

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
22-029

PHARMACOLOGY REVIEW(S)



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: 22-029
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 02/27/06
PRODUCT: FS-67 Patch (SALONPAS®) (10% **b(4)**
Methyl Salicylate & 3% l-Menthol Topical Patch)
INTENDED CLINICAL POPULATION: Proposed use is for the temporary relief of mild to moderate aches & pains of muscles & joints associated with: arthritis, simple backache, strains, bruises, and sprains.
SPONSOR; Hisamitsu Pharmaceutical Co., Inc.
DOCUMENTS REVIEWED: Vol. 1, 7-27 of 155
REVIEW DIVISION: Division of Anesthesia, Analgesia & Rheumatology Products (HFD-170)
PHARM/TOX REVIEWER: BeLinda A. Hayes, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob Rapport, M.D.
PROJECT MANAGER: Lisa Basham Cruz

Date of review submission to Division File System (DFS): December 13, 2006

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the nonclinical pharmacology and toxicology perspective, NDA 22-029 is considered approvable.

B. Recommendation for nonclinical studies

The existing reproductive toxicology studies for methyl salicylate demonstrate evidence of skeletal anomalies and variations at all doses tested in the rat, although only the high dose produced statistically significant changes. These studies did not include a toxicokinetic evaluation of exposure nor do we have clear human pharmacokinetic exposures in order to provide a complete assessment of any potential safety margin for these changes. The sponsor should be asked to determine an exposure margin for these reproductive changes based upon additional pharmacokinetic studies in pregnant rats and in the clinical setting. As the lack of these data does not change the recommended pregnancy category, which will remain a C, these studies could be completed as a Phase 4 Commitment, if there are not other approvable issues in this cycle.

C. Recommendations on labeling

The standard NSAID labeling should be included in this drug product label:

If pregnant or breast-feeding, ask a health professional before use. It is especially important not to use BRANDNAME during the last 3 months of pregnancy unless definitely directed to do so by a doctor because it may cause problems in the unborn child or complications during delivery.

If this NDA were for Rx status rather than OTC status, the suggested labeling for the teratogenesis section of the label should read as follows:

PREGNANCY

TERATOGENIC EFFECTS

Pregnancy Category C

Methyl salicylate was shown to be teratogenic in rats. Developmental effects were observed when administered subcutaneously to rats during

the period of organogenesis at doses up to 200 mg/kg/day. Methyl salicylate also exerted suppressive effects on fetal growth and delayed ossification at a dose of 200 mg/kg/day via subcutaneous injection. A statistically significant increase in incidence of skeletal anomalies and variations were observed in rats dosed with 200 mg/kg/day from gestation day 6 to day 21 of lactation. Fetal NOAEL was < 60 mg/kg based upon decrease birth index, growth suppression and increase incidence of skeletal anomalies and variation in the F₁ offspring of the high-dose group and the increase in the incidence of skeletal variations in F₁ offspring in the low-dose group. Teratogenic effects were not observed in rabbits when methyl salicylate was administered at subcutaneous doses up to 300 mg/kg/day from day 6 to 18 of gestation. It is not known how the exposures in these animal studies compare to the human exposures following recommended use of this product.

There were no evidences of teratogenicity when l-menthol was administered subcutaneously in rabbits or rats during the period of organogenesis at doses up to 600 mg/kg/day or 1000 mg/kg/day, respectively. Reproductive studies were not conducted with the SALONPAS _____

There are no adequate and well-controlled studies of dermal l-menthol, methyl salicylate or SALONPAS _____ in pregnant women. SALPONPAS _____ should be used during pregnancy only if potential benefit justifies the potential risk to the fetus. b(4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

In support of the NDA for Salonpas Plus Patch (10% methyl salicylate, 3% menthol), the sponsor conducted the full battery of genetic and reproductive toxicology studies with methyl salicylate and l-menthol. In addition to these studies, toxicology studies were conducted in rabbits, rats, mice and guinea pigs to investigate the toxic potential of FS 67, styrene-isoprene-styrene, and _____

The patch product, FS 67, containing 10% methyl salicylate _____ and 3% l-menthol _____ as the active ingredient was evaluated for the potential to induce skin irritation, sensitization, phototoxicity, and photosensitization in rabbits and guinea pigs. The safety profile of the excipients styrene-isoprene-styrene and _____ was evaluated in dogs, guinea pigs, rabbits, mice and rats. These studies were evaluated by María I. Rivera in November of 2001. Her review is attached in Appendix 1. The key findings of these studies are reproduced verbatim from that review below: b(4)

The preclinical studies suggest that FS 67A may cause "slight" skin irritation after a single exposure or a 14-day continuous exposure. FS 67A may not cause skin photoirritation and photosensitization after UV exposure. Whether exposure to the full UV Visible light spectrum may cause skin photoirritation or photosensitization was not determined.

The two excipients analyzed, SIS block copolymer and _____ did not show evidence of having significant skin toxicity. Studies were also conducted to address the potential systemic toxicity of these excipients after dermal application or oral administration. Both compounds did not produce major systemic toxicity. However, the Sponsor did not measure whether these excipients (or contaminating monomers) reached systemic circulation. In Toxicology Information Amendment N-010, the Sponsor stated that TK analysis was not possible due to technical difficulties related to the nature of the polymers (for details refer to Section IX of current review). High molecular weight polymers themselves are considered inert, the monomers and impurities resulting from the manufacturing process comprise the biological risk. This issue may not be of concern if the levels of contaminating monomers and impurities are negligible or kept within acceptable limits.

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l-Menthol was negative for the *in vitro* bacterial reverse mutation assay (Ames test), *in vitro* chromosome aberrations assay, both with and without metabolic activation, and micronucleus test.

B. Pharmacologic activity

Menthol, a cyclic terpene alcohol, is found naturally in the plant of the *Mentha* species, and demonstrates mild analgesic properties. The exact mechanism of action is not entirely clear, however, studies using the mouse hot-plate model of antinociception (thermal pain) and mouse abdominal constriction assay (chemical-induced pain) suggest that the antinociceptive effects of menthol may be mediated through selective activation of κ -opioid receptors (Galeotti, et al., 2002).

The perceived cooling sensation from menthol has been reported to be mediated via cold- and menthol-sensitive receptor-1 (CMR1) or transient receptor potential channel M8 (TRPM8) receptor (Rohacs, et al., 2005; Xing, et al., 2006; McKemy, et al., 2002; Tsuzuki, et al., 2004). CMR1 is a member of the transient receptor potential channel subfamily. Menthol functions as a chemical agonist of this thermally responsive receptor. Menthol interactions with this receptor result in the activation of a calcium-permeable channel, resulting in the release of Ca^{2+} from sensory neurons which in turn facilitate glutamate release and the modulation of neuronal transmission at sensory synapses.

Methyl salicylate is a nonsteroidal anti-inflammatory with analgesic and anti-inflammatory properties. The mechanism of action of methyl salicylate is thought to be mediated by inhibition of prostaglandin synthesis. Salicylate, the primary metabolite of methyl salicylate, inhibits the synthesis of prostaglandins by irreversibly acetylating and inactivating cyclooxygenase.

C. Nonclinical safety issues relevant to clinical use

The existing reproductive toxicology studies for methyl salicylate demonstrate evidence of skeletal anomalies and variations at all doses tested in the rat, although only the high dose produced statistically significant changes. These studies did not include a toxicokinetic evaluation of exposure nor do we have clear human pharmacokinetic exposures in order to provide a complete assessment of any potential safety margin for these changes. The sponsor should be asked to determine an exposure margin for these reproductive changes based upon additional pharmacokinetic studies in pregnant rats and in the clinical setting. As the lack of these data does not change the recommended pregnancy category, which will remain a C, these studies could be completed as a Phase 4 Commitment, if there are not other approvable issues in this cycle.

Carcinogenicity studies for l-menthol and methyl salicylate were not submitted with the NDA. Carcinogenicity studies are not required because the proposed drug product is not indicated for long-term use. The proposed indicated use of this product includes aches and pains associated with arthritis. It is recognized that arthritis is a chronic indication, and the potential exists that this product could be used in an intermittent manner that would exceed 6 month duration. However, following discussion with the Office of Non-Prescription Drug Products and the Associate Director of Pharmacology and Toxicology in the Immediate Office, the Agency determined that dermal carcinogenicity studies for both methyl salicylate and menthol will not be required for this drug product, based upon the extensive clinical experience with similar products.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-029 (FS-67 Topical Patch)
Review number: 1
Sequence number/date/type of submission: N 000/ March 1, 2006/NDA
Information to sponsor: Yes (X) No ()
Sponsor and/or agent: Hisamitsu Pharmaceutical Co., Inc.
 Tashiro Daikan-machi 408
 Tosu, Japan 841-0017
Manufacturer for drug substance: Methyl Salicylate: _____
 Japan
 I-Menthol: _____
 Japan

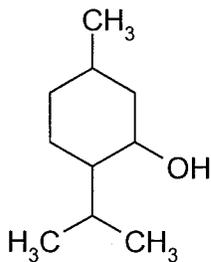
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Reviewer name: BeLinda A. Hayes, Ph.D.
Division name: Anesthesia, Analgesia & Rheumatology Products
HFD #: 170
Review completion date: November 28, 2006

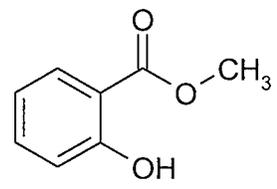
Drug:

Trade name: Salonpas _____ (proposed name)
Generic name: 10% Methyl Salicylate & 3% I-Menthol Patch
Code name: FS-67 Topical Patch
Chemical name:
 Methyl Salicylate: 2-Hydroxybenzoic acid methyl ester
 I-Menthol: 5-Methyl-2-(1-methylethyl)-cyclohexanol
CAS registry number: Methyl Salicylate: 119-36-8 I-Menthol: 2216-51-5
Molecular formula/molecular weight:
 Methyl Salicylate: C₈H₈O₃/152.15
 I-Menthol: C₁₀H₂₀O I-Menthol: /156.27
Structure:

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L-Menthol



Methyl salicylate

Relevant INDs/NDAs/DMFs:

There are no approved NDAs for either menthol or methyl salicylate listed either in Drugs@FDA or the Orange Book Online.

Although not approved under an NDA, there are several commercially available over-the-counter formulations that contain the combination of menthol and methyl salicylate, including the following (Nonprescription PDR; Clinical Pharmacology Online; Google):

<i>Drug Name</i>	<i>Menthol</i>	<i>Methyl salicylate</i>	<i>Indication</i>	<i>Company</i>
<u>Arthritis Hot® Pain Relief Creme</u>	10%	15%	For temporary relief of minor aches and pains of muscles and joints associated with: <ul style="list-style-type: none"> • arthritis • simple backache • strains • sprains 	G & W Laboratory, Inc.
<u>BenGay® Original Ointment</u>	16%	18.3%	Temporarily relieve minor aches and pains of muscles and joints associated with: <ul style="list-style-type: none"> • simple backache • arthritis • strains • bruises • sprains 	Pfizer Consumer Healthcare
<u>BenGay® Greaseless Pain Relieving Cream</u>	10%	15%		
<u>BenGay® Arthritis Formula Nongreasy Pain Relieving Cream</u>	8%	30%		
<u>BenGay® Ultra Strength NonGreasy Pain Relieving Cream (4% Camphor)</u>	10%	30%		
<u>BenGay® Menthol Vanishing Scent NonGreasy Pain Relieving Gel</u>	2.5%	--		
<u>BenGay® Pain Relieving Patch</u>	1.4%	--		
<u>BenGay® Ultra Pain Relieving Patch</u>	5%	--		
<u>Icy Hot® Extra Strength Pain Relieving Cream</u>	10%	30%	For temporary relief of: <ul style="list-style-type: none"> • minor arthritis pain, • sore muscles • bursitis • tendonitis • simple backache • strains • sprains • bruise • cramps 	Chatterm, Inc.
<u>Icy Hot® Chill Stick</u>				
<u>Icy Hot® Extra Strength Pain Relieving Cream</u>	10%	30%		
<u>Icy Hot® Extra Strength Medicated Patches</u>	5%	--		
<u>Icy Hot® Extra Strength Pain Relieving Balm</u>	7.6%	29%		
<u>Icy Hot® Maximum Strength Medicated Sleeve for Knee</u>	16%		Temporarily relieves minor pain associated with: <ul style="list-style-type: none"> • arthritis • bursitis • tendonitis • muscle strains • muscle sprains • bruises • cramps 	

Thera-Gesic Plus Topical Analgesic Creme	4%	25%	Temporary relief of minor aches and pains of muscles and joints associated with: <ul style="list-style-type: none"> • arthritis • simple backaches • strains • bruises • sprains 	Mission Pharmacal Company
Thera-Gesic® Maximum Strength Pain Relieving Cream	1%	15%		

IND#	Status	Division	Indication	Stamp Date	Sponsor
62,735	Active	170	Temporary relief of minor aches/pains of muscles/joints associated with simple backache/arthritis/strains/sprains	12-June-2001	Hitsamitsu Pharm

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
			3-Sept-2001	LOA supplied CMC Reviewer: Adequate
			11-May-2001	LOA supplied CMC Reviewer: Adequate
			10-Sept-2001	LOA supplied CMC Reviewer: Adequate
			16-May-2001	LOA supplied CMC Reviewer: Adequate
			30-Mar-2001	LOA supplied CMC Reviewer: Adequate
			27-Feb-2001	LOA supplied CMC Reviewer: Adequate
			07-Aug-2001	LOA supplied CMC Reviewer: Adequate
			11-Aug-2001	LOA supplied CMC Reviewer: Adequate
			22-Mar-2001	LOA supplied CMC Reviewer: Adequate

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Drug class: Analgesic combination product containing and NSAID and counterirritant.

Intended clinical population: The product is intended for patients requiring temporary relief of mild to moderate aches and pains of muscles and joints associated with arthritis, simple backache, strains, bruises, and sprains.

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Proposed Drug Substance Specifications for Methyl Salicylate				
Attributes	Method	Finished Products Specification	ICHQ3A Qualification Threshold	Acceptability
Related Substances	HPLC	NMT NMT NMT NMT NMT NMT	0.15% or 1.0 mg TDI, whichever is lower	Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable

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The — specified impurities that in the l-menthol drug substance technically exceed the ICHQ3A drug substance qualification threshold of NMT — instead of 0.15%. In the original

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NDA application, the sponsor did not provide any safety qualification for these impurities. Upon request for data to support the safety of these impurities, the sponsor notes that — these impurities are allowed as direct food additives under 21 CFR §172.515 (Synthetic flavoring substances and adjuvants). Acceptability as a flavoring agent alone does not provide adequate safety qualification, since the route of administration for this drug product is dermal rather than oral. Even though these impurities exceed ICH prescribed level, the potential risk should be minimal. The chemical structures of these impurities are closely related — (see images below), which was not genotoxic and these impurities would likely show comparable toxicity. There are no structural alerts for mutagenicity in — the — compounds, and the impurities have likely been in this product during preclinical and clinical studies, as well as in other currently marketed but unapproved drug products such as BenGay and IcyHot. Also, menthol has a long history of use following this route of administration.

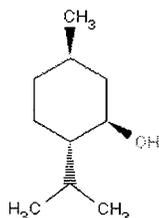
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Proposed Drug Substance Specifications for l-Menthol				
Attributes	Method	Finished Products Specification	ICHQ3A Qualification Threshold	Acceptability
Related Substances	GC	NMT NMT NMT NMT	0.15% or 1.0 mg TDI, whichever is lower	Acceptable Acceptable

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Levomenthol
L-Menthol
2216-51-5



As noted in the diagrams above, _____ l-menthol; therefore, the toxicological profile is not likely to be different from the drug substance.

The qualification thresholds for drug product impurities as per ICHQ3B(R2) and the proposed drug product specifications are listed in the table below:

Proposed Drug Product Specifications for FS-67 Topical Patch				
Attributes	Method	Finished Products Specification	ICH Q3B(R2)	Acceptability
Related Substances	HPLC	NMT NMT NMT	0.2% or 3.0 mg TDI, whichever is lower	Acceptable

b(4)

Although _____ is technically above the qualification threshold, this compound is an approved drug product and therefore does not pose any greater safety risk than the methyl salicylate. It is also a major metabolite of methyl salicylate and therefore is adequately qualified for safety.

Route of administration: Topical

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

[For (b)(2) applications:

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-029 are owned by Hisamitsu Pharmaceutical Co., Inc. or are data for which Hisamitsu Pharmaceutical Co., Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 22-029 that Hisamitsu Pharmaceuticals Co., Inc. 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 Hisamitsu Pharmaceuticals Co., Inc. 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 NDA 22-029.

Studies reviewed within this submission:

Genetic Toxicology Studies

- 15-32. A Bacterial Reverse Mutation Test for – Methyl Salicylate b(4)
- 15-33. A Chromosomal Aberration Test of Methyl Salicylate in Cultured Mammalian Cells (CHL/IU)
- 15-34. A Micronucleus Test of Methyl Salicylate in Rats
- 15-35. A Bacterial Reverse Mutation Test for l-Menthol
- 15-36. A Chromosomal Aberration Test of l-Menthol in Cultured Mammalian Cells (CHL/IU)
- 15-37. A Micronucleus Test of l-Menthol in Rats

Reproductive Toxicology Studies

- Final Report 40106. Reproductive and Developmental Toxicity Study of Methyl Salicylate in Rats by Subcutaneous Administration – Study of Fertility and Embryonic Development to Implantation b(4)
- Final Report 40108. Reproductive and Developmental Toxicity Study of Methyl Salicylate in Rats by Subcutaneous Administration – Study for Effects on Pre- and Postnatal Development, Including Maternal Function
- Final Report 40110. Reproductive and Developmental Toxicity Study of Methyl Salicylate in Rats by Subcutaneous Administration – Study for Effects on Embryo-Fetal Development
- Final Report 40113. Reproductive and Developmental Toxicity Study of Methyl Salicylate in Rabbits by Subcutaneous Administration – Study for Effects on Embryo-Fetal Development b(4)
- Final Report 40115. Reproductive and Developmental Toxicity Study of l-Menthol in Rats by Subcutaneous Administration

— Final Report 40117. Reproductive and Developmental Toxicity Study of l-Menthol in Rats by Subcutaneous Administration

b(4)

— Final Report 40122. Reproductive and Developmental Toxicity Study of l-Menthol in Rabbits by Subcutaneous Administration – Study for Effects on Embryo-Fetal Development

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Studies not reviewed within this submission:

The following studies have been previously reviewed by Dr. María I. Rivera for IND 62,735 (N000/Initial IND). Dr. Rivera's review has been appended to this document as Appendix 1.

General Toxicology Studies with FS-67

1540: A Primary Skin Irritation Study of FS-67-10.3 in Rabbits

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1541: A 14-Day Cumulative Skin Irritation Study of FS-67-10.3 in Rabbits

1542: A Skin Sensitization Study of FS-67-10.3 in Guinea Pigs

1543: A Skin Phototoxicity Study of FS-67-10.3 in Guinea Pigs

1544: A Skin Photosensitization Study of FS-67-10.3 in Guinea Pigs

Toxicology Studies with Styrene-Isoprene-Styrene

443: A Percutaneous Single Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Rats

4451: An Oral Single Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Beagle Dogs

1379A Primary Skin Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits

b(4)

1380: A 14-Day Cumulative Skin Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits

1378: A Primary Eye Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits

1381: A Skin Sensitization Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs

1382: A Skin Phototoxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs

1383: A Skin Photosensitization Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs

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4582: A 4-Weeks Percutaneous Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Rats with a Recovery Period of 4 Weeks

-4467: An Oral 4-Week Repeated Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Dogs with a 4-Week Recovery Period

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Toxicology Studies with Alicyclic Saturated Hydrocarbon Resin

-4446: A Percutaneous Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats

-80-20,21: The Acute Toxicity Test of

b(4)

-4567: An Oral Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats

-4452: An Oral Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Beagle Dogs

-80-001: The Primary Skin Irritation Test of

-80-002: The Cumulative Skin Irritation (3 Weeks) Test of

-1384: A Primary Eye Irritation Study of Alicyclic Saturated Hydrocarbon Resin in Rabbits

-80-7279: Allergenic Study of

-1503: A Skin Phototoxicity Study of Alicyclic Saturated Hydrocarbon Resin in Guinea Pigs

b(4)

-1389: A Skin Photosensitization Study of Alicyclic Saturated Hydrocarbon in Guinea Pigs

-4468: An Oral 4-Week Repeated Dose Toxicity Study of Alicyclic Saturated Hydrocarbon in Beagle Dogs with a 4-Week Recovery Period

-44568: A 4-Week Oral Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats with a Recovery Period of 4-Weeks

Genetic Toxicology Studies with Styrene-Isoprene-Styrene

-1046: A Reverse Mutation Test of Styrene-Isoprene-Styrene Block Copolymer Using Bacteria

b(4)

-1407: A Chromosomal Aberration Test of Styrene-Isoprene-Styrene Block Copolymer using CHL Cells

3B/84749/2: Microbial Metabolic Activation Test to Assess the Potential Mutagenic Effect of Resin A:

4A/841081: Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *In Vitro* and Treated with Resin A:

b(4)

1068: A Micronucleus Test of Alicyclic Saturated Hydrocarbon Resin in Mice

The following studies have been previously reviewed by Dr. María I. Rivera for IND 62,735 (N038/Toxicology Amendment). Dr. Rivera's review has been appended to this document as Appendix 2.

Genetic Toxicology Studies with Contained in the Patch Backing Cloth

19-37: A Bacterial Reverse Mutation Test

68-63: A Gene Mutation Assay in Mouse Lymphoma Cells

b(4)

19-38: A Chromosomal Aberration Test in Cultured Mammalian Cells

19-39: A Micronucleus Test in Rat Bone Marrow Cells

68-62: A Bacterial Reverse Mutation Test of FS-67 Backing Cloth

The following studies have been previously reviewed by Dr. María I. Rivera for IND NOTE: IND was submitted by Hisamitsu for a different drug product; however, these studies are owned by Hisamitsu and were submitted to this NDA. However, these studies were previously reviewed and were not reevaluated for this NDA. Dr. Rivera's summaries of these studies were included in her review of N038 for IND 62,735 has been appended to this document as Appendix 2. Dr. Rivera's assessment of the risk associated with these was described in her review, which is attached as Appendix 3.

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Genetic Toxicology Studies with the Patch Backing Cloth

19-29: A Bacterial Reverse Mutation Test

19-30: A Chromosomal Aberration Test in Cultured Mammalian Cells

19-31: A Micronucleus Test

b(4)

19-33: A Bacterial Reverse Mutation Test c _____

19-34: A Chromosomal Aberration Test _____

19-35: A Micronucleus Test _____

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The FS-67 topical patch contains two active ingredients, methyl salicylate and l-menthol. Methyl salicylate is an analgesic and anti-inflammatory compound. l-Menthol is a vasodilator and a mild analgesic substance. l-Menthol also induces coolness or sensation of freshness when applied to the oral cavity or skin. Both drug substances have a long history of use. They have been used in rubs, liniments, and patch for the relief of pain of muscular aches, neuralgia, rheumatism, arthritis, and sprains.

The sponsor did not perform any pharmacology studies on methyl salicylate or l-menthol. The sponsor submitted numerous articles from the publish literature, and therefore the NDA was submitted under Section 505(b)(2) of the FDC Act. Many of the articles submitted were not specifically related to the pharmacology of l-menthol or methyl salicylate. The following discussion was obtained from some of the references submitted by the sponsor or from a literature search performed by the reviewer.

2.6.2.2 Primary pharmacodynamics

l-Menthol

Primary pharmacodynamics: Menthol, a cyclic terpene alcohol, is found naturally in the plant of the *Mentha* species, and made synthetically. It possesses mild analgesic properties.

Mechanism of action: Studies evaluating menthol mode action have been published (Rohacs, et al., 2005; Xing, et al., 2006; McKemy, et al., 2002; Tsuzuki, et al., 2004; Galeotti, et al., 2002). Studies using the mouse hot-plate model of antinociception (thermal pain) and mouse abdominal constriction assay (chemical-induced pain) suggest that menthol analgesic effects may be mediated through selective activation of κ -opioid receptors (Galeotti, et al., 2002). In the standard mouse hot-plate model of antinociception, the oral administration of (-)-menthol (3-10 mg/kg) produced a dose-dependent increase in pain threshold. The antinociceptive effects of (-)-menthol was antagonized by both the unselective opioid antagonist naloxone and the selective κ -opioid antagonist nor-NBI. The μ antagonist (CTOP) and the δ_1 (7-bevzylidenenal-trexone) and δ_2 antagonist (naltriben) did not attenuate (-)-menthol antinociception. Similar results were found in the mouse abdominal constriction test. (-)-Menthol produced dose-dependent antinociceptive effects following oral (3-10 mg/kg) and intracerebroventricularly (10 μ g). Both the nonselective and selective κ -opioid antagonist prevented (-)-menthol antinociception.

The perceived cooling sensation from menthol has been reported to be mediated via cold- and menthol-sensitive receptor-1 (CMR1) or transient receptor potential channel M8

(TRPM8) receptor (Rohacs, et al., 2005; Xing, et al., 2006; McKemy, et al., 2002; Tsuzuki, et al., 2004). CMR1 is a member of the transient receptor potential channel subfamily. Menthol functions as a chemical agonist of this thermally responsive receptor. Menthol interactions with this receptor result in the activation of a calcium-permeable channel, resulting in the release of Ca^{2+} from sensory neurons which in turn facilitate glutamate release and the modulation of neuronal transmission at sensory synapses.

Drug activity related to proposed indication: The analgesic effect of l-menthol occurs, in part, through interaction with the κ -opioid receptor.

Methyl Salicylate

Primary pharmacodynamics: Methyl salicylate is a nonsteroidal anti-inflammatory with analgesic and anti-inflammatory properties.

Mechanism of action: The mechanism of action of methyl salicylate is thought to be mediated by inhibition of prostaglandin synthesis. Salicylate inhibits the synthesis of prostaglandins by irreversibly acetylating and inactivating cyclooxygenase.

Drug activity related to proposed indication: Relief of pain due to muscle aches and sprains.

2.6.2.3 Secondary pharmacodynamics

No Secondary Pharmacodynamic Studies or information from the literature were submitted in support of this NDA application.

2.6.2.3 Safety pharmacology

I-Menthol

No Safety Pharmacology Studies or information from the literature were submitted in support of this NDA application. The long history of use precludes the need for such studies.

Methyl Salicylate

No Safety Pharmacology Studies were submitted in support of this NDA application. The Safety Pharmacology information below is obtained from articles submitted with the NDA or online resource (AHFS Drug Information®, 2006).

Neurological effects: Significant neurological effects have not been associated with the use of salicylate.

Cardiovascular effects: Salicylate has no direct cardiovascular effects.

Pulmonary effects: "Salicylate may cause moderate to severe noncardiogenic pulmonary edema, principally with chronic or acute intoxication."

Renal effects: Significant renal toxicity has not been associated with use of salicylate.

Gastrointestinal effects: Adverse reactions to salicylates primarily involve the gastrointestinal tract. Salicylates can cause gastric mucosal irritation or damage. Symptoms associated with gastrointestinal (GI) irritation include GI disturbances (e.g., dyspepsia, heartburn, epigastric distress or nausea), GI bleeding, and/or mucosal lesions (i.e., erosive gastritis, gastric ulcers).

Abuse liability: Dependence does not occur with repeated use of methyl salicylate.

Otic System: Salicylate in large dosages and/or long-term therapy can cause tinnitus and hearing loss. Tinnitus and hearing loss are dose-dependent and reversible. Studies performed in animals suggest that salicylate-induced ototoxicity is due to actions on the inner ear and the central nervous system. Electrophysiologic and ultrastructural evidence in animals suggest that salicylate impairs the sensory hair of the cochlea (Didier, et al., 1993). In a study in which 11 healthy cats were treated with 200 mg/kg (i.p.) of sodium salicylate, a significant decrease in the spontaneous local field potential spindle frequencies in the auditory cortex were observed compared to the control group (Kenmochi and Eggermont, 1997).

2.6.2.5 Pharmacodynamic drug interactions

ADME information was obtained from literature references submitted by the sponsor.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not submitted by the sponsor.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The sponsor did not conduct any pharmacokinetic/toxicokinetic studies.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

Formal studies were not submitted.

Methyl salicylate: [¹⁴C]-Methyl salicylate is rapidly absorbed when applied to the skin of hairless mice (Maruta, et al., 1977). Serum levels of salicylates reached a peak at 2 to 4 hours after application. Approximately 40% of the dose was percutaneously absorbed at 48 hours post application.

In a study that compared the percutaneous absorption of commercially available topical products containing methyl salicylate or salicylate in rats, it was shown that salicylate was absorbed into the systemic circulation (Megwa, et al., 1995). Of particular relevant to this NDA, is the product Goanna rub (Herron Pharmaceutical Pty, Ltd. Queensland, Batch № 7257) which contains 10% methyl salicylate as the proposed Salonpas product. The salicylate concentration in plasma and underlying tissue was measured following the application of the Goanna rub (equivalent of 18.9 mg/cm of salicylic acid) to the abdominal skin of male Wistar rats. Appreciable amount of methyl salicylate was measured in top muscle layer on the treated site and in deep tissues on the treated and contralateral sides. NOTE: Goanna rub is not an FDA approved drug product.

2.6.4.4 Distribution

Formal studies were not submitted.

2.6.4.5 Metabolism

Formal studies were not submitted.

Methyl salicylate: Methyl salicylate is hydrolyzed to salicylate, an active metabolite. Salicylate is metabolized principally in the liver by the microsomal enzyme system. Salicylate is conjugated with glycine to form salicyric acid or conjugated with glucuronic acid to form salicyl phenolic glucuronide and salicyl acyl glucuronide.

Menthol: It is not know if menthol is metabolized in the skin. Metabolism of orally administered menthol in rats primary involves both glucuronidation and oxidation (Yamaguchi, et al., 1994). The proposed metabolic pathway in the rat is reproduced from Yamaguchi et al. below:

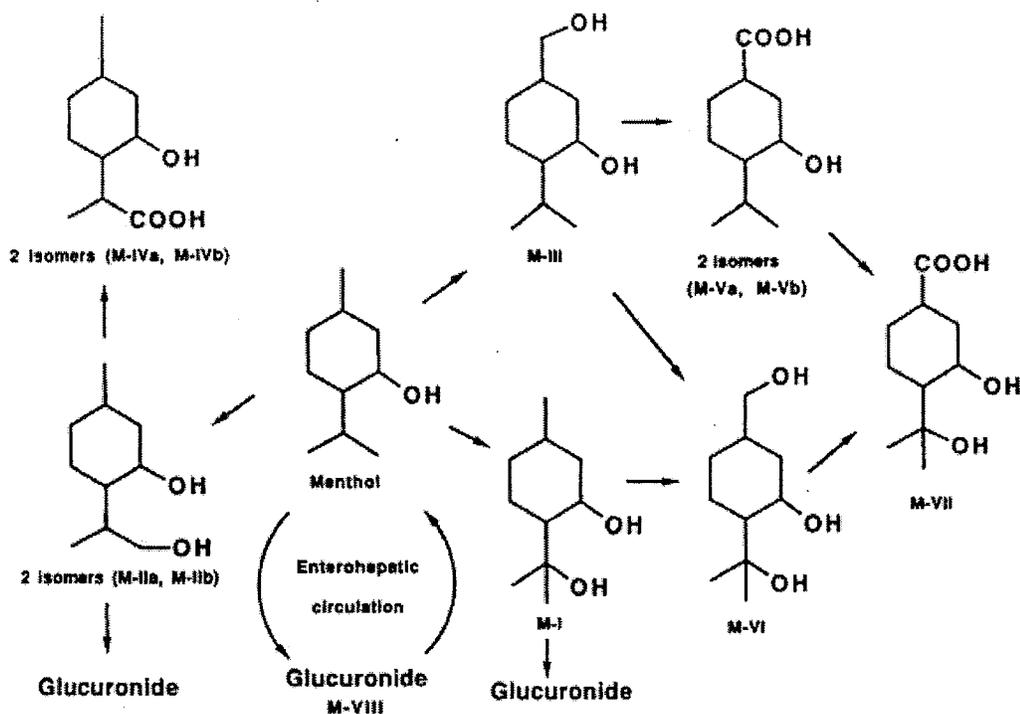


FIG. 9. Proposed metabolic pathways of l-menthol in the rat.

2.6.4.6 Excretion

Formal studies were not submitted.

Methyl Salicylate: Salicylate and its metabolites are rapidly and almost completely excreted in the urine.

Menthol: Following absorption, menthol is excreted in the urine and bile, primarily as the glucuronide salt. Metabolism and excretion studies have been reported in the published literature (Yamaguchi, et al., 1994). A single oral dose of [^3H]-l-menthol (500 mg/kg) was administered to intact and bile duct-cannulated male Fischer rats to evaluate the metabolic fate of l-menthol. Excreta were collected up to 48 hours post-dosing and the metabolites in urine and feces were characterized. At 48-hours, approximately 71% of the dose was recovered in the feces and urine of intact rats; approximately equal amount in the urine and feces. In the bile-duct cannulated male Fischer, 67% of the dose was recovered in the bile and 7% in the urine. Menthol glucuronide was the major biliary metabolite. The urinary metabolites were a series of mono- and dihydroxymenthols and carbonic acids. Menthol glucuronide was also detected in the urine.

2.6.4.7 Pharmacokinetic drug interactions

Formal studies were not submitted.

2.6.4.8 Other Pharmacokinetic Studies

Formal studies were not submitted.

2.6.4.9 Discussion and Conclusions

Methyl salicylate is rapidly and well absorbed from the skin. Following dermal application to the skin of hairless mice, peak serum levels were measured at 2-4 hours. Methyl salicylate is rapidly metabolized to salicylate in the liver and mainly excreted in urine.

Menthol is absorbed from the skin following dermal application. Metabolism of orally administered menthol involves both glucuronidation and oxidation. The metabolites are excreted in the urine and bile, primarily as the glucuronide salt.

