated for the percentage of PCE as well as the frequency of MPCE. Data were analyzed separately for males and females. The incidence of MPCE per 2000 PCEs for each dose group was determined. Statistical significance was determined using the Kastenbaum-Bowman tables which are based on the binomial distribution (Kastenbaum and Bowman, 1970). A significant reduction in percent PCE ($\geq$ 20% vs. vehicle) was used as an indication of toxicity. The test article would be considered to show a positive response if a dose-responsive increase in the incidence of MPCEs was observed and one or more doses were statistically elevated relative to the concurrent vehicle control values ($p \leq 0.05$, Kastenbaum-Bowman Tables) at any sampling time. Values that are statistically significant but do not exceed the range of historical negative control values will be considered to be not biologically relevant. The test article will be judged as negative if there is no statistically significant increase in the incidence of MPCEs above the concurrent vehicle control values and there is no evidence of dose response.

Reviewer's comment:

The sponsor does not state how a positive dose-response would be determined, specifically which statistical test would be utilized.

Results

Study validity: This study is valid. The incidence of the MPCEs in the vehicle control group was within the historical control range and the positive control group was significantly increased relative to the vehicle.

Study outcome: Phentolamine mesylate did not cause *in vivo* chromosomal damage in the Micronucleus Assay and is therefore not considered to be a clastogenic agent. The results summary table from the sponsor is reproduced below (Table 2). The percentage of PCE and the MPCE frequency were determined from bone marrow preparations from mice euthanized approximately 24 and 48 hours after test article administration. There were no statistically significant increases in the number of MPCE in the treated groups at any dose level for either time point as compared to the vehicle groups.

Table 2

Summary of Bone Marrow Micronucleus Analysis
Following a Single Intraperitoneal Dose of Phentolamine mesylate in ICR Mice

Treatment (10 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
0.9% sodium chloride	M	24	5	0.481 ± 0.02	---	0.2 ± 0.27	2 / 10000
for injection, USP	F	24	5	0.547 ± 0.07	---	0.3 ± 0.45	3 / 10000
Phentolamine mesylate							
25 mg/kg	M	24	5	0.557 ± 0.06	16	0.1 ± 0.22	1 / 10000
	F	24	5	0.536 ± 0.04	-2	0.2 ± 0.27	2 / 10000
50 mg/kg	M	24	5	0.525 ± 0.06	9	0.1 ± 0.22	1 / 10000
	F	24	5	0.541 ± 0.07	-1	0.1 ± 0.22	1 / 10000
100 mg/kg	M	24	5	0.536 ± 0.03	11	0.0 ± 0.00	0 / 10000
	F	24	5	0.539 ± 0.06	-1	0.1 ± 0.22	1 / 10000
Cyclophosphamide							
50 mg/kg	M	24	5	0.394 ± 0.04	-18	13.1 ± 2.53	*131 / 10000
	F	24	5	0.383 ± 0.04	-30	17.1 ± 3.11	*171 / 10000
0.9% sodium chloride	M	48	5	0.609 ± 0.05	---	0.1 ± 0.22	1 / 10000
for injection, USP	F	48	5	0.592 ± 0.05	---	0.1 ± 0.22	1 / 10000
Phentolamine mesylate							
100 mg/kg	M	48	5	0.529 ± 0.08	-13	0.0 ± 0.00	0 / 10000
	F	48	5	0.587 ± 0.04	-1	0.2 ± 0.27	2 / 10000

[1]*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables).

2.6.6.5 Carcinogenicity

The sponsor did not conduct a carcinogenicity assessment for phentolamine mesylate. The ICH M3 Guideline indicates that carcinogenicity assessment would not be required for a drug that would not be used either continuously for ≥ 6 months or intermittently over a lifetime such that the total exposure would add up to approximately six months time. For this indication, carcinogenicity assessment is not required.

There is one report in the literature by Poulet *et al.* describing a 24-month carcinogenicity study which shows development of hibernomas in rats dosed with phentolamine mesylate. Phentolamine mesylate was administered daily to rats by oral gavage at doses of 10, 50, and 150 mg/kg for 24 months. A dose-related increase in mortality was observed at high doses which the authors attribute to exaggerated pharmacological effects. Clinical signs included rales and labored breathing as a result of the significant hemodynamic effects induced by phentolamine. A statistically significant increase in incidence of hibernomas, a rare neoplasm of brown adipose tissue, was reported at all dose groups in males and the low dose group in females. Hepatocellular neoplasms and pituitary gland adenomas were also observed in treated groups but were within historical control ranges. Because phentolamine is not genotoxic and induces profound hemodynamic alterations, the authors put forth the hypothesis that the mechanism of tumor formation results epigenetically from the chronic adaptive responses of the rat secondary to the pharmacodynamic effects of phentolamine mesylate at the doses tested (Poulet, et al., 2004). Due to the acute indication sought in this NDA, the results from the Poulet *et al.* study will not be considered clinically relevant and therefore do not need to be included in the product labeling.

2.6.6.6 Reproductive and developmental toxicology

The sponsor is relying upon the Agency's previous findings of safety for Regitine® to support this drug product. Segment II studies are described in the current Regitine® drug product labeling; however, the current label lacks information on fertility and prenatal and post natal development. Based on current policy established at the office level of OND, no additional reproductive and developmental toxicology studies are required to support this NDA application. Regardless, the sponsor has submitted a Segment I male fertility study (A Rat Male Fertility Study with Phentolamine Mesylate in the Rat by Oral Gavage Administration; #96-047). This study has been previously reviewed by Jeri El-Hage, Ph.D. in the Division of Reproductive and Urologic Drug Products under another NDA. Results will be summarized here and the full review will be included as Appendix 1.

This study was conducted according to GLP by [redacted] — Briefly, male Sprague Dawley rats were treated with 0, 10, 75 and 150 mg/kg/day phentolamine mesylate orally by gavage in distilled water for 9 weeks; 4 weeks prior to mating and 3 weeks during the mating period, and 2 weeks after mating prior to sacrifice. Males were mated with untreated females. Male mating and fertility indices (caudal epididymal sperm counts, sperm morphology and motility) were determined. Females were killed on Day 15 of gestation and the following parameters were evaluated: live and dead fetuses, early and late resorptions, implantation sites, and number of corpora lutea. b(4)

No adverse effects in male rats on mating, fertility indices, or sperm parameters were observed in this study. Similarly, no drug-related effects were observed on reproductive parameters in untreated females mated with the treated males. The NOEL for reproductive toxicity and fertility in males was 150 mg/kg/day. The 150 mg/kg/day dose produces drug exposures (C_{max}) approximately 143 times human therapeutic exposure levels.

Species	*dose mg/kg*	*day*	*AUC_{0-24} ng.ml/hr*	*C_{max} ng/ml*	*multiple of human C_{max}*
rat	150	1	1963	389	143
rat	150	13	4489	707	259
human	2 cartridges	1	3.29 ($AUC_{0-8.5}$)	2.73	-

Note: the drug exposure comparison between rat and human was made between C_{max} values only. Direct comparisons could not be made between AUC levels because of different sample durations.

2.6.6.7 Local tolerance
The local tissue response was assessed in the dog model, as described in the acute toxicology section of this review.

2.6.6.8 Special toxicology studies
To provide data to support the safety of impurities/degradation products that exceed the ICH Q3A(R2)/Q3B(R2) safety qualification thresholds, the sponsor conducted the following genetic toxicology studies.

Study title: Bacterial Reverse Mutation Assay (Ames Test) with ———— b(4)

Key findings: Under the conditions of this study, ———— was concluded to be negative in the Bacterial Reverse Mutation Assay.

Study no.: ————

Volume #, and page #: eCTD 000 M4.2.3.7.6.1
Conducting laboratory and location: ————
Date of study initiation: October 26, 2005
GLP compliance: Yes b(4)
QA reports: yes (X) no ( )
Drug, lot #, and % purity: ———— Lot # M41063, Purity 99.6% (by HPLC, per COA from ————

Methods
Six to eight doses of test article as well as DMSO vehicle and positive controls were plated in triplicate with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9 using

the plate incorporation method. Positive controls were appropriate for each tester strain and metabolic activation condition. Five hundred µL of S9 or sham mix, 100 µL of tester strain and 50 µL of vehicle, test article dilution or positive control were added to 2.0 mL of molten selective top agar, vortexed and overlaid onto the surface of 25 mL minimal bottom agar. After solidification of the overlay, plates were inverted and incubated for approximately 48-72 hours at 37 ± 2°C. Plates that could not be counted immediately after incubation were stored at 2-8°C until colony counting could be conducted. The condition of the bacterial background lawn was evaluated for evidence of toxicity using a dissecting microscope. Precipitate was evaluated by the naked eye. Revertant colonies for a given tester strain and activation condition, with the exception of positive controls, were counted either entirely by automated colony counter or entirely by hand unless plates exhibited signs of toxicity.