2.6.4.10 Tables and figures to include comparative TK summary

No formal tables submitted by sponsor.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

The sponsor did not supply tabulated summaries of pharmacokinetic data.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: The sponsor did not conduct any general toxicology studies with the active constituents l-menthol or methyl salicylate. However, some general toxicology studies were conducted with the FS- 67 patch and two novel excipients to support their safe use in the drug product. These studies were reviewed by Dr. Rivera and are summarized in the tables below:

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Table 1. Studies Performed with FS-67

Study Type	Species/ Strain	Dosing Route	Duration of Dosing	Dose	Study Number
Irritation/Sensitization Studies					
Primary Skin Irritation	Rabbits	Topical	24 hours	6.25 cm ² Strips	1540
Skin Sensitization	Guinea Pigs	Topical	Once/week	4.0 cm ² Strips	1542
Phototoxicity	Guinea Pigs	Topical	One hour	2.25 cm ² Strips	1543
Photosensitization	Guinea Pigs	Topical	5 days	8.0 cm ² Strips	1544
Repeated Dose Studies					
14-Day Study	Rabbits	Topical	14 days	6.25 cm ² Strips	1541

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Table 4. Summary of Toxicology Studies for SIS Copolymer

Study Type	Species/ Strain	Dosing Route	Duration of Dosing	Dose (mg/kg)	Study Number
Single Dose Toxicity Studies					
Dermal Toxicity	Rats	Topical	24 hours	10% body surface	4443
Oral Toxicity	Dogs	Oral	Once	2000	4451
Irritation/Sensitization Studies					
Skin Irritation	Rabbits	Topical	24 hours	6.25 cm ² Strips	1379
Eye irritation	Rabbits	Eye	Once	Strip	1378
Sensitization	Guinea Pigs	Topical	Once/week	4.0 cm ² Strips	1381
Phototoxicity	Guinea Pigs	Topical	One hour	2.25 cm ² Strips	1382
Photosensitization	Guinea pigs	Topical	5 days	8.0 cm ² Strips	1383
Repeated Dose Toxicity Studies					
4-Week Dermal	Rats	Topical	4 weeks	Up to 10% body surface	4582
14-Day Dermal	Rabbits	Topical	14 days	6.25 cm ² Strips	1380
4-Week Oral	Dogs	Oral	4 weeks	80, 400 and 2000	4467
Mutagenicity Studies					
Ames Test	<i>S. typhi</i> and <i>E. coli</i>	In Vitro (extract)		Up to 5000 µg/plate	1046
In Vitro Cytogenetics	CHL cells	In Vitro (extract)	6, 24, & 48 hours	Up to 160 µg/mL	1047
Micronucleus	Mice	IP with extract	Once	50 mL/kg	1133

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Table 5. Summary of Toxicology Studies for

Study Type	Species/ Strain	Dosing Route	Duration of Dosing	Dose (mg/kg)	Study Number
Single Dose Toxicity Studies					
Dermal Toxicity	Rats	Topical	24 hours	2000	4446
Oral Toxicity	Rats	Oral	Once	5000 and 10,000	80-20,21
Oral Toxicity	Rats	Oral	Once	500 to 2000	4567
Oral Toxicity	Dogs	Oral	Once	2000	4452
Irritation/Sensitization Studies					
Skin Irritation	Rabbits	Topical	4 hours	0.5 mL of 10% sol'n	80-001
Eye irritation	Rabbits	Eye	Once	0.1 mL	1384
Sensitization	Guinea Pigs	Intradermal Topical	Once Once	0.05 mL of 10% sol'n	80-7279
Phototoxicity	Guinea Pigs	Topical	One hour	50 mg	1503
Photosensitization	Guinea pigs	Topical	5 days	100 mg	1389
Repeated Dose Toxicity Studies					
3-Week Dermal	Rabbits	Topical	3 weeks	0.25 mL	80-002
4-Week Oral	Rats	Oral	4 weeks	250, 500 and 1000	4568
4-Week Oral	Dogs	Oral	4 weeks	80, 400 and 2000	4468
Mutagenicity Studies					
Ames Test	<i>S. typhi</i> and <i>E. coli</i>	In Vitro		Up to 5000 µg/plate	3B/84749/2
In Vitro Cytogenetics	CHO cells	In Vitro (Extract)	2 & 20 hours	Up to 10 µL/mL	4A/841081
Micronucleus	Mice	Oral	Once	500, 1000 and 2000	1068

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Genetic toxicology: A standard battery of genetic toxicology studies was completed for the active substances, l-menthol and methyl salicylate, in the FS 67 patch. l-Menthol tested negative in the *in vitro* bacterial reverse mutation assay (Ames test) at concentrations of 15.6, 31.3, 62.5, 125, 250, and 500 µg/plate. l-Menthol also tested negative for the induction of structural and numerical chromosome aberrations in the *in*

in vivo chromosome aberration test using Chinese hamsters fibroblastic cell lines. In the *in vivo* rat micronucleus test, l-menthol also was negative for DNA damage.

The genotoxic potential of methyl salicylate was evaluated in these same batteries of genetic toxicology studies. Methyl salicylate was negative in the *in vitro* Ames test at concentrations of 46.9, 93.8, 187.5, 375, 750, and 1500 µg/plate. When evaluated for its clastogenic potential in the *in vivo* chromosome aberration test using Chinese hamster fibroblastic cell lines, methyl salicylate tested negative for the induction of structural and numerical aberrations. Methyl salicylate also tested negative for clastogenic potential in the *in vivo* rat micronucleus test.

The standard battery of genetic toxicology studies were also conducted with the novel excipients, SIS and _____ contained in the backing cloth. These studies were reviewed by Dr. María Rivera. The genetic toxicology studies performed with the novel excipients were listed above. The genetic toxicology studies conducted with _____ in the backing cloth are summarized below:

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Table 7. Summary of Mutagenicity Studies with [redacted] the Backing Cloth

Study Type	Species/ Strain	Dosing Route	Duration of Dosing	Dose (mg/kg)	Study Number
Studies with [redacted]					
Ames Test	<i>S. typhi and E. coli</i>	In Vitro		Up to 5000 µg/plate	19-29
In Vitro Cytogenetics	CHL cells	In Vitro	6, 24, & 48 hours	Up to 2893 µg/mL	19-30
Micronucleus	Rats	IP	Twice	500, 1000 and 2000	19-31
Studies with [redacted]					
Ames Test	<i>S. typhi and E. coli</i>	In Vitro		Up to 5000 µg/plate	19-33
In Vitro Cytogenetics	CHL cells	In Vitro	6, 24, & 48 hours	Up to 3313 µg/mL	19-34
Micronucleus	Rats	IP	Twice	500, 1000 and 2000	19-35
Studies with [redacted]					
Ames Test	<i>S. typhi and E. coli</i>	In Vitro		Up to 5000 µg/plate	19-37
In Vitro Cytogenetics	CHL cells	In Vitro	6, 24, & 48 hours	Up to 5000 µg/mL	19-38
Mouse Lymphoma	L5178Y mouse lymphoma cells	In Vitro	Short term 24 hours	Up to 3000 µg/mL Up to 37 µg/mL	68-63
Micronucleus	Rats	IP	Twice	500, 1000 and 2000	19-39
Studies on Extracts of Backing Cloth					
Ames Test	<i>S. typhi and E. coli</i>	In Vitro (extract)		Up to 100% of DMSO extract	68-62

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SIS copolymer and [redacted] "In general, the assays did not show the excipients have a strong genetic toxicity risk. Limitations were found in two of the studies: Ames

test for SIS copolymer, and *in vivo* micronucleus assay. Technical difficulties were encountered with solubility or with measuring systemic exposure, respectively. Taking into consideration the topical route intended for administration into humans, and the high molecular weight of these polymers, it is not expected that these excipients *per se* may pose a genotoxic risk. As indicated above for systemic toxicity, "levels of contaminating monomers above regulatory standards may pose a risk."

The FS-67 patch contains [redacted] in the patch backing cloth not previously used in US marketed drug product [redacted]

[redacted] Genotoxicity studies were conducted with these [redacted] and were reviewed by Dr. María I. Rivera ([redacted] FS 67A, N-038/8-3-05/Toxicology Information Amendment). Her review is attached in Appendix 2. Her conclusions are reproduced verbatim from that review.

"The results of the Ames test and MLA suggest that the [redacted] may have potential to be mutagenic in humans. Two other assays, the chromosomal aberration in CHL/IU cells and micronucleus test in rats, were negative. Given the difference in sensitivity between assays, the two negative assays do not negate a potential risk to humans. [redacted] increased the frequency of polyploid cells without inducing structural aberrations in CHL/IU cells. Although the toxicological relevance of this finding is not clear (given the absence of structural aberrations and the negative results in the *in vivo* micronucleus test), an increase in polyploidy may indicate a potential to induce aneuploidy. Aneuploidy is a frequent cause of mental retardation, congenital malformations, and abortions in humans and has been recognized as involved in the process of carcinogenesis.

[redacted] was negative in all assays. However, as stated below, the NTP and CCRIS databases contain positive genotoxic results for this [redacted]

In all *in vitro* assays submitted in the current amendment, all [redacted] precipitated at very low concentrations. Therefore, except for [redacted] which showed positive results in 2 *in vitro* assays, it is not known if a sufficient amount of the [redacted] accumulated intracellularly and subsequently, was able to reach the DNA. In other words, the negative results may reflect low intracellular exposure.

In order to prove that no mutagens are eluded from the backing cloth, the sponsor conducted an Ames Test using a DMSO extract of the FS-67 backing cloth. The DMSO extract was negative in the Ames test. The sponsor did not quantitate the content of [redacted] in the extract used in the Ames test."

Carcinogenicity: The sponsor did not conduct any carcinogenicity studies. The information below was obtained from a reference article submitted with the NDA.

Carcinogenicity - Menthol.

The carcinogenic potential of dl-menthol was evaluated in Fischer 344 rats and B6C3F1 mice by the Carcinogenesis Testing Program of the National Cancer Center of NIH (DHEW Publication No. (NIH) 79-1348, 1978). The rats (n=50/dose/sex) were administered dl-menthol in their feed at doses of 3,750 (188 mg/kg/day) and 7,500 (375 mg/kg/day) ppm and mice (n=50/dose/sex) were administered dl-menthol in their feed at doses of 2,000 (334 mg/kg /day) and 4,000 (667 mg/kg/day) ppm for 103 weeks. The data suggested that under the condition of the study, dl-menthol was not carcinogenic in either Fischer 344 rats or B6CF1 mice. No carcinogenic effects were observed in any organs of both male and female rats. Also, there were no treatment-related effects on survival rate. In male mice, an increase incidence of hepatocellular carcinoma was observed in the high dose group (14/48, 29%) compared to the control group (8/47, 17%). An increase incidence of alveolar/bronchial adenoma or carcinoma was observed in the females in the high dose group (5/48, 10%) relative to the control group (1/49, 2%). In both rats and mice, these incidences were not considered to be treatment-related because they were not statistically significant and were within the range of the laboratory historical-control groups.

Reproductive toxicology: Reproductive studies were completed in support of the FS 67 patch. The standard battery has been completed for both active substances, l- menthol and methyl salicylate.

Reproductive toxicology - Menthol:

In the segment I study in rats (fertility and embryonic development), l-menthol was administered to males for 2 weeks prior to mating and continued throughout mating until one day prior to euthanasia. Female rats were treated for a total of 14 days prior to mating, throughout mating and through gestation day 6. The results indicated that reproductive performance in males and females was not altered by l-menthol treatment under the conditions tested. l-Menthol had no effects on mean epididymal sperm count, sperm production rate, and sperm motility or morphology compared to control. The NOAEL for maternal toxicity was < 100 mg/kg/day based on the body weight. Based upon the presence of maternal toxicity (i.e., reduction in body weight), the study is considered valid.

The definitive segment II (embryofetal development) study was completed in the rabbit model. Female rabbits were treated with l-menthol (150, 300, and 600 mg/kg/day) from gestation day 6 to gestation day 18. Two dams aborted. One dam in the mid-dose group aborted on gestation day 29, and one dam aborted in the high-dose group on gestation day 27. Significant reduction in body weight gain was observed in all l-menthol treatment groups. There was no difference in mean fetal weight in the treatment groups compared to the control animals. l-Menthol was not teratogenic under the conditions tested; there were no significant malformations (external, visceral or skeletal) or variations between treatment groups. The NOAEL for maternal toxicity was not established and should be considered to be < 150 mg/kg/day based upon reduction in body weight gain. Based upon the presence of maternal toxicity, the study is considered valid. The NOAEL for fetal development was not established.

In a definitive segment III study in rats, female rats (F₀) were treated with l-menthol (100, 300, and 1000 mg/kg/day) from gestation day 6 to gestation day 21 of lactation. Results indicated that there was a significant reduction in mean body weight gain in the F₀ females in all l-menthol treatment groups throughout the gestation period.

Developmental landmarks in the F₁ males indicated that balanopreputial separation in males in the 100, 300, and 1000 mg/kg/day groups were delayed compared to the control. No treatment-related effects on the development of the F₁ pups were observed. There were no treatment-related effects in the test for function, emotional behavior, motor coordination, or learning ability. Reproductive performance in the F₁ generation was not altered by F₀ generation l-menthol treatment in any dose level tested.

Reproductive toxicology – Methyl Salicylate:

In the fertility and embryonic development study, methyl salicylate (30, 100, and 300 mg/kg/day) was administered subcutaneously to male rats for 2 weeks prior to mating and continued throughout mating until one day prior to euthanasia. Female rats were treated for a total of 14 days prior to mating, throughout mating and through gestation day 6. Reproductive performance in males and females were unchanged by methyl salicylate treatment under the conditions tested. However, early embryonic development was significantly altered by methyl salicylate treatment. Specifically, the mean number of corpora lutea was significantly reduced only in the mid dose group (100 mg/kg/day). There were no significant changes in the number of viable embryos and dead embryos. The NOAEL for maternal toxicity was 100 mg/kg/day based on depression of body weight gain.

Embryofetal development was evaluated in both rats and the rabbit models. Female rats were treated with methyl salicylate (50, 100, and 200 mg/kg/day) from gestation day 6-17 in the definitive segment II study. No treatment-related mortalities or overt clinical signs were observed. Methyl salicylate was teratogenic; there were significant malformations (external and skeletal) between the high-dose group and control group. A slight increase in the number of live fetuses with craniorhachischisis and gastroschisis were observed. Developmental toxicity was noted in the high-dose group based upon decreased mean fetal body weight. Therefore, the NOAEL for developmental toxicity was 100 mg/kg/day. A NOAEL for maternal toxicity was 100 mg/kg/day based on the reduction in body weight gain in the high-dose group.

In the definitive segment II study in the rabbit, female rabbits were treated with methyl salicylate from gestation day 7 through gestation day 18. One dam aborted in the high-dose group. No treatment-related effects on mean body weight or body weight gain were observed. In contrast to the results observed in rats, methyl salicylate was not teratogenic in rabbits. No significant effects on fetal development were noted. Also, no significant treatment-related effects on the incidence of fetal malformations were observed. The NOAEL for maternal toxicity was 100 mg/kg/day based on the one incidence of abortion in the high-dose group. A NOAEL for fetal toxicity was < 30 mg/kg/day.

Prenatal and postnatal development was evaluated in female rats (F₀) treated subcutaneously with methyl salicylate (20, 60, and 200 mg/kg/day) from gestation day 6 to lactation day 21. Two females in the high-dose group died prior to the scheduled necropsy. One female exhibited vaginal hemorrhaging prior to her death. Upon necropsy, it was revealed that there were 14 dead fetuses in her uterus. No clinical signs were observed in the females that survived until scheduled necropsy. Mean body weight gain in the F₀ females were significantly reduced during the gestation period. In surviving F₀ females, there were no significant differences in the mean litter size, mean number of live fetuses, mean number of dead fetuses, and number of implantations between groups. However, the birth index was significantly decreased in the F₁ generation born to the high-dose F₀ females. Offspring mean body weight during lactation and maturation periods were significantly reduced in the 200 mg/kg/day treatment groups compared to the control. Developmental landmarks in the F₁ males indicated that balanopreputial separation in males in the 200 mg/kg/day group was delayed compared to controls. There were no differences in the mean acquisition of vaginal patency in F₁ females between treatment groups. Males and females in the high-dose group exhibited a delay in incisor eruptions. There were no treatment-related effects of methyl salicylate in neurobehavioral evaluation (rotarod performance, water maze, and open field) and function test (righting reflex, ipsilateral flexor, visual pacing reflex, and Preyer's reflex). Methyl salicylate was teratogenic. A significant increase incidence in skeletal anomalies (fusion of cervical vertebra and misshapen sternebra), skeletal variations (full supernumerary ribs, accessory lumbarization, 7 lumbar vertebra), and incomplete ossification of the cervical, thoracic and lumbar vertebrae were observed in F₁ pups in the high-dose group. Also, there was a slight increase in the incidence of fetuses with variations (cervical rib, incomplete ossification of thoracic and caudal vertebrae); however these were not statistically significant. Reproductive performance in the F₁ generation was not altered by F₀ generation methyl salicylate treatment at any dose level. Maternal NOAEL was 60 mg/kg based upon the depression of body weight gain, decrease food consumption, and reduction in the gestation index in the F₀ females in the 200 mg/kg/day group. Fetal NOAEL was < 60 mg/kg based upon decrease birth index, growth suppression and increase incidence of skeletal anomalies and variation in the F₁ offspring of the high-dose group and the increase in the incidence of skeletal variations in F₁ offspring in the low-dose group.

Special toxicology: Acute, subacute, genotoxic, skin phototoxicity and skin sensitization studies were performed with styrene-isoprene-styrene (SIS) and salicyclic saturated hydrocarbon resin (————), two excipients, to establish their safety as components in the drug product. These studies were reviewed by Dr. María I. Rivera (IND 62,735 FS 67A, N-000, N-009, N-010, N-011). Her review is attached in Appendix 1. Dr. Rivera's conclusions are reproduced verbatim from the reviews.

“The two excipients analyzed, SIS block copolymer and ———— did not show evidence of having significant skin toxicity. Studies were also conducted to address the potential systemic toxicity of these excipients after dermal application or oral administration. The findings were consistent with both excipients having low potential to produce systemic toxicity; the HEDs used were at least 100 fold

higher than the expected dose if only one patch is used. However, the Sponsor did not provide any PK or TK data to demonstrate systemic exposure to these polymers (or contaminating monomers).”

Dermal Toxicity – Methyl Salicylate

Published data in the scientific literature indicates that methyl salicylate is not a skin irritant in rats (Arts, et al., 1998; Arts, et al., 1997). To determine whether methyl salicylate possess sensitizing properties, Brown Norway rats received 150 µl of 5% methyl salicylate on each shaved flank (approximately 12 cm²). Seven days after the first application, 75 µl (at 50% of the initial concentration) was applied on the dorsum of both ears of each rat. Blood was drawn on days 8 and 14 to measure serum IgE levels. Methyl salicylate did not change serum IgE levels. Arts and colleagues reported similar findings in 1998. Using a similar experimental design in Brown Norway and Wistar rats, dermal application of methyl salicylate did not produce any skin irritation or reaction and the serum level of IgE was not increased at day 20 or 21.

2.6.6.2 Single-dose toxicity

The sponsor did not conduct any single dose toxicity studies with the individual active drug substances. The sponsor did conduct an acute dermal irritation study and skin sensitization study with the drug product formulation FS-67 patch. These studies were previously reviewed by Dr. Rivera for the IND.

2.6.6.3 Repeat-dose toxicity

The sponsor did not conduct any repeat-dose toxicity studies with the individual active drug substances. The sponsor did conduct a 14-day repeat dose toxicology study with the drug product formulation FS-67 patch, which was previously reviewed by Dr. Rivera for the IND.

2.6.6.4 Genetic toxicology

Genetic Toxicology Studies with l-Menthol

Study title: A Bacterial Reverse Mutation Test of l-Menthol

Key findings: l-Menthol was evaluated in the Ames Reverse Mutation Assay at concentrations of 15.6, 31.3, 62.5, 125, 250, and 500 µg/plate. l-Menthol at a concentrations \geq 250 µg/plate was cytotoxic. Under the conditions of the study, l-menthol was negative in the bacterial reverse mutation assay.

Study no. 15-35

Volume #, and page #: 11, 001

Conducting laboratory and location: _____

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Date of study initiation: November 5, 2001

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: l-Menthol, lot # 55-050, 100%

Methods

Strains/species/cell line: *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 and *Escherichia coli* WP2uvrA

Doses used in definitive study: 15.6, 31.3, 62.5, 125, 250, and 500 µg/plate.

Basis of dose selection: A dose-range finding study was conducted to select the appropriate doses to use in the mutagenicity assay.

Negative controls: DMSO

Positive controls: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), N-ethyl-N-nitro-N-nitrosoguanidine (ENNG), 9-aminoacridine (9AA), and 2-aminoanthracene (2AA) were selected for positive controls based on the bacterial strain as indicated in the following table:

Test Strain	Positive Control Substance (µg/plate)	
	-S9	+S9
TA100	AF2 (0.01)	2AA (1.0)
TA1535	ENNG (5.0)	2AA (2.0)
TA98	AF-2 (0.1)	2AA (0.5)
TA1537	9AA (80)	2AA (2.0)
WP2uvrA	ENNG (2.0)	2AA (10.0)

Incubation and sampling times: The mutagenicity test was performed according to the plate-incorporation procedures. The S9 metabolic activator, the tester strain, and the test article were combined in molten agar which is overlaid onto the minimal agar plate. The agar plate was incubated at 37°C for 48 hours. The vehicle control, positive control and all doses of the test articles were plated in duplicates.

Analysis:

- > Mutation frequencies: Expressed as mean number of revertants per plates. Revertants colonies were measured twice per plate and the means of these values were the measured value.

- Cytotoxicity: Background lawn was observed macroscopically or with a stereoscope (x 40)
- Counting Method: The number of revertant colonies was counted manually.

Criteria for positive results: “The results were judged positive (+) if the mean number of revertant colonies per plate increased two-fold or more compared to that of the negative control and dose-dependency was found; and if the results showed reproducibility between the dose finding test and the main test.”

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears to be valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced reliable results.

Study outcome: l-Menthol was evaluated at the following concentrations: 15.6, 31.3, 62.5, 125, 250, and 500 µg/plate in the mutagenicity assay. In the mutagenicity assay, the positive controls induced mutation frequencies as expected; the mean revertants per plate were within historical control data for the laboratory. l-Menthol did not induce mutation frequencies in any of the *Salmonella typhimurium* strains (TA987, TA100, TA1535 or TA1537) or the WP2*uvrA* in the presence or absence of the metabolic activator; no increase in revertants was observed at any dose. However, there was cytotoxicity (killing of the bacterial strain) at ≥ 250 µg/plate in TA98, TA100, and TA1537, and at 500 µg/plate in WP2*uvrA* without metabolic activation. Cytotoxicity was also observed in all tester strains at 500 µg/plate in the presence of metabolic activation.

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Study title: A Chromosomal Aberration Test of l-Menthol in Cultured Mammalian Cells (CHL/IU)

Key findings: l-Menthol showed no evidence of mutagenic potential in the chromosomal aberration assay. l-Menthol did not induce structural or numerical chromosomal aberrations in the Chinese hamster fibroblast cell lines in the presence or absence of metabolic activation.

Study no. — 15-36

Volume #, and page #: 11,027

Conducting laboratory and location: _____

Date of study initiation: November 5, 2001

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: l-Menthol, Lot №. 55-050, 100% pure

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Methods

Strains/species/cell line: Chinese lung hamster fibroblast cell lines (CHL/IU)

Doses used in definitive study:

Short-term treatment (6 hours) with and without metabolic activation: 173.6, 208.3, 250, 300, and 360 µg/mL

Continuous treatment for 24-hours: 49.4, 74.1, 111.1, 166.7, and 250 µg/mL

Continuous treatment for 48 hours: 144.7, 173.6, 208.3, 250, and 300 µg/mL

Basis of dose selection: It was based on a cell growth inhibition test at concentrations of 24.4, 48.8, 97.5, 195, 390, 780, and 1560 µg/mL for all treatment conditions. The 50% growth inhibition was estimated to be 323 µg/mL in short-term treatment without metabolic activation, 333 µg/mL in short-term treatment with metabolic activation; and 220 µg/mL and 259 µg/mL in continuous treatment for 24 hours and 48 hours, respectively. The highest concentration selected for the chromosomal aberration test was determined by taking the 50% growth inhibition dose as an index.

Negative controls: Vehicle (DMSO)

Positive controls: Mitomycin C (MMC) and Benzo(a)pyrene (B(a)P) were positive controls for the chromosomal aberration test. In the short-term treatment without metabolic activation, and for the continuous for 24 and 48 hour, MMC was used as positive control after dissolution in physiological saline to a fixed concentration (0.15 µg/mL). B(a)P served as the positive control for the short-term treatment with metabolic activation after dissolution in DMSO to a fixed concentration (20 µg/mL).

Incubation and sampling times: The cell suspensions were incubated for 72 hours, after which the cells were harvested using 0.25% trypsin solution at 37°C. The cells were

suspended at 1×10^4 cells per mL in the culture medium and the Petri dishes were seeded with 5 mL of the cell suspension and incubated for 72 hours. After the 72 hour incubation period, 2.5 mL of the culture medium was removed and 0.5 mL of the culture medium was added for the short-term test without metabolic activation and with metabolic activation. The test solution, positive control and negative control were added to the medium and incubated for 6 hours. For the continuous treatment test, after the 72 hour incubation period, the test solution, the negative control, and the positive control was added to the Petri dish and incubated for 24 or 48 hours.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): According to the sponsor, the results were judged positive if: 1) the frequency of cells with a structural or numerical aberrations were significantly higher in the test article treatment groups than in the negative control group, and 2) dose-dependency or reproducibility could be conformed. *Counting Method*– Under blind condition, 100 well-spread metaphases per slide (200 metaphase per dose) were analyzed. Chromosomal aberrations were recorded as structural or numerical aberrations. Cells with 25 ± 2 chromosomes were examined for structural aberrations. *Validity*– the study appears valid as the appropriate positive controls were used, an acceptable number of mitotic cells were evaluated and the protocol was consistent with those described in the OECD Guideline 473: *In vitro* Mammalian Chromosome Aberration Test.

Study outcome: Results from the definitive chromosomal aberration tests are presented in the sponsor's tables 2 and 3 reproduced on the following pages. In comparison to the negative control, l-menthol was void of significant clastogenic activity in the short-term test in both the absence and presence of metabolic activation (sponsor's table 2). In the absence of metabolic activity, the percentage of cells with structural aberrations following exposure to l-menthol at concentrations of 173.6 $\mu\text{g/mL}$, 208.3 $\mu\text{g/mL}$, and 250 $\mu\text{g/mL}$ was 1.0%, 1.0%, and 2.5%, respectively. At the two highest concentrations (300 and 360 $\mu\text{g/mL}$), l-menthol was cytotoxic.

In the presence of metabolic activation, the percentage of cells with structural aberrations following exposure to l-menthol at concentrations of 173.6 $\mu\text{g/mL}$, 208.3 $\mu\text{g/mL}$, 250 $\mu\text{g/mL}$, 300 $\mu\text{g/mL}$, and 360 $\mu\text{g/mL}$ was 0.5%, 0.5%, 1.5%, 1.5%, and 2.5%, respectively. Twenty-two percent of the cells treated with the positive control (B(a)P had structural aberrations.

As indicated in the sponsor's table 3, l-menthol also did not display any clastogenic activity in the continuous treatment assay in the presence or absence of metabolic activation. In comparison to the negative control, the frequency of cells with structural aberrations was in the range of 0.0 – 1.0% and 0.0% - 1.5% for the 24 hours and 48 hours treatment, respectively. Dose-dependent cytotoxicity was observed in both the 24 and 48 hours treatment.

Table 2 Results of Chromosomal Aberration Test (Short-term Treatment)

15-36

Treatment time (h)	S 9 Mts	Dose levels (µg/ml)	No. of cells observed	Number of cells with structural aberrations (frequency %)						Number of gaps	Cell Proliferation Ratio (%)	Number of cells with numerical aberrations (frequency %)				
				Chromatid break	Chromatid exchange	Chromosome break	Chromosome exchange	Others	Total			No. of cells observed	Polyploidy	endo-reduplication	Total	
6 -- 18	-	Negative control (DMSO)	100	0	0	0	0	0	0	0	100	100	0	0	0	
			100	0	0	0	0	0	0	0		0	100	0	0	0
			200	0	0	0	0	0	0	0		0	200	0	0	0
6 -- 18	-	173.6	100	0	0	0	0	0	0	0	92.2	100	0	0	0	
			100	2	0	0	0	0	2	1 (0.5)		0	200	0	0	1 (0.5)
			200	0	0	0	0	0	0	0		0	100	0	0	0
6 -- 18	-	208.3	100	0	0	0	0	0	0	0	82.6	100	0	0	0	
			100	1	0	0	0	0	1	1 (1.0)		0	200	0	0	2 (1.0)
			200	1	0	0	0	0	1	1 (1.0)		0	100	0	0	0
6 -- 18	-	250	100	0	0	0	0	0	0	0	59.1	100	0	0	0	
			100	2	0	0	0	0	2	2 (2.5)		0	200	0	0	3 (1.5)
			200	2	0	0	0	0	2	2 (2.5)		0	100	0	0	0
6 -- 18	-	300	TOX	---	---	---	---	---	---	---	16.5	TOX	---	---	---	
			---	---	---	---	---	---	---	---		---	---	---	---	
			---	---	---	---	---	---	---	---		---	---	---	---	
6 -- 18	-	360	TOX	---	---	---	---	---	---	---	9.6	TOX	---	---	---	
			---	---	---	---	---	---	---	---		---	---	---		
			---	---	---	---	---	---	---	---		---	---	---		
6 -- 18	-	Positive control (MMC) 0.15	100	4	18	0	0	0	19	0	65.2	100	0	0	0	
			100	3	18	0	0	0	18	0		200	0	0	0	
			200	7	36	0	0	0	37 (18.5)	0		100	0	0	0	
6 -- 18	+	Negative control (DMSO)	100	0	0	0	0	0	0	0	100	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
6 -- 18	+	173.6	100	0	0	0	0	0	0	0	97.0	100	0	0	0	
			100	1	0	0	0	0	1	1 (0.5)		0	200	0	0	1 (0.5)
			200	0	0	0	0	0	0	0		0	100	0	0	0
6 -- 18	+	208.3	100	0	0	0	0	0	0	0	88.1	100	0	0	0	
			100	1	0	0	0	0	1	1 (0.5)		0	200	0	0	1 (0.5)
			200	1	0	0	0	0	1	1 (0.5)		0	100	0	0	0
6 -- 18	+	250	100	0	0	0	0	0	0	0	67.8	100	0	0	0	
			100	1	0	0	0	0	1	1 (1.5)		0	200	0	0	3 (1.5)
			200	1	0	0	0	0	1	1 (1.5)		0	100	0	0	0
6 -- 18	+	300	100	0	0	0	0	0	0	0	43.4	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
6 -- 18	+	360	100	1	1	0	0	0	2	0	37.8	100	0	0	0	
			100	1	2	0	0	0	3	3 (2.5)		0	200	0	0	3 (1.5)
			200	2	3	0	0	0	5	5 (2.5)		0	100	0	0	0
6 -- 18	+	Positive control (BAP) 20	100	2	23	0	0	0	25	0	70.6	100	0	0	0	
			100	1	20	0	0	0	21	0		200	0	0	0	
			200	3	43	0	0	0	46 (21.5)	0		100	0	0	0	

Remarks: Final concentration of S 9 was 5%, gpp; chromosid and chromosome gap. Treatment time: treatment time - recovery time.
 The data of each plate per dose fill in line 1 & 2, and the total in line 3. #: no. of viable cell count %
 -S 9 Mts; without metabolic activation, + S 9 Mts; with metabolic activation, DMSO; Dimethyl Sulfoxide, MMC; Mitomycin C, BAP; Benz(a)pyrene, TOX; Metaphase cells were not observed due to cytotoxicity.
 No statistically significant difference was noted when compared with the negative control group (p<0.05).

Table 3 Results of Chromosomal Aberration Test (Continuous Treatment)

15-36

Treatment time (h)	Dose levels (µg/ml)	No. of cells observed	Number of cells with structural aberrations (frequency %)						Number of gaps	Cell Proliferation Ratio (%)	Number of cells with numerical aberrations (frequency %)					
			Chromatid break	Chromatid exchange	Chromosome break	Chromosome exchange	Others	Total			No. of cells observed	Polyploidy	endo-reduplication	Total		
24 -- 0	-	Negative control (DMSO)	100	0	0	0	0	0	0	0	100	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
24 -- 0	-	49.4	100	0	0	0	0	0	0	0	92.9	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
24 -- 0	-	74.1	100	0	0	0	0	0	0	0	85.8	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
24 -- 0	-	111.1	100	1	0	0	0	0	1	0	64.6	100	0	0	0	
			100	1	0	0	0	0	1	1 (1.0)		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
24 -- 0	-	166.7	100	0	0	0	0	0	0	0	41.7	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
24 -- 0	-	250	TOX	---	---	---	---	---	---	---	33.9	TOX	---	---	---	
			---	---	---	---	---	---	---	---		---	---	---		
			---	---	---	---	---	---	---	---		---	---	---		
24 -- 0	-	Positive control (MMC) 0.05	100	7	21	0	0	0	28	0	65.4	100	0	0	0	
			100	4	21	0	0	0	25	0		200	0	0	0	
			200	11	42	0	0	0	48 (24.0)	0		100	0	0	0	
48 -- 0	-	Negative control (DMSO)	100	0	0	0	0	0	0	0	100	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
48 -- 0	-	144.7	100	0	0	0	0	0	0	0	92.6	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	0	0	0	0	0	0	0		0	100	0	0	0
48 -- 0	-	173.6	100	0	0	0	0	0	0	0	76.7	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	1	0	0	0	0	1	1 (0.5)		0	100	0	0	0
48 -- 0	-	208.3	100	0	0	0	0	0	0	0	57.1	100	0	0	0	
			100	0	0	0	0	0	0	0		0	200	0	0	0
			200	2	1	0	0	0	3	3 (1.5)		0	100	0	0	0
48 -- 0	-	250	TOX	---	---	---	---	---	---	26.4	TOX	---	---	---		
			---	---	---	---	---	---	---		---	---	---			
			---	---	---	---	---	---	---		---	---	---			
48 -- 0	-	300	TOX	---	---	---	---	---	---	11.0	TOX	---	---	---		
			---	---	---	---	---	---	---		---	---				
			---	---	---	---	---	---	---		---	---				
48 -- 0	-	Positive control (MMC) 0.05	100	7	23	0	0	0	30	0	75.5	100	0	0	0	
			100	6	22	0	0	0	28	0		200	0	0	0	
			200	13	45	0	0	0	48 (24.0)	0		100	0	0	0	

Remarks: gpp; chromosid and chromosome gap. Treatment time: treatment time - recovery time.
 The data of each plate per dose fill in line 1 & 2, and the total in line 3. #: no. of viable cell count %
 DMSO; Dimethyl Sulfoxide, MMC; Mitomycin C, TOX; Metaphase cells were not observed due to cytotoxicity.
 No statistically significant difference was noted when compared with the negative control group (p<0.05).

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Study title: A Micronucleus Test of l-Menthol in Rats.

Key findings: The results indicate that l-menthol tested negative in the *in vivo* rat micronucleus test under the conditions of the assay.

Study no.: 15-37

Volume #, and page #: 11, 057

Conducting laboratory and location:

Date of study initiation: November 6, 2001

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: l-menthol, lot # 55-050, 100%

Methods

Strains/species/cell line: Crj:CD (SD) IGS rats, male

Doses used in definitive study: 250, 500, 1000, and 2000 mg/kg; l-menthol was subcutaneously administered twice at 24 hours interval.

Basis of dose selection: Tested dose of 250, 500, 1000, and 2000 mg/kg l-menthol via subcutaneous injection (10 mL/kg) in preliminary dose-range finding study of toxicity (n=5/group). Animals were observed for mortality and clinical signs at 0.5, 1, 2, 6, 24 and 48 hours after dosing.

Negative controls: Corn oil (10 mL/kg; vehicle)

Positive controls: Cyclophosphamide (20 mg/kg, orally)

Incubation and sampling times: Animals were euthanized by CO₂ inhalation 24 hours after the final dosing.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): **No. of replicates** – A total of 2000 immature erythrocytes (IE) were examined per animals. The IE was scored for the presence of micronucleated immature erythrocytes (MNIE). Five hundred erythrocytes (IE + mature erythrocytes: ME) per animal were also counted. **Counting methods** – Slides were analyzed under a fluorescent microscopic. The ratio of MNIE was calculated. The ratio of IE to all erythrocytes was determined as an indicator of suppression of the increase in bone marrow cells. **Criteria for positive results** – The test article was considered to induce a positive response if it produced a significant increase in the MNIE at a value of $p < 0.05$ (Kastenbaum and Bowman's tables) over the negative control. The test article was judged to be an inhibitor of bone

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marrow proliferation if the IE ratio showed a significant difference ($p < 0.01$) against the negative control. **Study validity** – The study appears to be valid for the following reasons: 1) the study design was consistent with the OECD guidelines and 2) the positive control cyclophosphamide produced a statistically significant increase in the number of micronucleated immature erythrocytes and immature erythrocytes.

Study outcome: l-Menthol did not produce any clinical signs or death at 250, 500, 1000, and 2000 mg/kg. Analysis of the bone marrow cells collected at 24 hours after treatment is presented in the sponsor's table 5 below. As expected, cyclophosphamide at a dose of 20 mg/kg significantly increased the frequency of micronucleated immature erythrocytes and immature erythrocytes. The results indicated no l-menthol-induced bone marrow toxicity in male rats. Also, there was no indication of significant difference in frequency of MNIE and IE in comparison to vehicle. Thus, the sponsor concluded that the clastogenicity of l-menthol was judged as negative. Reviewer concurs with the sponsor's conclusions.

Table 5 Group results of micronucleus test in rats

Test article	Dose levels	Treatment frequency	Administration route	Mortality of animals	IE %			MNIE %		
					Mean ± S.D.	Minimum-Maximum value	Total*	Mean ± S.D.	Minimum-Maximum value	Total*
Corn oil	-	2	s.c.	0/6	49.03 ± 4.50	43.4 - 55.0	20	0.17 ± 0.05	0.10 - 0.25	
l-Menthol	250 mg/kg	2	s.c.	0/10	52.68 ± 3.31	47.8 - 57.2	20	0.10 ± 0.05	0.05 - 0.20	
l-Menthol	500 mg/kg	2	s.c.	0/10	49.86 ± 4.23	42.8 - 57.8	22	0.11 ± 0.06	0.05 - 0.20	
l-Menthol	1000 mg/kg	2	s.c.	0/10	47.32 ± 4.83	40.2 - 54.8	22	0.11 ± 0.05	0.05 - 0.20	
l-Menthol	2000 mg/kg	2	s.c.	0/10	45.98 ± 2.81	42.6 - 51.2	31	0.16 ± 0.09	0.05 - 0.30	
CP	20 mg/kg	1	p.o.	0/6	40.20 ± 2.72 *	37.4 - 44.0	395	3.29 ± 0.79 *	2.10 - 4.30	

IE %: Incidence of immature erythrocytes

MNIE %: Incidence of micronucleated immature erythrocytes

CP: Cyclophosphamide monohydrate

*: Significantly different from negative control ($p < 0.01$, student-t test)

#: Significantly different from negative control ($p < 0.05$, Kastenbaum and Bowman's method)

#: Total number of MNPCE per group

Remarks) Bone marrow samples were collected at 24 hours following the final treatment with negative control, test article, or positive control.

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Genetic Toxicology Studies with Methyl Salicylate

Study title: A Bacterial Reverse Mutation Test of Methyl Salicylate

Key findings: Methyl salicylate was evaluated in the Ames Reverse Mutation Assay at concentrations of 46.9, 93.8, 187.5, 375, 750, and 1500 µg/plate. Methyl salicylate at a concentrations \geq 750 µg/plate was cytotoxic. Under the conditions of the study, methyl salicylate was negative in the bacterial reverse mutation assay.

Study no.: 15-32

Volume #, and page #: 15, 001

Conducting laboratory and location:

Date of study initiation: October 30, 2001

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Methyl salicylate, lot # Y619E, 100%

Methods

Strains/species/cell line: *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 and *Escherichia coli* WP2uvrA

Doses used in definitive study: 46.9, 93.8, 187.5, 375, 750, and 1500 µg/plate.

Basis of dose selection: A dose-range finding study was conducted to select the appropriate doses to use in the mutagenicity assay.

Negative controls: DMSO

Positive controls: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), N-ethyl-N-nitro-N-nitrosoguanidine (ENNG), 9-aminoacridine (9AA), and 2-aminoanthracene (2AA) were selected for positive controls based on the bacterial strain as indicated in the following table:

Test Strain	Positive Control Substance (µg/plate)	
	-S9	+S9
TA100	AF2 (0.01)	2AA (1.0)
TA1535	ENNG (5.0)	2AA (2.0)
TA98	AF-2 (0.1)	2AA (0.5)
TA1537	9AA (80)	2AA (2.0)
WP2uvrA	ENNG (2.0)	2AA (10.0)

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Incubation and sampling times: The mutagenicity test was performed according to the plate-incorporation procedures. The S9 metabolic activator, the tester strain, and the test article were combined in molten agar which is overlaid onto the minimal agar plate. The agar plate was incubated at 37°C for 48 hours. The vehicle control, positive control and all doses of the test articles were plated in duplicates.

Analysis:

- Mutation frequencies: Expressed as mean number of revertants per plates. Revertants colonies were measured twice per plate and the means of these values were the measured value.
- Cytotoxicity: Background lawn was observed macroscopically or with a stereoscope (x 40)
- Counting Method: The number of revertant colonies was counted manually.

Criteria for positive results: “The results were judged positive (+) if the mean number of revertant colonies per plate increased two-fold or more compared to that of the negative control and dose-dependency was found; and if the results showed reproducibility between the dose finding test and the main test.”

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears to be valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced reliable results.

Study outcome: Methyl salicylate was evaluated at the following concentrations: 46.9, 93.8, 187.5, 375, 750, and 1500 µg/plate in the mutagenicity assay. In the mutagenicity assay, the positive controls induced mutation frequencies as expected; the mean revertants per plate were within historical control data for the laboratory. Methyl salicylate did not induce mutation frequencies in any of the *Salmonella typhimurium* strains (TA98, TA100, TA1535 or TA1537) or the WP2*uvrA* in the presence or absence of the metabolic activator; no increase in revertants was observed at any dose. However, there was cytotoxicity (growth inhibition) at concentrations ≥ 750 µg/plate in TA98, TA100, TA1537, and at 1500 µg/plate in WP2*uvrA* with and without metabolic activation.

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Study title: A Chromosomal Aberration Test of Methyl Salicylate in Cultured Mammalian Cells (CHL/IU)

Key findings: Methyl salicylate showed no evidence of mutagenic potential in the chromosomal aberration assay. Methyl salicylate did not induce chromosomal aberrations in the Chinese hamster fibroblast cell lines in the presence or absence of metabolic activation.

Study no.: → 15-33

Volume #, and page #: 15,027

Conducting laboratory and location:

Date of study initiation: October 30, 2001

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Methyl Salicylate, Lot №. Y619E, 100% pure

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Methods

Strains/species/cell line: Chinese lung hamster fibroblast cell lines (CHL/IU)

Doses used in definitive study:

Short-term treatment (6 hours) with and without metabolic activation: 350, 400, 500, 550, and 600 µg/mL

Continuous treatment for 24-hours: 300, 350, 400, 450, 500, and 550 µg/mL

Continuous treatment for 48 hours: 300, 350, 400, 450, 500, and 550 µg/mL

Basis of dose selection: It was based on a cell growth inhibition test at doses of 23.8, 47.5, 95, 190, 380, 760, and 1520 µg/mL for all treatment conditions. The 50% growth inhibition was estimated to be 568 µg/mL in short-term treatment without metabolic activation, 549 µg/mL in short-term treatment with metabolic activation; and 519 µg/mL and 515 µg/mL in continuous treatment for 24 hours and 48 hours, respectively. The highest dose selected for the chromosomal aberration test was determined by taking the 50% growth inhibition dose as an index.

Negative controls: Vehicle (DMSO)

Positive controls: Mitomycin C (MMC) and Benzo(a)pyrene (B(a)P) were positive controls for the chromosomal aberration test. In the short-term treatment without metabolic activation, and for the continuous for 24 and 48 hour, MMC was used as positive control after dissolution in physiological saline to a fixed concentration (0.15 µg/mL). B(a)P served as the positive control for the short-term treatment with metabolic activation after dissolution in DMSO to a fixed concentration (20 µg/mL).

Incubation and sampling times: The cell suspensions were incubated for 72 hours, after which the cells were harvested using 0.25% trypsin solution at 37°C. The cells were

suspended at 1×10^4 cells per mL in the culture medium and the Petri dishes were seeded with 2 mL of the cell suspension and incubated for 72 hours. Two plastic Petri dishes were used per dose. After the 72 hour incubation period, 1.0 mL of the culture medium was removed and 0.2 mL of the culture medium was added for the short-term test without metabolic activation and with metabolic activation. The test solution, positive control and negative control were added to the medium and incubated for 6 hours. Afterward, the culture medium was poured out, the cells were washed once with saline, 2 mL of fresh culture medium was added and then incubated for another 18 hours. For the continuous treatment test, after the 72 hour incubation period, the test solution, the negative control, and the positive control was added to the Petri dish and incubated for 24 or 48 hours.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): According to the sponsor, the results were judged positive if: 1) the frequency of cells with a structural or numerical aberrations were significantly higher in the test article treatment groups than in the negative control group, and 2) dose-dependency or reproducibility could be conformed. *Counting Method* – Under blind condition, 100 well-spread metaphases per slide (200 metaphase per dose) were analyzed. Chromosomal aberrations were recorded as structural or numerical aberrations. Cells with 25 ± 2 chromosomes were examined for structural aberrations. *Validity* – the study appears valid as the appropriate positive controls were used, an acceptable number of mitotic cells were evaluated and the protocol was consistent with those described in the OECD Guideline 473: *In vitro* Mammalian Chromosome Aberration Test.

Study outcome: Results from the definitive chromosomal aberration tests showed that methyl salicylate was void of significant clastogenic activity in the short-term test in both the absence and presence of metabolic activation. In the absence of metabolic activity, the percentage of cells with structural aberrations following exposure to methyl salicylate at concentrations of 350 $\mu\text{g/mL}$, 400 $\mu\text{g/mL}$, 450 $\mu\text{g/mL}$, 500 $\mu\text{g/mL}$, 550 $\mu\text{g/mL}$ and 600 $\mu\text{g/mL}$ was 0.5%, 0.5%, 1.0%, 1.0%, 0.5%, 1.5%, and 2.5%, respectively. For the positive control, MMC, the percentage of cells with structural aberrations was 24.5%. Cytotoxicity was not observed at any concentrations tested.

In the presence of metabolic activation, the percentage of cells with structural aberrations following exposure to methyl salicylate at concentrations of 350 $\mu\text{g/mL}$, 400 $\mu\text{g/mL}$, 450 $\mu\text{g/mL}$, 500 $\mu\text{g/mL}$, 550 $\mu\text{g/mL}$ and 600 $\mu\text{g/mL}$ was 0.5%, 1.0%, 0.5%, 1.5%, 0.5%, 2.5%, and 3.0%, respectively. Twenty-five percent of the cells treated with the positive control (B(a)P had structural aberrations.

Study title: A Micronucleus Test of Methyl Salicylate in Rats.

Key findings:

1. Methyl salicylate at doses up to 1000 mg/kg was not clastogenic to rat bone marrow. No significant increase in the percentage of micronucleated immature erythrocytes was observed at any of the doses of methyl salicylate evaluated.
2. Decrease in the percentage of immature erythrocytes in the mid- and high-dose groups; indicate that the bone marrow was exposed to methyl salicylate.

b(4)

Study no.: — 15-34

Volume #, and page #: 15, 057

Conducting laboratory and location:

Date of study initiation: October 31, 2001

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Methyl salicylate, lot № Y619E, 100%

Methods

Strains/species/cell line: Crj:CD (SD) IGS rats, male

Doses used in definitive study: 125, 250, 500, and 1000 mg/kg; methyl salicylate was subcutaneously administered twice at 24 hours interval.

Basis of dose selection: Tested dose of 30, 100, 300, and 1000 mg/kg methyl salicylate via subcutaneous injection (10 mL/kg) in preliminary dose-range finding study of toxicity (n=3/group). Animals were observed for mortality and clinical signs at 0.5, 1, 2, 6, 24 and 48 hours after dosing.

Negative controls: Corn oil (10 mL/kg; vehicle)

Positive controls: Cyclophosphamide (20 mg/kg, orally)

Incubation and sampling times: Animals were euthanized by CO₂ inhalation 24 hours after the final dosing.

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Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

No. of replicates – A total of 2000 immature erythrocytes (IE) were examined per animal. The IE was scored for the presence of micronucleated immature erythrocytes (MNIE). Five hundred erythrocytes (IE + mature erythrocytes: ME) per animal were also counted.

Counting methods – Slides were analyzed under a fluorescent microscopic. The ratio of MNIE was calculated. The ratio of IE to all erythrocytes was determined as an indicator of suppression of the increase in bone marrow cells.

Criteria for positive results – The test article was considered to induce a positive response if it produced a significant increase in the MNIE at a value of $p < 0.05$ (Kastenbaum and Bowman's tables) over the negative control. The test article was judged to be an inhibitor of bone marrow proliferation if the IE ratio showed a significant difference ($p < 0.01$) against the negative control.

Study validity – The study appears to be valid for the following reasons: 1) observation of toxicity (death) demonstrated systemic exposure, 2) cyclophosphamide produced a statistically significant increase in the number of micronucleated immature erythrocytes compared to the control, and 3) dosing appeared adequate based upon the results of the dose-ranging study.

Study outcome: Methyl salicylate did not produce any overt clinical signs or death at 30, 100, and 300 mg/kg. However, four deaths (4/10) occurred in the 1000 mg/kg group on day 2. Analysis of the bone marrow cells collected at 24 hours after final treatment is presented in the sponsor's table 5 below. As expected, cyclophosphamide at a dose of 20 mg/kg significantly increase ($p < 0.05$) micronucleated immature erythrocytes. Methyl salicylate at all dose levels evaluated did not significantly increase the number of micronucleated immature erythrocytes per 2000 polychromatic erythrocytes analyzed. However, methyl salicylate at doses of 500 mg/kg (IE% = 40.54%) and 1000 mg/kg (IE% = 33.80%) did significantly ($p < 0.01$) reduced the number of immature erythrocytes.

Table 5 Group results of micronucleus test in rats

Test article	Dose levels	Treatment frequency	Administration route	Mortality of animals	IE %		Total [¶]	MNIE %	
					Mean ± S.D.	Minimum-Maximum value		Mean ± S.D.	Minimum-Maximum value
Corn oil	-	2	s.c.	0/6	49.43 ± 4.61	41.2 - 53.6	14	0.12 ± 0.07	0.00 - 0.20
Methyl Salicylate	125 mg/kg	2	s.c.	0/10	48.72 ± 1.64	46.8 - 51.2	18	0.09 ± 0.06	0.00 - 0.20
Methyl Salicylate	250 mg/kg	2	s.c.	0/10	45.02 ± 2.13	41.6 - 48.2	17	0.08 ± 0.06	0.00 - 0.20
Methyl Salicylate	500 mg/kg	2	s.c.	0/10	40.54 ± 1.70 *	36.4 - 42.2	17	0.09 ± 0.05	0.00 - 0.15
Methyl Salicylate	1000 mg/kg	2	s.c.	4/10	33.80 ± 3.45 *	30.6 - 39.2	11	0.09 ± 0.07	0.00 - 0.20
CP	20 mg/kg	1	p.o.	0/6	39.47 ± 1.48 *	37.2 - 41.2	429	3.58 ± 0.28 *	3.20 - 4.00

IE %: Incidence of immature erythrocytes

MNIE %: Incidence of micronucleated immature erythrocytes

CP: Cyclophosphamide monohydrate

*: Significantly different from negative control ($p < 0.01$, student-t test)

¶: Significantly different from negative control ($p < 0.05$, Kastenbaum and Bowman's method)

¶: Total number of MNPCE per group

Remarks) Bone marrow samples were collected at 24 hours following the final treatment with negative control, test article, or positive control.

2.6.6.5 Carcinogenicity

Carcinogenicity studies for l-menthol and methyl salicylate were not submitted with the NDA. Carcinogenicity studies are not required because the proposed drug product is not indicated for long-term use.

The proposed indicated use of this product includes aches and pains associated with arthritis. It is recognized that arthritis is a chronic indication, and the potential exists that this product could be used in an intermittent manner that would exceed 6 month duration. However, following discussion with the Office of Non-Prescription Drug Products and the Associate Director of Pharmacology and Toxicology in the Immediate Office, the Agency determined that dermal carcinogenicity studies for both methyl salicylate and menthol will not be required for this drug product, based upon the extensive clinical experience with similar products.

2.6.6.6 Reproductive and developmental toxicology

Reproductive Toxicology Studies with Methyl Salicylate

Fertility and early embryonic development

Study title: Reproductive and Developmental Toxicity Study of Methyl Salicylate in Rats by Subcutaneous Administration.

Key study findings: Methyl salicylate was administered to male rats for 2 weeks prior to mating and continued throughout mating until euthanasia. Female rats were treated for a total of 2 weeks prior to mating, throughout mating and through gestation day 6. The following findings regarding fertility and early embryonic development were obtained:

1. The study appears to be valid as significant depression of body weight gain were observed in both female and male rats in the high dose (300 mg/kg/day) group.
2. Reproductive performance in males was not altered under the condition tested.
3. Methyl salicylate had no effects on mean epididymal sperm count, sperm production rate, motility or morphology compared to control.
4. Reproductive performance in females was not altered by any dose of l-menthol tested.
5. Early embryonic development was altered by methyl salicylate. Specifically, the mean number of corpora lutea was significantly reduced by 12.8% and in the 100 mg/kg/day group. However, there were no significant changes in the number of viable embryos and dead embryos.
6. **Maternal toxicity NOAEL:** Due to depression of body weight gain in the dams in the 300 mg/kg/day group, the **NOAEL was 100 mg/kg/day.**
7. **NOAEL** for reproductive performance in male was less than 100 mg/kg/day.

8. NOAEL for reproductive performance in female was 30 mg/kg/day due to a decrease in corpora lutea.

Study no.: 40106

Volume #, and page #: 1.7, 070

Conducting laboratory and location: _____

Date of study initiation: March 27, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Methyl Salicylate/ Lot Y096/100.1%

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Methods

Doses: 30, 100, and 300 mg/kg/day

Species/strain: Rats/Crj:CD (SD) IGS

Number/sex/group: 20/sex/group

Route, formulation, volume, and infusion rate: subcutaneous. Dissolved in corn oil, dose volume of 1.0 mL/kg

Satellite groups used for toxicokinetics: 3/sex/group

Study design: Toxicology males were dosed daily 2 weeks prior to mating, throughout mating period and up to one day prior to euthanasia. Toxicology female rats were dosed daily from 2 weeks prior to mating, throughout the mating period and up to Gestation Day (GD) 6.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily (before administration and 4 hours after administration) for clinical signs and mortality during the dosing period and once during the other periods.

Body weights: Male body weights were recorded twice weekly throughout the dosing period until euthanasia. Female body weights were measured twice weekly from the start of dosing to the start of mating and daily during gestation.

Food Consumption: Male's food consumption was recorded twice weekly throughout the dosing period until euthanasia. Female's food consumption was measured twice weekly from the start of dosing to the start of mating and daily during gestation.

Toxicokinetics: Blood was collected, from three males and three females per group, for toxicokinetic analysis 4 hours after dosing first day of dosing (day 0) and final day of dosing (day 13).

Estrous Cycle: Vaginal smears for determining the stage of estrous were evaluated daily from the start of drug administration and continued until evidence of copulation was observed.

Mating and fertility indices were calculated as follows:

Mating and Fertility Endpoints: At the end the 2 weeks mating period, the mating and fertility indices were calculated as follows:

Copulation Index = (number of animals with confirmed copulation/number of animals mated) x 100

Male Fertility Index = (number of pregnant females/number of males with confirmed copulation) x 100

Female Fertility Index = (number of pregnant females/number of females with confirmed copulation) x 100

Gestation Day 13 Uterine Examinations: Female rats were euthanized by exsanguination from the lateral iliac artery on gestation day 13. The uterus and ovaries were examined. The numbers of corpora lutea were recorded. The number of dead and live embryos was counted. Also, the pre-implant loss index (number of corpora lutea–number of implants/number of corpora lutea x 100 and dead embryo index (number of dead embryos/number of implants x 100) were calculated.

Spermatogenic endpoints. Sperm were collected from the right caudal epididymis. Sperm count and motility were examined microscopically using a hemocytometer. Sperm morphology was also determined.

Spermatogenic indices were calculated as follows:

Sperm Motility = (number of mobile sperm/number of sperm examined) x 100

Sperm form anomalies index = (number of abnormal sperm/ number of sperms examined) x 100

Necropsy: Macroscopic examination was completed on all animals at the scheduled euthanasia. Necropsy included examination of the organs and tissues. The testes, left epididymis, ovaries and skin of the treated site were preserved for histopathological examination. Also, organs with lesions were preserved for histopathological examination.

Organ Weights: Weights of the following organs were obtained: testes, epididymis. The relative organs weight was calculated from the body weight measured the day prior to the necropsy.

Statistics: Statistical analysis was conducted using significance levels of p<0.01 and p<0.05 for comparison of treated groups with the control group. The following statistical tests were used:

Statistical Test	Parameters
Barlett's Method	Body Weights, Body Weight Gain, Food Consumption, Organ Weight, Estrous Cycle, Estrus Count, Number of Days Required to for Successful Copulation, Sperm Count, Number of Corpora Lutea, Implants, and Live Embryos
Chi-Square Test	Copulation Index, Male fertility Index, Female Fertility Index
Wilcoxon's Rank Sum Test	Sperm Motility, Sperm Form Anomalies Index, Pre-implant Loss Index, and Dad Embryo

Results

Mortality: No treatment-related death occurred in the females. However, one death occurred in the male high dose group on the fourth day of drug administration. On the day prior to this rat death, he exhibited hypoactivity, bradypnea, hypothermia and blanching.

Clinical signs: No significant treatment-induced overt clinical signs were observed in either the males or females in the three treatment groups. However, on day 9 of drug dosing to day 15 of gestation, the treated site of one female in the high dose group developed crust at the site and there was a loss of hair.

Body weight: Treatment-induced effects on body weight were observed in both males and females in the high dose group. At the high dose, the males mean body weight and body weight gain was significantly lower throughout the treatment period (i.e. days 1 thru 49). As depicted in the table below, the mean body weights gains were decreased by 20.0% or greater following treatment with 300 mg/kg/day during treatment period.

Day of Treatment	Vehicle Control	Mean Body Weight Gain (g) ± SD in Males in the High Dose Group	
		300 mg/kg/day	% Change of Control
1	5.3 ± 3.7	-6.3 ± 7.7**	↓219%
3	17.4 ± 4.7	-3.1 ± 19.8**	↓117.8.0%
7	40.0 ± 7.9	16.1 ± 13.4**	59.8%
10	55.4 ± 11.0	29.2 ± 16.0**	47.3%
14	74.7 ± 13.1	47.8 ± 16.5**	36.0%
17	83.4 ± 14.0	52.9 ± 18.7**	36.6%
21	97.7 ± 16.7	68.6 ± 19.9**	29.8%
24	114.5 ± 17.3	83.4 ± 21.9**	27.2%
28	126.3 ± 95.3	95.3 ± 24.9**	24.5%
31	140.7 ± 20.6	107.8 ± 26.1**	23.4%
35	153.4 ± 21.7	120.5 ± 30.8**	21.0%
38	163.3 ± 24.0	126.6 ± 31.9**	22.5%
42	174.9 ± 24.0	137.4 ± 32.1**	21.4%
45	182.3 ± 24.1	145.5 ± 34.9**	20.2%
49	194.1 ± 24.2	154.9 ± 37.0*	20.2%

** Significantly different from vehicle control (p<0.01)

Similarly, the mean bodyweight gain of females in the high dose group was significantly lower than the control on treatment days 1 (↓96.2%), 3 (↓8.9%), 10 (39.2%), and 14 (34%). Compared to control, this reduction in mean body weight gain continued through gestation. This observation is depicted in sponsor's figure 6 below.

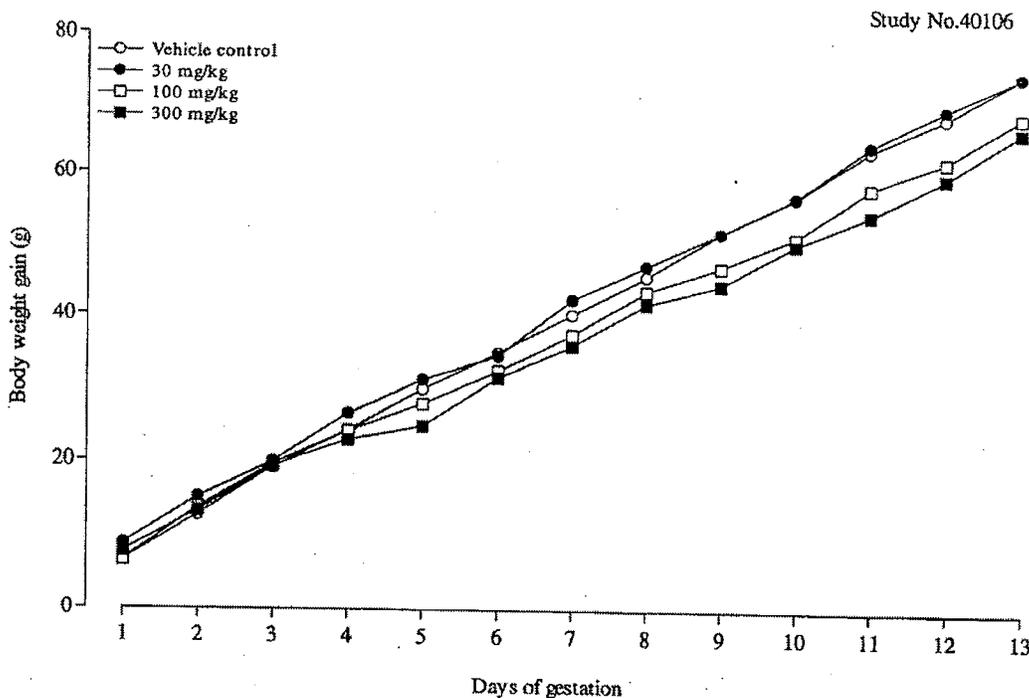


Fig.6 Body weight gains in Fo dams.

Food consumption: Food consumption was significantly lower in males in the high dose group on day 1 (27%) of treatment. Females in the high dose group also showed a significant reduction in food consumption on days 1 (28.7%), and 3 (17.1%). During gestation period relative to control on days 4 and 5, food consumption was 9% and 11% lower, respectively.

Toxicokinetics: Blood was drawn from three males and three females per group at four hours post-dosing on days 0 (first day of dosing) and 13 (final day of dosing). The toxicokinetic results are presented in the table below:

Day of Dosing	Dose (mg/kg/day)	Mean Plasma Concentration (± SD) (µg/mL)	
		Male (n=3)	Female (n=3)
0	30	46.4 (5.5)	53.5 (8.9)
	100	147 (8)	164 (28)
	300	239 (23)	277 (32)
13	30	46.1 (1.3)	47.7 (7.2)
	100	126 (6)	144 (31)
	300	290 (16)	300 (44)

Plasma concentration was dose-dependent; increasing the dose of methyl salicylate produced an increase in the plasma concentration of drug. There were no apparent differences in blood plasma levels between the first day of treatment and final treatment day. Also, no clear gender differences were observed between the females and males.

Necropsy: Necropsy of both males and females suggest that the only potentially treatment-related changes were note in the subcutis area of the injection site. All males and females in the control and methyl salicylate groups retained an oily fluid in the subcutis of the treated site. This oily fluid is most likely the vehicle; corn oil was the vehicle.

Organ Weights: There were no treatment-related effects on testes and epididymides weight.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): As indicated in the table below, no treatment-related effects on either male mating index or male fertility index was noted. Male copulation index was calculated by dividing the number of males with evidence of mating by the total number of males used for mating (x100). Male fertility index was calculated by dividing the number of pregnant females/the number of males with confirmed copulation (x100). In the mid-dose and high-dose treatment groups, the copulation index and fertility index were the result of the one and two out of 20 subjects, respectively, not copulating.

Parameter	Summary of Male Reproductive Performance (n = 19-20)			
	Dose (mg/kg)			
	0	30	100	300
Male Copulation Index (%)	100%	100%	95%	94.74%
Male Fertility Index (%)	100%	90%	94.74%	94.44%

Spermatogenic Endpoints: As depicted in the table below, there were no effects of methyl salicylate at the doses tested on mean sperm numbers, motility, and morphology compared to control animals.

Dose (mg/kg/day)	N _e Examined	Spermatogenic Endpoints (Mean ± S.D.)		
		Count of Sperm (x10 ⁶ /g)	Sperm Motility (%)	Sperm Form Anomalies Index (%)
0	20	583.71 ± 149.75	92.24 ± 21.69	7.25 ± 19.94
30	20	632.62 ± 128.30	97.22 ± 2.04	3.97 ± 7.76
100	20	615.37 ± 120.48	97.86 ± 2.07	2.40 ± 2.30
300	19	559.60 ± 184.64	87.62 ± 29.78	10.04 ± 9.02

In females, methyl salicylate had no effects on female mating index or female fertility index; these indices were not changed by the treatment. The 94.7% female fertility index was the result of the one male that died in this group. There were no significant differences between the control and methyl salicylate groups in the count of estrus or estrous cycle.

Parameter	Summary of Female Reproductive Performance (n = 20)			
	Dose (mg/kg)			
	0	100	300	1000

Female Mating Index (%)	100%	100%	100%	100%
Female Fertility Index (%)	100%	90%	94.74%	94.44%
Mean Count of Estrous \pm S.D.	3.70 \pm 0.47	3.70 \pm 0.73	3.90 \pm 0.31	3.70 \pm 0.80
Mean Estrus Cycle Length \pm S.D.	4.10 \pm 0.24	4.0 \pm 0.0	4.13 \pm 0.56	4.46 \pm 1.83

Mean embryonic data is presented in the tables below. No treatment-related effects on the number of implantation sites and pre-implantation loss were observed. The number of implantation sites and pre-implantation loss was comparable among the four treatment groups. However, the number of corpora lutea was statistically significantly lower in the mid-dose group (100 mg/kg/day). This change, however, was not dose-related and therefore, does not appear to be biologically relevant. There were no significant changes in the number of viable embryos or dead embryos.

Effects of methyl salicylate on the mean number of corpora lutea, implantation site, percent of rats pregnant and placenta appearance.

Dose (mg/kg)	# Pregnant/Total (%)	(Total №) MEAN (\pm S.D.)		№ (% of the corpora lutea)
		Corpora Lutea	Implantation Site	Pre-implantation loss
0	20/20 (100%)	312 (15.60 \pm 1.60)	297 (14.85 \pm 1.57)	15 (4.81%)
30	18/20 (90%)	264 (14.67 \pm 2.93)	254 (14.11 \pm 2.93)	10 (3.79%)
100	18/20 (94.74%)	272 (14.32 \pm 1.49)*	267 (14.05 \pm 1.54)	5 (1.84%)
300	17/19 (89.5%)	277 (14.58 \pm 1.54)	268 (14.11 \pm 1.52)	9 (3.25%)

*: Significantly different from control group ($p < 0.05$)

Effects of methyl salicylate on litter (i.e., number of viable ad embryos).