All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion of the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion of the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls (TA98: 10-50; TA100: 80-240; TA1535: 5-45; TA1537: 3-21; WP2 *uvr*A: 10-60). Test strain culture titers must be $\geq 0.3 \times 10^9$ cells/mL in order to ensure that appropriate numbers of bacteria are plated on each plate. The mean of each positive control must exhibit at least a 3-fold increase in the number of revertants as compared to vehicle. A minimum of three non-toxic dose levels is also required to evaluate the assay data. Toxicity is described as a ≥ 50% reduction in the mean number of revertants per plate as compared to vehicle accompanied by an abrupt dose-dependent drop in the revertant count and/or a moderate reduction in the background lawn.

Positive responses would be indicated by a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Mean number of revertants per plate for TA1535 and TA1537 must be ≥ 3-fold the mean of the vehicle and TA98, TA100 and WP2 *uvr*A must be ≥ 2-fold the mean of the vehicle to be considered a positive response.

Strains/species/cell line: *Salmonella typhimurium* histidine auxotrophs utilized included: TA98, TA100, TA1535 and TA1537. *Escherichia coli* tryptophan auxotroph utilized: WP2 *uvr*A

Doses used in definitive study: vehicle (DMSO), 15, 50, 150, 500, 1500 and 5000 µg/plate

Basis of dose selection: The initial toxicity-mutation assay tested 1.5, 5.0, 15, 50, 150, 1500, 5000 µg/plate of the test article in DMSO vehicle and did not demonstrate precipitation or positive mutagenic response. Toxicity, as evidenced by bacterial lawn clearing, was observed at 1500 and 5000 µg/plate with most test conditions. Based on the initial toxicity-mutation assay, the maximum dose utilized in the definitive study was 5000 µg/plate.

Negative controls: The vehicle, DMSO, was used as the negative control.

Positive controls: The positive controls utilized for the respective strain are indicated in the sponsor's table below (Table 1).

Table 1

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	Rat	2-aminoanthracene [redacted] Lot No. 15216JA Exp. Date 10-Mar-2007 CAS No. 613-13-8 Purity 97.3%	1.0
WP2 *uvrA*			10
TA98	None	2-nitrofluorene [redacted] Lot No. 087088HS Exp. Date 08-Mar-2006 CAS No. 607-57-8 Purity 99.9%	1.0
TA100, TA1535		sodium azide [redacted] Exp. Date 31-Jul-2008 CAS No. 26628-22-8 Purity 99.9%	1.0
TA1537		9-aminoacridine [redacted] Lot No. 106F06582 Exp. Date 08-Nov-2009 CAS No. 90-45-9 Purity >97%	75
WP2 *uvrA*		methyl methanesulfonate [redacted] Exp. Date 27-Jan-2007 CAS No. 66-27-3 Purity 99.9%	1,000

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Reviewer's comment:
In deviation from the protocol, the plates were incubated for 17 hours longer than the upper limit of the range of 48 to 72 hours. Vehicles and positive controls used in the assay demonstrated that the test system was functioning correctly. The results were also consistent with the initial trial in which the plates were incubated for the protocol-specified duration. The Study Director reviewed the data and determined that this deviation did not have an adverse impact on the integrity of the data or the validity of the study conclusion.

Results
Study validity: The study is valid. The definitive and confirmatory studies utilized suitable numbers of replicate plates and appropriate counting methods. The positive controls demonstrated clear increases in tester strain revertants while the negative control (vehicle) was within historical range for the tester strains with this vehicle.

Study outcome: Both the definitive and confirmatory studies with [redacted] were negative. The sponsor summarized the results of the confirmative assay in the table below (Table 2). The data in the table indicate negative mutagenic responses in the presence and absence of exogenous metabolic activation with S9. No precipitate was observed but toxicity occurred beginning at 1500 or 5000 µg per plate.

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Table 2

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay

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Test Article Id : [redacted]
Study Number : [redacted] Experiment No : 82

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	32 ± 7	122 ± 7	44 ± 8	12 ± 1	15 ± 2
15	31 ± 9	130 ± 23	37 ± 4	14 ± 1	17 ± 4
50	31 ± 7	122 ± 9	36 ± 11	10 ± 2	14 ± 1
150	31 ± 4	126 ± 5	37 ± 6	11 ± 4	17 ± 4
500	30 ± 4	118 ± 16	35 ± 4	15 ± 2	14 ± 4
1500	29 ± 4	94 ± 9	34 ± 2	21 ± 2	12 ± 7
5000	35 ± 11	0 ± 0	0 ± 0	5 ± 9	15 ± 3
Positive	96 ± 3	466 ± 69	451 ± 10	598 ± 285	108 ± 19

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	34 ± 10	97 ± 10	21 ± 3	16 ± 4	15 ± 5
15	45 ± 7	107 ± 10	21 ± 1	17 ± 2	17 ± 4
50	38 ± 13	123 ± 23	22 ± 3	15 ± 4	18 ± 3
150	39 ± 2	108 ± 20	19 ± 6	13 ± 5	18 ± 8
500	38 ± 11	93 ± 21	19 ± 4	13 ± 2	17 ± 2
1500	36 ± 7	79 ± 6	17 ± 3	15 ± 3	13 ± 6
5000	23 ± 3	0 ± 0	10 ± 2	0 ± 0	19 ± 5
Positive	155 ± 30	303 ± 11	73 ± 5	52 ± 16	109 ± 10

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 50 µL

Reviewer's note:
The value of the positive control for the TA1535 plus rat liver microsomes condition is 73. It is difficult to read in the figure.

Study title: Bacterial Reverse Mutation Assay (Ames Test) with [redacted]

Key findings: Under the conditions of this study, [redacted] was concluded to be negative in the Bacterial Reverse Mutation Assay.

b(4)

Study no.: [redacted]

Volume #, and page #: eCTD 000 M4.2.3.7.6.1
Conducting laboratory and location: [redacted]
Date of study initiation: October 26, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )

Drug, lot #, and % purity: [redacted] Lot # 05-OC-FP-010, Purity 100% (by HPLC, per COA from [redacted] b(4)

Methods

Six to eight doses of test article as well as DMSO vehicle and positive controls were plated in triplicate with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9 using the plate incorporation method. Positive controls were appropriate for each tester strain and metabolic activation condition. Five hundred µL of S9 or sham mix, 100 µL of tester strain and 50 µL of vehicle, test article dilution or positive control were added to 2.0 mL of molten selective top agar, vortexed and overlaid onto the surface of 25 mL minimal bottom agar. After solidification of the overlay, plates were inverted and incubated for approximately 48-72 hours at 37 ± 2°C. Plates that could not be counted immediately after incubation were stored at 2-8°C until colony counting could be conducted. The condition of the bacterial background lawn was evaluated for evidence of toxicity using a dissecting microscope. Precipitate was evaluated by the naked eye. Revertant colonies for a given tester strain and activation condition, with the exception of positive controls, were counted either entirely by automated colony counter or entirely by hand unless plates exhibited signs of toxicity.

All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion of the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion of the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls (TA98: 10-50; TA100: 80-240; TA1535: 5-45; TA1537: 3-21; WP2 *uvr*A: 10-60). Test strain culture titers must be $\geq 0.3 \times 10^9$ cells/mL in order to ensure that appropriate numbers of bacteria are plated on each plate. The mean of each positive control must exhibit at least a 3-fold increase in the number of revertants as compared to vehicle. A minimum of three non-toxic dose levels is also required to evaluate the assay data. Toxicity is described as a ≥ 50% reduction in the mean number of revertants per plate as compared to vehicle accompanied by an abrupt dose-dependent drop in the revertant count and/or a moderate reduction in the background lawn.

Positive responses would be indicated by a dose-related increase in the mean number of revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Mean number of revertants per plate for TA1535 and TA1537 must be ≥ 3-fold the mean of the vehicle and TA98, TA100 and WP2 *uvr*A must be ≥ 2-fold the mean of the vehicle to be considered a positive response.