Dose (mg/kg)	№ of Viable Embryos (Mean \pm S.D.)	№ of Dead Embryos (% of the # implants)
0	279 (13.95 \pm 1.70)	18 (4.81)
30	239 (13.28 \pm 2.82)	15 (5.91)
100	253 (13.32 \pm 1.77)	14 (5.24)
300	260 (13.68 \pm 1.57)	8 (2.99)

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Embryofetal development

Study title: Reproductive and Developmental Toxicity Study of Methyl Salicylate in Rabbits by Subcutaneous Administration – Study for Effects on Embryo-Fetal Development

Key study findings:

1. There were no treatment-related mortalities noted in the study.
2. One dam aborted in the high-dose group.
3. No treatment-related effects on mean body weight or mean body weight gain were observed.
4. No significant treatment-related effects on fetal development were noted.
5. No significant treatment-related effects on the incidence of fetal malformation were observed.
6. **NOAEL** maternal toxicity was 100 mg/kg/day based on the one incidence of abortion in the 300 mg/kg/day group.
7. **NOAEL** fetal toxicity was < 30 mg/kg/day.

Study no.: 401113

Volume #, and page #: 1.9, 218

Conducting laboratory and location:

Date of study initiation: March 18, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Methyl Salicylate, Lot № Y096, 100.1%

b(4)

Methods

Doses: 30, 100, 300 mg/kg/day

Species/strain: New Zealand White Rabbits//Kbs:NZW

Number/sex/group: See table below on group assignments

Group	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Females
1	Methyl Salicylate	0	0	1.0	22
2	Methyl Salicylate	30	30	1.0	20
3	Methyl Salicylate	100	100	1.0	21
4	Methyl Salicylate	300	300	1.0	20

Route, formulation, volume, and infusion rate: subcutaneous, dissolved in corn oil

Satellite groups used for toxicokinetics: None

Study design: Animals were dosed from day 6 to 18 of gestation. The study design consisted of one control vehicle group and three treatment groups. Day of successful copulation was designated as gestation day 0.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily (before administration and 4 hours after administration) for mortality during the administration period and once during other periods.

Body weights: Body weights were recorded on days 0, 3, 6-19, 23, 26, and 29 of gestation. Body weight gain was calculated from the body weight on gestation day 6.

Food Consumption: Food consumption was recorded on days 1, 3, 6-19, 23, 26, and 29.

Necropsy: All surviving rats were euthanasia by exsanguination on day 29 of gestation. Their organs and tissues were examined macroscopically after removal of the ovaries and uterus.

Examination of Embryo and Fetuses:

Cesarean Section: The number of corpora lutea, implants, early and late resorptions, and dead and live fetuses were recorded. The placenta was examined macroscopically.

Fetal Observations: Each fetus was weighed and examined for external anomalies.

Visceral Examination: Each fetus was sexed, sectioned into the head, chest, and abdomen, and examined macroscopically. The brain, kidneys and heart were removed and fixed in Bouin's solution and subsequently examined for visceral anomalies. The other organs were removed and fixed in 10% neutral buffered formalin solution and preserved.

Skeletal Examination: Skeletal anomalies, variations and progress of ossification were assessed after staining with Alizarin Red S.

Toxicokinetic Analysis: Blood was collected four hours post-dosing on days 6 and 18 of gestation. Blood samples were collected from the auricular vein.

Statistics: Statistical analysis was conducted using significance levels of $p < 0.01$ and $p < 0.05$ for comparison of treated groups with the control group. The following statistical tests were used:

Statistical Test	Parameters
Barlett's Method	Body weights, Body weight gain, Food consumption, Number of corpora lutea, Implants, Live fetuses, Vertebral bodies and arches, and Body weight of the live fetuses
Chi-Square Test	Sex ratio of fetuses
Wilcoxon's Rank Sum Test	Indices of pre-implant loss, early and late resorption, dead fetuses, total dead fetuses, placental (by type), anomalies, external (by type) anomalies, visceral (by type) and progress of ossification

Results

Mortality (dams): No treatment-related deaths occurred in the females.

Clinical signs (dams): Clinical signs were observed daily. Overt clinical signs were observed in one rabbit in the mid-dose group, and in one rabbits in the high-dose group. Summary of these clinical signs are presented in the table below.

Summary of Clinical Signs

Rabbit №	Dose (mg/kg/day)	Day of Occurrence	Observed Clinical Sign(s)	Outcome	Necropsy Results
318	100	Gestation days 14 - 25	Crust at treatment site		
		Gestation Day 26-29	Loss of hair		
415	300	Gestation Day 18	Blanching Vaginal hemorrhage Abortion		Uterus: Retention of bloody material; Vagina: Retention of bloody material Subcutis (treated and untreated site): Retention of oily fluid All fetuses in the uterus had died (late resorption)

Body weight (dams): No treatment-related effects were observed. The mean body weights and mean body weight gain were comparable to control body weights during gestation.

Food consumption (dams): On gestation days 1, 3, 6, 7, 9, 10, 14, and 16 mean food consumption for the 100 mg/kg/day group was statistically significantly higher than the control. Relative to the control, mean food consumption was 28.9%, 18.0%, 16.1%, 12.6%, 13.5%, 15.0%, 16.5% and 25.6% higher on days 1, 3, 6, 7, 9, 10, 14, and 16, respectively.

Toxicokinetics: Blood was drawn from four dams per group at four hours post-dosing on gestations days 6 and 18. The toxicokinetic results are presented in the table below:

Day of Gestation	Dose (mg/kg/day)	Plasma Concentration (\pm SD) (μ g/mL)
6	30	24.3 (2.4)
	100	62.5 (4.7)
	300	142(18)
18	30	16.5 (2.1)
	100	47.8(6.7)
	300	98.4(26.2)

Plasma concentration was dose-dependent; increasing the dose of methyl salicylate produced an increase in the plasma concentration of drug. There was a slight decrease in the blood plasma levels of methyl salicylate between gestation day 6 and gestation day 18. This difference could be the result in the alteration in the metabolism of methyl salicylate under the conditions tested.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Maternal Necropsy: Necropsy of all females suggests that the only potentially treatment-related changes were noted in the subcutis area of the injection site. All females in the control and methyl salicylate groups retained an oily fluid in the subcutis of the treated site. This oily fluid is most likely the vehicle; corn oil was the vehicle.

Gestation Day 29 Data: No treatment-related effects on the number of corpora lutea, number of dead fetuses, number of live fetuses, early resorption, late resorption, body weight of live fetuses, number of fetuses with external anomalies, and number of live fetuses with placenta anomalies were observed. However, the number of pre-implant loss in the low dose group was significantly lower (66.7%) than the control group. The sex ratio of the high dose group differed significantly ($\uparrow 44.4\%$) from the control group; the ratio of males was higher.

Offspring (malformations, variations, etc.): There were no significant differences in the number of malformations, that is external, soft tissue or skeletal, between treatment groups.

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Study title: Reproductive and Developmental Toxicity Study of Methyl Salicylate in Rats by Subcutaneous Administration – Study for Effects on Embryo-Fetal Development

Key study findings: Female rats were treated with methyl salicylate from gestation day 6 to gestation day 17 in a definitive segment II study. The key findings were:

1. There were no treatment-related mortalities noted in the study.
2. There were no clinical overt signs of toxicity.
3. Methyl salicylate exerted suppressive effects on fetal growth. Significant reduction in body weight gain was observed during gestation in the high-dose treatment group.
4. Significant reduction in food consumption was observed during gestation in the high dose treatment group.
5. There was a significant decrease in mean fetal body weight in the high-dose animals (22%) compared to the control.
6. There were significant malformations (external and skeletal) between the high-dose group and control group, indicating that methyl salicylate was teratogenic.
7. Consistent with methyl salicylate suppressive effects on fetal growth, a delay in the progress of ossification was observed.
8. **NOAEL** maternal toxicity was 100 mg/kg/day based on the one reduction in body weight gain in the 200 mg/kg/day group.
9. **NOAEL** fetal development was 100 mg/kg/day.

Study no.: 40110

Volume #, and page #: 1.9, 041

Conducting laboratory and location:

Date of study initiation: April 11, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Methyl Salicylate, Lot № Y096, 100.1%

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Methods

Doses: 50, 100, 200 mg/kg/day

Species/strain: Rats/Crj:CD (SD) IGS

Number/sex/group: See table below on group assignments

Group	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/ml)	Dosage Volume (ml/kg)	Number of Females
1	Methyl Salicylate	0	0	1.0	20
2	Methyl Salicylate	50	50	1.0	20
3	Methyl Salicylate	100	100	1.0	20
4	Methyl Salicylate	200	200	1.0	20

Route, formulation, volume, and infusion rate: subcutaneous, dissolved in corn oil

Satellite groups used for toxicokinetics: None

Study design: Animals were dosed from days 6 to 17 of gestation. The study design consisted of one control vehicle group and three treatment groups. Day of successful copulation was designated as gestation day 0.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily (before administration and 4 hours after administration) for mortality during the administration period and once during other periods.

Body weights: Body weights were recorded on days 0, 3, and 6-20 of gestation. Body weight gain was calculated from the body weight on gestation day 6.

Food Consumption: Food consumption was recorded on days 1, 3, 6-20 of gestation.

Necropsy: All surviving rats were euthanasia by exsanguination on day 20 of gestation. Their organs and tissues were examined macroscopically after removal of the ovaries and uterus.

Examination of Embryo and Fetuses:

Cesarean Section: The number of corpora lutea, implants, early and late resorptions, and dead and live fetuses were recorded. The placenta was examined macroscopically.

Fetal Observations: Each fetus was weighed and examined for external anomalies.

Visceral Examination: Visceral exams were performed on the fetus of the control and high (200 mg/kg/day) groups only.

Skeletal Examination: Skeletal anomalies, variations and progress of ossification were assessed after staining with Alizarin Red S.

Statistics: Statistical analysis was conducted using significance levels of $p < 0.01$ and $p < 0.05$ for comparison of treated groups with the control group. The following statistical tests were used:

Statistical Test	Parameters
Barlett's Method	Body weights, body weight gain, food consumption, number of corpora lutea, implants, live fetuses, vertebral bodies and arches, and body weight of the live fetuses
Chi-Square Test	Sex ratio of fetuses
Wilcoxon's Rank Sum Test	Indices of pre-implant loss, early and late resorption, dead fetuses, total dead fetuses, placental (by type), anomalies, external (by type) anomalies, visceral (by type) and progress of ossification

Results

Mortality (dams): No treatment-related deaths occurred in the females.

Clinical signs (dams): No treatment-related overt clinical signs were observed.

Body Weight: Treatment-induced effects on body weight were observed in the high dose group. Mean body weights in the 200 mg/kg/day group were statistically significantly lower compared to the control dams on gestation days 7 (3.5%), 8 (4.2%), 9 (3.4%), 10 (3.9%), 12 (3.4%), and 13 (3.6%). As depicted in the table below, the mean body weight gains were decreased by 10% or greater throughout gestation. Also, the mean body weight gain in the 100 mg/kg/day group were significantly ($p < 0.01$) lower than the control dams on gestation day 7 (125%).

Day of Treatment	Vehicle Control	Mean Body Weight Gain (g) \pm SD in F ₀	
		200 mg/kg/day	% Change of Control
7	3.5 \pm 2.7	-7.5 \pm 4.6**	314.3%%
8	8.7 \pm 5.8	-4.8 \pm 6.1**	155.2%
9	12.7 \pm 4.0	1.4 \pm 5.3**	89%
10	19.2 \pm 4.8	6.1 \pm 8.3**	68%
11	23.0 \pm 5.5	12.0 \pm 7.6**	47.8%
12	30.3 \pm 6.1	18.6 \pm 5.7**	38.6%
13	35.2 \pm 5.5	22.4 \pm 8.7**	36.4%
14	40.7 \pm 5.7	30.0 \pm 9.1**	24.1%
15	48.4 \pm 5.9	37.9 \pm 7.1**	21.2%
16	58.2 \pm 7.6	48.2 \pm 8.2 **	17.2%
17	71.1 \pm 7.9	62.5 \pm 9.2**	12.1%
18	88.0 \pm 10.4	76.6 \pm 10.2**	13%
19	103.7 \pm 11.5	93.1 \pm 11.4*	10.2%
20	120.5 \pm 12.2	106.4 \pm 13.1**	11.7%

* Significantly different from vehicle control ($p < 0.05$)

** Significantly different from vehicle control ($p < 0.01$)

Food consumption (dams): On gestation days 6, 7, and 8, mean food consumption for the 200 mg/kg/day group was statistically significantly lower than the control. Relative to the control, mean food consumption was 5%, 25.7%, and 18.2%, respectively. On gestation day 7, the mean food consumption of the dams in the 100 mg/kg group was significantly lower (11.6%) than the control dams.

Terminal and necropsic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Maternal Necropsy: Necropsy of all females suggests that the only potentially treatment-related changes were noted in the subcutis area of the injection site. All females in the control and methyl salicylate groups retained an oily fluid in the subcutis of the treated site. This oily fluid is most likely the vehicle; corn oil was the vehicle.

Gestation Day 29 Data: No treatment-related effects on the number of corpora lutea, number of dead fetuses, number of live fetuses, early resorption, late resorption, pre-implant loss, number of fetuses with external anomalies, and number of live fetuses with placenta anomalies were observed. However, the mean body weight of live male and female fetuses in the high-dose group was significantly lower than the control group.

The mean body weight of both the male and female fetuses were 22% lower than the control, respectively. Morphologically, some external malformations or developmental variations were observed in the high-dose group. Compared to the control groups, there was a slight increase in the number of live fetuses with craniorachischisis (8 fetuses, 2.86%) and gastroschisis (1 fetus, 0.36%).

Offspring (malformations, variations, etc.): There were no significant differences in the number of skeletal anomalies between the fetuses in the three methyl salicylate treatment groups and vehicle group. However, examination of the F₁ fetus in the 200 mg/kg/day demonstrated an increase in the incidence of several skeletal variations; an increase in incidence of short and full supernumerary rib, splitting of the thoracic and lumbar, 7 lumbar vertebrae and incomplete ossification of thoracic centrum. Fetuses in the high-dose group also differ significantly from the control in the progress of ossification in the vertebrae, sternebra, metatarsus, and phalanges. Also, compared to the control group there were no significant difference in the number of skeletal anomalies in the fetus in the 50 and 100 mg/kg/day groups.

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SKELETAL EXAMINATIONS		
Parameter	Dose (mg/kg/day)	
	0	200
No litters examined skeletally	132	129
	SKELETAL VARIATIONS Number of Subjects (percentage of the fetuses examined)	
No of offsprings with skeletal variations (%)	14 (10.61)	97 (75.19)**
Cervical rib	0	1 (0.78)
Short supernumerary rib	12 (9.09)	43 (33.33)**
Full supernumerary rib	0	59 (45.74)**
Asymmetry of the sternbra	0	2 (1.55)
Splitting of the sternbra	0	3 (2.33)
Splitting of the thoracic vertebral body	3 (2.270)	42 (32.56)**
Splitting of the lumbar vertebral body	0	15 (11.63)**
7 lumbar vertebrae	0	42 (32.56)**
In complete ossification of thoracic centrum	0	10 (7.75)*
In complete ossification of lumbar centrum	0	1 (0.78)
	PROGRESS OF OSSIFICATION Mean (\pm S.D.) or Number of ossification (%) ^A	
VERTRBRAE		
Cervical (body)	0.89 (0.89)	0.08 (0.15)**
Thoracic (body)	12.98 (0.070)	12.69 (0.49)**
Lumbar		
- Arch (R)	6.0 (0.0)	6.33 (0.44)**
- Arch (L)	6.0 (0.0)	6.33 (0.44)**
- Body	6.0 (0.0)	6.33 (0.44)**
Sacrocaudal		
- Arch (R)	5.94 (0.36)	5.61 (0.47)*
- Arch (L)	5.95 (0.36)	5.60 (0.49)*
- Body	7.90 (0.72)	6.91 (0.76)**
STERNEBRAE		
- 6 th	130 (98.48) ^A	92 (72.32) ^A **
METACARPUS		
- Right	513 (77.73) ^A	406 (62.95) ^A **
- Left	513 (77.73) ^A	394 (61.56) ^A **
PHALANGES OF HINDLIMBS		
- Distal	640 (98.46) ^A	614 (95.19) ^A **
METATARSUS		
- Right	540 (82.44) ^A	509 (79.53) ^A **
- Left	546 (82.73)	515 (79.84) ^A *

*: p < 0.05, significantly different from control

** : p < 0.01, significantly different from control

A: represent the number of ossification (ossification percentage)

Prenatal and postnatal development

Study title: Reproductive and developmental Toxicity Study of Methyl Salicylate in Rats by Subcutaneous Administration – Study for Effects on pre- and Postnatal development, including maternal Function

Key study findings: Female rats were treated subcutaneously with methyl salicylate (20, 60, and 200 mg/kg/day) from gestation day 6 to day 21 of lactation in the definitive Segment III study. The key findings of the study were:

1. F₀ mortality was noted in the 200 mg/kg/day group; two females died on day 23 of gestation. One female that died prior to scheduled necropsy exhibited vaginal hemorrhaging.
2. No treatment-related clinical signs were observed in the females that survived until scheduled sacrifice
3. Mean body weight gain in the F₀ females was significantly reduced during the gestation period.
4. In surviving F₀ females, there were no significant differences in the mean litter size, mean number of live fetuses, mean number of dead fetuses, and number of implantation.
5. The birth index was significantly decreased in the F₁ generation born to the high dose F₀ females.
6. Offspring, both males and females, mean body weight during lactation and maturation periods were significantly reduced in the 200 mg/kg/day treatment groups compared to the control.
7. Methyl salicylate suppressed postnatal development. Developmental landmarks in the F₁ males indicated that balanopreputial separation in males from the 200 mg/kg/day group was delayed compared to controls. Also, F₁ males and F₁ females in the 200 mg/kg group had a delay in incisor eruption. Eyelid separation in F₁ females in this high dose group was also delayed compared to the control group.
8. There were no treatment-related effects of methyl salicylate in behavioral evaluation and function tests.
9. Reproductive performance in the F₁ generation was not altered by F₀ generation methyl salicylate treatment at any level tested.
10. Methyl salicylate was teratogenic. An increase incidence in skeletal anomalies (i.e., fusion of cervical vertebra and misshapen sternebra) and variations (i.e., full supernumerary ribs, accessory, lumbarization, 7 lumbar vertebra, and incomplete ossification of the cervical, thoracic and lumbar vertebrae) were observed in the 200 mg/kg/day group.
11. **Maternal NOAEL was 60 mg/kg** based upon the depression of body weight gain, decrease food consumption, and reduction in the gestation index in the F₀ females in the 200 mg/kg/day group.
12. **Fetal NOAEL was < 60 mg/kg** based upon decrease in birth index, growth suppression (decreased mean body weight during the lactation and maturation periods), and increase incidence of skeletal anomalies and variation the F₁

offsprings of the 200 mg/kg/day group. While not statistically significant, there was a slight increase in the incidence of fetuses with variations (cervical rib, incomplete ossification of thoracic and caudal vertebrae) in the low dose group. Since historical control data were not submitted, one could not rule out that this is not treatment-related.

Study no.: 40108

Volume #, and page #: 1.8, 001

Conducting laboratory and location: _____

Date of study initiation: June 10, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Methyl Salicylate, Lot # Y096, 200.1%

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Methods

Doses: 20, 60, and 200 mg/kg/day

Species/strain: Rats/Crj:CD (SD) IGS 1

Number/sex/group: 20/females/group

Route, formulation, volume, and infusion rate: subcutaneous, dissolved in corn oil, Volume was 1.0 mL/kg for all groups

Satellite groups used for toxicokinetics: None

Study design: F₀ female rats were dosed once a day on gestation day 6 to day 21 of lactation (delivery day was designated day 0 of lactation). Dosing was based on the results of a dose-range finding study in pregnant female rats (40107). All females were allowed to deliver naturally and rear their young to weaning (postnatal day 22). To reduce variability among the litters, eight pups per litter (4 per sex) were randomly selected on postnatal day 4.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily (before administration and 4 hours after administration) for mortality during the administration period and once during other periods.

Body weights: Body weights were recorded on days 3, 6, 9, 12, 15, 18, and 20 of gestation, and on days 0, 4, 7, 10, 14, 17, and 21 of lactation. Body weight gain was calculated for the gestation period and lactation period.

Food Consumption: Food consumption was recorded on days 1, 3, 6, 9, 12, 15, 18, and 20 of gestation and on days 1, 4, 7, 10, 14, 17, and 20 of lactation.

Necropsy: All surviving F₀ female rats were euthanized by exsanguination on day 22 after delivery. Their organs and tissues were examined macroscopically. After extraction of the ovaries and uterus, the number of implantation traces in the uterus was counted. Any F₀ female dams failing to deliver by day 24 of gestation were necropsied after obtain body weight. For all animals, the skin of the treated site and the ovaries and the uterus were fixed in 10% neutral buffered formalin solution and preserved. Examination of offsprings:

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At birth: The following delivery observations were performed: number of litter, number of stillborns, number of live newborns, and the still birth index was calculated (number of stillborns/number of litter).

Live offsprings: Weighted, sexed and examined for external anomalies.

Stillborns: Using the floating test, their extracted lungs were evaluated to determine whether they had breathed or not. All stillborns were fixed in pure ethanol by litter and stored.

Viability and Weanling Measures:

Viability Index: = (# of live newborns on PND 4/# of live newborns) x 100

Weaning Index = (# of live weanling/# of live newborns after culling) x 100

Observations during lactation and after weaning included:

Function Test: righting reflex and ipsilateral flexor (postnatal (PND) 5), visual pacing reflex (PND 6), Preyer's reflex (PND 28).

Postnatal Differentiation test: All newborns were examined for pinna detachment (PND 4), incisor eruption (PND 10), gait and eyelid separation (PND 15), descensus testis (PND 31), cleavage of the balanopreputial gland (PND 42), and vaginal opening (PND 42).

Birth to PND 22: Testing of the F₁ litter included daily examination for clinical signs and mortality. Body weight of pups was recorded on day 0, 7, 14, and 21 after birth.

Organ Weight: 1 offspring of each sex from a dam was euthanasia on PND 22. The following organs were isolated and weighed: heart, lungs, liver, kidneys, adrenals, brain, spleen, thymus, and testes and ovaries.

Skeletal Examination: On PND 22, 2 offspring of each sex from a dam were euthanized under ether anesthesia. Their organs and tissues were observed macroscopically. Skeletal anomalies and variations were assessed after staining with alizarin red S.

PND 22 (weaning) and until mating: Offspring were observed daily for clinical signs and mortality. Body weight and food consumption were recorded once a week. Neurobehavioral evaluation included: rotarod performance (5 weeks of age), water maze (6 weeks of age), and open field (8 weeks of age) were performed.

F₁ Reproductive Capacity: At 12 to 13 weeks, the F₁ capacity to reproduce was evaluated. Duration of mating required for copulation, copulation index, and male and female fertility indices were assessed.

Results

F₀ in-life:

Mortality: Two F₀ females in the 200 mg/kg/day group died on day 23 of gestation

Clinical Signs: One female that died in the high-dose group had vaginal hemorrhaging at the time of death. The other female had delivered 4 live pups and 11 stillborns when she was found dead.

No clinical signs were observed in the females that survived until scheduled sacrifice.

Body weight: The mean bodyweight of females in the high dose group was significantly different from the control on gestation days 12 (↓3.7%), 15 (↑3.8%), 18 (↑5.1%), and 20 (↓4.6%). Treatment-related effects on body weight gain were observed in the high-dose group throughout gestation period. The mean body weight gain of females in the high-dose group was statistically significantly lower than the control on gestation days 9 (40.8%), 12 (36.2%), 15 (22.8%), 18 (20.4%) and 20 (15.7%). No treatment-related effects on mean body weight or mean body weight gain were observed during lactation.

Food Consumption: On gestation day 9, mean food consumption of the dams in the 200 mg/kg/day treatment group was significantly lower (10.2%) than the control. Mean food consumption was also significantly lower than the control during lactation. Relative to the control, mean food consumption was 42.9%, 15.4%, 16.3%, 11.8%, 10.3%, and 21.9% lower on days 1, 4, 7, 14, 17, and 21, respectively.

Toxicokinetics: Not performed.

F₀ necropsy: Necropsy of all females surviving to the scheduled sacrifice suggests that the only potentially treatment-related changes were noted in the subcutis area of the injection site. All females in the control and methyl salicylate groups retained an oily fluid in the subcutis of the treated site. This oily fluid is most likely the vehicle; corn oil is the vehicle.

The two females that were found dead on gestation day 23 also had retention of oily fluid in the subcutis at the injection site. The female that displayed vaginal hemorrhaging prior to her death also had a dark red macula in the stomach and 14 dead fetuses in her uterus.

F₁ physical development: There were no significant differences in the mean litter size, mean number of live fetuses, mean number of dead fetuses, and number of implantation. As depicted in the sponsor table 7, these parameters in the methyl salicylate group were comparable to those in the control group. Also, there were no significant differences in the weanling index among the 4 treatment groups.

A significant decrease in the birth index (6%) and lower body weight (9.2%) in the male newborns were observed in the 200 mg/kg/group.

Offspring mean body weight of both F₁ males and F₁ females during lactation and maturation periods were significantly reduced in the 200 mg/kg/day group.

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Table 7 Terminal delivery in P₀ dams and F₁ offspring

Group and dose	Vehicle Control	20 mg/kg	60 mg/kg	200 mg/kg
No. of dams	20	20	20	20 g)
Gestational days a)	21.60 ± 0.50	21.90 ± 0.55	21.95 ± 0.22*	21.94 ± 0.42
No. of implantations b)	278(13.90 ± 2.00)	295(14.75 ± 1.41)	292(14.60 ± 2.06)	251(13.94 ± 2.13)
No. of litter b)	270(13.50 ± 2.16)	281(14.05 ± 1.57)	279(13.95 ± 2.50)	215(11.94 ± 3.33)
Gestation index c)	100	100	100	90.00
No. of live newborns b)	268(13.40 ± 2.09)	279(13.95 ± 1.47)	277(13.85 ± 2.52)	208(11.56 ± 3.36)
Birth index d)	96.40	94.58	94.86	82.87*
Sex ratio of live newborns e)	1.00(134/134)	0.94(135/144)	0.95(135/142)	0.94(101/107)
Body weight of live newborns (g) a)				
Male	6.5 ± 0.5	6.7 ± 0.6	6.6 ± 0.4	5.9 ± 0.6**
Female	6.0 ± 0.4	6.3 ± 0.5	6.3 ± 0.5	5.6 ± 0.7
No. of stillborns f)				
Male	0	2	0	2
Female	2	0	2	5
Total	2(0.74)	2(0.71)	2(0.72)	7(3.26)
No. of live newborns with external anomalies	0	0	0	0

*: P<0.05, **: P<0.01 (significantly different from vehicle control).

a) Values are mean ± S.D.

b) Values in parentheses represent mean ± S.D.

c) Values in represent percentages to the number of pregnant animals.

d) Values in represent percentages to the number of implantations.

e) Values in parentheses represent number of male/female live newborns.

f) Values in parentheses represent percentages to the number of litters.

Craniorachischisis was observed in 4 stillborns in the 200 mg/kg group and vestigial tail and anal atresia were observed in the 1 stillborn in the 60 mg/kg group.

g) Dams with live newborns were 18 among 20 dams.

At scheduled necropsy on PND 22 for pups not selected for further evaluation, there were no internal findings that could be attributed to maternal care. However, examination of the fetuses for variations demonstrated an increase in the incidence of several skeletal anomalies and variations. Although there was a slight increase in the incidence of F₁ pups with fusion of the sternebra, fusion of thoracic vertebra, variation of cervical rib, short supernumerary rib, extra frontal ossification site, 8 lumbar vertebra, and incomplete ossification of caudal vertebra, these changes were not statistically significant. However, F₁ pups in the 200 mg/kg/day group had a statistically significant increase in the incidence of fusion of the cervical vertebra, misshapen sternebra, full supernumerary rib, accessory sternebra, lumbarization, 7 lumbar vertebra, incomplete ossification of cervical vertebra, incomplete ossification of thoracic vertebra, and incomplete ossification of lumbar vertebra.

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Skeletal Examination in F ₁ Offsprings			
	Dose (mg/kg/day)		
Parameter	0	60	200
№ litters examined skeletally	77	75	62
SKELETAL ANOMALIES			
№ of offsprings with skeletal anomalies (%)	3 (3.90)	6 (8.0)	20 (32.26)**
Nodulated ribs	0	1 (1.33)	0
Fusion of cervical vertebra	0	0	8 (12.90)**
Fusion of sternebra	3 (3.90)	5 (6.67)	11 (17.74)
Fusion of thoracic vertebra	0	0	2 (3.23)
Misshapen sternebra	0	0	5 (8.06)*
SKELETAL VARIATIONS			
№ of offsprings with skeletal variations (%)	20 (25.97)	30 (40)	58 (93.55)**
Cervical rib	1 (1.30)	5 (6.67)	0
Short supernumerary rib	0	0	2 (3.23)
Full supernumerary rib	0	0	45 (72.58)**
Extra frontal ossification site	0	0	2 (3.23)
Accessory sternebra	11 (14.29)	12 (16.0)	44 (70.97)**
Lumbarization	0	0	4 (6.45)*
Shortened 13 th rib	2 (2.60)	0	0
7 lumbar vertebrae	0	0	39 (62.90)**
8 lumbar vertebrae	0	0	1 (1.61)
Incomplete ossification of cervical vertebrae	1 (1.30)	2 (2.67)	24 (38.71)*
Incomplete ossification of thoracic vertebrae	6 (7.79)	10 (13.33)	43 (69.35)**
Incomplete ossification of lumbar vertebrae	0	0	22 (35.48)**
Incomplete ossification of caudal vertebrae	1 (1.30)	4 (5.33)	3 (4.84)

*: p < 0.05, significantly different from control

** : p < 0.15, significantly different from control

Developmental landmarks in the F₁ pups were examined. F₁ Males: Balanopreputial separation was delayed in the treatment groups compared to the control group. A significant decrease in the differentiation of the balanopreputial separation was observed on PND 43 for the males in the 200 mg/kg/day group; 11.11% of the males achieved balanopreputial separation compared to 40% of the control males. All males in the control group, 2, 60, and 200 mg/kg/day treatment groups, achieved balanopreputial separation on PND 46, PND 47, PND 60, and PND 72, respectively. On PND 10 (2.90% of offspring), PND 11 (24.64% of offspring), and PND 12 (63.77% of offspring), a significant decrease in the differentiation of incisor eruption was observed in the high dose group. On PND 12 and PND 15, incisor eruption was observed in 100% of the offspring in the control group and 200 mg/kg group, respectively. There was no difference in the mean day of acquisition of pinna detachment, piliation, gait, and descensus tests in the males between treatment groups. F₁ females: There was no difference in the mean day of vaginal patency, pinna detachment, piliation, and gait.

Developmental Landmarks Parameter	Day of Acquisition (for 100% of the pups)			
	0	20	60	200
	Males			
Balanopreputial Separation	45	47	61	72
Incisor Eruption	12	23	12	15
	Females			
Eye lid Separation	15	16	15	17
Incisor Eruption	12	13	13	16

F₁ behavioral evaluation: Behavioral evaluations included the water maze learning test, motor coordination, and open field. There were no treatment-related effects of methyl salicylate treated animals that were significantly different from control.

No treatment-related effects in the righting reflex, ipsilateral flexor reflex; visual placing or Preyer's reflex were observed. The results of these function tests in the methyl salicylate groups were comparable to those in the control group.

F₁ reproduction: Reproductive performance in the F₁ generation was not altered by F₀ maternal treatment. There were no differences in female or male mating indices or fertility indices. Similarly, there were no differences in the pre-coital interval.

F₂ findings: Cesarean section data of the F₂ litters were not altered by F₀ maternal treatment at any dose level tested. Cesarean section parameters evaluated included number of corpora lutea, number of implantation, pre-implantation loss, and viable fetuses.

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Reproductive Toxicology Studies with l-Menthol**Fertility and early embryonic development****Study title: Reproductive and Developmental Toxicity Study of l-Menthol in Rats by Subcutaneous Administration**

Key study findings: l-Menthol was administered to male rats for 2 weeks prior to mating and continued throughout mating until euthanasia. Female rats were treated for a total of 2 weeks prior to mating, throughout mating and through gestation day 6. The following findings regarding fertility and early embryonic development were obtained:

1. The study appears to be valid as significant depression of body weight gain were observed in both female and male rats in the high dose (1000 mg/kg/day) group.
2. Dose-dependent decrease in body weight gain was observed in both male and female rats.
3. Reproductive performance in males was not altered under the condition tested.
4. l-Menthol had no effects on mean epididymal sperm count, sperm production rate, motility or morphology compared to control.
5. Reproductive performance in females was not altered by any dose of l-menthol tested.
6. **Maternal NOAEL** could not be determined in this study since reduction in body weight was observed in the dams in the lower dose of 100 mg/kg/day. **The NOAEL was less than 100 mg/kg/day.**

Study no.: 40115

Volume #, and page #: 1.3, 071

Conducting laboratory and location:

Date of study initiation: April 26, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: l-Menthol, /55-058/100%

b(4)

Methods

Doses: 100, 300, and 1000 mg/kg/day @ 0.5, 1.5, and 5 mL/kg, respectively

Species/strain: Rats/Crj:CD(SD)IGS

Number/sex/group: 20/sex/group

Route, formulation, volume, and infusion rate: subcutaneous, solution

Satellite groups used for toxicokinetics:

Study design: Daily dosing for males 2 weeks prior to mating and throughout mating; female rats 2 weeks prior to mating during mating, through Gestation Day (GD) 6.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily (before administration and 4 hours after administration) for mortality and moribundity,

Body weights: Male body weights were recorded twice weekly throughout the dosing period until euthanasia. Female body weights were measured daily during gestation.

Food Consumption: Food consumption of the female rats was recorded daily.

Toxicokinetics: Blood was collected, from three males and three females per group, for toxicokinetic analysis 4 hours after dosing first day of dosing (day 0) and final day of dosing (day 13).

Estrous Cycle: Vaginal smears for determining the stage of estrous were evaluated daily from the start of drug administration and continued until evidence of copulation was observed.

Mating and fertility indices were calculated as follows:

Mating and Fertility Endpoints: At the end the 2 weeks mating period, the mating and fertility indices were calculated as follows:

Copulation Index = (number of animals with confirmed copulation/number of animals mated) x 100

Male Fertility Index = (number of pregnant females/number of males with confirmed copulation) x 100

Female Fertility Index = (number of pregnant females/number of females with confirmed copulation) x 100

Gestation Day 13 Uterine Examinations: Female rats were euthanized by exsanguination from the lateral iliac artery on gestation day 13. The uterus and ovaries were examined. The numbers of corpora lutea were recorded. The number of dead and live embryos was counted. Also, the pre-implant loss index (number of corpora lutea – number of implants/number of corpora lutea x 100 and dead embryo index (number of dead embryos/number of implants x 100) were calculated.