Strains/species/cell line: *Salmonella typhimurium* histidine auxotrophs utilized included: TA98, TA100, TA1535 and TA1537. *Escherichia coli* tryptophan auxotroph utilized: WP2 *uvr*A

Doses used in definitive study: vehicle (DMSO), 50, 150, 500, 1500 and 5000 µg/plate

Basis of dose selection: The initial toxicity-mutation assay tested 1.5, 5.0, 15, 50, 150, 1500, 5000 µg/plate of the test article in DMSO vehicle and did not demonstrate precipitation or positive mutagenic response. No toxicity was observed but reductions in revertant cell counts were seen at 5000 µg/plate with some test conditions. Based on the initial toxicity-mutation assay, the maximum dose utilized in the definitive study was 5000 µg/plate.

Negative controls: The vehicle, DMSO, was used as the negative control.

Positive controls: The positive controls utilized for the respective strain are indicated in the sponsor's table below (Table 1).

Table 1

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	Rat	2-aminoanthracene (—————) Lot No. 132163A Exp. Date 10-Mar-2007 CAS No. 613-13-8 Purity 97.3%	1.0
WP2 *uvrA*			10
TA98	None	2-nitrofluorene (—————) Lot No. 085/08/ES Exp. Date 08-Mar-2006 CAS No. 607-57-8 Purity 99.9%	1.0
TA100, TA1535		sodium azide (—————) Lot No. 073K0171Y Exp. Date 31-Jul-2008 CAS No. 26628-22-8 Purity 99.9%	1.0
TA1537		9-aminoacridine (—————) Lot No. [illegible] Exp. Date 08-Nov-2009 CAS No. 90-45-9 Purity >97%	75
WP2 *uvrA*		methyl methanesulfonate (—————) CAS No. [illegible] Exp. Date 27-Jan-2007 CAS No. 66-27-3 Purity 99.9%	1,000

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Results

Study validity: The study is valid. This study utilized suitable numbers of replicate plates and appropriate counting methods. The positive controls demonstrated clear increases in tester strain revertants while the negative control (vehicle) was within historical range for the tester strains with this vehicle.

Study outcome: Both definitive and confirmatory studies with ————— are negative. The sponsor summarized the results of the confirmative assay in the table below (Table 2). The data in the table indicate negative mutagenic responses in the presence and absence of

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exogenous metabolic activation. No precipitate or appreciable toxicity was observed, but reductions in revertant counts were observed at 5000 µg per plate with some test conditions.

Table 2

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay

b(4)

Test Article Id : [redacted]
Study Number [redacted] Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	15 ± 2	96 ± 4	18 ± 4	7 ± 1	17 ± 6
50	12 ± 1	101 ± 6	16 ± 1	9 ± 2	13 ± 4
150	17 ± 1	105 ± 6	22 ± 5	7 ± 1	15 ± 3
500	17 ± 3	93 ± 11	25 ± 5	7 ± 1	17 ± 4
1500	10 ± 2	42 ± 15	17 ± 5	6 ± 3	11 ± 3
5000	11 ± 4	11 ± 3	8 ± 2	3 ± 1	3 ± 2
Positive	97 ± 12	311 ± 13	242 ± 16	909 ± 55	83 ± 14

Liver Microsomes: Rat Liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	17 ± 5	126 ± 7	12 ± 5	4 ± 2	20 ± 3
50	26 ± 4	114 ± 14	11 ± 3	6 ± 1	12 ± 3
150	20 ± 2	133 ± 12	15 ± 1	7 ± 3	12 ± 1
500	21 ± 5	124 ± 10	15 ± 3	5 ± 2	13 ± 6
1500	20 ± 3	93 ± 15	18 ± 1	5 ± 1	10 ± 3
5000	11 ± 4	1 ± 2	6 ± 2	4 ± 2	9 ± 2
Positive	199 ± 26	380 ± 26	71 ± 5	30 ± 2	229 ± 46

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 50 µL

Study title: *In vitro* Mammalian Chromosome Aberration Test with [redacted]

Key findings: Under the conditions of this study [redacted] was concluded to be negative for clastogenicity in the *in vitro* Mammalian Chromosome Aberration Test in CHO cells.

Study no.: [redacted]

b(4)

Volume #, and page #: eCTD 000 M4.2.3.7.6.1
Conducting laboratory and location: [redacted]
Date of study initiation: October 24, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: [redacted] Lot # M41063, Purity 99.6% (by HPLC, per COA from [redacted]

Methods

CHO-K_1 cells were seeded at ~5 x 10^5 cells per flask incubated at 37 ± 1°C in a humidified 5 ± 1% CO_2 in air for 16-24 hours. Duplicate cultures of CHO-K_1 cells were exposed to vehicle or test article in the presence or absence of S9 metabolic activation for 4 hours with a 16-hour recovery time or in the absence of S9 activation for 20 hours continuous exposure before harvesting. Two hours prior to harvesting, the metaphase arrestor Colcemid® was added to all conditions at a final concentration of 0.1 µg/mL and the cells were returned to the incubator. At the completion of the incubation time cells were harvested and an aliquot of was removed from each culture for a concurrent toxicity test. The presence of test article precipitate was determined using the unaided eye and the cells were counted and viability was assessed by trypan blue exclusion. The remainder of the cells was centrifuged and the pellet was resuspended in 2-4 mL 0.075 M KCl and allowed to stand at room temperature for 4-8 minutes. Cells were collected by centrifugation, supernatant was aspirated and the cells were fixed with two washes with ~2 mL Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v) and stored overnight at 2-8°C. To prepare the slides, an aliquot of cells was dropped onto a slide and allowed to air dry. The slides were then stained with 5% Giemsa, air dried and permanently mounted. The selection of dose levels for analysis of chromosome aberrations was based on the results of the concurrent toxicity test. The highest dose level selected for evaluation was the dose which induced ≥ 50% toxicity, as measured by cell growth inhibition, and included a sufficient number of scorable metaphase cells. Two additional lower doses were included in the analysis. The mitotic index was determined for each treatment group (percentage cells in mitosis per 500 cells scored). Metaphase cells with 20 ± 2 centromeres were examined under oil immersion. A minimum of 200 metaphase spreads were examined and scored for chromatid-type (including chromatid and isochromatid breaks and exchange figures such as quadriradials, triradials and complex rearrangements) and chromosome-type (chromosome breaks and exchange figures such as dicentrics and rings) aberrations. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome) and were considered part of an incomplete exchange if they were observed with an exchange figure. Severely damaged cells (≥ 10 aberrations) and pulverized chromosomes or cells were also recorded. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test and the Cochran-Armitage test was used to measure dose-responsiveness.

The frequency of cells with structural chromosomal aberrations in the vehicle group must be within the range of the historical vehicle control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to vehicle. The test article would be considered to have a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). A reproducible and significant increase at the high concentration only with no dose-response or a reproducible significant increase at one dose level other than the high dose with no dose-response would be considered positive. Test articles not demonstrating a statistically significant increase in aberrations would be considered negative.

Strains/species/cell line: Chinese hamster ovary (CHO-K_1) cells

Doses used in definitive study:

-S9 4-hr/16 hr: DMSO vehicle; ——— 50, 100, 175 µg/mL; or positive control Mitomycin C, 0.2 µg/mL

+S9 4-hr/16 hr: DMSO vehicle; ——— 50, 100, 150 µg/mL; or positive control Cyclophosphamide, 10 µg/mL b(4)

-S9 20-hr: DMSO vehicle ——— 12.5, 25, 75 µg/mL; or positive control Mitomycin C, 0.1 µg/mL

Basis of dose selection: Dose levels for the chromosome aberration assay were selected based on the results from a preliminary toxicity assay which examined a reduction of cell growth with exposure to the test article relative to vehicle. CHO-K_1 cells were exposed to vehicle or the test article (0.299-2990 µg/mL) in the presence or absence of S9-induced metabolic activation for 4 hours with a 16 hour recovery time before harvesting and counting. Cells were also exposed to vehicle or the same range of doses of test article continuously for 20 hours in the absence of S9. The osmolality and pH of the highest concentration of test article were equivalent to vehicle. At the conclusion of the incubation period, a visible precipitate was observed at 2990 µg/mL dose of treatment article. Substantial toxicity, as defined as at least 50% cell growth inhibition, was observed at dose levels ≥ 299 µg/mL in both the –S9 and +S9 4-hour exposure groups and at dose levels ≥ 89.7 µg/mL in the non-activated 20-hour continuous exposure group.

The dose levels chosen for the concurrent toxicity test for the chromosome aberration assay are listed in the sponsor's table below (Table 1):

Table 1

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	25, 50, 100, 125, 150, 175, 200, 225, 250
	20 hr	0 hr	6.25, 12.5, 25, 50, 75, 100, 125, 150
S9-activated	4 hr	16 hr	25, 50, 100, 125, 150, 175, 200, 225, 250

Negative controls: The vehicle, DMSO, was used as the negative control.