Spermatogenic endpoints. Sperm was collected from the right caudal epididymis. Sperm count and motility were examined microscopically using a hemocytometer. Sperm morphology was also determined.

Spermatogenic indices were calculated as follows:

Sperm Motility = (number of mobile sperm/number of sperms examined) x 100

Sperm form anomalies index = (number of abnormal sperm/number of sperms examined) x 100

Necropsy: Macroscopic examination was completed on all animals at the scheduled euthanasia. Necropsy included examination of the organs and tissues. The testes, left epididymis, ovaries and skin of the treated site were preserved for histopathological examination. Also, organs with lesions of dead animal were preserved for histopathological examination.

Organ Weights: Weights of the following organs were obtained: testes, epididymis. The relative organs weight was calculated from the body weight measured the day prior to the necropsy.

Statistics: Statistical analysis was conducted using significance levels of $p < 0.01$ and $p < 0.05$ for comparison of treated groups with the control group. The following statistical tests were used:

Statistical Test	Parameters
Barlett's Method	Body Weights, Body Weight Gain, Food Consumption, Organ Weight, Estrous Cycle, Estrus Count, Number of Days Required to for Successful Copulation, Sperm Count, Number of Corpora Lutea, Implants, and Live Embryos
Chi-Square Test	Copulation Index, Male Fertility Index, Female Fertility Index,
Wilcoxon's Rank Sum Test	Sperm Motility, Sperm Form Anomalies Index, Pre-implant Loss Index, and Dead Embryo

Results

Mortality: No treatment-related deaths occurred in the females. However, one death occurred in the male high dose group on the seventh day of drug administration. On the day prior to this rat death, he exhibited epileptoid convulsions and temporary coma during dosing on day 6. After drug administration, decrease activity was seen in this rat.

Disposition	Summary of Animal Disposition (N=20/sex/group)							
	Males (mg/kg)				Females (mg/kg)			
	0	100	300	1000	0	100	300	1000
Found Dead	0	0	0	1	0	0	0	0
Scheduled Necropsy	20	20	20	19	20	20	20	20

Clinical signs: No-treatment induced clinical signs were observed in either the males or females in the 100 and 300 mg/kg treatment groups. Treatment-related findings at 1000 mg/kg included prone or lateral position, hypoactivity, bradypnea and hypothermia. These signs were observed in 1 (5%) male and 2 (10%) female at 4 hours post-dosing on day 1 of drug treatment. These signs were also observed in 10% and 5% of the females on days 2 and 3 respectively. On days 8 and 10, treatment-related changes at the injection site began to appear in the females and males, respectively. These changes included crust at the site of injection and hair loss. The number of rats exhibiting these changes increased with the number of days of treatment.

Body weight: Male Rats. As depicted in Table 4 (copied from the sponsor's submission), the mean body weight of the l-menthol-treated male rats was significantly

lower than the controls. The reduced body weight became evident on days 10, 10, and 7 for the 100, 300, and 1000 mg/kg/day treatment groups, respectively, and remained reduced for the remainder of the study.

Table 4 Body weights in male rats

Group and dose		Vehicle control		100 mg/kg		300 mg/kg		1000 mg/kg	
Days of treatment	0	375.6 ± 10.0	(20)	376.0 ± 11.2	(20)	376.7 ± 11.1	(20)	376.4 ± 13.9	(20)
	1	385.4 ± 11.7	(20)	380.6 ± 11.2	(20)	382.0 ± 10.6	(20)	382.0 ± 13.8	(20)
	3	400.4 ± 13.7	(20)	392.6 ± 12.6	(20)	394.7 ± 11.7	(20)	390.5 ± 18.9	(20)
	7	426.7 ± 16.2	(20)	414.9 ± 14.0	(20)	416.1 ± 16.6	(20)	419.9 ± 21.8*	(19)
	10	445.8 ± 18.9	(20)	428.9 ± 16.0*	(20)	428.0 ± 18.1*	(20)	419.7 ± 26.7**	(19)
	14	469.9 ± 22.3	(20)	446.3 ± 17.3**	(20)	443.9 ± 19.2**	(20)	436.3 ± 25.4**	(19)
	17	483.6 ± 21.4	(20)	454.5 ± 17.5**	(20)	448.1 ± 20.0**	(20)	447.8 ± 26.6**	(19)
	21	508.3 ± 22.7	(20)	489.3 ± 18.2**	(20)	465.7 ± 22.6**	(20)	463.3 ± 27.8**	(19)
	24	525.2 ± 26.8	(20)	480.5 ± 19.8**	(20)	475.4 ± 25.7**	(20)	474.8 ± 27.6**	(19)
	28	545.5 ± 29.6	(20)	494.6 ± 22.4**	(20)	492.0 ± 25.7**	(20)	493.1 ± 30.4**	(19)
	31	562.7 ± 29.0	(20)	503.2 ± 22.7**	(20)	499.1 ± 28.0**	(20)	500.3 ± 31.6**	(19)
	35	581.9 ± 30.5	(20)	513.4 ± 24.1**	(20)	514.9 ± 30.2**	(20)	519.9 ± 33.8**	(19)
	38	597.7 ± 37.8	(20)	522.4 ± 25.0**	(20)	524.0 ± 32.0**	(20)	532.5 ± 36.9**	(19)
	42	614.5 ± 40.8	(20)	539.1 ± 27.5**	(20)	533.8 ± 31.1**	(20)	547.6 ± 39.1**	(19)
	45	629.5 ± 42.6	(20)	537.8 ± 28.3**	(20)	541.8 ± 33.4**	(20)	558.7 ± 40.4**	(19)
	49	645.4 ± 41.1	(20)	547.2 ± 27.9**	(20)	553.2 ± 35.1**	(20)	573.2 ± 40.1**	(19)

*: P<0.05, **: P<0.01 (significantly different from vehicle control). Values are mean±S.D. and the values in parentheses represent the number of animals. One animal (1000 mg/kg) died on day 7 of administration.

The body weight gain data are presented in Table 7 (copied from the sponsor's submission). Compared to the control group, a significant reduction of body weight gain was observed in all three treatment groups throughout the treatment period. By the end of the study period (day 49), the body weight gain observed in the groups was 36.5%, 34.5%, and 26.7% less than the control in the 100, 300, and 1000 mg/kg/day groups, respectively.

Table 7 Body weight gains in male rats

Group and dose		Vehicle control		100 mg/kg		300 mg/kg		1000 mg/kg	
Days of treatment	1	8.8 ± 4.2	(20)	4.6 ± 4.5**	(20)	5.4 ± 3.6**	(20)	5.7 ± 3.7*	(20)
	3	24.6 ± 5.5	(20)	16.6 ± 4.1**	(20)	18.6 ± 4.4**	(20)	14.1 ± 10.3**	(20)
	7	58.7 ± 9.7	(20)	39.0 ± 6.3**	(20)	39.4 ± 6.5**	(20)	32.4 ± 11.4**	(19)
	10	76.3 ± 12.4	(20)	52.9 ± 8.4**	(20)	51.3 ± 10.7**	(20)	44.8 ± 14.9**	(19)
	14	94.2 ± 16.7	(20)	70.4 ± 10.4**	(20)	67.3 ± 12.3**	(20)	69.8 ± 14.2**	(19)
	17	108.0 ± 18.5	(20)	78.5 ± 12.2**	(20)	71.4 ± 12.5**	(20)	71.4 ± 12.5**	(19)
	21	132.1 ± 17.4	(20)	93.3 ± 13.9**	(20)	89.8 ± 14.6**	(20)	87.9 ± 11.3**	(19)
	24	148.5 ± 21.0	(20)	104.5 ± 15.2**	(20)	98.1 ± 17.4**	(20)	99.2 ± 16.4**	(19)
	28	168.9 ± 24.5	(20)	118.7 ± 17.4**	(20)	115.3 ± 17.1**	(20)	113.6 ± 19.6**	(19)
	31	182.1 ± 24.0	(20)	127.2 ± 17.4**	(20)	122.5 ± 19.2**	(20)	124.8 ± 22.2**	(19)
	35	204.3 ± 24.3	(20)	137.4 ± 18.4**	(20)	138.2 ± 22.0**	(20)	144.4 ± 23.2**	(19)
	38	223.1 ± 21.3	(20)	146.4 ± 19.2**	(20)	147.4 ± 23.5**	(20)	152.0 ± 28.0**	(19)
	42	238.5 ± 26.1	(20)	158.1 ± 21.6**	(20)	157.1 ± 22.9**	(20)	172.1 ± 28.6**	(19)
	45	253.1 ± 27.2	(20)	161.8 ± 22.9**	(20)	185.2 ± 25.3**	(20)	183.2 ± 30.1**	(19)
	49	269.6 ± 25.3	(20)	171.3 ± 22.2**	(20)	176.5 ± 27.4**	(20)	192.8 ± 30.5**	(19)

*: P<0.05, **: P<0.01 (significantly different from vehicle control). Values are mean±S.D. and the values in parentheses represent the number of animals. One animal (1000 mg/kg) died on day 7 of administration.

Female Rats. As depicted in Table 5 and 6 from the sponsor's submission, females in the low-dose (100 mg/kg/day) and mid-dose (300 mg/kg/day) groups' mean body weight was significantly lower when compared to the control group during treatment and during gestation. Similar findings were noted with body weight gain (Tables 8 and 9 from the sponsor's submission). Significant reduction of body weight gain was also observed in the low-dose and mid-dose groups during treatment and gestation.

Table 5 Body weights in female rats

Group and dose		Vehicle control		100 mg/kg		300 mg/kg		1000 mg/kg	
		Body weight (g)							
Days of treatment	0	239.5 ± 10.8	(20)	243.4 ± 10.7	(20)	239.8 ± 10.3	(20)	238.5 ± 10.4	(20)
	1	241.3 ± 9.8	(20)	242.6 ± 8.4	(20)	240.6 ± 10.0	(20)	243.1 ± 9.5	(20)
	3	251.9 ± 9.4	(20)	249.0 ± 10.4	(20)	243.7 ± 9.8	(20)	246.4 ± 10.5	(20)
	7	266.3 ± 10.1	(20)	261.6 ± 12.4	(20)	259.4 ± 13.0	(20)	263.3 ± 10.8	(20)
	10	274.3 ± 8.8	(20)	266.6 ± 12.3	(20)	265.4 ± 12.0	(20)	268.9 ± 10.9	(20)
	14	287.1 ± 9.7	(20)	271.4 ± 14.3**	(20)	273.1 ± 15.1**	(20)	279.0 ± 11.3	(20)

*: P<0.05, **: P<0.01 (significantly different from vehicle control). Values are mean±S.D. and the values in parentheses represent the number of animals.

Table 6 Body weights in Fo dams

Group and dose		Vehicle control		100 mg/kg		300 mg/kg		1000 mg/kg	
		Body weight (g)							
Days of gestation	0	293.0 ± 13.3	(20)	279.5 ± 14.2**	(20)	322.0 ± 15.5**	(20)	295.7 ± 17.1	(20)
	1	307.5 ± 13.8	(20)	287.8 ± 13.5**	(20)	350.5 ± 15.3**	(20)	305.0 ± 15.4	(20)
	2	316.4 ± 13.3	(20)	296.4 ± 13.0**	(20)	358.4 ± 16.0**	(20)	312.7 ± 15.2	(20)
	3	323.5 ± 13.2	(20)	301.4 ± 12.6**	(20)	363.0 ± 15.5**	(20)	319.0 ± 17.0	(20)
	4	330.3 ± 10.8	(20)	307.2 ± 12.2**	(20)	368.9 ± 15.4**	(20)	323.9 ± 16.8	(20)
	5	339.0 ± 13.0	(20)	312.5 ± 11.5**	(20)	372.8 ± 14.9**	(20)	329.8 ± 16.5	(20)
	6	343.1 ± 14.7	(20)	317.8 ± 12.1**	(20)	376.4 ± 16.3**	(20)	335.4 ± 15.4	(20)
	7	347.4 ± 12.7	(20)	322.5 ± 12.3**	(20)	377.5 ± 15.3**	(20)	340.1 ± 17.7	(20)
	8	353.4 ± 12.4	(20)	328.7 ± 11.2**	(20)	378.3 ± 16.0**	(20)	344.4 ± 17.5	(20)
	9	356.7 ± 15.3	(20)	331.6 ± 12.6**	(20)	379.5 ± 17.9**	(20)	347.9 ± 16.5	(20)
	10	361.4 ± 11.9	(20)	338.3 ± 11.8**	(20)	378.5 ± 17.6**	(20)	355.0 ± 17.4	(20)
	11	370.3 ± 13.2	(20)	346.4 ± 12.6**	(20)	383.0 ± 17.5**	(20)	359.6 ± 17.7	(20)
	12	378.0 ± 12.4	(20)	351.1 ± 12.0**	(20)	389.8 ± 17.5**	(20)	366.5 ± 17.6	(20)
	13	380.6 ± 11.5	(20)	358.0 ± 17.9**	(20)	394.4 ± 17.1**	(20)	371.9 ± 17.8	(20)

** : P<0.01 (significantly different from vehicle control). Values are mean±S.D. and the values in parentheses represent the number of dams.

Table 8 Body weight gains in female rats

Group and dose		Vehicle control		100 mg/kg		300 mg/kg		1000 mg/kg	
Days of treatment	1	1.7 ± 8.6	(20)	1.3 ± 4.9	(20)	0.8 ± 5.0	(20)	3.6 ± 6.5	(20)
	3	12.4 ± 6.7	(20)	7.5 ± 4.0	(20)	8.4 ± 6.0	(20)	6.9 ± 8.6	(20)
	7	26.7 ± 6.3	(20)	20.2 ± 5.7*	(20)	19.6 ± 8.7**	(20)	23.8 ± 8.5	(20)
	10	34.7 ± 8.1	(20)	25.2 ± 7.3**	(20)	25.6 ± 8.7**	(20)	30.4 ± 9.9	(20)
	14	47.6 ± 9.0	(20)	29.9 ± 9.7**	(20)	33.3 ± 9.3**	(20)	39.5 ± 10.0*	(20)

*: P<0.05, **: P<0.01 (significantly different from vehicle control). Values are mean±S.D. and the values in parentheses represent the number of animals.

Table 9 Body weight gains in Fo dams

Group and dose		Vehicle control		100 mg/kg		300 mg/kg		1000 mg/kg	
Days of gestation	1	9.5 ± 4.0	(20)	8.3 ± 4.4	(20)	7.5 ± 3.3	(20)	9.2 ± 4.9	(20)
	2	18.4 ± 4.8	(20)	17.0 ± 5.6	(20)	15.3 ± 3.6	(20)	16.9 ± 4.7	(20)
	3	25.5 ± 5.9	(20)	22.0 ± 6.2	(20)	20.6 ± 5.1*	(20)	23.3 ± 5.8	(20)
	4	32.3 ± 5.3	(20)	27.7 ± 8.5	(20)	23.9 ± 6.3**	(20)	28.2 ± 6.6	(20)
	5	38.0 ± 8.2	(20)	35.0 ± 8.3*	(20)	29.6 ± 5.3**	(20)	34.1 ± 5.7	(20)
	6	45.1 ± 7.2	(20)	38.4 ± 7.9*	(20)	33.4 ± 5.8**	(20)	39.7 ± 7.3*	(20)
	7	49.7 ± 7.3	(20)	43.1 ± 8.5*	(20)	39.4 ± 6.6**	(20)	44.4 ± 9.0	(20)
	8	55.2 ± 6.8	(20)	48.2 ± 8.3*	(20)	43.3 ± 8.1**	(20)	48.7 ± 7.6*	(20)
	9	58.2 ± 6.8	(20)	52.1 ± 9.6	(20)	47.5 ± 8.6**	(20)	52.2 ± 8.3	(20)
	10	63.9 ± 5.3	(20)	58.9 ± 10.5	(20)	52.4 ± 7.6**	(20)	57.9 ± 8.3	(20)
	11	72.9 ± 6.2	(20)	66.7 ± 8.8	(20)	60.0 ± 8.7**	(20)	63.8 ± 8.9**	(20)
	12	78.0 ± 7.8	(20)	71.1 ± 10.7	(20)	67.8 ± 7.9**	(20)	70.8 ± 10.3*	(20)
	13	82.6 ± 8.5	(20)	78.5 ± 11.0	(20)	71.4 ± 9.3**	(20)	76.2 ± 10.4	(20)

*: P<0.05, **: P<0.01 (significantly different from vehicle control). Values are mean±S.D. and the values in parentheses represent the number of dams.

Food consumption: No significant treatment-related effects on food consumption were observed in either the male or female rats in the low- and mid-dose groups. However, a decrease in food consumption was observed in both males and females in the 1000 mg/kg group.

Toxicokinetics: Plasma levels of l-menthol are reproduced in the sponsor's table below. The results indicate that the rats were exposed to a measurable amount of l-menthol. The

plasma levels were dose-dependent; levels increased with increasing dose in both genders. There were no clear sexual differences observed between the results of the male and female rats. Plasma concentration was slightly affected by repeated dosing in both males and females. The mean plasma concentration of l-menthol was lower on day 13 in the males treated with 300 and 1000 mg/kg/day. In contrast, the mean plasma concentration was higher on day 13 in the females administered 1000 mg/kg/day of l-menthol. These results could be due to a difference in the metabolism of l-menthol after repetitive administration.

Table 21. Plasma concentrations of l-Menthol in male and female rats

Days of administration	Dose (mg/kg)		male (n=3)		female (n=3)	
			Plasma concentrations (µg/mL)		Plasma concentrations (µg/mL)	
			Time after administration		Time after administration	
			4 hr		4 hr	
0	100	Mean	0.905		0.997	
		SD	0.051		0.189	
	300	Mean	2.99		2.11	
		SD	2.34		0.59	
	1000	Mean	7.45		4.39	
		SD	0.51		0.81	
13	100	Mean	1.02		0.720	
		SD	0.04		0.227	
	300	Mean	1.95		1.84	
		SD	0.40		0.42	
	1000	Mean	5.51		6.22	
		SD	0.97		0.55	

Values less than limit of quantitation (LLOQ: 0.05 µg/mL) were measured to be 0

Necropsy: The results are presented in the table below. The only potentially treatment-related changes were noted in the subcutis area of the injection site. An oily fluid was retained in the treatment site. In the males, the degree of retention (severe) was highest for the control group and the 1000 mg/kg group; 63% of the subjects were graded severe. Females in the control group and highest treatment group presented with retention of an oily fluid in the subcutis of the treated site. In the control group and the 1000 mg/kg group, 50% and 45% of the subjects had a moderate degree of fluid retention, respectively. In the highest treatment group, 55% of the females were scored with a slight amount of retention of oily fluid. In addition, crust and loss of hair was also observed in the males and females.

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Number Examined	Incidence of Gross Necropsy Observations							
	№ Males				№ Females			
	20	20	20	19	20	20	20	20
Parameters	Dose Group (mg/kg/day)				Dose Group (mg/kg/day)			
	0	100	300	1000	0	100	300	1000
Epididymis								
<u>Nodule, light yellow</u>								
Slight	1	1	0	0				
Moderate	0	0	0	0				
Severe	0	0	0	0				
Integument (treated site)								
<u>Loss of hair</u>								
Slight	0	0	0	4	0	0	0	2
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Crust								
Slight	0	0	0	4	0	20	20	11
Moderate	0	0	0	0	10	0	0	9
Severe	0	0	0	0	10	0	0	0
Subcutis (treated site)								
<u>Retention, fluid, oily</u>								
Slight	0	20	12	0	0	20	20	11
Moderate	2	0	8	7	10	0	0	9
Severe	18	0	0	12	10	0	0	0

Organ Weights: The effect of l-menthol on absolute and relative organ weights in male is summarized in the sponsor’s table copied below. A significant (p<0.01) increase in the relative weights of the testis and epididymides was noted in the l-menthol treated males compared to the control. No changes were observed in the absolute weights of the testis and epididymides. The reviewer concurs with the sponsor statement that “these changes were considered to be attributable to the low final body weight and not be related to the l-menthol.”

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): As indicated in the table below, no treatment-related effects on either male mating index or male fertility index was noted. Male copulation index was calculated by dividing the number of with evidence of mating by the total number of males used for mating (x100). In the highest dose treatment group, the copulation index and fertility index were the result of the one male out of 19 subjects not copulating.

Parameter	Summary of Male Reproductive Performance (n = 19-20)			
	Dose (mg/kg)			
	0	100	300	1000
Male Copulation Index (%)	100%	100%	100%	94.74%
Male Fertility Index (%)	100%	100%	100%	94.74%

Spermatogenic Endpoints: As depicted in the table below, there were no effects of l-menthol at the doses tested on mean sperm number, motility, and morphology compared to control animals.

Dose (mg/kg/day)	№ Examined	Spermatogenic Endpoints (Mean ± S.D.)		
		Count of Sperm (x10 ⁶ /g)	Sperm Motility (%)	Sperm Form Anomalies Index (%)
0	20	665.69 ± 90.86	98.57 ± 1.05	1.70 ± 1.54
100	20	696.13 ± 139.06	97.84 ± 2.12	2.10 ± 1.46
300	20	634.62 ± 116.50	97.70 ± 2.11	1.55 ± 0.93
1000	19	652.36 ± 113.03	97.88 ± 1.82	1.70 ± 1.18

In females, l-menthol had no effects on female mating index or female fertility index; these indices were not changed by the treatment. The 94.7% female fertility index was the result of the one male that died in this group. There were no significant differences between the control and l-menthol groups in the count of estrus or estrous cycle.

Parameter	Summary of Female Reproductive Performance (n = 20)			
	Dose (mg/kg)			
	0	100	300	1000
Female Mating Index (%)	100%	100%	100%	100%
Female Fertility Index (%)	100%	100%	100%	94.74%
Mean Count of Estrous ± S.D.	3.80 ± 0.41	3.90 ± 0.31	3.90 ± 0.64	3.85 ± 0.37
Mean Estrus Cycle Length ± S.D.	4.07 ± 0.23	4.0 ± 0.0	4.0 ± 0.0	4.24 ± 0.68

Mean embryonic data is presented in the tables below. No treatment-related effects on the number of corpora lutea and implantation sites were observed. The number of corpora lutea and implantation sites was comparable among the four treatment groups. However, the number of pre-implantation loss was significantly higher in the low-dose group (100 mg/kg/day) group. The low-dose was also associated with an increase in number of viable embryo. The observed increase number of dead embryo at this dose was not statistically significant.

Effects of l-menthol on the mean number of corpora lutea, implantation site, percent of rats pregnant and placenta appearance.

Dose (mg/kg)	# Pregnant/Total (%)	(Total №) MEAN (± S.D.)		№ (% of the corpora lutea)
		Corpora Lutea	Implantation Site	Pre-implantation loss
0	20/20 (100%)	307 (15.3 ± 1.23)	300 (15.0 ± 1.03)	7 (2.28%)
100	20/20 (100%)	329 (16.45 ± 1.39)	308 (15.40 ± 1.39)	21 (6.38%)*
300	20/20 (100%)	307 (15.35 ± 1.39)	299 (14.95 ± 1.61)	8 (2.61%)
1000	20/20 (100%)	307 (15.35 ± 1.90)	290 (14.50 ± 1.54)	17 (5.54%)

*: Significantly different from control group (p<0.05)

Effects of l-menthol on litter (i.e., number of live and dead embryos).

Dose (mg/kg)	N ^o of Live Embryos (Mean ± S.D.)	N ^o of Dead Embryos (% of the # implants)
0	288 (14.40 ± 1.39)	12 (4.0)
100	276 (13.8 ± 2.14)	32 (10.39)
300	286 (14.30 ± 1.75)	13 (4.35)
1000	271 (13.55 ± 1.88)	19 (6.55)

Embryofetal development**Study title: Reproductive and Developmental Toxicity Study of l-Menthol in Rabbits by Subcutaneous.**

Key study findings: Female New Zealand rabbits were treated with l-menthol from gestation day 6 to gestation day 18 in a definitive segment II study. The key findings were:

1. Two dams aborted. One dam in the mid-dose group aborted on gestation day 29, and one dam in the high-dose group aborted on gestation day 27.
2. Significant reduction in body weight gain was observed in all l-menthol treatment groups; thus a **NOAEL** for maternal toxicity was not established and should be considered to be <150 mg/kg/day.
3. There was no difference in mean fetal weight in the treatment groups compared to the control animals.
4. There were no significant malformations (external, visceral or skeletal) or variations between treatment groups, indicating that l-menthol was not teratogenic under the conditions tested.
5. A **NOAEL** for developmental toxicity was not established and should be considered to be < 150 mg/kg/day.

Study no.: 40122

Volume #, and page #: 1.5, 218

Conducting laboratory and location:

Date of study initiation: April 11, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: l-Menthol, 55-058, 100%

Methods

Doses: 150, 300, and 600 mg/kg/day

Species/strain: New Zealand White Rabbits/Kbs:NZW

Number/sex/group: See table below on group assignments

b(4)

Group	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/ml)	Dosage Volume (ml/kg)	Number of Females
1	l-Menthol	0	0	3	20
2	l-Menthol	150	200	0.75	20
3	l-Menthol	300	200	1.5	21
4	l-Menthol	600	200	3.0	20

Route, formulation, volume, and infusion rate: subcutaneous, dissolved in corn oil

Satellite groups used for toxicokinetics: None

Study design: Animals were dosed from day 6 to 18 of gestation. The study design consisted of one control vehicle group and three treatment groups. Day of successful copulation was designated as gestation day 0.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily (before administration and 4 hours after administration) for mortality during the administration period and once during other periods.

Body weights: Body weights were recorded on days 0, 3, 6-19, 23, 26, and 29 of gestation.

Food Consumption: Food consumption was recorded on days 1, 3, 6-19, 23, 26, and 29.

Necropsy: All surviving rats were euthanasia by exsanguination on day 29 of gestation. Their organs and tissues were examined macroscopically after removal of the ovaries and uterus.

Examination of Embryo and Fetuses:

Cesarean Section: The number of corpora lutea, implants, early and late resorptions, and dead and live fetuses were recorded. The placenta was examined macroscopically.

Fetal Observations: Each fetus was weighed and examined for external anomalies.

Visceral Examination: Each fetus was sexed, sectioned into the head, chest, and abdomen, and examined macroscopically. The brain, kidneys and heart were removed and fixed in Bouin's solution and subsequently examined for visceral anomalies. The other organs were removed and fixed in 10% neutral buffered formalin solution and preserved.

Skeletal Examination: Skeletal anomalies, variations and progress of ossification were assessed after staining with Alizarin Red S.

Toxicokinetic Analysis: Blood was collected four hours post-dosing on days 6 and 18 of gestation. Blood samples were collected from the auricular vein.

Results

Mortality (dams): One female died on gestation 28 in the low-dose group (150 mg/kg/day). This dam presented with paralysis of the hind limbs on gestation 15. One dam in the mid-dose group (300 mg/kg/day) and one dam in the high dose group (600 mg/kg/day) aborted on gestation day 29 and gestation day 27, respectively. This finding could be attributed to the l-menthol treatment.

Clinical signs (dams): Clinical signs were observed daily. Overt clinical signs were observed in one rabbit in the low-dose group, one rabbit in the mid-dose group, and in 3 rabbits in the high-dose group. Summary of these clinical signs are presented in the table below.

Summary of Clinical Signs

Rabbit №	Dose (mg/kg/day)	Day of Occurrence	Observed Clinical Sign(s)	Outcome	Necropsy Results
219	150	Gestation day 13	Paralysis of hind limbs	Died on gestation day 28	Dislocation of the lumbar vertebra
306	300	Gestation Day 29	Had an abortion		Subcutis (nontreated and treated sites): Retention of oily fluid
404	600	Gestation Day 15	Lateral position, bradypnea, and hypothermia	Sacrificed on gestation day 15	Gall Bladder: Spot, mucosa, black Subcutis (nontreated site): Retention of oily fluid
411	600	Gestation Day 18	Bradypnea, hypothermia, and hypoactivity	Died on gestation day 19	Thymus: Spot, dark red Vagina: Coloration, mucosa, dark red Subcutis (treated site): Retention of oily and; coloration and dark red
420	600	Gestation Day 27	Had an abortion		Subcutis (treated site): Retention of oily

Body weight (dams): The mean body weights were comparable to control body weights during gestation. Treatment-related effects on body weight gain were observed during gestation. As depicted in Figure 2 (copied from the sponsor submission), a reduction on body weight gain occurred in the treatment groups. On gestation days 11 thru 23, a significant reduction in body weight gain was observed in the low dose group. The mean body weight gain in the low dose group ranged from 31% to 55% lower than the control group. A significant reduction in body weight gain was observed in the mid dose and high dose groups on gestation days 17 thru 26 (56%-62% lower than control) and gestation days 9 thru 14 (115-49% lower than control) and on day 17 (58% lower than control), respectively.

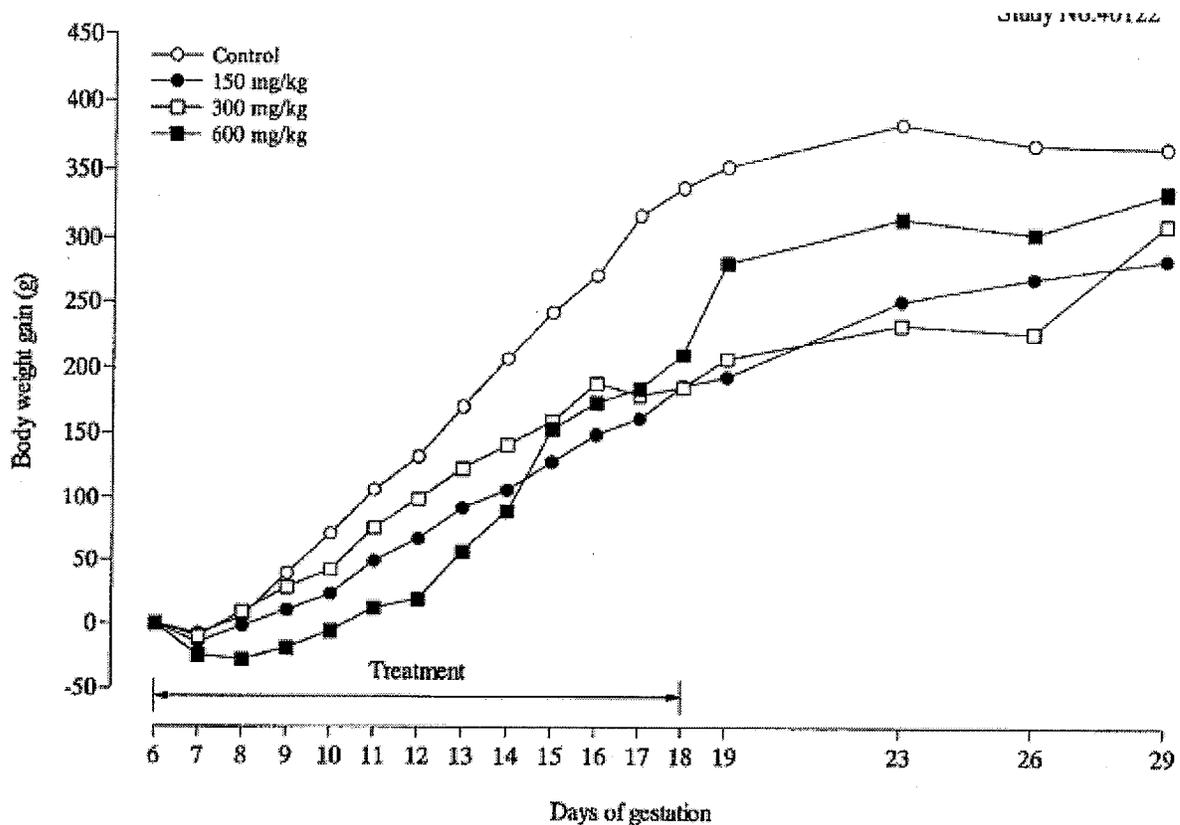


Fig.2 Body weight gains in Fo dams.

Food consumption (dams): No significant treatment-related effects on food consumption were observed during gestation. The mean food consumption for the treatment group was comparable to the control group.

Toxicokinetics: Blood was drawn from four dams per group at four hours post-dosing on gestations days 6 and 18. The toxicokinetic results are presented in the table below:

Day of Gestation	Dose (mg/kg/day)	Plasma Concentration (± SD) (µg/mL)
6	150	1/79 (0.44)
	300	3.59 (0.73)
	600	7.84 (3.85)
18	150	1.64 (0.70)
	300	3.45 (0.89)
	600	8.55 (1.73)

Plasma concentration was dose-dependent; increasing the dose of menthol produced an increase in the plasma concentration of drug. There were no apparent differences in blood plasma levels between the gestation day 6 and gestation day 18.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): One female in the 150 mg/kg/day group died on gestation day 28. Dislocation of the lumbar vertebra was observed during the necropsy; this is incidental and not treatment-related. One female in the 600 mg/kg/day group died on gestation 19.

All rabbits that survived to the scheduled necropsy on gestation day 29 showed treatment-related findings. Retention of an oily substance in the subcutis of the treated site and non-treated site (i.e., breast, axillary region, abdomen, or inguinal region) was observed in all dams in the control and l-menthol treatment groups. The degree of retention ranged from slight to moderate. This finding is attributable to the vehicle since it was also observed in the control group.

Data from cesarean section are presented in the sponsor's table 7 presented below. No treatment-related effects on the number of implants, number of pre-implants loss, and placental anomalies were observed. The number of corpora lutea in the low dose and high dose groups was comparable to the control group. A significant decrease ($p < 0.05$) in the number of corpora lutea was observed in the mid dose group. As depicted in Table 7, no treatment-related effects were observed in litter size, number of live and dead fetuses, number of early and late resorptions, and fetal body weight. While the sex ratio of live fetuses in the mid and high dose groups were comparable to the control group, the sex ratio of live fetuses in the low dose group was significantly different from the control group. There were a higher number of males born.

Table 7 Observation on cesarean section in Po dams

Study No. 40122

Group and dose	Control	150 mg/kg	300 mg/kg	600 mg/kg
No. of dams	20	19	17	17
No. of corpora lutea a)	206 (10.30 ± 2.39)	191 (10.05 ± 1.61)	145 (8.53 ± 1.87)*	164 (9.65 ± 1.50)
No. of implants a)	164 (8.20 ± 3.07)	159 (8.37 ± 3.04)	130 (7.65 ± 2.32)	128 (7.53 ± 2.70)
No. of pre-implant loss b)	42 (20.39)	32 (16.75)	15 (10.34)	36 (21.95)
No. of total dead fetuses c)	18 (10.98)	11 (6.92)	14 (10.77)	5 (3.91)
Early resorptions	14 (8.54)	7 (4.40)	12 (9.23)	2 (1.56)
Late resorptions	4 (2.44)	4 (2.52)	2 (1.54)	2 (1.56)
Dead fetuses	0	0	0	1 (0.78)
No. of live fetuses a)	146 (7.30 ± 3.34)	148 (7.79 ± 2.76)	116 (6.82 ± 1.98)	123 (7.24 ± 2.70)
Sex ratio of live fetuses d)	0.80 (65/ 81)	1.35 (85/ 63)*	1.15 (62/ 54)	0.86 (57/ 66)
Body weight of live fetuses (g) e)				
Male	39.21 ± 10.15	44.99 ± 7.81	43.64 ± 5.06	42.96 ± 6.77
Female	40.87 ± 10.52	43.75 ± 6.45	41.73 ± 6.30	41.89 ± 6.15
No. of live fetuses with external anomalies f)	2 (1.37)	1 (0.68)	1 (0.80)	0
Meningoencephalocele	0	0	1 (0.80)	0
Open eyelid	0	0	1 (0.80)	0
Cleft lip	1 (0.68)	0	0	0
Short tail	1 (0.68)	1 (0.68)	0	0
No. of live fetuses with placental anomalies	0	0	0	0

*: $P < 0.05$ (significantly different from control).
a) Values in parentheses represent mean ± S.D.
b) Values in parentheses represent percentages to the number of corpora lutea.
c) Values in parentheses represent percentages to the number of implants.
d) Values in parentheses represent number of male/female fetuses.
e) Values are mean ± S.D.
f) Values in parentheses represent percentages to the number of live fetuses.

No treatment-related gross abnormalities were observed. Several non-treatment gross abnormalities were observed: 1) One fetus in the mid-dose group presented with meningoencephalocele; 2) One fetus in the low-dose group presented with a short tail; 3) One fetus in the control group presented with a cleft lip while another one had a short tail.

Offspring (malformations, variations, etc.): The sponsor's analysis of fetuses malformations are copied below. Skeletal examination did not detect any significant treatment-related skeletal variations or malformations among the fetuses examined. Examinations did reveal some external, visceral and/or skeletal abnormalities and skeletal variations in the l-menthol treatment groups. However, there was no particular pattern of appearance and no significant difference between the control and l-menthol groups. Skeletal anomalies were observed in 3 (2.08%), 5 (3.40%), 2 (1.74%), and 4 (3.25%) fetuses in the control, 150, 300, and 600 mg/kg/day groups, respectively. Skeletal variations were observed in 76 (52.78%), 73 (49.66%), 46 (40.0%), and 83 (67.48%) fetuses in the control, 150, 300, and 600 mg/kg/day groups, respectively.

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Prenatal and postnatal development

Study title: Reproductive and Developmental Toxicity Study of l-Menthol in rats by Subcutaneous Administration

Key study findings: Female rats were treated subcutaneously with l-menthol (100, 300, and 1000 mg/kg/day) from gestation day 6 to day 21 of lactation in the definitive segment III study. The key findings of the study were:

1. **Maternal NOAEL** could not be determined in this study since reduction in mean body weight gain was observed in the dams in the lowest dose of 100 mg/kg/day. **The NOAEL was less than 100 mg/kg/day.**
2. F₀ mortality was noted in 1 of 20 in the 1000 mg/kg/day group. This dam displayed epileptoid convulsions prior to death.
3. No treatment-related clinical signs were observed in the dams that survived to the scheduled sacrifice.
4. Mean body weight gain in the F₀ females were significantly reduced throughout the gestation period in all l-menthol treatment groups.
5. In surviving F₀ females, there were no differences between the litter size, number of pups born, number of implantations, birth index, sex ratio, birth index, and body weights.
6. No treatment-related effects on the development of the F₁ were observed. l-Menthol had no effects in the test for function, motor coordination, learning ability, emotional behavior, reproductive performance.
7. Offspring mean body weight during the pre-weaning and post-weaning was comparable to controls.
8. There were no treatment-related findings on the PND 22 pups not selected for further study at necropsy.
9. Developmental landmarks in the F₁ males indicated that balanopreputial separation in males from the 100, 200 and 1000 mg/kg/day groups was delayed compared to controls.

Study no.: 40117

Volume #, and page #: 1.4, 001

Conducting laboratory and location. _____

Date of study initiation: July 11, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

b(4)

Drug, lot #, and % purity: l-Menthol, Lot No 5-058, 100%

Methods

Doses: 100, 300, and 1000 mg/kg/day @ 0.5, 1.5, and 5.0 mL/kg, respectively

Species/strain: Rats/Crj:CD(SD)IGS

Number/sex/group: 20/females/group

Route, formulation, volume, and infusion rate: subcutaneous, dissolved in corn oil

Satellite groups used for toxicokinetics: No toxicokinetic study was performed.

Study design: F₀ female rats were dosed once a day on gestation day 6 to day 21 of lactation. Dosing was based on the results of a dose-range finding study in pregnant female rats (40116). All females were allowed to deliver naturally and rear their young to weaning (postnatal day 22). To reduce variability among the litters, eight pups per litter (4 per sex) were randomly selected on postnatal day 4.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily (before administration and 4 hours after administration) for mortality during the administration period and once during other periods.

Body weights: Body weights were recorded on days 3, 6, 9, 12, 15, 18, and 22 of gestation, and on days 0, 4, 7, 10, 14, 17, and 21 of lactation. Body weight gain was measured on day 0 after delivery during the lactation period.

Food consumption: Food consumption was recorded on days 1, 3, 6, 9, 12, 15, 18, and 20 of gestation and on days 1, 4, 7, 10, 14, 17, and 20 of lactation.

Necropsy: All surviving F₀ female rats were euthanized by exsanguination on day 22 after delivery. Their organs and tissues were examined macroscopically. After extraction of the ovaries and uterus, the number of implantation traces in the uterus was counted. Any F₀ female dams failing to deliver by day 24 of gestation were necropsied after obtain body weight. For all animals, the skin of the treated site and the ovaries and the uterus were fixed in 10% neutral buffered formalin solution and preserved.

Examination of offspring:

At birth: The following delivery observations were performed: number of litter, number of stillborns, number of live newborns, and the still birth index was calculated (number of stillborns/number of litter).

Live offspring: Weighted, sexed and examined for external anomalies.

Stillborns: Using the floating test, their extracted lungs were evaluated to determine whether they had breathed or not. All stillborns were fixed in pure ethanol by litter and stored.

Observations during lactation and after weaning included:

Function test: righting reflex and ipsilateral flexor (postnatal (PND) 5), visual pacing reflex (PND 6), Preyer's reflex (PND 28).

Postnatal differentiation test: All newborns were examined for pinna detachment (PND 4), incisor eruption (PND 10), gait and eyelid separation (PND 15), descensus testis (PND 31), cleavage of the balanopreputial gland (PND 42), and vaginal opening (PND 42).

Birth to PND 22: Testing of the F₁ litter included daily examination for clinical signs and mortality. Body weight of pups was recorded on day 0, 7, 14, and 21 after birth.

Organ weight: 1 offspring of each sex from a dam was euthanized on PND 22. The following organs were isolated and weighed: heart, lungs, liver, kidneys, adrenals, brain, spleen, thymus, and testes and ovaries.

Skeletal examination: On PND 22, 2 offsprings of each sex from a dam were euthanized under ether anesthesia. Their organs and tissues were observed macroscopically. Skeletal anomalies and variations were assessed after staining with alizarin red S.

PND 22 (weaning) and until mating: Offsprings were observed daily for clinical signs and mortality. Body weight and food consumption were recorded once a week. Neurobehavioral evaluation included: rotarod performance (5 weeks of age), water maze (6 weeks of age), and open field (8 weeks of age) were performed.

F₁ Reproductive capacity: At 12 to 13 weeks, the F₁ capacity to reproduce was evaluated. Duration of mating required for copulation, copulation index, and male and female fertility indices were assessed.

Results

F₀ in-life:

Mortality: One F₀ female in the 1000 mg/kg/day group died on day 12.

Clinical signs: The female in the 1000 mg/kg/day group that died early displayed epileptoid convulsions during drug administration on day 12. No treatment-related clinical signs were observed in the dams that survived until scheduled sacrifice.

Body weight: The sponsor's table 2 for mean body weights and table 3 for mean body weight gain of the F₀ generation are reproduced below. Mean body weights in the 300 and 1000 mg/kg/day group were significantly ($p < 0.05$) reduced compared to the controls beginning on gestation day 15. Mean body weights in the 1000 mg/kg/day group were significantly ($p < 0.05$) lower than the control dams on gestation day 20. Body weight changes were still evident in the l-menthol group during lactation. A significantly ($p < 0.01$ or $p < 0.05$) lower mean body weight was observed on lactation day 0-21 in the F₀ dams in the 100, and 300 mg/kg/day group compared to the control dams. At 1000 mg/kg/day, there was a statistically significant decrease in body weight on lactation days 0, 7, and 14-21. Body weight gains were statistically significantly decreased throughout the gestation period in the 100, 300, and 1000 mg/kg/day groups. During lactation period, a significant depression of body weight gains was observed throughout the lactation period in the 1000 mg/kg/day group and on lactation days 7-21 in the 300 mg/kg/day group.

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Study No. 40117

Table 2 Body weights in F₀ dams

Group and dose		Vehicle Control		100 mg/kg		300 mg/kg		1000 mg/kg				
Days of gestation	0	262.5±	11.1	(19)	262.1±	12.7	262.4±	14.1	(19)	262.2±	11.1	(20)
	3	283.6±	10.9	(19)	282.3±	10.7	282.8±	16.6	(19)	280.3±	12.0	(20)
	6	297.6±	10.3	(19)	296.7±	10.8	296.7±	17.8	(19)	295.4±	12.4	(20)
	9	313.8±	12.4	(19)	309.0±	13.1	308.4±	19.5	(19)	305.0±	14.4	(20)
	12	332.0±	13.8	(19)	323.4±	13.6	322.7±	19.7	(19)	321.0±	14.5	(20)
	15	352.7±	16.2	(19)	339.3±	14.6	338.5±	23.2*	(19)	336.0±	18.5*	(19)
	18	391.6±	17.5	(19)	375.5±	20.0	371.6±	26.6*	(19)	370.7±	22.2*	(19)
	20	420.9±	20.9	(19)	400.5±	22.1*	399.1±	30.0*	(19)	399.7±	22.0*	(19)

+: P<0.05 (significantly different from vehicle control).
 Values are mean±S.D. and the values in parentheses represent the number of dams.
 One dam in the 1000 mg/kg group died on day 12 of gestation.

Table 2 - continued Body weights in F₀ dams

Study No. 40117

Group and dose		Vehicle Control		100 mg/kg		300 mg/kg		1000 mg/kg				
Days of lactation	0	340.1±	14.5	(19)	323.2±	18.6*	320.1±	26.5*	(19)	318.0±	20.6**	(19)
	4	356.5±	12.9	(19)	326.8±	16.6**	331.7±	31.1**	(19)	329.3±	20.8	(19)
	7	365.6±	12.2	(19)	330.2±	16.1**	333.6±	25.2**	(19)	347.9±	21.7*	(19)
	10	375.7±	14.8	(19)	334.7±	19.1**	341.9±	27.5**	(19)	363.8±	19.8	(19)
	14	383.1±	13.4	(19)	335.2±	17.5**	341.2±	20.5**	(19)	363.4±	19.0**	(19)
	17	386.0±	13.8	(19)	333.0±	15.0**	337.8±	20.6**	(19)	366.3±	17.9**	(19)
	21	375.7±	12.1	(19)	317.5±	14.8**	325.5±	18.0**	(19)	359.5±	17.8**	(19)

+: P<0.05, **: P<0.01 (significantly different from vehicle control).
 Values are mean±S.D. and the values in parentheses represent the number of dams.

Table 3 Body weight gains in F₀ dams

Study No. 40117

Group and dose		Vehicle Control		100 mg/kg		300 mg/kg		1000 mg/kg				
Days of gestation	9	16.2±	4.5	(19)	12.2±	3.8*	11.7±	5.6*	(19)	9.6±	7.3*	(20)
	12	34.4±	7.3	(19)	26.7±	6.1**	26.0±	5.7**	(19)	25.6±	7.5**	(20)
	15	55.2±	8.8	(19)	42.6±	6.4**	41.8±	9.7**	(19)	41.4±	10.1**	(19)
	18	83.9±	10.9	(19)	78.8±	13.4**	75.1±	13.5**	(19)	76.0±	13.2**	(19)
	20	123.3±	13.6	(19)	103.7±	15.4**	102.4±	16.1**	(19)	105.0±	12.3**	(19)

+: P<0.05, **: P<0.01 (significantly different from vehicle control).
 Values are mean±S.D. and the values in parentheses represent the number of dams.
 One dam in the 1000 mg/kg group died on day 12 of gestation.

Table 3 - continued Body weight gains in F₀ dams

Study No. 40117

Group and dose		Vehicle Control		100 mg/kg		300 mg/kg		1000 mg/kg				
Days of lactation	4	16.4±	12.2	(19)	3.6±	9.7**	11.7±	14.7	(19)	21.9±	9.7	(19)
	7	25.8±	10.6	(19)	7.0±	10.7**	13.6±	10.1**	(19)	30.0±	12.9	(19)
	10	35.8±	11.7	(19)	11.5±	10.1**	21.8±	16.4**	(19)	45.9±	11.9*	(19)
	14	43.1±	10.6	(19)	12.0±	10.7**	21.1±	12.9**	(19)	45.4±	14.9	(19)
	17	46.0±	11.6	(19)	9.8±	14.2**	17.7±	15.1**	(19)	48.3±	12.0	(19)
	21	35.7±	13.8	(19)	-6.7±	12.6**	5.4±	15.4**	(19)	41.6±	16.1	(19)

+: P<0.05, **: P<0.01 (significantly different from vehicle control).
 Values are mean±S.D. and the values in parentheses represent the number of dams.

Food consumption: Food consumption was statistically significantly lower on gestation day 9 at 300 mg/kg/day (9%) and 1000 mg/kg/day (21%). A significant increase in food consumption was observed in the 1000 mg/kg/day group on lactation days 7 (9%) and 10 (9%).

Toxicokinetics: Not performed.

F₀ necropsy: All F₀ dams that survived to the scheduled sacrifice on lactation day 22 had retention of oily fluid in the subcutis of the treated site. The F₀ dams in the control and 1000 mg/kg/group had the highest degree of retention. This was not considered to be a treatment-related finding. The F₀ dam in the 1000 mg/kg/treatment group that died on gestation day 12 had retention of oily fluid and crust in the treated site.

F₁ physical development: No treatment-related effects on litter size, number of live fetuses, number of dead fetuses, sex ratio, number of implantations, birth index, and body weights. As depicted in the sponsor's table 7, these parameters in the l-menthol treatment groups were comparable to those in the control group.

Table 7 Terminal delivery in F₀ dams and F₁ offspring

Study No. 40117

Group and dose	Vehicle Control	100 mg/kg	300 mg/kg	1000 mg/kg
No. of dams	19	20	19	19
Gestational days a)	21.89 ± 0.32	21.45 ± 0.51**	21.74 ± 0.45	21.84 ± 0.50
No. of implantations b)	273(14.37 ± 2.11)	285(14.25 ± 2.05)	276(14.53 ± 1.43)	270(14.21 ± 1.47)
No. of litter b)	257(13.53 ± 1.93)	258(12.90 ± 2.81)	259(13.63 ± 1.74)	252(13.26 ± 2.18)
Gestation index c)	100	100	100	100
No. of live newborns b)	254(13.37 ± 1.95)	258(12.90 ± 2.81)	258(13.58 ± 1.71)	249(13.11 ± 2.16)
Birth index d)	93.84	90.53	93.48	92.22
Sex ratio of live newborns e)	0.97(125/129)	1.22(142/116)	0.80(116/143)	0.82(112/137)
Body weight of live newborns (g) a)				
Male	6.6 ± 0.6	6.4 ± 0.5	6.6 ± 0.6	6.6 ± 0.6
Female	6.2 ± 0.5	6.1 ± 0.5	6.2 ± 0.5	6.4 ± 0.5
No. of stillborns f)				
Male	1	0	0	2
Female	2	0	1	1
Total	3(1.17)	0	1(0.39)	3(1.19)
No. of live newborns with external anomalies	0	0	0	0

** P<0.01 (significantly different from vehicle control).

a) Values are mean ± S.D.

b) Values in parentheses represent mean ± S.D.

c) Values in represent percentages to the number of pregnant animals.

d) Values in represent percentages to the number of implantations.

e) Values in parentheses represent number of male/female live newborns.

f) Values in parentheses represent percentages to the number of litters.

One dam in the 1000 mg/kg group was excluded from an evaluation, since the dam died on day 12 of gestation

There were no treatment-related effects on the mean body weights of the F₁ males and females during the pre-weaning (i.e., lactation) and post-weaning (i.e., maturation) periods.

Necropsy conducted on F₁ males and females pups on day 22 after birth failed to identify any treatment-related findings that could be attributed to treatment of the F₀ generation.

Morphologically, there were no treatment-related skeletal anomalies. The examination in the l-menthol 1000 mg/kg/day group was comparable to those in the control group.

Developmental landmarks in the F₁ pups were examined. Balanopreputial separation was delayed in males in the control group compared to the treatment groups. Pups in all treatment groups reached balanopreputial separation earlier than the control group. A significant increase in the differentiation of the balanopreputial; separation was observed on PND 43 for the males in the 300 and 1000 mg/kg/group; 57.89% (p < 0.01) and 31.58% (p < 0.05) of the males in the 300 and 1000 mg/kg/day groups reached balanopreputial, respectively compared to 15.79% in the control group. On PND 10, a significant increase in the differentiation of incisor eruption was observed for the males (70.67% compared to 39.4% for the control group) and females (74.03% compared to 40.54% for the control group) in the 1000 mg/kg/group. On PND 15, a significant

decrease in the differentiation index for eyelid separation was observed for males (88.98% compared to 98.72% for the control group) and females (92.31% compared to 100% for the control group) in the 100 mg/kg/group.

There was no difference in the mean day of acquisition of pinna detachment, piliation, gait, and descensus of testis in the males between treatment groups. For the females, there was no difference in the mean day of acquisition of vaginal patency, pinna detachment, and piliation.

Developmental Landmarks Parameter	Day of Acquisition (for 100% of the pups)			
	Dose (mg/kg)	0	100	300
Males				
Balanopreputial Separation	54	47	49	49
Eyelid Separation	16	16	16	22
Incisor Eruption	13	13	13	12
Females				
Eyelid Separation	15	22	16	15
Incisor Eruption	14	14	13	12

F₁ behavioral evaluation: No treatment-related effects in the righting reflex, ipsilateral flexor reflex, visual placing or Preyer’s reflex were observed. The results of these function tests in the l-menthol groups were comparable to those in the control group.

F₁ reproduction: Reproductive performance in the F₁ generation was not altered by F₀ maternal treatment. There were no differences in female or male mating indices for fertility indices. Similarly, there were no differences in the pre-coital interval.

F₂ findings: Cesarean section data of the F₂ litters were not altered by F₀ maternal treatment at any dose level tested. Cesarean section parameters evaluated included number of corpora lutea, number of implantation, pre-implantation loss, and viable fetuses.

2.6.6.7 Local tolerance

Local tolerance studies for l-menthol and methyl salicylate were not submitted.

2.6.6.8 Special toxicology studies

Special toxicology studies were conducted to evaluate the potential dermal toxicity associated with the novel excipients in the drug product and the FS 67A patch. These studies were reviewed by Dr. María Rivera. In her review, Dr. Rivera concluded that SIS copolymer and _____ did not show evidence of having significant skin toxicity. Results from the studies conducted with the patch suggested that the patch formulation

b(4)

may cause "slight" skin irritation after a single exposure or a 14-day continuous exposure. FS 67A did not cause skin photoirritation and photosensitization. However, as Dr. Rivera pointed out in her review, the studies did not mimic the solar spectrum as they were conducted with only UVA light exposure. Whether FS 67A may cause skin photoirritation or photosensitization after exposure to UVB or Visible light is not known.

2.6.7 TOXICOLOGY TABULATED SUMMARY

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

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The sponsor has provided a full battery of genetic toxicology studies and reproductive and developmental toxicology studies in support of the NDAs for Salonpas. These studies evaluated the genotoxic potential and potential reproductive toxic effects of the active constituents (i.e., l-menthol and methyl salicylate) in the patch. In addition to these studies, genotoxicity, dermal toxicity, phototoxicity, and skin sensitization studies were conducted with the novel excipients SIS copolymer and

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Pertinent to the proposed indication, the potential for dermal toxicity or systemic toxicity from the patch is low. Based on the results from the toxicity study in rabbits and guinea pigs, the potential dermal toxicity was minimal. As demonstrated in the rabbit primary irritation study, it produced slight irritation following single application or following use for 14 days. The patch did not elicit phototoxicity or photosensitization in guinea pigs.

The two novel excipients, SIS block copolymer and , were not shown to be a skin irritant following a single or 14 day dosing in rats and/or rabbits. These excipients also did not cause phototoxicity or photosensitization. SIS block copolymer and are not genotoxicants. They tested negative in the Ames Test and Chromosomal Aberration Test in CHL cells. also tested negative in the Micronucleus Test in mice.

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Reproductive studies showed that l-menthol was not a reproductive toxicant. l-Menthol did not affect reproductive function in male and female rats. l-Menthol also did not cause malformations in developmental toxicity studies in rats or rabbits.

Reproductive studies showed that methyl salicylate did not affect reproductive function in male and female rats. Developmental studies in rats showed that methyl salicylate was teratogenic. However, the safety margin for the teratogenic effects could not be determined from the studies because some skeletal variations were observed in some of the F₁ offspring in the low dose group.

Unresolved toxicology issues (if any): None

Recommendations: From a pharmacology/toxicology perspective, based upon the review of the non-clinical data, NDA 22-029 is considered to be approved.

Suggested labeling: The non-clinical section of the labeling, if this NDA were for Rx status rather than OTC status, should read as follows:

TERTATOGENIC EFFECTS

Pregnancy Category C

Methyl salicylate was shown to be teratogenic in rats. Developmental effects were observed when administered subcutaneously to rats during the period of organogenesis at doses up to 200 mg/kg/day. Methyl salicylate also exerted suppressive effects on fetal growth and delayed ossification at a dose of 200 mg/kg/day. A significant increase incidence of skeletal anomalies and variations were observed in rats dosed with 200 mg/kg/day from gestation day 6 to day 21 of lactation. Teratogenic effects were not observed in rabbits when methyl salicylate was administered at subcutaneous doses up to 300 mg/kg/day from day 6 to 18 of gestation.

There were no evidences of teratogenicity when l-menthol was administered subcutaneously in rabbits or rats during the period of organogenesis at doses up to 600 mg/kg/day or 1000 mg/kg/day, respectively. Reproductive studies were not conducted with the SALONPAS

b(4)

There are no adequate and well-controlled studies of dermal l-menthol, methyl salicylate or SALONPAS PATCH in pregnant women. SALONPAS PATCH should be used during pregnancy only if potential benefit justifies the potential risk to the fetus.

Signatures (optional):

Reviewer Signature BeLinda A. Hayes, Ph.D.

Supervisor Signature R. Daniel Mellon, Ph.D. Concurrence Yes X No

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Reference List

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APPENDIX/ATTACHMENT 1:

Dr. María I. Rivera's Review of IND 62,735 (N000)

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 62735

Review number: 001

Sequence number/date/type of submission: N-000/6-12-01/Initial IND; N-009/9-24-01/IT; N-010/10-17-01/IT; N-011/10-22-01/IT

Information to sponsor: Yes

Sponsor and/or agent: Hisamitsu Pharmaceutical Co., Inc.

Manufacturer for drug substance : _____

***l*-Menthol:** _____

Methyl Salicylate: _____

b(4)

Reviewer name: Maria I. Rivera

Division name: Analgesic, Anti-Inflammatory and Ophthalmic Drug Products

HFD #: 550

Review completion date: November 3, 2001

Drug:

Trade name: FS 67A topical patch

Generic name: 10% Methyl Salicylate/3% *l*-Menthol topical patch

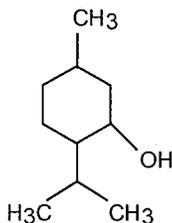
l-Menthol

Chemical name: 5-methyl-2-(1-methylethyl)-cyclohexanol

CAS registry number: 2216-51-5

Molecular formula/molecular weight: C₁₀H₂₀O/156.27

Structure:



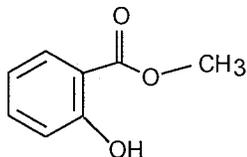
Methyl Salicylate

Chemical name: 2-Hydroxybenzoic acid methyl ester

CAS registry number: 119-36-8

Molecular formula/molecular weight: C₈H₈O₃/152.15

Structure:



Relevant DMFs:

b(4)

Drug class: Analgesic

Indication: Temporary relief of minor aches and pain of muscles and joints associated with simple backache, arthritis, strain, bruises and sprains.

Clinical formulation:

Ingredients	% per patch	mg per patch
Methyl salicylate	10.00	
Menthol	3.00	
Styrene-isoprene-styrene block copolymer		
Polyisobutylene 1,200,000		
Polyisobutylene		
Mineral oil		
Synthetic aluminum silicate		
Alicyclic saturated hydrocarbon resin		
Backing cloth		
Film		

b(4)

Route of administration: topical

Proposed clinical protocol: Six Phase I protocols designed to assess the safety of the FS 67A topical patch product. Four protocols are directed to assess the potential for irritation and sensitization of FS 67A. Two protocols are designed to assess the percutaneous absorption of methyl salicylate and *l*-menthol from the patch compared to ointment preparations containing either 60% methyl salicylate or 16% *l*-menthol.

Study Name	# days of patch application
A 14-Day Cumulative Irritation Study of FS 67A in Healthy Volunteers	14
Repeated Insult Patch Test of FS 67A in Healthy Volunteers (Modified Draize Test)	10
Evaluation of Phototoxicity in Humans	1
Evaluation of Human Photoallergy by Repeated Insult Patch Test	7
A Randomized Single-Dose Three Treatment Crossover Evaluation Designed to Compare the Percutaneous Absorption of <i>l</i> -Menthol Following Topical Application of FS 67A and Two Reference Ointments in Healthy Volunteers	1
A Randomized Single-Dose Three Treatment Crossover Evaluation Designed to Compare the Percutaneous Absorption of Methyl Salicylate Following Topical Application of FS 67A and Two Reference Ointments in Healthy Volunteers	1

Previous clinical experience: There is no previous human experience with the specific methyl salicylate/menthol formulation in the FS 67A patch product presented in this IND. However, these same active ingredients are found in currently available OTC topical products such as Bengay and Thera-gesic. Hisamitsu has an original patch product, Salonpas (US 69), which was introduced in the USA in the 1950's. Each Salonpas patch contains 6.3% methyl salicylate (132

mg), 5.7% *l*-menthol (120 mg) and 1.2% *dl*-camphor (26 mg). It is available for OTC use as an external analgesic drug product.

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

Introduction and drug history:

IND 62735 describes a patch product, FS 67A, containing 10% methyl salicylate (mg) and 3% menthol () as the active ingredients, and the indication is the treatment of muscle and joint pain. The active ingredients (as single ingredients or in combination) have been reviewed by the Expert Panel for OTC Topical Analgesic Products and were granted GRAS/E status in 1979. The Tentative Final Monograph for OTC External Analgesic Drug Products (TMF 48 FR 5852) was published in 1983 but it did not include topical patches. Therefore, the Sponsor plans to submit a New Drug Application for FS 67A.

This review evaluates a series of nonclinical studies conducted to investigate FS 67A potential to induce skin irritation, sensitization, phototoxicity and photosensitization. The review also evaluates nonclinical studies conducted on two excipients, styrene-isoprene-styrene (SIS) block copolymer and alicyclic saturated hydrocarbon resin () to establish its safety as components of the proposed product. These excipients are listed in 21CFR as indirect food additives under the following sections:

21CFR177.1810: SIS copolymers for use as articles or components that contact food.

21CFR176.180: Alicyclic Saturated Hydrocarbon resins as components of paper and paperboard in contact with dry food.

Studies reviewed within this submission:

- A Primary Skin Irritation Study of FS-67-10.3 in Rabbits
- A 14-Day Cumulative Skin Irritation Study of FS-67-10.3 in Rabbits
- A Skin Sensitization Study of FS-67-10.3 in Guinea Pigs
- A Skin Phototoxicity Study of FS-67-10.3 in Guinea Pigs
- A Skin Photosensitization Study of FS-67-10.3 in Guinea Pigs
- A Percutaneous Single Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Rats
- An Oral Single Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Beagle Dogs
- A Primary Skin Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits
- A 14-Day Cumulative Skin Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits
- A Primary Eye Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits
- A Skin Sensitization Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs
- A Skin Phototoxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs

- A Skin Photosensitization Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs
- A 4-Weeks Percutaneous Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Rats with a Recovery Period of 4 Weeks
- An Oral 4-Week Repeated Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Dogs with a 4-Week Recovery Period
- A Percutaneous Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats
- The Acute Toxicity Test _____
- An Oral Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats
- An Oral Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Beagle Dogs
- The Primary Skin Irritation Test _____
- The Cumulative Skin Irritation (3 Weeks) Test of _____
- A Primary Eye Irritation Study of Alicyclic Saturated Hydrocarbon Resin in Rabbits
- Allergenic Study of _____
- A Skin Phototoxicity Study of Alicyclic Saturated Hydrocarbon Resin in Guinea Pigs
- A Skin Photosensitization Study of Alicyclic Saturated Hydrocarbon in Guinea Pigs
- An Oral 4-Week Repeated Dose Toxicity Study of Alicyclic Saturated Hydrocarbon in Beagle Dogs with a 4-Week Recovery Period
- A 4-Week Oral Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats with a Recovery Period of 4-Weeks
- A Reverse Mutation Test of Styrene-Isoprene-Styrene Block Copolymer Using Bacteria
- A Chromosomal Aberration Test of Styrene-Isoprene-Styrene Block Copolymer using CHL Cells
- Microbial Metabolic Activation Test to Assess the Potential Mutagenic Effect of Resin A: _____
- Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *In Vitro* and Treated with Resin A: _____
- A Micronucleus Test of Alicyclic Saturated Hydrocarbon Resin in Mice

b(4)

b(4)

Studies not reviewed within this submission: none

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

II. SAFETY PHARMACOLOGY:

III. PHARMACOKINETICS/TOXICOKINETICS:

Note: The Pharmacology, Safety Pharmacology and Pharmacokinetics of methyl salicylate and menthol are well established; additional preclinical studies were not considered necessary at this time.

IV. GENERAL TOXICOLOGY:

TOXICOLOGY STUDIES WITH FS-67

Study title: A Primary Skin Irritation Study of FS-67-10.3 in Rabbits

Objective: Evaluate the primary skin irritant effects of FS-67-10.3.

Key study findings:

- FS-67 induced slight erythema of grade 1 in both intact and abraded sites, which cleared 48 hours post-application
- The Primary Irritation Index = 0.1 and therefore, FS-67-10.3 was classified as slightly irritant.

Study no: 1540

Volume #, and page #: 4, 436

Conducting laboratory and location: _____

Date of study initiation: August 23, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: FS-67-10.3, FS071J, *l*-menthol (96.2%), methyl salicylate (96.0%)

Reference Control Article: □ adhesive tape was chosen as the reference control article because of its wide use in primary skin irritation studies.

Methods: Each rabbit received a 2.5 cm² strip of FS-67-10.3 on one intact and one abraded site. On the other intact and abraded site, a similar sized piece of □ adhesive tape was placed as the reference material. Test and reference materials were held on place for 24 hours with an occlusive dressing. Skin irritation was scored according to the method of Draize.

Dosing:

Species/strain: Rabbits/Japanese White

#/sex/group or time point (main study): 6 females/group (one group)

Age: 17 weeks

Weight: 3.28 – 3.55 kg

Doses in administered units: Two 2.5 cm² pieces of each the test article and the reference control

b(4)

b(4)

Route, form, volume, and infusion rate: Topical application on both intact and abraded sites on the back of each rabbit.

Observations and times:

Clinical signs: Hourly until 6 hours after application and once daily thereafter

Body weights: On the day of application and on the final day of observation

Skin reactions: 24, 48 and 72 hours after application

Results:

Mortality: No death was reported.

Clinical signs: No abnormal clinical signs were observed.

Body weights: No abnormalities were observed.

Skin reactions:

- FS-67-10.3 induced erythema of grade 1 in intact skin and abraded skin 24 hours after application in 2 animals and 3 animals, respectively. The erythema disappeared by 48 hours after application. The primary irritation index was 0.1.
- — adhesive tape induced erythema of grade 1 or 2 in both intact skin and abraded skin 24 hours after application in all animals. The erythema disappeared by 2 to 7 days after application. The primary irritation index was 1.0.
- The irritation potential of FS-67-10.3 was weaker than that of — adhesive tape under the conditions of this study.

b(4)

Sponsor Conclusions: The primary skin irritant effect of FS-67-10.3 was classified as “slightly irritant” under the conditions of the study.

Reviewer Comments: The reviewer concurs with the Sponsor conclusion. However, the following two points are worth mentioning:

- 1) The Sponsor did not mention whether the FS-67 strip was moistened before application to ensure good contact with the skin
- 2) The value of using the —-tape is not clear.

b(4)

Study title: A 14-Day Cumulative Skin Irritation Study of FS-67-10.3 in Rabbits

Objective: Evaluate FS-67-10.3 potential for cumulative skin irritation.