Positive controls: Mitomycin C (MMC) was used as the positive control at 0.2 µg/mL for the 4-hour and at 0.1 µg/mL for the 20-hour non-activated groups. Cyclophosphamide (CP) was used as the positive control at 10 µg/mL for the 4-hour S9-activated group.

Results

Study validity: This study is valid. It utilizes appropriate replicates and cell counting/viability methodology. The vehicles and positive controls for the activated and non-activated groups with 4-hour incubation are within the range of the historical data set and the positive controls are significantly higher than vehicle controls. No historical data is provided by the sponsor for the 20-hour incubation group. However, for these data the vehicle and all doses of the test article are within the range of the 4-hour incubation historical dataset. The positive control is significantly higher than the vehicle control.

Study outcome: The tables provided by the sponsor are reproduced below (Tables 2 and 3). The highest concentration used in each condition reached a level which produced adequate evidence of cytotoxicity characterized by cell counts and trypan blue exclusion. No evidence of a concentration-dependent increase in chromosomal aberrations was found. Therefore, under the conditions of this assay ——— is considered to be negative for clastogenicity. b(4)

Table 2

Treatment Time	Recovery Time	Harvest Time	S9	Toxicity* at highest dose scored (µg/mL)	Mitotic Index Reduction **	LED[1] for Structural Aberrations µg/mL	LED[1] for Numerical Aberrations µg/mL
4 hr	16 hr	20 hr	-	56% at 175	10%	None	None
20 hr	0 hr	20 hr	-	56% at 75	21%	None	None
4 hr	16 hr	20 hr	+	56% at 150	17%	None	None

Table 3

SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	7.9	200	200	0.010	±0.100	2.5	1.0
[redacted]									
50	-S9	4	7.7	200	200	0.005	±0.071	1.5	0.5
100	-S9	4	7.6	200	200	0.010	±0.100	1.0	1.0
175	-S9	4	7.1	200	200	0.015	±0.122	4.0	1.5
MMC, 0.2	-S9	4	9.1	200	100	0.220	±0.484	0.5	19.0**
DMSO	+S9	4	9.4	200	200	0.010	±0.100	1.5	1.0
[redacted]									
50	+S9	4	8.8	200	200	0.005	±0.071	2.0	0.5
100	+S9	4	8.7	200	200	0.035	±0.307	2.5	2.0
150	+S9	4	7.8	200	200	0.005	±0.071	1.0	0.5
CP, 10	+S9	4	7.1	200	100	0.350	±0.796	1.5	20.0**
DMSO	-S9	20	10.2	200	200	0.005	±0.071	6.5	0.5
[redacted]									
12.5	-S9	20	9.6	200	200	0.005	±0.071	4.5	0.5
25	-S9	20	9.6	200	200	0.005	±0.071	2.0	0.5
75	-S9	20	8.1	200	200	0.010	±0.100	6.5	1.0
MMC, 0.1	-S9	20	7.8	200	100	0.390	±0.875	2.0	24.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's exact test.

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b(4)

Study title: *In vitro* Mammalian Chromosome Aberration Test with [redacted]

Key findings: Under the conditions of this study, [redacted] was concluded to be positive for clastogenicity in the *in vitro* Mammalian Chromosome Aberration Test in CHO cells.

Study no.: [redacted]

Volume #, and page #: eCTD 000 M4.2.3.7.6.1
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: October 24, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: _____ Lot # 05-OC-FP-010, Purity 100% (by HPLC, per COA from _____ b(4)

Methods

CHO-K_1 cells were seeded at ~5 x 10^5 cells per flask incubated at 37 ± 1°C in a humidified 5 ± 1% CO_2 in air for 16-24 hours. Duplicate cultures of CHO-K_1 cells were exposed to vehicle or test article in the presence or absence of S9 metabolic activation for 4 hours with a 16 hour recovery time or in the absence of S9 activation for 20 hours continuous exposure before harvesting. Two hours prior to harvesting, the metaphase arrestor Colcemid® was added to all conditions at a final concentration of 0.1 µg/mL and the cells were returned to the incubator. At the completion of the incubation time cells were harvested and an aliquot was removed from each culture for a concurrent toxicity test. The presence of test article precipitate was determined using the unaided eye and the cells were counted and viability was assessed by trypan blue exclusion. The remainder of the cells was centrifuged and the pellet was resuspended in 2-4 mL 0.075 M KCl and allowed to stand at room temperature for 4-8 minutes. Cells were collected by centrifugation, supernatant was aspirated and the cells were fixed with two washes with ~2 mL Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v) and stored overnight at 2-8°C. To prepare the slides, an aliquot of cells was dropped onto a slide and allowed to air dry. The slides were then stained with 5% Giemsa, air dried and permanently mounted. The selection of dose levels for analysis of chromosome aberrations was based on the results of the concurrent toxicity test. The highest dose level selected for evaluation was the dose which induced ≥ 50% toxicity, as measured by cell growth inhibition, and included a sufficient number of scorable metaphase cells. Two additional lower doses were included in the analysis. The mitotic index was determined for each treatment group (percentage cells in mitosis per 500 cells scored). Metaphase cells with 20 ± 2 centromeres were examined under oil immersion. A minimum of 200 metaphase spreads were examined and scored for chromatid-type (including chromatid and isochromatid breaks and exchange figures such as quadriradials, triradials and complex rearrangements) and chromosome-type (chromosome breaks and exchange figures such as dicentrics and rings) aberrations. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome) and were considered part of an incomplete exchange if they were observed with an exchange figure. Severely damaged cells (≥ 10 aberrations) and pulverized chromosomes or cells were also recorded. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test and the Cochran-Armitage test was used to measure dose-responsiveness.

The frequency of cells with structural chromosomal aberrations in the vehicle group must be within the range of the historical vehicle control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to vehicle. The test article would be considered to have a positive response when the percentage of cells with aberrations is increased in a dose-

responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). A reproducible and significant increase at the high concentration only with no dose-response or a reproducible significant increase at one dose level other than the high dose with no dose-response would be considered positive. Test articles not demonstrating a statistically significant increase in aberrations would be considered negative.

Reviewer's comment:
The sponsor has provided a table with historical control data for vehicle and positive control substances at the four hour time point only.

Strains/species/cell line: Chinese hamster ovary ($CHO\text{-}K_1$) cells

Doses used in definitive study:

-S9 4-hr/16 hr: DMSO vehicle; ——— 150, 300, 600 µg/mL; or positive control Mitomycin C, 0.2 µg/mL

+S9 4-hr/16 hr: DMSO vehicle, ——— 5, 50, 100 µg/mL; or positive control Cyclophosphamide, 10 µg/mL

-S9 20-hr: DMSO vehicle; ——— 5, 150, 300 µg/mL; or positive control Mitomycin C, 0.1 µg/mL

b(4)

Basis of dose selection: Dose levels for the chromosome aberration assay were selected based on the results from a preliminary toxicity assay which examined a reduction of cell growth with exposure to the test article. $CHO\text{-}K_1$ cells were exposed to vehicle or the test article (0.296-2960 µg/mL) in the presence or absence of S9-induced metabolic activation for 4 hours with a 16-hour recovery time before harvesting and counting. Cells were also exposed to vehicle or the same range of doses of test article continuously for 20 hours in the absence of S9. The osmolality and pH of the highest concentration of test article were equivalent to vehicle in all groups. At the conclusion of the incubation period, a visible precipitate was observed at $\geq$ 888 µg/mL concentration of treatment article in the non-activated and S9-activated 4-hour exposure groups and a visible precipitate was observed at $\geq$ 2960 µg/mL concentration of treatment article in the non-activated 20-hour exposure group. The color of the media changed from red to rust due to the presence of the test article in the media at dose levels $\geq$ 888 µg/mL (pH=7.5). Substantial toxicity, as defined as at least 50% cell growth inhibition, was observed at concentration levels $\geq$ 888 µg/mL in the non-activated 4-hour group and at dose levels $\geq$ 296 µg/mL in the S9-activated 4-hour and non-activated 20-hour exposure groups.

The dose levels chosen for the concurrent toxicity test for the chromosome aberration assay are listed in the sponsor's table below (Table 1):

Table 1

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	75, 150, 300, 600, 650, 700, 750, 800, 850
	20 hr	0 hr	75, 150, 300, 600, 650, 700, 750, 800, 850
S9-activated	4 hr	16 hr	25, 50, 100, 200, 225, 250, 275, 300

Negative controls: The vehicle, DMSO, was used as the negative control.

Positive controls: Mitomycin C (MMC) was used as the positive control at 0.2 µg/mL for the 4-hour and at 0.1 µg/mL for the 20-hour non-S9 activated groups. Cyclophosphamide (CP) was used as the positive control at 10 µg/mL for the 4-hour S9-activated group.

Results

Study validity: This study is valid. It utilizes appropriate replicates and cell counting/viability methodology. The vehicles and positive controls for the activated and non-activated groups with 4-hour incubation are within the range of the historical data set and the positive controls are significantly higher than vehicle controls. No historical data is provided by the sponsor for the 20-hour incubation group. The positive control is significantly higher than the vehicle control for each condition.

Study outcome: The percentage of cells with structural aberrations in the non-activated 4-hour exposure group was significantly increased over vehicle at 300 µg/mL ($p \leq 0.05$, Fisher's exact test) but was negative for a dose response using the Cochran-Armitage test ($p \geq 0.05$). However, the percentage of cells with structural aberrations (3.5%) was within the historical control values (0-5%). Therefore, it is not considered to be biologically significant. The percentage of cells with numerical aberrations in the test article group for all conditions and exposure times was not significantly increased above vehicle ($p \geq 0.05$, Fisher's exact test). The percentage of cells with structural aberrations in the S9-activated 4-hour exposure group was significantly increased over vehicle at 100 µg/mL ($p \leq 0.01$, Fisher's exact test) and showed a positive dose response using the Cochran-Armitage test ($p \leq 0.05$). However, the percentage of cells with structural aberrations (4.5%) was within the historical control values (0-5%). Therefore, it is not considered to be biologically significant. The percentage of cells with structural aberrations in the non-activated 20-hour continuous exposure group was significantly increased over vehicle at 300 µg/mL ($p \leq 0.01$, Fisher's exact test) and showed a positive dose response using the Cochran-Armitage test ($p \leq 0.05$). The percentage of cells with structural aberrations (6.0%) was outside the historical control values (0-5%). Therefore, it is considered to be positive. The tables provided by the sponsor are reproduced below (Tables 2 and 3). The highest concentration used in each condition reached a level which produced adequate evidence of cytotoxicity characterized by cell counts, trypan blue exclusion and mitotic index. Therefore, under the conditions of this assay [redacted] is considered to be positive for clastogenicity. b(4)

Table 2

Treatment Time	Recovery Time	Harvest Time	S9	Toxicity* at highest dose scored (µg/mL)	Mitotic Index Reduction **	LED[1] for Structural Aberrations µg/mL	LED[1] for Numerical Aberrations µg/mL
4 hr	16 hr	20 hr	-	35% at 600	70%	None	None
20 hr	0 hr	20 hr	-	53% at 300	25%	None	None
4 hr	16 hr	20 hr	+	28% at 100	51%	None	None

* cell growth inhibition
** relative to solvent control at high dose evaluated for chromosome aberrations
[1] LED= lowest effective dose

Reviewer's note:
In the above table, a ≥50% reduction in mitotic index was used as the measure of adequate toxicity for the 4 h conditions in the presence and absence of S9. At the level of ≥50% cell growth inhibition there were too few scorable cells.

Table 3

SUMMARY - REPEAT ASSAY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-S9	4	8.3	200	200	0.005	±0.071	0.5	0.5
150	-S9	4	7.0	200	200	0.015	±0.122	1.0	1.5
300	-S9	4	4.6	200	200	0.040	±0.221	2.5	3.5*
600	-S9	4	2.5	200	200	0.005	±0.071	0.5	0.5
MMC, 0.2	-S9	4	4.0	200	100	0.250	±1.048	0.5	16.0
DMSO	+S9	4	8.8	200	200	0.005	±0.071	2.0	0.5
25	+S9	4	10.2	200	200	0.005	±0.071	0.5	0.5
50	+S9	4	10.6	200	200	0.025	±0.186	1.0	2.0
100	+S9	4	4.3	200	200	0.045	±0.208	1.5	4.5**
CP, 10	+S9	4	3.8	200	100	0.300	±1.106	1.5	17.0**
DMSO	-S9	20	7.7	200	200	0.010	±0.100	1.0	1.0
75	-S9	20	7.5	200	200	0.000	±0.000	1.0	0.0
150	-S9	20	6.6	200	200	0.015	±0.122	1.5	1.5
300	-S9	20	5.8	200	200	0.060	±0.238	2.0	6.0**
MMC, 0.1	-S9	20	5.8	200	100	0.200	±0.449	1.5	18.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's exact test.

b(4)

Reviewer's comment: I believe the asterisk(s) denoting statistical significance was inadvertently omitted by the sponsor for the Percent Structural Aberrations in the MMC positive control group 4-hour exposure non-activated group.

Study title: Mammalian Erythrocyte Micronucleus Test with ———— b(4)

Key findings: Under the conditions of this study, ———— did not cause chromosomal damage in bone marrow cells and is therefore concluded to be negative in the *in vivo* Micronucleus Assay.

Study no.: [redacted]

Volume #, and page #: eCTD 000 M4.2.3.7.6.1
Conducting laboratory and location: [redacted]
Date of study initiation: January 5, 2007 b(4)
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: [redacted] lot # 07-OC-FP-001; 99.2% per COA

Methods
After analysis of the stock solutions of [redacted] rom the definitive study, it was determined that the mice in the high dose (40 mg/mL) group did not receive the full dose of [redacted] The low and mid dose stock concentrations were found to be adequate. The definitive micronucleus test was repeated with the vehicle, high dose, and positive control. The solutions for the repeat study were found to be adequate. The low and mid dose results in the initial assay and the high dose results in the repeat assay, with their respective vehicles and positive controls, will be considered in this review. b(4)

Strains/species/cell line: 6-8 week old ICR mice; Male weight range: 25.9-28.9 g, repeat study: 32.0-36.8 g; Female weight range: 23.7-27.9 g, repeat study: 24.6-27.9 g; [redacted]

Doses used in definitive study: vehicle (0.9% saline), 100, 200, and 400 mg/kg b(4)

Basis of dose selection: A toxicity study was conducted using a single i.p. injection of [redacted] at 300, 500 and 750 mg/kg. Mortality was observed at doses of 500 mg/kg (2/6) and 750 mg/kg (5/6). Lethargy was seen in all mice at all doses and piloerection was seen in all mice at 300 and 500 mg/kg and 2/6 mice at 750 mg/kg. Ataxia was seen in all mice at 500 mg/kg. Convulsions (6/6) and irregular breathing (2/6) were observed at 750 mg/kg. The maximum tolerated dose was estimated to be 400 mg/kg and was selected as the high dose in the definitive study.

Negative controls: The vehicle, 0.9% NaCl (saline), was used as the negative control.

Positive controls: Cyclophosphamide (CP), which induces micronucleus formation, was used at 50 mg/kg at a concentration of 2.5 mg/mL. Sterile water was used to dissolve the CP.

Incubation and sampling times: The test article and vehicle were administered intraperitoneally at 10 mL/kg body weight. The definitive micronucleus study was conducted using established and validated procedures (Heddle, 1973;Hayashi, et al., 1994;Mavournin, et al., 1990). The mice were randomized and placed into seven treatment groups of five/sex. An additional group of five mice per sex were administered the high dose to be used as a replacement group in the event of mortality. The study design is outlined in Table 1 which was provided by the sponsor.

Table 1

Treatment (10 mL/kg)	Number of Mice/Sex Dosed	Number of Mice/Sex Used for Bone Marrow Collection After Dose Administration	
		24 hr	48 hr
Initial Study			
Vehicle Control: 0.9% sodium chloride (saline)	10	5	5
Test Article: [redacted]			
Low dose (100 mg/kg)	5	5	0
Mid dose (200 mg/kg)	5	5	0
High dose (400 mg/kg)**	15*	5	5
Positive Control: CP (50 mg/kg)	5	5	0
Repeat study			
Vehicle Control: 0.9% sodium chloride (saline)	10	5	5
Test Article: [redacted]			
High dose (400 mg/kg)	10	5	5
Positive Control: CP (50 mg/kg)	5	5	0

b(4)

*Including 5 replacement mice/sex to ensure the availability of five mice for micronucleus analysis.
**Based on the dose formulation analysis, a concentration of 40 mg/mL was outside of the acceptable range (± 20 %), therefore a dose of 400 mg/kg was not achieved.