Key study findings:

- FS-67 produced mild skin irritation following application to intact and abraded skin for 14 consecutive days.
- Histopathological examination revealed slight thickening of the epidermis and cell infiltration of the corium.
- Total mean scores varied from 1.0-2.0 during the 14-day observation period, suggesting that the irritant effects were not cumulative.

Study no: 11541

Volume #, and page #: 4, 467

Conducting laboratory and location: _____

Date of study initiation: August 23, 2000

b(4)

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: FS-67-10.3, FS07J, *l*-menthol (96.2%), methyl salicylate (96.0%)

Reference Control Article: [] adhesive tape was chosen as the reference control article because of its wide use in primary skin irritation studies.

b(4)

Methods: Each rabbit received a 2.5 cm² strip of FS-67-10.3 on one intact and one abraded site. On the other intact and abraded site, a similar sized piece of [] adhesive tape was placed as the reference material. After 24 hours, the materials were removed, the sites wiped with cotton soaked in sterile water, and a new piece of the appropriate test material applied. This procedure was repeated for 14 consecutive days. Skin irritation was scored according to the method of Draize.

Dosing:

Species/strain: Rabbits/Japanese White

#/sex/group or time point (main study): 6 female/group (one group)

Age: 17 weeks

Weight: 3.36 – 3.50 kg

Doses in administered units: Two 2.5 cm² pieces of each the test article and the reference control

Route, form, volume, and infusion rate: Topical application on both intact and abraded sites on the back of each rabbit.

Observations and times:

Clinical signs: Once daily (1 hr after each application)

Body weights: Day 1, 8, and 15

Skin reactions: Daily, each time the applied pieces were removed

Histopathology (skin at the irritation site): At termination (Day 15)

Results:

Mortality: No death was reported.

Clinical signs: No abnormalities were observed.

Body weights: A decrease was observed in all the animals on day 8 or 15 of application.

Mean +/- S.D. values are given below:

<u>Day 1</u>	<u>Day 8</u>	<u>Day 15</u>
3.44 ± 0.05	3.26 ± 0.11	3.16 ± 0.15

The decrease was mild and the Sponsor concluded that it was a procedure-related effect and not test-material related.

Skin reactions:

- FS-67-10.3: Slight erythema (score 1) on both intact and abraded sites from day 2 to end of 14-day period. No edema was observed. The mean scores on days 2, 4, 8, 11, and 15 were 1.8, 2.0, 1.6, 1.0 and 1.6, respectively.

- | tape: The Sponsor did not include the data for the | adhesive tape. However, the Sponsor stated in the summary that the adhesive tape caused slight to moderate to well defined erythema and became more severe as the study progressed. The mean scores on days 2, 4, 8, 11, and 15 were 2.9, 5.4, 5.7, 4.0 and 4.4, respectively.

b(4)

Histopathology:

- FS-67-10.3: In the intact skin, slight thickening of epidermis and cell infiltration in the corium in 5 animals was observed. In the abraded skin, slight thickening of the epidermis in 3 animals and slight cell infiltration in the corium in 2 animals.
- | adhesive tape: In the intact and abraded skin, slight to mild thickening of the epidermis and infiltration of the corium in all animals.

b(4)

Sponsor Conclusions: FS-67-10.3 had no cumulative skin irritability and repeated irritation potential was less than | adhesive tape under the conditions of this study.

Reviewer Comments: The reviewer concurs with the Sponsor conclusion. However, the following two points are worth mentioning:

b(4)

- The Sponsor did not mention whether the FS-67 strip was moistened before application to ensure good contact with the skin
- The value of using the | tape is not clear.

Study title: A Skin Sensitization Study of FS-67-10.3 in Guinea Pigs

Objective: Evaluate whether FS-67-10.3 has skin sensitization potential (allergic contact dermatitis).

Key study findings:

- FS-67 had no skin sensitization potential under the conditions used on the study.

Study no: 1542

Volume #, and page #: 4, 505

Conducting laboratory and location:

Date of study initiation: August 11, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: FS-67-10.3, FS07J, *l*-menthol (96.2%), methyl salicylate (96.0%)

Positive control article: 2,4-Dinitrochlorobenzene (DNCB)

b(4)

Methods: Buehler Method. Three test groups were included as described in the table below.

Test group	No. animals	Sensitization	Challenge
Test article	10	FS-67-10.3	FS-67-10.3
Positive control	10	1% DNCB	0.25% DNCB
Control	10	Placebo patch	FS-67-10.3 or 0.25% DNCB

Dosing:

Species/strain: Guinea Pigs/Hartley

#/sex/group or time point (main study): 10 females/group

Age: 5 weeks

Weight: 331 – 401 g

Doses in administered units: A 2.0 x 2.0 cm piece of FS-67-10.3 patch or a 2.0 x 2.0 cm patch containing DNCB (1% or 0.25% in ethanol).

Route, form, volume, and infusion rate: Topical application on the back (sensitization phase) or left abdominal lateral region (challenge phase).

Observations and times:

Clinical signs: Daily

Body weights: Starting day of sensitization (Day 0), day of final sensitization (Day 14), day of challenge exposure (Day 28), and final day of observation (Day 30)

Skin reactions: 24 and 48 hr after removal of application

Results:

Mortality: No death was reported.

Clinical signs: No abnormalities were observed.

Body weights: A decrease was observed in 1 animals on each FS-67-10.3- and DNCB-treated groups on day 28. There were no abnormalities in the general condition of the animals; the Sponsor judged the change in body weight was stress-related.

Skin reactions:

- FS-67-10.3: No signs of skin irritation following challenge application.
- DNCB positive control: Erythema of score 1 – 3 in all of the animals 24 and 48 hr following challenge application; edema of score 1 in 7 animals and in 5 animals 24 and 48 following challenge application, respectively. The mean scores were 2.9 and 2.4 at 24 and 48 hr, respectively.
- Control group: No skin responses.

Sponsor Conclusions: FS-67-10.3 had no skin sensitization potential under the conditions of this study.

Reviewer Comments: The reviewer concurs.

Study title: A Skin Phototoxicity Study of FS-67-10.3 in Guinea Pigs

Objective: Evaluate the skin phototoxicity potential of FS-67-10.3.

Key study findings:

- FS-67 did not induce skin phototoxicity in guinea pigs after UVA light exposure.

Study no: 1543

Volume #, and page #: 4, 548

b(4)

Conducting laboratory and location**Date of study initiation:** August 11, 2001**GLP compliance:** Yes**QA report:** Yes**Drug, lot #, and % purity:** FS-67-10.3, FS07J, *l*-menthol (96.2%), methyl salicylate (96.0%)**Positive control article:** 8-Methoxypsoralen (8-MOP)

b(4)

Methods: FS-67 was applied to shaved backs of each guinea pig and the site covered for one hour with surgical tape. 8-MOP was applied to the contralateral site to serve as a positive control. The materials were removed 1 hr after application and the application sites exposed to 14 joules/cm² of UV light (320 – 420 nm, max. = 350 nm) at a distance of 10 cm. Skin irritation was scored by the method of Draize.

Dosing:

Species/strain: Guinea Pigs/Hartley

#/sex/group or time point (main study): 7 females/group (one group)

Age: 5 weeks

Weight: 309 – 377 g

Doses in administered units: 1.5 x 1.5 cm pieces of FS-67-10.3 or 0.02 ml of 0.02% (w/v) 8-MOP solution in ethanol were applied to two 1.5 x 1.5 cm application sites.

Route, form, volume, and infusion rate: Topical

Observations and times:

Clinical signs: Daily

Body weights: Day of application/irradiation and on the final day of observation

Skin reactions: 24, 48 and 72 hr after irradiation

Results:

Mortality: No death was reported.

Clinical signs: No abnormalities were observed.

Body weights: No treatment-related effects.

Skin reactions:

- FS-67-10.3: No skin reactions in the UV(+) or UV(-) areas up to 72 hr post-irradiation.
- 8-MOP: Erythema of score 3 and edema of score 2 at 24 hr after irradiation in all animals. The mean scores 24, 48 and 72 hr after irradiation were 5.0, 3.9, and 2.9, respectively. Skin reactions were not observed in the UV(-) area.

Sponsor Conclusions: FS-67-10.3 had no skin phototoxicity potential under the conditions of the study.

Reviewer Comments: The reviewer concurs with the conclusion that under the conditions of the study, FS-67-10.3 was not phototoxic. However, it is noteworthy that only UVA exposure was used in this study. It is preferable to conduct studies with exposure to UVA-UVB-Visible light (280-700 nm) to mimic solar conditions. This may not be necessary if the drug product does not absorb in the UV-Visible light region or if the patch is opaque to light.

Study title: A Skin Photosensitization Study of FS-67-10.3 in Guinea Pigs**Objective:** Evaluate the skin photosensitization potential of FS-67-10.3**Key study findings:**

- FS-67 did not induce photosensitization in guinea pigs after UVA light exposure.

Study no: 1544**Volume #, and page #:** 4, 579**Conducting laboratory and location.****Date of study initiation:** August 11, 2000**GLP compliance:** Yes**QA report:** Yes**Drug, lot #, and % purity:** FS-67-10.3, FS07J, *l*-menthol (96.2%), methyl salicylate (96.0%)**Formulation/vehicle:** none**Positive control article:** 6-Methylcoumarin (6-MC)

b(4)

Methods: FS-67 was applied to shaved areas in the cervico-dorsal region of each guinea pig. The site had been prepared by intradermal injection of Freund's Complete Adjuvant and stripping of the skin surface with cellophane tape to produce abrasion of the epidermal surface. One hour after application, the materials were removed and the application sites exposed to 10 Joules/cm² of UV light (320-420 nm, max. = 350 nm) at a distance of 10 cm. This procedure was repeated for five consecutive days. Another group of animals received 6-MC as a positive control. A third group served as a control group and was not exposed to test materials during the induction phase. Two weeks after the fifth application, animals were shaved at the dorsal area, and received another one hour application of either FS-67 or the positive control material. On the control group, the same animal received the test article and 6-MC in separate areas. Sites were irradiated as described above.

Dosing:

Species/strain: Guinea pigs/Hartley

#/sex/group or time point (main study): 10 females/group

Age: 5 weeks

Weight: 301 – 345 g

Doses in administered units: 2 x 4 cm piece of FS-67-10.3 or 0.1 ml of 2% (w/v) 6-MC solution in ethanol were applied to a 2 x 4 cm application site.

Route, form, volume, and infusion rate: Topical application on the cervico-dorsal area (sensitization phase) or dorsal area (challenge phase) of each of 10 guinea pigs.

Observations and times:

Clinical signs: Daily

Body weights: Starting day of photosensitization (Day 0), day of final photosensitization (Day 4), day of photochallenge (Day 21), and final day of observation (Day 23)

Skin reactions: 24 and 48 hr after irradiation for photochallenge exposure

Results:

Mortality: No death was reported.

Clinical signs: No abnormalities were observed.

Body weights: A decrease was observed in one animal in the test article group on the day of final photosensitization. However, there were no abnormalities in the general condition of the animal and therefore, the Sponsor judged the decrease in body weight as stress-related.

Skin reactions:

- FS-67: No skin reactions in the UV(+) or UV(-) sites.
- 6-MC positive control: Grade 1 erythema was observed at the UV(+) site of 3 animals. The group mean scores 24 and 48 hr after irradiation for challenge exposure were 2.1 and 1.9, respectively. There were no skin reactions at the UV(-) site of the animals.
- Control group: No skin reactions following photochallenge exposure at the UV(+) or UV(-) sites.

Sponsor Conclusions: FS-67-10.3 had no skin photosensitization potential under the conditions of the study.

Reviewer Comments: The reviewer concurs with the conclusion that under the conditions of the study, FS-67-10.3 induced no photosensitization. However, it is noteworthy that only UVA exposure was used in this study. It is preferable to conduct studies with exposure to UVA-UVB-Visible light (280-700 nm) to mimic the solar conditions. This may not be necessary if the drug product does not absorb in the UV-Visible light region or if the patch is opaque to light.

TOXICOLOGY STUDIES WITH STYRENE-ISOPRENE-STYRENE

Study title: A Percutaneous Single Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Rats

Objective: Determine the systemic toxicity of styrene-isoprene-styrene (SIS) block copolymer following a percutaneous single dose in rats.

Key study findings:

- No toxic effects were seen following application of SIS block copolymer to 10% of rat's surface area.
- The Sponsor did not provide evidence of systemic exposure after dermal application of the SIS block copolymer.

Study no: | -4443

Volume #, and page #: 5, 626

Conducting laboratory and location: _____

Date of study initiation: December 28, 1999

b(4)

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS block copolymer, lot # 912062, % purity was not specified

Methods: A sufficient amount of SIS copolymer sheet was applied to shaved areas to cover approximately 10% of the skin surface of the rat. The test article sites were occluded with lint sheet and surgical tape and held in place for 24 hours. Afterwards, the test material was removed and the animals observed for 14 days. Animals in the control group were clipped and shaved in the same manner and surgical tape affixed.

Dosing:

Species/strain: Rats/Sprague-Dawley

#/sex/group or time point (main study): 5 rats/sex/group

Age: 6 weeks

Weight: 182 – 190 g

Doses in administered units: One application area of 10% body surface area of the rat, which is thought to be the largest practicable area. The group composition is shown in the following table.

Group	Application area	Sex	Actual application area (cm ²)	Dose (mg/kg)*
Control	0 cm ²	M	0	0
		F	0	0
Treatment	10% body surface area	M	2.904 – 3.024	3380 – 3421
		F	2.400 – 2.475	3716 - 3845

*Calculated from weight of cut sheets and body weight on day of administration.

Route, form, volume, and infusion rate: Topical administration (clipped dorsal area) for 24 hr

Observations and times:

Clinical signs: Immediately to 5 min after, 15 and 30 min after, 1, 2, 4, and 6 hr after administration, and once daily, thereafter.

Body weights: Before dosing and on days 1, 2, 3, 7 and 14

Gross pathology: Termination (Day 14); organs examined: external appearance, brain, pituitary, thyroid, salivary gland, thymus, heart, lung, liver, spleen, kidney, adrenal, stomach, small intestine, large intestine, pancreas, gonad, urinary bladder, lymph node, application site, other tissues or organs

Results:

Mortality: No deaths occurred in any group.

Clinical signs: No abnormalities were observed.

Body weights: No treatment-related effects.

Gross pathology: No abnormalities were found.

Sponsor's Conclusions: The percutaneous single toxicity in rats of the SIS block copolymer was suggested to be low.

Reviewer’s Comments: The reviewer agrees that SIS copolymer appeared to be non-toxic to rats treated with a single dermal application. However, the study has the following deficiencies:

- The Sponsor did not conduct a PK analysis to determine whether the SIS copolymer (or contaminating monomers) penetrated the skin and was capable to reach systemic circulation.
- The Sponsor did not conduct a full assessment of toxicological parameters (no clinical chemistry, hematology, urinalysis, histopathology, etc).

Study title: An Oral Single Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Beagle Dogs

Objective: Determine the systemic toxicity of SIS block copolymer following a single oral dose in dogs.

Key study findings:

- The single oral administration of styrene-isoprene-styrene block copolymer to beagle dogs at 2000 mg/kg was not associated with marked acute toxic changes.
- The Sponsor did not provide evidence of systemic exposure after oral administration of SIS copolymer.

Study no: V-4451

Volume #, and page #: 5, 676

Conducting laboratory and location: _____

Date of study initiation: December 16, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS block copolymer, lot # 0902-2, % purity was not specified

b(4)

Methods: SIS copolymer was cut into small pieces and placed in gelatin capsules based upon individual animal body weights. Six gelatin capsules/animal were used to administer the copolymer dose. Animals were observed for 14 days.

Dosing:

Species/strain: Dogs/Beagle

#/sex/group or time point (main study): 3 dogs/sex/group

Age: approximately 6.5 months

Weight: 7.6 – 7.8 kg (male), 7.9 – 8.4 kg (female)

Doses in administered units: 2000 mg/kg, *maximum dose level described in the toxicity study guidelines for pharmaceuticals*

Route, form, volume, and infusion rate: oral, capsules

Observations and times:

Clinical signs: Immediately to 5 min after dosing, 10 and 30 min after dosing, and 1, 2, 3, 4, 5 and 6 hr after dosing, and once daily thereafter

Body weights: Days 1, 3, 7, 10, 13 and on the day of necropsy

Food Consumption: Daily

Gross pathology: Termination. The Sponsor did not list the specific organs but used the following categories: cranial, thoracic, abdominal, and other regions

Organs weighed: Brain, adrenal, spleen, heart, lung (including bronchial tubes), liver (with gallbladder), kidney

Results:

Mortality: No deaths occurred.

Clinical signs: Feces containing yellowish white granular material like the test article in all animals on the day following administration. Vomiting containing test article yellowish white material in one male 4 hr after administration, and soft feces and vomiting of white foamy material in one female on days 9 and 13 after administration.

Body weights: There were no treatment-related effects.

Food consumption: Decreased food consumption was observed in one male animal from day 5 after administration onward. This animal showed no abnormalities in general condition, body weight, or necropsy.

Organ weights: No abnormalities were observed in absolute or relative organ weight.

Gross pathology: No treatment-related abnormalities.

Sponsor's Conclusions: SIS block copolymer was considered to be nontoxic to dogs after single oral dose of 2000 mg/kg.

Reviewer's Comments: The reviewer agrees that SIS copolymer caused no toxicity to dogs after a single 2000 mg/kg oral dose. However, the study has the following deficiencies:

- The Sponsor did not conduct a PK analysis to determine whether the SIS copolymer (or contaminating monomers) was capable to reach systemic circulation.
- The Sponsor did not conduct a full assessment of toxicological parameters (no clinical chemistry, hematology, urinalysis, histopathology, etc).

Study title: A Primary Skin Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits

Objective: Evaluate the primary skin irritant effects of SIS copolymer.

Key study findings:

- SIS copolymer induced no signs of skin irritation.

Study no: 11379

Volume #, and page #: 5, 711

Conducting laboratory and location:

Date of study initiation: December 13, 1999

GLP compliance: The Sponsor mentioned the study was done according to two GLP guidelines but a GLP certificate was not submitted.

QA report: Yes, it did not have the director signature.

b(4)

Drug, lot #, and % purity: SIS block copolymer sheet, lot # 912062, % purity was not specified
Reference control article: | adhesive tape was chosen as the reference control article because of its wide use in primary skin irritation studies.

Methods: Rabbits received a 2.5 cm² sheet of SIS copolymer on one intact and one abraded site. On the other intact and abraded site, a similar sized piece of | adhesive tape was placed as the reference material. Test and reference materials were held on place for 24 hours with an occlusive dressing. Skin irritation was scored according to the method of Draize. b(4)

Dosing:

Species/strain: Rabbits/Japanese White

#/sex/group or time point (main study): 6 females/group (one group)

Age: 17 weeks

Weight: 2.61 – 2.88 kg

Doses in administered units: Two 2.5 cm² pieces of each SIS copolymer and the | tape. b(4)

Route, form, volume, and infusion rate: Topical application in both intact and abraded sites on the back of each rabbit.

Observations and times:

Clinical signs: Hourly until 6 hr after application and once daily thereafter.

Body weights: On the day of application and on the final day of observation.

Skin reactions: 24, 48 and 72 hours after application

Results:

Mortality: None was reported.

Clinical signs: No abnormal clinical signs were observed.

Body weights: A slight decrease in body weight was observed in 3 animals. There were no abnormalities in general condition of the animals.

Skin reactions:

- SIS copolymer: No skin reactions in either intact or abraded sites in any animal.
- | Tape: Grade 1 or 2 erythema in both intact and abraded skin 24 or 48 hr after application which disappeared by 72 hr after application. The primary skin irritation index was 0.5 (slightly irritant). b(4)

Sponsor Conclusions: SIS copolymer was classified as non-irritant.

Reviewer Comments: The reviewer concurs with the Sponsor conclusion. However, the following two points are worth mentioning:

- The Sponsor did not indicate whether the FS-67 strip was moistened before application to ensure good contact with the skin
- The value of using the | tape is not clear. b(4)

Study title: A 14-Day Cumulative Skin Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits

Objective: Evaluate SIS copolymer potential to cause cumulative skin irritation.

Key study findings:

- SIS copolymer was not associated with skin reactions following application to intact or abraded skin for 14 consecutive days.
- Histopathological examination revealed slight acanthosis and slight to mild dermal inflammatory cell infiltration following application of SIS copolymer.

Study no: 1380

Volume #, and page #: 5, 741

Conducting laboratory and location:

Date of study initiation: December 13, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS copolymer sheet, lot # 912062, % purity was not specified

Reference Control Article: I adhesive tape was chosen as the reference control article because of its wide use in primary skin irritation studies.

Methods: Rabbits received a 2.5 cm² sheet of SIS copolymer on one intact and one abraded site. On the other intact and abraded site, a similar sized piece of I adhesive tape was placed as the reference material. After 24 hours, the materials were removed, the sites wiped with a cotton soaked in sterile water, and a new piece of the appropriate test material applied. This procedure was repeated for 14 consecutive days. Skin irritation was scored according to the method of Draize.

Dosing:

Species/strain: Rabbits/Japanese White

#/sex/group or time point (main study): 6 females/group (one group)

Age: 17 weeks

Weight: 2.39 – 2.86 kg

Doses in administered units: Two 2.5 cm² pieces of each the test article and the reference control

Route, form, volume, and infusion rate: Topical application on both intact and abraded sites on the back of each rabbit.

Observations and times:

Clinical signs: Once daily (1 hr after each application)

Body weights: Day 1, 7, and 14

Skin reactions: Daily

Histopathology (skin at the irritation site): At termination (Day 14)

Results:

Mortality: None was reported.

Clinical signs: No abnormalities were observed.

b(4)

b(4)

Body weights: A mild decrease was observed in all the animals from starting day of application to day 7 of application. The Sponsor concluded that the effect was procedure-related and not test-material related.

Skin reactions:

- SIS copolymer: No signs of skin irritation in the intact or abraded site following application for 14 consecutive days. b(4)
- Adhesive tape: Grade 1 or 2 erythema in all animals on day 1 after application, which gradually became more severe thereafter. Grade 1 edema was observed in 3 animals on day 8 – 14 after the application. The mean scores were 2.2, 2.3, 2.5, 2.0 and 4 on days 1, 4, 7, 10 and 14, respectively.

Histopathology:

- FS-67-10.3: In the intact skin, slight acanthosis and dermal inflammatory cell infiltration in 2 and 5 animals, respectively. In the abraded skin, slight acanthosis in 3 animals, and slight to mild dermal inflammatory cell infiltration in 4 and 2 animals, respectively. b(4)
- Adhesive tape: In the intact skin, slight and mild acanthosis in 4 and 2 animals, respectively, and dermal inflammatory cell infiltration in all the animals. In the abraded skin, slight and mild acanthosis in 3 and 1 animals, respectively, and slight dermal inflammatory cell infiltration in 4 animals.

Sponsor Conclusions: SIS copolymer had no cumulative skin irritation following application to intact and abraded skin for 14-consecutive days. The incidence and severity of histopathological changes in adhesive tape were similar to those observed in the animals to which SIS copolymer was applied. b(4)

Reviewer Comments: The reviewer concurs.

Study title: A Primary Eye Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits

Objective: Evaluate the irritant effects of SIS copolymer to the eye.

Key study findings:

- SIS copolymer was not an eye irritant under the conditions of the study.

Study no: 1378 b(4)

Volume #, and page #: 5, 780

Conducting laboratory and location: _____

Date of study initiation: December 13, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS copolymer sheet, lot # 0901-1, % purity was not specified

Methods: SIS copolymer was cut into small pieces using a sterilized scissors and placed into the conjunctival sac of the left eye of each rabbit. The right eye remained untreated and served as control. A separate group of rabbits had a similar treatment but their eyes were washed with 0.2 ml of water 30 sec after treatment. Eye irritation was scored according to the method of Kay and Calandra².

Dosing:

Species/strain: Rabbits/Japanese White

#/sex/group or time point (main study): 6 females/unwashed group, 3 females/washed group

Age: 14 weeks

Weight: 2.57 – 2.97 kg

Doses in administered units: 0.1 ml of SIS copolymer. The Sponsor mentioned that “*the SIS copolymer was measured with a 0.1 ml vessel and the actual weight of the test article was determined and recorded*” but did not specify the weight.

Route, form, volume, and infusion rate: conjunctival sac

Observations and times:

Clinical signs: Hourly until 6 hr after application and once daily thereafter.

Body weights: On the day of application and at the end of the observation period.

Eye irritation: Examined macroscopically and with an ophthalmoscope 1, 24, 48 and 72 hr after application for abnormalities in the cornea, iris, and conjunctiva. The eyes were also examined by fluorescein staining.

Results:

Mortality: None was reported.

Clinical signs: No abnormalities were observed.

Body weights: No abnormalities were observed.

Primary Eye Irritation:

- There were no changes in the cornea, iris, or conjunctiva in the unwashed group or washed group.
- The mean total score (MTS) was 0 at 1, 24 and 72 hr after application.
- Lid closure was observed as an ocular change immediately after application only in two animals.

Sponsor’s Conclusions: SIS block copolymer was classified as “nonirritating” on the rabbit eye in accordance to the Kay and Calandra’s² evaluation criteria.

Reviewer’s Comments: The reviewer concurs. However, the Sponsor did not specify how much of the copolymer (in weight units) was applied to the eye.

Study title: A Skin Sensitization Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs

² Kay, J.H. and Calandra, J.C. Interpretation of eye irritation test. *J. Soc. Cosm. Chem.*, **13**, 281(1962).

Objective: Evaluate whether SIS copolymer has skin sensitization potential (allergic contact dermatitis).

Key study findings:

- No signs of skin reactions were observed after challenge exposure to SIS copolymer and there was no evidence of skin sensitization.

Study no: 1381

Volume #, and page #: 5, 826

Conducting laboratory and location:

Date of study initiation: December 15, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS copolymer sheet, lot # 912062, % purity was not specified

Positive control article: 2,4-Dinitrochlorobenzene (DNCB)

b(4)

Methods: Buehler Method. Three test groups were included as described in the table below.

Test group	No. animals	Sensitization	Challenge
Test article	10	SIS copolymer	SIS copolymer
Positive control	10	1% DNCB	0.25% DNCB
Control	10	Placebo patch	SIS copolymer or 0.25% DNCB

Dosing:

Species/strain: Guinea Pigs/Hartley

#/sex/group or time point (main study): 10 female/group

Age: 7 weeks

Weight: 342 – 409 g

Doses in administered units: A 2.0 x 2.0 cm piece of SIS copolymer or a 2.0 x 2.0 cm patch containing 0.2 ml of DNCB (1% or 0.25% in ethanol).

Route, form, volume, and infusion rate: Topical application on the back (sensitization phase) or left abdominal lateral region (challenge phase) of each of 10 guinea pigs.

Observations and times:

Clinical signs: Daily

Body weights: Starting day of sensitization (Day 0), day of final sensitization (Day 14), day of challenge exposure (Day 28), and final day of observation (Day 30)

Skin reactions: 24 and 48 hr after removal of application

Results:

Mortality: None was reported.

Clinical signs: No abnormalities were observed.

Body weights: No abnormalities were observed.

Skin reactions:

- SIS copolymer: No signs of skin irritation following challenge application.

- DNCB positive control: Erythema of grade 2 or 3 and edema of grade 1 in all of the animals 24 hr following challenge application; erythema of grade 1 or 2 in all animals and edema of grade 1 in 5 animals 48 hr following challenge application. The mean scores were 3.6 and 2.4 at 24 and 48 hr, respectively.
- Control group: No skin responses.

Sponsor Conclusions: SIS copolymer had no skin sensitization potential under the conditions of this study.

Reviewer Comments: The reviewer concurs.

Study title: A Skin Phototoxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs

Objective: Evaluate the skin phototoxicity potential of SIS copolymer.

Key study findings:

- SIS copolymer did not induce skin phototoxicity in guinea pigs after UVA light exposure.

Study no: 1382

Volume #, and page #: 6, 871

Conducting laboratory and location: _____

Date of study initiation: December 15, 1999

GLP compliance: The Sponsor mentioned the study was done according to two GLP guidelines but a GLP certificate was not submitted.

QA report: Yes, it did not have the director signature.

Drug, lot #, and % purity: SIS copolymer sheet, lot # 912062, % purity was not specified

Positive control article: 8-Methoxypsoralen (8-MOP)

Methods: SIS copolymer was applied to shaved backs of guinea pigs and the site covered for one hour with surgical tape. 8-MOP was applied to the contralateral site to serve as a positive control. The materials were removed 1 hr after application and the application sites exposed to 14 joules/cm² of UV light (320 – 420 nm, max. = 350 nm) at a distance of 10 cm. Skin irritation was scored by the method of Draize.

Dosing:

Species/strain: Guinea Pigs/Hartley

#/sex/group or time point (main study): 7 females/group (one group)

Age: 6 weeks

Weight: 347 – 375 g

Doses in administered units: 1.5 x 1.5 cm pieces of SIS copolymer or 0.02 ml of 0.02% (w/v) 8-MOP solution in ethanol were applied to two application sites.

Route, form, volume, and infusion rate: Topical application on the back of each guinea pig.

b(4)

Observations and times:

Clinical signs: Daily

Body weights: Day of application/irradiation and on the final day of observation

Skin reactions: 24, 48 and 72 hr after irradiation

Results:

Mortality: None was reported

Clinical signs: No abnormalities were observed.

Body weights: No treatment-related effects.

Skin reactions:

- SIS copolymer: No skin reactions in the UV(+) or UV(-) areas up to 72 hr post-irradiation.
- 8-MOP: Erythema of score 3 and edema of score 1 or 2 at 24 hr after irradiation in all animals. The mean scores 24, 48 and 72 hr after irradiation were 4.6, 2.6, and 2.4, respectively. Skin reactions were not observed in the UV(-) area.

Sponsor Conclusions: SIS block copolymer had no skin phototoxicity potential under the conditions of the study.

Reviewer Comments: The reviewer concurs with the conclusion that under the conditions of the study, SIS copolymer was not phototoxic. However, it is noteworthy that only UVA exposure was used in this study. It is preferable to conduct studies with exposure to UVA-UVB-Visible light spectrum (280 – 700 nm) to mimic solar conditions. This may not be necessary if the test material does not absorb in the UV-Visible region or the patch is opaque to light.

Study title: A Skin Photosensitization Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs

Objective: Evaluate the skin photosensitization potential of SIS copolymer.

Key study findings:

- SIS copolymer did not induce skin photosensitization in guinea pigs after UVA light exposure.

Study no: 1383

Volume #, and page #: 6, 902

Conducting laboratory and location:

Date of study initiation: December 27, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS copolymer sheet, lot # 912062, % purity was not specified

Formulation/vehicle: The test article was cut into pieces of appropriate sizes and used for application.

Positive control article: 6-Methylcoumarin (6-MC)

b(4)

Methods: SIS copolymer sheet was applied to shaved areas in the cervico-dorsal region of each guinea pig. The site had been prepared by intradermal injection of Freund's Complete Adjuvant and stripping of the skin surface with cellophane tape to produce abrasion of the epidermal surface. One hour after application, the materials were removed and the application sites exposed to 10 Joules/cm² of UV light (320-420 nm, max. = 350 nm) at a distance of 10 cm. This procedure was repeated for five consecutive days. Another group of 10 animals received 6-MC as a positive control. A third group served as a control group and was not exposed to test materials during the induction phase. Two weeks after the fifth application, animals were shaved at the dorsal area, and received another one hour application of either SIS copolymer sheet or the positive control material. On the control group, the same animal received the test article and 6-MC in separate areas. Sites were evaluated at 24 and 48 hr after photochallenge exposure.

Dosing:

Species/strain: Guinea pigs/Hartley

#/sex/group or time point (main study): 10 females/group

Age: 6 weeks

Weight: 314 – 424 g

Doses in administered units: 2 x 4 cm piece of SIS copolymer or 0.1 ml of 2% (w/v) 6-MC solution in ethanol were applied to a 2 x 4 cm application site.

Route, form, volume, and infusion rate: Topical application on the cervico-dorsal area of each of 10 guinea pigs.

Observations and times:

Clinical signs: Daily

Body weights: Starting day of photosensitization (Day 0), day of final photosensitization (Day 4), day of photochallenge (Day 21), and final day of observation (Day 23)

Skin reactions: 24 and 48 hr after irradiation after photochallenge exposure

Results:

Mortality: None was reported.

Clinical signs: No abnormalities were observed.

Body weights: No abnormalities were observed.

Skin reactions:

- SIS copolymer: No skin reactions in the UV(+) or UV(-) sites.
- 6-MC positive control: Erythema of grade 1 or 2 was observed at the UV(+) site in all animals and edema of grade 1 in 7 animals at 24 hr after irradiation. The group mean scores 24 and 48 hr after irradiation for challenge exposure were 3.2 and 2.5, respectively. The UV(-) site showed no skin reactions.
- Control group: No skin reactions following photochallenge exposure at the UV(+) or UV(-) sites.

Sponsor Conclusions: SIS copolymer had no skin photosensitization potential under the conditions of the study.

Reviewer Comments: The reviewer concurs with the conclusion that under the conditions of the study, SIS-copolymer induced no photosensitization. However, it is noteworthy that only UVA exposure was used in this study. It is preferable to conduct studies with exposure to UVA-UVB-Visible light spectrum (280 – 700 nm) to mimic solar conditions. This may not be necessary if the test material does not absorb in the UV-Visible region or the patch is opaque to light.

Study title: A 4-Week Percutaneous Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Rats with a Recovery Period of 4 Weeks

Objective: Determine the dose-response for the occurrence of toxic signs and the nature of the toxicity.

Key study findings:

- SIS block copolymer was not toxic to rats when applied at levels up to 10% of the rat's body surface area for 4 weeks.
- The Sponsor did not provide evidence of systemic exposure after dermal application of the SIS block copolymer.