The mice were euthanized by CO_2 asphyxiation approximately 24 or 48 hours after drug administration. Five male and five female mice were treated with the positive control and euthanized at the 24 hour time point. After each mouse was euthanized, the groin area was cleansed with 70% ethanol and the femurs were exposed, separated just above the kneecaps and the heads of the femur were removed with a scissors. The bone marrow was flushed from the femurs using a syringe into a culture tube containing fetal bovine serum. The tubes were centrifuged at approximately 100 x g for 5 minutes and the supernatant was aspirated leaving approximately 0.1 mL of serum. The cells were resuspended and a small drop was placed on a microscope slide and spread along the length of the slide. The slides were fixed with methanol and stained with May-Gruenwald-Giemsa stain, permanently mounted and scored in a blind fashion. The number of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) among 200 erythrocytes (PCE + NCE) per mouse were determined. The number of micronucleated polychromatic erythrocytes (MPCE) were then determined for 2000 PCE per mouse. The dose group means were calculated for the percentage of PCE as well as the frequency of MPCE. Data were analyzed separately for males and females. The incidence of MPCE per 2000 PCEs for each dose group was determined. Statistical significance was determined using the Kastenbaum-Bowman tables which are based on the binomial distribution (Kastenbaum and Bowman, 1970). A significant reduction in percent PCE ($\geq$ 20% vs. vehicle) was used as an indication of

toxicity. The test article would be considered to show a positive response if a dose-responsive increase in the incidence of MPCEs was observed and one or more doses were statistically elevated relative to the concurrent vehicle control values ($p \leq 0.05$, Kastenbaum-Bowman Tables) at any sampling time. Values that are statistically significant but do not exceed the range of historical negative control values will be considered to be not biologically relevant. The test article will be judged as negative if there is no statistically significant increase in the incidence of MPCEs above the concurrent vehicle control values and there is no evidence of dose response.

Reviewer's comment:
The sponsor does not state how a positive dose-response would be determined, specifically which statistical test would be utilized.

Results
Study validity: This study is valid. The incidence of the MPCEs in the vehicle control group was within the historical control range and the positive control group was significantly increased relative to the vehicle.

Study outcome: ——— did not cause *in vivo* chromosomal damage in the Micronucleus Assay and is therefore not considered to be a clastogenic agent. The summary tables for the definitive and repeat definitive studies provided by the sponsor are reproduced below (Tables 2 and 3). The percentage of PCE and the MPCE frequency were determined from bone marrow preparations from mice euthanized approximately 24 and 48 hours after test article administration. There were no statistically significant increases in the number of MPCE in the treated groups at any dose level for either time point as compared to the vehicle groups.

b(4)

Table 2

Table 8.0-5: Initial Study - Summary of Bone Marrow Micronucleus Analysis Following a Single Intraperitoneal Dose of [redacted] in ICR Mice

Treatment (10 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
0.9% sodium chloride (saline)							
	M	24	5	0.594 ± 0.02	---	0.4 ± 0.22	4 / 10000
	F	24	5	0.557 ± 0.03	---	0.3 ± 0.27	3 / 10000
[redacted]							
100 mg/kg	M	24	5	0.486 ± 0.06	-18	0.1 ± 0.22	1 / 10000
	F	24	5	0.502 ± 0.04	-10	0.3 ± 0.45	3 / 10000
200 mg/kg	M	24	5	0.345 ± 0.09	-42	0.1 ± 0.22	1 / 10000
	F	24	5	0.501 ± 0.03	-10	0.0 ± 0.00	0 / 10000
400 mg/kg (targeted)**	M	24	5	0.478 ± 0.04	-20	0.3 ± 0.45	3 / 10000
	F	24	5	0.495 ± 0.08	-11	0.1 ± 0.22	1 / 10000
Cyclophosphamide							
50 mg/kg	M	24	5	0.361 ± 0.06	-39	11.9 ± 1.56	*119 / 10000
	F	24	5	0.344 ± 0.04	-38	13.9 ± 3.27	*139 / 10000
0.9% sodium chloride (saline)							
	M	48	5	0.492 ± 0.04	---	0.3 ± 0.27	3 / 10000
	F	48	5	0.511 ± 0.09	---	0.1 ± 0.22	1 / 10000
[redacted]							
400 mg/kg (targeted)**	M	48	5	0.478 ± 0.06	-3	0.1 ± 0.22	1 / 10000
	F	48	5	0.518 ± 0.06	1	0.2 ± 0.27	2 / 10000

*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables).

**Based on the dose formulation analysis, a concentration of 40 mg/mL was outside of the acceptable range (± 20%), therefore, a dose of 400 mg/kg was not achieved.

b(4)

Table 3

: Repeat Definitive Study - Summary of Bone Marrow Micronucleus Analysis Following a Single Intraperitoneal Dose of [redacted] in ICR Mice

Treatment (10 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
0.9% sodium chloride (saline)							
	M	24	5	0.441 ± 0.13	---	0.3 ± 0.27	3 / 10000
	F	24	5	0.528 ± 0.04	---	0.6 ± 0.42	6 / 10000
[redacted]							
400 mg/kg	M	24	5	0.418 ± 0.12	-5	0.5 ± 0.50	5 / 10000
	F	24	5	0.461 ± 0.07	-13	0.7 ± 0.76	7 / 10000
Cyclophosphamide							
50 mg/kg	M	24	5	0.377 ± 0.07	-15	7.4 ± 2.07	*74 / 10000
	F	24	5	0.397 ± 0.04	-25	7.0 ± 1.37	*70 / 10000
0.9% sodium chloride (saline)							
	M	48	5	0.525 ± 0.07	---	0.1 ± 0.22	1 / 10000
	F	48	5	0.549 ± 0.06	---	0.6 ± 0.42	6 / 10000
[redacted]							
400 mg/kg	M	48	5	0.495 ± 0.05	-6	0.0 ± 0.00	0 / 10000
	F	48	5	0.480 ± 0.04	-13	0.4 ± 0.42	4 / 10000

*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables).

b(4

2.6.6.9 Discussion and Conclusions

General toxicology: A single dose local toxicology study was performed with the clinical formulation of OraVerse® and the — impurities, [redacted] at doses up to ten times the to-be-marketed formulation. No test article-related changes were observed in any of the parameters examined with the exception of the histopathology of local tissues. Minimal to mild inflammation was seen in the injection site of all groups. The 1X clinical formulation group showed muscle degeneration and fibrosis which was not seen at the higher doses. Minimal to moderate hemorrhaging in lymph nodes and minimal inflammation in lymph nodes and salivary glands was observed in both vehicle and treated animals. Several vehicle and 10X clinical formulation group dogs showed minimal inflammation and degeneration in the trigeminal ganglia. Nerve fiber degeneration was observed in the superior alveolar nerve of one 1X clinical formulation group dog but nerve fiber degeneration was not observed at higher doses.

b(4)

In the absence of intact (un-injected) control dogs, it is not possible to determine whether the pathologies observed in nerves, muscle and surrounding tissues seen in the vehicle group are due to pre-existing lesions or to needle placement. This drug product will be administered via a commonly used dental needle. Any potential pathology resulting from needle placement would be no greater than an injection of the dental anesthetic and is

therefore of no toxicologic concern. It is concluded that phentolamine mesylate, ——— ———————— , at doses up to ten times the intended human dose, did not cause considerably greater levels of toxicity than the vehicle injection in this study. b(4)

Genetic toxicology: The genotoxic potential of phentolamine mesylate was evaluated in the *in vitro* Bacterial Reverse Mutation Assay (Ames Test), the *in vitro* Chromosome Aberration Assay using CHO cells and the *in vivo* Mouse Micronucleus Assay. The sponsor submitted two separate studies for each test. The first Ames Test submitted did not utilize a high enough concentration of the test article and was concluded to be invalid. Phentolamine mesylate was negative in the second Ames Test. Phentolamine mesylate was negative in the first *in vitro* Chromosome Aberration Assay in both the presence and absence of metabolic activation and negative in the second assay in the presence of metabolic activation. In the second assay in the absence of metabolic activation, the high concentration showed an equivocal result. In both *in vivo* Micronucleus Assays, phentolamine mesylate was negative. The weight of evidence suggests phentolamine mesylate is most likely not mutagenic or clastogenic.

The levels of — impurities, ———————— exceed ICH guidelines. The sponsor conducted the Bacterial Reverse Mutation Assay (Ames Test) and the *in vitro* Mammalian Chromosome Aberration Assay for — compounds. ——— was negative in both assays and ——— was negative in the Ames Test and positive in the *in vitro* Mammalian Chromosome Aberration Assay. However, in IT submission 073, the sponsor concluded that ——— was *negative* in the *in vitro* Mammalian Chromosome Aberration Assay. We consulted Dr. David Jacobson-Kram and he concurred that the result was positive. To further assess the clastogenic potential of ——— was evaluated in the *in vivo* Micronucleus Test where it yielded a negative result. Neither ———————— are concluded to be genotoxic. b(4)

Carcinogenicity: The sponsor did not conduct a carcinogenicity assessment for phentolamine mesylate. The ICH M3 Guideline indicates that carcinogenicity assessment would not be required for a drug that would not be used either continuously for ≥ 6 months or intermittently over a lifetime such that the total exposure would add up to approximately six months time. For the proposed acute indication, carcinogenicity assessment is not required for the drug product. There is one report in the literature by Poulet *et al.* describing a 24-month carcinogenicity study which shows development of hibernomas in rats dosed with phentolamine mesylate (Poulet, et al., 2004). Due to the acute indication sought in this NDA, the results from the Poulet *et al.* study will not be considered relevant for the product labeling.

Reproductive toxicology: The sponsor has submitted a male fertility study with phentolamine mesylate. At concentrations up to 143 times human therapeutic exposure levels, phentolamine mesylate was shown to have no adverse effects on male fertility in the rat.

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

Test Article: Phentolamine mesylate

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number/ (Reference)	Report/ Reference Location
Single Dose Toxicity	Mouse	IV	Single dose	75[a]	No		(NIOSH 1995)	Section 4.3
	Mouse	PO	Single dose	1000[a]	No		(Phentolamine Mesylate for Injection, USP Label)	Section 4.3
	Rat	IV	Single dose	75[b]	No		(NIOSH 1995)	Section 4.3
	Rat	SC	Single dose	275[b]	No		(NIOSH 1995)	Section 4.3
	Rat	PO	Single dose	1250[a]	No		(Phentolamine Mesylate for Injection, USP Label)	Section 4.3
	Rabbit	IV	Single dose	35[b]	No		(NIOSH 1995)	Section 4.3
	Rabbit	SC	Single dose	200[b]	No		NIOSH 1995)	Section 4.3
	Beagle dog	Intraoral injection	Single dose	0, 0.024, 0.24	Yes		44-001	Section 4.2.3.1
	Rhesus monkey	Cisterna magna infusion	Single dose	4, 8, 16	No		Martins & Wiley 1973)	Section 4.3
Repeat Dose Toxicity	Rhesus monkey	Cisterna magna infusion	4 days	1, 2, 4	No		Martins & Wiley 1973)	Section 4.3

IV = intravenous; PO = oral; SC = subcutaneous; NIOSH = National Institute of Occupational Safety and Health; USP = United States Pharmacopeia

[a]LD_{50}; [b]minimum lethal dose

b(4)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg[a])	GLP Compliance	Testing Facility	Study Number/ (Reference)	Report/ Reference Location
Genotoxicity	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	*In vitro*	Not applicable	10, 33, 100, 333, 1000 µg/plate; 33, 100, 333, 1500, 2000 µg/plate with S-9	Yes		T5796.502	Section 4.2.3.3
	CHO cells	*In vitro*	Not applicable	13, 25, 50, 100, 200 µg/mL; 100, 200, 400, 450, 500, 550, 600, and 800 µg/mL with S9	Yes		T8199.337	Section 4.2.3.3
	Swiss-Webster mice	IP	Single dose	6.32, 11.2, 20.0, 35.5, and 63.2	Yes		96067	Section 4.2.3.3
Carcinogenicity	Sprague-Dawley rat	PO	24 months	0, 10, 50, 150	Yes		(Poulet et al. 2004)	Section 4.3
Reproductive & Developmental Toxicology	Sprague-Dawley rat	PO	4 weeks during mating until G15	0, 10, 75, 150	Yes		96-4097	Section 4.2.3.5
Local Tolerance	Beagle dog	Intraoral injection	Single dose	0, 0.024, 0.24	Yes		044-001	Section 4.2.3.1

b(4)

CHO = Chinese hamster ovary; IP = intraperitoneal; PO = oral; G = gestation day

[a]mg/kg unless otherwise indicated

Type of Study	Test System	Method of Administration	Doses (mg/kg)	GLP Compliance	Study Number/ (Reference)	Report/ Reference Location
Carcinogenicity	Sprague-Dawley rat	Oral gavage	0, 10, 50, 150	Yes	(Poulet et al. 2004)	Section 4.3

Test Article: ———

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg*)	GLP Compliance	Testing Facility	Study Number/ (Reference)	Report/ Reference Location
Single Dose Toxicity and Local Tolerance	Beagle dog	Intraoral injection	Single dose	0, 0.00054, 0.0054	Yes		044-001	Section 4.2.3.7
Genotoxicity	*S. typhimurium* strains and *E. coli* WP2 uvrA	*In vitro*	Not applicable	0, 1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate	Yes		AB16SB.503.B TL	Section 4.2.3.7
	CHO cells	*In vitro*	Not applicable	0, 6.25, 12.5, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250 µg/mL	Yes		AB16SB.331.B TL	Section 4.2.3.7

CHO = Chinese hamster ovary
*mg/kg unless otherwise indicated

b(4)

Test Article: ———

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg*)	GLP Compliance	Testing Facility	Study Number/ (Reference)	Report/ Reference Location
Single Dose Toxicity and Local Tolerance	Beagle dog	Intraoral injection	Single dose	0, 0.0004, 0.004	Yes		044-001	Section 4.2.3.6
Genotoxicity	*S. typhimurium* strains and *E. coli* WP2 uvrA	*In vitro*	Not applicable	0, 50, 150, 500, 1500, 5000 µg/plate	Yes		AB16SC.503.B TL	Section 4.2.3.7
	CHO cells	*In vitro*	Not applicable	0, 25, 50, 75, 100, 150, 200, 225, 250, 275, 300, 600, 650, 700, 750, 800, 850 µg/mL	Yes		AB16SC.331.B TL	Section 4.2.3.7
Micronucleus test	ICR mice	*In vivo*	Single dose	0, 100, 200 400 mg/kg	Yes		AB16SC.123.B TL	Section 4.2.3.7

CHO = Chinese hamster ovary
*mg/kg unless otherwise indicated

b(4)

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: In support of the NDA for OraVerse®(phentolamine mesylate), the sponsor conducted a single-dose local tolerance study and a battery of genetic toxicology studies with phentolamine mesylate and ——impurities/degradants found in the drug product, ——————→ A Segment I male fertility study with oral administration of phentolamine mesylate was also included in the NDA. Repeat-dose toxicology, reproductive and developmental toxicology and carcinogenicity studies are not required for this application.

b(4)

In the single dose local toxicology study, no test article-related changes were observed in any of the parameters examined with the exception of the histopathology of local tissues. Minimal to mild inflammation was seen in the injection site of all groups. The 1X clinical formulation group showed muscle degeneration and fibrosis which was not seen at the higher doses. Minimal to moderate hemorrhaging in lymph nodes and minimal inflammation in lymph nodes and salivary glands was observed in both vehicle and treated

animals. Several vehicle and 10X clinical formulation dogs showed minimal inflammation and degeneration in the trigeminal ganglia. Nerve fiber degeneration was observed in the superior alveolar nerve of one 1X clinical formulation dog but nerve fiber degeneration was not observed at higher doses. In the absence of intact (un-injected) control dogs, it is not possible to determine whether the pathologies observed in nerves, muscle and surrounding tissues seen in the vehicle group are due to pre-existing lesions or to needle placement. This drug product will be administered via a commonly used dental needle. Any potential pathology resulting from needle placement would be no greater than an injection of the dental anesthetic and is therefore of no toxicologic concern. It is concluded that phentolamine mesylate, ——— at doses up to ten times the intended human dose, did not cause considerably greater levels of toxicity than the vehicle injection in this study. b(4)

The sponsor conducted several studies to assess the genotoxic potential of phentolamine and — mpurities found in the drug product, ——— The weight of evidence suggests that none of the compounds are genotoxic. b(4)

The sponsor did not conduct a carcinogenicity assessment for phentolamine mesylate, as none was required. However, there is one report in the literature by Poulet *et al.* describing a 24-month carcinogenicity study which shows development of hibernomas in rats dosed with phentolamine mesylate (Poulet, et al., 2004). Due to the acute indication sought in this NDA, the results from the Poulet *et al.* study will not be considered relevant and not be included in the label.

Although no reproductive toxicology studies were required for this application, the sponsor has submitted a male fertility study with phentolamine mesylate. At concentrations up to 143 times human therapeutic exposure levels, phentolamine mesylate was shown to have no adverse effects on male fertility in the rat.

Unresolved toxicology issues (if any): none

Recommendations: From a pharmacology/toxicology perspective, based upon review of the non-clinical data, NDA 22-159 may be approved.

Suggested labeling:
The table below contains the draft labeling submitted by the sponsor, the proposed changes and the rationale for the proposed changes.
Please note that there is one outstanding issue. We have requested that the sponsor provide updated exposure values for the Pregnancy section (5.2) of the label. The exposure values in the current draft of the label are the same values found in the original Regitine® label and do not seem to be updated for the OraVerse® product. An addendum will be submitted when the sponsor provides the updated values.

sponsor's labeling	*proposed changes*	*rationale*

b(4)

b(5)

[redacted]

b(4)

b(5)

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

Appendix 1
The following review by Jeri El-Hage, Ph.D. (Division of Reproductive and Urologic Drug Products) is reproduced verbatim.

Male Fertility Study with Phentolamine Mesylate in the Rat (# 96-4097)
The study was conducted according to GLP by [redacted] b(4)
from 12-20-96 to February 24, 1997 using lot # 96502. The final QA'd report is in volume 1.19.

Male Sprague Dawley rats (n = 25/dose) were treated with 0, 10, 75 and 150 mg/kg/day phentolamine mesylate orally by gavage in distilled water for 9 weeks; 4 weeks prior to mating and 3 weeks during the mating period, and 2 weeks after mating prior to sacrifice. Males were mated with untreated females.

Male animals were 60 days of age at the initiation of dosing and animals of both sexes were 90 days of age at the beginning the mating period. Male mating and fertility indices were determined. Females were killed on Day 15 of gestation and the following parameters were evaluated: live and dead fetuses, early and late resorptions, implantation sites, and number of corpora lutea. Evaluations of caudal epididymal sperm counts, sperm morphology and motility were assessed by [redacted] b(4)

Male data:
Mortality: 1/25 HD males died during dosing week 3
Clinical signs: unremarkable.
Body weight/food consumption: unremarkable
Testicular/epididymal weights: unaffected by treatment.

Sperm counts: No drug-related effects on counts, motility or morphology.

Sperm Parameters in Phentolamine Treated Rats

Parameter	Controls	10 mg/kg/day	75 mg/kg/day	150 mg/kg/day
Sperm count 10^6 sperm/g	934 ± 269	1063 ± 250	1154 ± 246	1024 ± 176
Sperm motility, % motile	96.2	93.9	96.0	96.0
Sperm morphology % abnormal	0.1%	0.1%	0.1%	0.1%

Male fertility: No drug-related effects on the male mating or fertility indices (see data below). The reduction in male fertility index in the high dose group was not considered treatment related since it was not statistically significant and it was in the historical control range (mean = 89.9%, range = 76.5 – 100%). This conclusion is supported by the fact that there were no differences in sperm assessment parameters (caudal epididymal count/motility/morphology).

Parameter	Control	10 mg/kg/day	75 mg/kg/day	150 mg/kg/day
Mating index (% days 1-4)	88%	91%	83%	95%
Male fertility index*	100%	92%	96%	83%

* number impregnated/number mated

Female data:

Body wt/gain: There were no differences in maternal body weight or wt gain between the female groups.

Cesarean Section Data

Parameter	Controls	10 mkd	75 mkd	150 mkd
# Pregnant/dams with viable fetuses	25/25	23/25	24/25	20/24
Corpora lutea, mean/animal	17.6	16.1	17.5	16.5
Implantation sites, mean # / animal	14.6	14.6	14.6	13.9
Preimplantation loss, #/ animal	3.0	1.5	2.9	2.5
Live fetuses, #/litter	13.9	14.0	13.9	13.0
Postimplantation loss, # / %	0.7 4.6%	0.6 4.4%	0.7 4.8%	0.9 7.5%
Dead fetuses/late resorptions	0	0	0	0

Toxicokinetics -evaluation was not performed in conjunction with this study but has been conducted for these dose levels in previous toxicity studies. Exposure comparisons between the dose levels in this study and human therapeutic exposures are summarized below.

Species/ Dose	AUC_{0-24}, ng.hr/ml	Multiple of Human AUC with 40 mg	with 80 mg
Male rat, 10 mg/kg/d	Not available		
Male rat, 75 mg/kg/day	1143 - 1703	> 25 X	> 10X
Male rat, 150 mg/kg/day	1963 - 4889	> 22 X	> 18 X
Man, 40 mg	44		
Man, 80 mg	104		

Conclusions: Male rats treated with 10, 75, and 150 mg/kg/day for 4 weeks prior to and 3 weeks during mating exhibited no adverse effects on mating or fertility indices, or sperm parameters (counts, motility, or morphology). Similarly, no drug-related effects were observed on reproductive parameters in untreated females mated with the treated males. The NOEL for reproductive toxicity and fertility in males was 150 mg/kg/day. The 150 mg/kg/day dose produces drug exposures (AUC) in rats greater than 18 times human therapeutic exposures . The 150 mg/kg/day dose is sufficiently high since mortality was observed in 1 male in this study and was observed at doses ≥ 150 mg/kg/day in the 1, 3 and 6 month rat oral toxicity studies.

Reference List

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Ref Type: Pamphlet

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/s/

Elizabeth Bolan
12/3/2007 05:10:59 PM
PHARMACOLOGIST

R. Daniel Mellon
12/3/2007 05:14:53 PM
PHARMACOLOGIST
I concur.

PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST
Division of Anesthesia, Analgesia, and Rheumatology Products

NDA Number: 22-159 **Applicant: Novalar** **Stamp Date: April 9, 2007**
Drug Name: phentolamine mesylate

IS THE PHARM/TOX SECTION OF THE APPLICATION FILEABLE? Yes [✔] No [ ]

The following parameters are necessary in order to initiate a full review, i.e., complete enough to review but may have deficiencies.

	Parameters	Yes	No	Comment
1	On its face, is the Pharmacology/Toxicology section of the NDA organized in a manner to allow substantive review to begin?	✔		This NDA was submitted electronically.
2	Is the Pharmacology/Toxicology section of the NDA indexed and paginated in a manner to allow substantive review to begin?	✔		
3	On its face, is the Pharmacology/Toxicology section of the NDA legible so that substantive review can begin?	✔		
4	Are **final reports** of ALL required* and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility*, juvenile studies, ocular toxicity studies*, acute adult studies*, chronic adult studies*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetic studies, etc)? Have electronic files of the carcinogenicity studies been submitted for statistical review?	✔		All final reports were submitted. The sponsor plans to rely on information from the Regitine label for Seg 2 and 3 repro tox.
5	If the formulation to be marketed is different from that used in the toxicology studies, has the sponsor made an appropriate effort to either repeat the studies with the to be marketed product or to explain why such repetition should not be required?	✔		The formulation used in the preclinical toxicology studies is the same formulation the sponsor intends to market.
6	Are the proposed labeling sections relative to pharmacology appropriate (including human dose multiples expressed in mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	✔		Multiples are expressed as "X times" the plasma level and mg are given.
	For a 505(b)(2) submission, has the sponsor identified a referenced product?	✔		The sponsor will rely upon the Agency's previous findings for Regitine (NDA 8-278).
	For a 505(b)(2) submission, has the sponsor submitted patent certification information to support the information referenced in the proposed drug product labeling?	✔		
7	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions?	✔		The requested *in vivo* micronucleus test was submitted.
	Based upon a cursory review, do the excipients appear to have been adequately qualified?	✔		All excipients are USP.
	Has the applicant submitted any studies or data to address any impurity or extractable issues (if any)?	✔		The cap and plunger are both made with [redacted] rubber (DMF [redacted] : ask CMC about extractable issues. b(4)
8	On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route?	✔		The sponsor has submitted a tocicology study in dogs using the intraoral injection route of administration. This study contains local toxicology data and histology.
9	Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	✔		All submitted studies are GLP.

	Has the sponsor submitted a statement(s) that the pharm/tox studies have been performed using acceptable, state-of-the-art protocols which also reflect agency animal welfare concerns?	✔ ✔		No statement has been submitted but the studies appear to be up to current nonclinical standards regarding GLP and animal welfare issues.
11	From a pharmacology perspective, is this NDA fileable? If "no", please state below why it is not.	✔		FILING ISSUES: none
12	If the NDA is fileable, are there any filing review issues that need to be conveyed to Sponsor? If so, specify:		✔	Filing review issues for the 74-day letter: none

Note: We will ask CMC about whether extractable studies have been conducted for the cap and plunger rubber.

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/s/

Elizabeth Bolan
5/23/2007 03:30:13 PM
PHARMACOLOGIST

R. Daniel Mellon
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I concur.