Study no: 4582

Volume #, and page #: 6, 952; Vol. 1, Toxicology Information Amendment N-009

Conducting laboratory and location: _____

Date of study initiation: June 8, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS copolymer sheet, lot # 005081, % purity was not specified. A table was submitted in Supplement 009 which indicates that styrene content was _____

b(4)

Methods: SIS copolymer (cut sheets) was applied to shaved skin, such that application was made to 2.5%, 5% and 10% of the rat's body surface area (BSA). Dose levels applied during the fourth week were 759, 1556 and 3059 mg/kg for the three groups of male rats, and 837, 1681 and 3339 mg/kg for the three groups of female rats. The **control group** was clipped and shaved in the same manner as the SIS-treated groups and only surgical tape affixed. This procedure was not applied to another group of animals, designated as the **non-treatment group**. Additional animals were added to the 2 control and 10% BSA groups and served as recovery animals (4 weeks).

Dosing:

Species/strain: Rats/Sprague-Dawley

#/sex/group or time point (main study): 12 rats/sex/group

Satellite group used as recovery: six rats/sex/group for an additional control and 10% BSA group

Age: 6 weeks

Weight: 131-183 g (males), 122 – 156 g (females)

Doses in administered units: 0 cm², 2.5%, 5%, 10% body surface area (BSA);

Route, form, volume, and infusion rate: Percutaneous, once daily, 7 times/week, the test article preparation was applied to the clipped dorsal area and covered with lint sheet and surgical tape

Observations and times:

Clinical signs: Three times daily during the administration period (prior, immediately after, and 2 hr after dosing), once daily during the recovery period.

Body weights: Days 1, 4 and 7 of administration period, twice a week thereafter; Days 1, 3, and 7 during the recovery period, and then twice a week.

Food consumption: Day before and every 3 or 4 days during the administration period; days 1- 3 and days 3 – 7, thereafter at every 3 - 4 days intervals.

Ophthalmoscopy: . Macroscopic and ophthalmoscopic examinations were done three times: day 11 of quarantine/acclimatization period, day 25 of administration, day 24 of recovery

Hematology: Termination

Clinical chemistry: Termination

Urinalysis: Termination

Gross pathology: Termination

Organs weighed: See Appendix

Histopathology: Termination (see Appendix for tissues examined).

Results:

Mortality: One female on the non-treatment group. This animal showed no abnormalities in clinical signs, at necropsy or histopathology. The cause of death was not clear.

Clinical signs: No abnormalities were observed during the administration or recovery period.

Body weights: **Administration Period:** No test-article related effects. However, body weight and body weight gain in the control group were lower than in the non-treatment group in males (Gain = 124 ± 24 vs. 208 ± 19 , respectively). **Recovery Period:** In males, no test-article related effects in males. Body weight in the control group was lower than that of the non-treatment group but the values were more similar by the end of the recovery period; In females, a statistically significant increase in body weight gain in the 10% BSA group compared to control group (49 ± 7 vs. 67 ± 12 , control vs. 10% BSA, respectively).

Food consumption: **Administration Period:** No test-article related effects. In males, food consumption in the control group was statistically significantly lower than that of the non-treatment group on days 4, 10 – 21, and 28 of administration. In females, food consumption was statistically significantly lower in the control than that of the non-treatment group on day 4, and higher than that of the non-treatment group on days 7 and 14 to 28 of administration. **Recovery Period:** In females, food consumption in the control group was higher than that of the non-treatment group on days 3, 7 and 17 of recovery.

Ophthalmoscopy: No test-article related effects.

Hematology: **At the end of Administration Period:** No test-article related effects. Comparing the control group and the non-treatment group, the following changes were statistically significant: In males, increases in red cell count, hemoglobin and hematocrit; In females, increase in mean corpuscular hemoglobin concentration, decrease in platelet count and lengthening of active partial thromboplastin time. **At the end of Recovery Period:** In males, a

slight but significant decrease in mean corpuscular hemoglobin in the 10% BSA group (4% decrease compared to control group). In females, a significant decrease in fibrinogen in the 10% BSA group (11% decrease compared to control group).

Clinical chemistry: **At the end of Administration Period:** No changes considered test-article related. Comparing the control group to the non-treatment group, the following changes were statistically significant: In males, increases in GOP and GPT activity and urea nitrogen, decreases in triglyceride, and glucose; In females, increases in GOP and GPT activity and urea nitrogen and a decrease in creatine. **At the end of Recovery Period:** There were no test-article-related changes in both males and females.

Urinalysis: **Week 4 of Administration:** In males, a significant decrease in protein was observed in the 2.5% BSA group but it was not dose-dependent. In females, a significant decrease in water intake was observed in the 10% BSA group (16% decrease compared to control). Comparing the control group and the non-treatment group, the following changes were statistically significant: In males, decreases in pH and water intake and increases in ketone bodies and urobilinogen. In females, increases in protein and ketone bodies. **Week 4 of Recovery:** a significant increase in urine volume was observed in the 10% BSA group in females (118%). In males, a decrease in pH and an increase in water intake were observed in the control group compared to the non-treatment group.

Organ weights: **At the end of Administration Period:** In males, no test-article related effects. In females, a significant increase in relative weight of the adrenals in the 10% BSA group (11% compared to control group). The following changes were observed in the control group compared to the non-treatment group: In males, increase in the relative weight of the pituitary, brain, adrenals and testes; a decrease in the absolute weight and an increase in the relative weight of the salivary glands, heart, lungs and kidneys; a decrease in the absolute weight of thymus, spleen, and seminal vesicle; a decrease in the absolute and relative weight of the liver; In females, increase in the relative weight of the salivary glands, heart, liver, kidneys, adrenals and ovaries; decrease in the absolute weight of the thymus and lungs. The Sponsor attributed these changes to the low body weight in the control compared to the non-treatment group at necropsy in both males and females. **At the end of Recovery Period:** No test-article-related changes. Some changes due to low body weight in the control group compared to the non-treatment group at necropsy were as follows: In males, a decrease in the absolute weight of the thymus and an increase in the relative weight of the testes; In females, increase in the pituitary and liver relative weight.

Gross pathology: **At the end of Administration Period:** The following lesions were observed but were judged to be incidental because of the incidence: lung - dark red focus in two males in the control group, 1 male in the 2.5% BSA group, and in one animal of each sex in the 10% BSA group; liver - white focus in one animal of each sex in the 2.5% BSA group; stomach - dark red focus in the glandular stomach in 4 males in the control group, 2 males and 3 females in the 2.5% BSA group, and in 1 male and 3 females in the 10% BSA group; large intestine - intussusception in the colon in 1 female in the 10% BSA group; testis - smallness (bilateral) in 1 animal in the control group. **At the end of Recovery Period:** Dark red focus in the glandular stomach was observed in 1 female in the 10% BSA group, but it was judged to be incidental because of the incidence.

Histopathology:

At the end of Administration Period:

1) Physical irritation at the application site with mild thickening of the epidermis in some animals at all dose levels.

2) Changes in the control and 10% BSA groups, considered incidental judging from the incidence and/or pathological nature: Adrenal: slight hypertrophy of the cortical cell in 2 females in the control group and 3 females of the 10% BSA group. Colon: Mild intussusception in 1 female in the 10% BSA group in which intussusception was also found at necropsy. Kidney: slight tubular basophilia in 1 female each on the control and non-treatment groups. Liver: mild granulomatous inflammation in 1 female of the control group; slight necrosis of the hepatocytes in 1 female in the 10% BSA group. Lung (bronchus): slight hemorrhage in 2 males in the control group and in 2 males and 1 female in the 10% BSA group in which dark red focus was found at necropsy. Pancreas: slight atrophy of the acinar cells was observed in 1 female in the 10% BSA group. Prostate: slight or mild cell infiltration in the interstitium in 1 animal each of the control and 10% BSA group and in 2 animals of the non-treatment group. Stomach: slight erosion in the glandular stomach in 4 males in the control group and in 1 male and 3 females of the 10% BSA group in which dark red focus in the glandular stomach was found at necropsy. Testis: slight immaturity in 1 animal in the control group in which smallness (unilateral) was found at necropsy. Thymus: slight or mild atrophy in 1 male and 2 females in the control group and in 3 animals of each sex in the 10% BSA group.

3) Gross lesions in the 2.5% and 5% BSA groups: Liver: mild agranulomatous inflammation in 1 male in the 2.5% BSA group in which white focus was found at necropsy; moderate necrosis of the hepatocytes in 1 female in the 2.5% BSA group in which white focus was found at necropsy. Lung (bronchus): slight hemorrhage in 1 male in the 2.5% group in which dark red focus was found at necropsy. Stomach: slight or mild erosion in the glandular stomach in 1 male and 2 females in the 2.5% BSA group in which dark red focus in the glandular stomach was found at necropsy; no abnormality was found in 1 animal of each sex in the 2.5% BSA group in which dark red focus in the glandular stomach was found at necropsy.

At the end of Recovery Period: Application site (dorsal skin): no abnormalities. Stomach: slight erosion in the glandular stomach was observed in 1 female in the 10% BSA group in which dark red focus in the glandular stomach was found at necropsy.

Sponsor's Conclusions:

- There were no changes attributed to SIS copolymer in clinical signs, body weight, food consumption, ophthalmology, urinalysis (including water intake), hematology or blood chemistry examinations.
- The pathological changes at the application site (dorsal skin) were considered to have no toxicological significance and thought to be due to physical irritation induced by the test article sheets when affixed directly to the skin.
- The increase in adrenal weight in the 10% BSA female group was considered stress-related because there was no histopathological change in this organ.
- The nontoxic dose level of styrene-isoprene-styrene block copolymer was 10% BSA of the rat/day for each sex in the present study.

Reviewer's Comment: The reviewer agrees that exposure of rats to SIS copolymer at 0.25%, 0.5% or 10% BSA for a month caused no serious systemic or skin toxicity. However, the Sponsor did not conduct a PK analysis to determine whether the SIS copolymer (or

contaminating monomers) penetrated the skin and was capable to reach systemic circulation. In most of the toxicological parameters measured, there were statistically significant differences between the control (surgical tape affixed) and the non-treatment group. The Sponsor did not specify why these differences occurred.

Study title: An Oral 4-Week Repeated Dose Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Dogs with a 4-Week Recovery Period

Objective: Determine the dose-response for the occurrence of toxic signs and the nature of the toxicity.

Key study findings:

- The oral administration of SIS block copolymer to dogs at doses up to 2000 mg/kg/day for 4-weeks did not produce any treatment related toxicity.
- The Sponsor did not provide evidence of systemic exposure after dermal application of the SIS block copolymer.

Study no: 14467

Volume #, and page #: 6, 986

Conducting laboratory and location: _____

Date of study initiation: January 21, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS copolymer sheet, lot # 0902-2, % purity was not specified

Formulation/vehicle:

Methods: The requisite amount of the test article, calculated based on the most recent body weight of each animal on the day of administration, was cut into small pieces using a sterilized scissors and was put into gelatin capsules of a ½ oz size. Eight capsules/animal were used to administered the test-article dose. Additional animals were included in the control and highest dose group, and were subjected to a four-week recovery period.

Dosing:

Species/strain: Dogs/Beagle

#/sex/group or time point (main study): 3 dogs/sex/group

Satellite groups used for recovery: 2 dogs/sex/group for an additional control and highest dose group

Age: 6 months

Weight: 7.3 – 9.0 kg

Doses in administered units: 0 (empty capsules), 80, 400 and 2000 mg/kg

Route, form, volume, and infusion rate: oral, capsules

Observations and times:

b(4)

Clinical signs: Week -1: once daily in the morning; administration period: prior to, 1, 3 hr after administration (prior to and 1 hr after administration only on weekends and holidays); recovery period: once daily in the morning

Body weights: Week -1: once daily in the morning; administration period: day 1 of administration, once a week during the administration period, day 28 of administration, day of necropsy; recovery period: day 1 of recovery, once a week during the recovery period, day 28 of recovery, day of necropsy

Food consumption: Daily from week -1.

Ophthalmoscopy: Week -1: once; administration period: week 3 of administration (prior to administration); recovery period: once in week 4 of recovery. Macroscopical examination of external appearance including appendages of eye, observation of cornea, conjunctiva, iris and lens, observation and photographing of fundus oculi and vitreous body

EKG: Week -2: once; administration period: week 4 of administration (prior to administration); recovery period: once in week 4 of recovery

Hematology: once each in weeks -2, -1, 2 and 4 of administration and week 4 of recovery

Clinical chemistry: once each in weeks -2, -1, 2 and 4 of administration and week 4 of recovery

Urinalysis: once each in weeks -2, -1, 2 and 4 of administration and week 4 of recovery

Gross pathology: Termination

Organs weighed: Refer to Appendix

Histopathology: Termination, refer to Appendix for tissues examined

Results:

Mortality: No deaths occurred.

Clinical signs: **Administration Period:** The most predominant sign was feces containing test article-like yellowish white granular substance observed in most animals since week 1. Some animals in the 400 mg/kg and 2000 mg/kg had vomits containing test-article like yellowish white substance. In addition, vomiting (ingesta, yellowish or whitish foamy) and soft or mucous feces were seen in each experimental group. The only sign observed in the control group was vomiting (ingesta, yellowish or whitish foamy) in 1 male and 1 female dog. **Recovery Period:** No noticeable changes except for presence of test-like material in the feces (as described above) during week 1 in the 2000 mg/kg group.

Body weights: No noticeable changes.

Food consumption: No noticeable changes.

Ophthalmoscopy: No test-article related effects.

Electrocardiography: **Administration Period:** The Sponsor recorded the following changes but considered they were incidental because of the feature of occurrence, relationship with doses or comparison with pretreatment control values.

Decrease of the QRS interval ($p < 0.05$) in males of the 400 mg/kg group in week 4.

High QTc value ($p < 0.05$) in males if the 80 mg/kg group in week 4.

Recovery Period: No noticeable changes.

Hematology: No test-article related effects.

Clinical chemistry: No test-article related effects.

Urinalysis: No test-article related effects.

Organ weights: No test-article related effects.

Gross pathology: No test-article related effects.
Histopathology: No test-article related effects.

Sponsor's Conclusions: The administration of SIS copolymer at doses up to 2000 mg/kg/day for 4-weeks failed to produce any treatment related systemic toxicity, and this dose is considered a no-effect level. The presence of material in the vomitus and feces is considered to be the unchanged polymeric test material, and is not considered an adverse effect.

Reviewer's Comments: The reviewer agrees that SIS copolymer caused no systemic or local toxicity after oral administration to dogs at doses up to 2000 mg/kg for 4-weeks. However, the Sponsor did not conduct a PK analysis to determine whether the SIS copolymer (or contaminating monomers) was capable to reach systemic circulation. The number of animals used in the recovery group was not appropriate (n=2). At least 3 animals should be used for statistically significant findings.

TOXICOLOGY STUDIES WITH ALICYCLIC SATURATED HYDROCARBON RESIN
b(4)

Study title: A Percutaneous Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats

Objective: Determine the systemic toxicity of alicyclic saturated hydrocarbon resin following a percutaneous single dose in rats.

b(4)

Key study findings:

- No toxic effects were seen following application of _____ to rats at a concentration of 2000 mg/kg.
- The Sponsor did not provide evidence of systemic exposure after dermal application of _____

Study no: -4446

Volume #, and page #: 7, 1224

Conducting laboratory and location: _____

b(4)

Date of study initiation: December 28, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, radiolabel, and % purity: _____, lot # A92201, % purity was not specified

b(4)

Methods: _____ was pulverized using a mortar, placed on a lint sheet (4 x 5 cm) and wetted with 0.5 ml liquid paraffin. It was applied to the shaved skin surface of the animals and covered with surgical tape. After application for 24 hours, the test material was removed and the animals observed for 14 days. Animals in the control group were treated with liquid paraffin (0.5 ml/animal) alone in the same manner.

Dosing:

Species/strain: Rats/Sprague-Dawley

#/sex/group or time point (main study): 5 rats/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: 6 weeks

Weight: 184 – 194 g

Doses in administered units: The toxicity of the article was expected to be low. Therefore, one dose level of 2000 mg/kg was selected in accordance to the *maximum dose level described in the toxicity study guideline for pharmaceuticals*.

Route, form, volume, and infusion rate: Percutaneous administration at the dorsal area for 24 hr

Observations and times:

Clinical signs: Immediately to 5 min after, 15 and 30 min after, 1, 2, 4, and 6 hr after administration, and once daily thereafter

Body weights: Before dosing and on days 1, 2, 3, 7 and 14

Gross pathology: Termination (Day 14). The Sponsor did not list the specific organs but used the following categories: cranial, thoracic, abdominal, and other regions

Results:

Mortality: No deaths occurred in any group.

Clinical signs: No abnormalities were observed.

Body weights: No treatment-related effects.

Gross pathology: No abnormalities were found.

Sponsor's Conclusions: The percutaneous single toxicity in rats of the _____ was suggested to be low. **b(4)**

Reviewer's Comments: The reviewer agrees that _____ caused no toxicity to rats after dermal application of a single 2000 mg/kg dose level. However, the Sponsor did not conduct a PK analysis did not evaluate whether _____ (or contaminating monomers) penetrated the skin and was capable to reach systemic circulation.

Study title: The Acute Toxicity Test of _____ **b(4)**

Objective: To determine the LD₅₀ of _____ in rats.

Key study findings:

- _____ was not toxic at doses of 5,000 or 10,000 mg/kg.
- The LD₅₀ of _____ in rats is greater than 10,000 mg/kg.

Study no: 80-20,21 **b(4)**

Volume #, and page #: 7, 1302

Conducting laboratory and location: not indicated

Date of study initiation: not indicated

GLP compliance: not specified

QA report: No

Drug, lot #, and % purity: _____ lot # B-15470, % purity was not specified

Formulation/vehicle: olive oil

b(4)

Methods: _____ was suspended in olive oil and administered by gavage. Animals were observed daily for 7 days.

Dosing:

Species/strain: Rats/Sprague-Dawley

#/sex/group or time point (main study): 10 rats/sex/group

Age: 4 weeks

Weight: not specified

Doses in administered units: 5,000 and 10,000 mg/kg

Route, form, volume, and infusion rate: gavage, 2 - 4 ml/100 g body weight

Observations and times:

Clinical signs: Daily

Body weights: Days 1, 3, and 7

Gross pathology: Termination (Day 7)

Results:

Mortality: No deaths occurred in any group.

Clinical signs*: Marked diarrhea was found from about 30 min after administration in all animals and gradually depressed locomotion activity. These signs disappeared on day 2.

Body weights: Body weight gain was slightly lower in female animals in the 10,000 mg/kg dose group compared to the 5000 mg/kg dose group.

Gross pathology*: No treatment-related abnormalities.

**The Sponsor did not submit the data for these observations. The information presented above was copied from the summary provided by the Sponsor in the Results Section.*

Sponsor's Conclusions: The LD₅₀ of _____ in the rat is considered to be over 10,000 mg/kg in both sexes.

b(4)

Reviewer's Comments: The Sponsor did not submit a full report. Some data and information are missing as indicated above. Based on the data provided, the reviewer cannot complete the safety evaluation. The reviewer asked the Sponsor to provide the data. The Sponsor responded that the data is no longer available (Toxicology Information Amendment N-009).

Study title: An Oral Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats

Objective: Determine the systemic toxicity of _____ following a single oral dose in rats.

b(4)

Key study findings:

- The single oral administration of _____ to rats at 2000 mg/kg was not associated with acute toxicity.
- The Sponsor did not provide evidence of systemic exposure after oral administration of _____.

b(4)

Study no: -4567

Volume #, and page #: 7, 1326

Conducting laboratory and location: _____

Date of study initiation: October 6, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: _____ lot # A98210, % purity was not specified

Formulation/vehicle: 2 % w/v *gummi arabicum pulveratum* (GAP) solution

b(4)

Methods: The requisite amount of _____ were weighed and suspended in GAP solution using a mortar to prepare test suspensions at each concentration. The suspensions were administered orally by gavage. The control group received 2% GAP solution in the same manner. Animals were observed for 14 days.

b(4)

Dosing:

Species/strain: Rats/Sprague-Dawley

#/sex/group or time point (main study): 5 rats/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: approximately 6 weeks

Weight: 153 – 166 g (males), 113 – 125 g (females)

Doses in administered units: 0, 500, 1000, and 2000 mg/kg, *maximum dose level described in the toxicity study guidelines for pharmaceuticals*

Route, form, volume, and infusion rate: oral, gavage

Observations and times:

Clinical signs: Immediately to 5 min after dosing, 10 and 30 min after dosing, and 1, 2, 4, and 6 hr after dosing, and once daily thereafter

Body weights: Before dosing and on days 1, 2, 3, 7, 10, and 14

Gross pathology: Termination (Day 14). The Sponsor did not list the specific organs but used the following categories: external appearance, cranial, thoracic, abdominal, and other regions

Results:

Mortality: No deaths occurred in any group.

Clinical signs: No abnormalities were observed.

Body weights: No treatment-related effects.

Gross pathology: No treatment-related abnormalities.

Sponsor's Conclusions: The oral single dose toxicity of _____ in rats was low and the approximate lethal dose was estimated to be more than 2000 mg/kg.

Reviewer's Comments: The reviewer agrees that _____ appeared non-toxic to rats after a single oral dose of 2000 mg/kg. However, the study has the following deficiencies:

b(4)

- The Sponsor did not conduct a PK analysis to determine whether _____ (or contaminating monomers) reached systemic circulation.
- The Sponsor did not conduct a full assessment of toxicological parameters (no clinical chemistry, hematology, urinalysis, histopathology, etc).

Study title: An Oral Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Beagle Dogs

Objective: Determine the systemic toxicity of _____ following a single oral dose in dogs.

b(4)

Key study findings:

- The single oral administration of _____ to beagle dogs at 2000 mg/kg was not associated with marked acute toxic changes.
- The Sponsor did not provide evidence of systemic exposure after oral administration of _____

Study no: - 4452

Volume #, and page #: 7, 1398

Conducting laboratory and location: _____

b(4)

Date of study initiation: December 16, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: _____ lot # A92201, % purity was not specified

Methods: The requisite amount of _____ were calculated base on the body weight of each animal, pulverized using a mortar and placed in gelatin capsules of a ½ oz size. Six capsules/animal were used to administered the test-article dose. Animals were observed for 14 days.

b(4)

Dosing:

Species/strain: Dogs/Beagle

#/sex/group or time point (main study): 3 dogs/sex/group (one group)

Age: approximately 6 months

Weight: 8.1 – 8.4 kg (male), 7.3 – 7.9 kg (female)

Doses in administered units: 2000 mg/kg, *maximum dose level described in the toxicity study guidelines for pharmaceuticals*

Route, form, volume, and infusion rate: oral, capsules

Observations and times:

Clinical signs: Immediately after to 5 min after dosing, 10 and 30 min after dosing, and 1, 2, 3, 4, 5 and 6 hr after dosing, and once daily thereafter
 Body weights: Days 1, 3, 7, 10, 13 after administration and on the day of necropsy
 Food Consumption: Daily
 Gross pathology: Termination. The Sponsor did not list the specific organs but used the following categories: cranial, thoracic, abdominal, and other regions
 Organs weighed: Brain, adrenal, spleen, heart, lung (including bronchial tubes), liver (with gallbladder), kidney

Results:

Mortality: No deaths occurred in any group.
 Clinical signs: feces containing test article-like white to light yellow granular material was observed in all animals on the day following administration. Vomiting of feed was observed in 1 male on day 12 administration.
 Body weights: There were no treatment-related effects.
 Food consumption: No abnormalities were observed.
 Organ weights: No abnormalities were observed in absolute or relative organ weight.
 Gross pathology: No treatment-related abnormalities.

Sponsor's Conclusions: The single oral administration of _____ to beagle dogs at 2000 mg/kg was not associated with marked acute toxic changes, although feces containing the test article were observed. Therefore, it was judged that its toxicity was low.

b(4)

Reviewer's Comments: The reviewer agrees that _____ caused no apparent toxicity to dogs after a single oral dose of 2000 mg/kg. However, the study has the following deficiencies:

- The Sponsor did not conduct a PK analysis to evaluate whether _____ (or contaminating monomers) reached systemic circulation.
- The Sponsor did not conduct a full assessment of toxicological parameters (no clinical chemistry, hematology, urinalysis, histopathology, etc).

Study title: The Primary Skin Irritation Test of _____

Objective: Evaluate the primary skin irritant effects of _____

b(4)

Key study findings:

- _____ (dissolved in olive oil) induced very slight erythema (grade 1) but his response was not different to that of the olive oil control.
- _____ was classified as nonirritant.

b(4)

Study no: -80-001

Volume #, and page #: 7, 1422

Conducting laboratory and location: not specified

Date of study initiation: not indicated

GLP compliance: Not indicated

QA report: No

Drug, lot #, and % purity: _____, lot # B-15470, % purity was not specified

Formulation/vehicle: Olive oil

b(4)

Methods: _____ (in olive oil) was applied to both intact and abraded sites on the backs of each rabbit. Olive oil was used as the reference material and was applied in a similar manner. Test and reference materials were held in place for 4 hours with an occlusive dressing. Skin irritation was scored according to the method of Draize.

Dosing:

Species/strain: Rabbits/Japanese White

#/sex/group or time point (main study): 6 males/group (one group)

Age: not indicated

Weight: 2.5 – 3.0 kg

Doses in administered units: 0.5 ml of 10% _____ on 2.5 x 2.5 cm piece of lint

Route, form, volume, and infusion rate: Topical application in both intact and abraded sites on the back of each rabbit.

Observations and times:

Skin reactions: 4, 24, 48 and 72 hours and on day 7 after application

Results:

Mortality: None was reported.

Skin reactions: Very slight erythema (grade 1) was observed in both the intact and abraded sites in olive oil and _____ treated animals that persisted up to 72 hr after application. One animal in the _____ treated group has a slight edema of grade 1, and it was also sustained up to 72 hr after application. All reactions disappeared by day 7. The primary irritation scores were 0.78 and 0.75 for the reference control olive oil and _____ respectively.

b(4)

Sponsor Conclusions: _____ was classified as non-irritant.

Reviewer Comments: The reviewer concurs.

Study title: The Cumulative Skin Irritation (3 Weeks) Test of _____

Objective: Evaluate _____ potential for cumulative skin irritation.

b(4)

Key study findings:

- Skin irritation reactions were similar in animals treated with _____ or olive oil vehicle.

Study no: -80-002

Volume #, and page #: 7, 1445

b(4)

Conducting laboratory and location: not indicated

Date of study initiation: not indicated

GLP compliance: not indicated

QA report: No

Drug, lot #, and % purity: _____ lot # B-15470, % purity was not specified

b(4)

Formulation/vehicle: Olive oil

Methods: Nine rabbits were selected, seven with intact and two with abraded sites. Each rabbit received 0.25 ml of _____ in olive oil in one site and 0.25 ml of olive oil in a second site. This procedure was repeated for 21 consecutive days. Skin irritation scored according to the method of Draize.

Dosing:

Species/strain: Rabbits/Japanese White

#/sex/group or time point (main study): 7 males/group (intact skin group), 2 males/group (abraded skin group)

Age: not indicated

Weight: 2.5 – 3.0 kg

Doses in administered units: 0.25 ml of 10% (w/v) _____ on two 5 x 5 cm areas

Route, form, volume, and infusion rate: Topical application in both intact and abraded sites on the back of the rabbits.

b(4)

Observations and times:

Skin reactions: Daily for signs of erythema and edema formation and other skin reactions.

Histopathology: At termination, application site

Results:

Mortality: No death was reported.

Skin reactions: Both _____ and the reference solution, olive oil, showed similar responses. Intact site: most animals showed very slight erythema; a few animals had severe erythema or severe erythema to slight eschar formation. Abraded site: very slight erythema or moderate to severe erythema were observed in both groups. Other reactions: Slight edema, atonia and desquamation were also observed in some animals in both intact and abraded sites.

Histopathology: Slight edema in the subcutaneous tissues including the dermis (The Sponsor did not submit the data).

b(4)

Sponsor Conclusions: _____ produced mild skin irritation following application to intact and abraded skin for 21 consecutive days, and was not more severe in nature than that seen with olive oil alone.

Reviewer Comments: The reviewer concurs.

Study title: A Primary Eye Irritation Study of Alicyclic Saturated Hydrocarbon Resin in Rabbits

Objective: Evaluate the irritant effects of _____ to the eye.

b(4)

Key study findings:

- _____ was minimally irritating to the rabbit eye.
- At 1 hr post-instillation, discharge was observed in all unwashed eyes and redness and chemosis in one animal.

Study no: 1384**Volume #, and page #:** 7, 1457**Conducting laboratory and location:** _____

b(4)

Date of study initiation: December 13, 1999**GLP compliance:** Yes**QA report:** Yes**Drug, lot #, and % purity:** _____ lot # A92201, % purity was not specified

b(4)

Methods: The test article was pulverized using a mortar and placed in the conjunctival sac of the left eye of each rabbit. The right eye remained untreated and served as control. A separate group of rabbits had a similar treatment but their eyes were washed with 0.2 ml of water 30 sec after treatment. Eye irritation was scored according to the method of Kay and Calandra².

Dosing:

Species/strain: Rabbits/Japanese White

#/sex/group or time point (main study): 6 females/unwashed group, 3 females/washed group

Age: 14 weeks

Weight: 2.46 – 2.86 kg

Doses in administered units: 0.1 ml of pulverized _____ The Sponsor mentioned that _____ *was measured with a 0.1 ml vessel and the actual weight of the test article was determined and recorded*² but did not specify the weight.

b(4)

Route, form, volume, and infusion rate: conjunctival sac

Observations and times:

Clinical signs: Hourly until 6 hr after application and once daily thereafter.

Body weights: On the day of application and at the end of the observation period.

Eye irritation: Examined macroscopically and with an ophthalmoscope 1, 24, 48 and 72 hr after application. Fluorescein staining was also performed.

Results:

Mortality: None was reported.

Clinical signs: No abnormalities were observed.

Body weights: A slight decrease in body weight was observed in 4 animals in the unwashed group and all animals in the washed group. There were no abnormalities in the general condition of the animals; the Sponsor judged the decrease in body weight was due to stress from restraint at the time of application.

² Kay, J.H. and Calandra, J.C. Interpretation of eye irritation test. *J. Soc. Cosm. Chem.*, 13, 281(1962).

Primary Eye Irritation:

- Unwashed group: 1 hr after application -eye discharge in the conjunctiva (grade 1) in all animals, chemosis and redness in one animal; 24 – 72 hr after application – no ocular changes were observed. The mean total score (MTS) 1 hr after application = 2.7 but was 0 both at 24 and 72 hr after application. Lid closure was observed in all animals immediately after application only.
- Washed group: no ocular reactions were observed.

Sponsor's Conclusions: _____ was classified as "minimally irritating" on the rabbit eye in accordance to the Kay and Calandra's² evaluation criteria. b(4)

Reviewer's Comments: The reviewer concurs. However, the Sponsor did not specify how much _____ (in weight units) was applied to the eye.

Study title: Allergenic Study of _____

Objective: Evaluate whether _____ has skin sensitizing potential (allergic contact dermatitis). b(4)

Key study findings:

- _____ induced no signs of skin irritation following challenge application.
- The Sponsor did not submit the data, and the reviewer could not confirm the findings.

Study no: _____ 80-7279

Volume #, and page #: 8, 1497

Conducting laboratory and location: not indicated

Date of study initiation: August 1980

GLP compliance: Not indicated b(4)

QA report: No

Drug, lot #, and % purity _____, lot # B-15470, % purity was not specified

Formulation/vehicle: olive oil

Positive control article: 2,4-Dinitrochlorobenzene (DNCB)

Method: Maximization Test. _____ was given as an intradermal injection (0.05 ml) into the shaved scapula of guinea pigs. Some sites also received intradermal injections of Freund's Complete Adjuvant (0.05 ml) or a mixture (0.1 ml) of Freund's Complete Adjuvant and _____. One week later, _____ in olive oil (0.2 ml) was applied topically on filter paper, which was then covered with tape. The material was left on place for 48 hr. The positive control group received a patch containing 0.2 ml of DNCB in olive oil. Two weeks later, animals received another application of _____ in white vaseline or DNCB in acetone, and the test-material left on place for 24 hr (closed application). As a control for the challenge exposure, groups untreated guinea-pigs each received lateroabdominal applications of the same quantity of _____ or DNCB. b(4)

Dosing:

Species/strain: Guinea Pigs/Hartley

#/sex/group or time point (main study): 5 females/group

Age: not indicated

Weight: 360 – 480 g

Doses in administered units: 10 % _____ or 0.1% DNCB

Route, form, volume, and infusion rate: Intradermal injection in the left and right scapula for initial sensitization phase; topical application on the scapular region for second sensitization phase; topical application in the latero-abdominal region for challenge phase

b(4)

Observations and times:

Skin reactions: 24 hr after removal of application

Results: *The Sponsor did not include the data. The following results were taken from the Summary provided by the Sponsor in the Results Section.*

Mortality: No death was reported.

Skin reactions:

- _____ No signs of skin irritation following challenge application with _____. However, redness, and swelling of eschar were seen after intradermal injection with the solution of Adjuvant and _____. The same symptoms were seen from intradermal injection of Adjuvant alone. Therefore, it is not thought that the symptoms seen at the sensitization site with the solution of Adjuvant and _____ were caused by _____.
- DNCB positive control: Moderate erythema of grade 2 after challenge exposure.
- Control group: No skin responses.

b(4)

Sponsor Conclusions: _____ was classified as a non-sensitizer in this animal model.

Reviewer Comments: The reviewer couldn't confirm the results reported because the Sponsor did not submit the data. The reviewer asked the Sponsor to provide the individual animal listings. The Sponsor response was that the data are no longer available (Toxicology Information Amendment N-009). The Sponsor submitted an additional table and photographs (photocopies) related to the study. The table shows that 10% _____ did not cause a skin sensitization reaction in any of the animals whereas all the animals in the positive control group (0.1% DNCB) were sensitized (n = 12 animals/group). The photocopies of the skin photographs were not clear enough to make an interpretation feasible.

b(4)

Study title: A Skin Phototoxicity Study of Alicyclic Saturated Hydrocarbon Resin in Guinea Pigs

Objective: Evaluate the skin phototoxicity potential of _____

b(4)

Key study findings:

- _____ did not induce skin phototoxicity in guinea pigs after UVA exposure.

Study no: 1503

Volume #, and page #: 8, 1511

Conducting laboratory and location: _____

Date of study initiation: February 28, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: _____ lot # A98210, % purity was not specified

Formulation/vehicle:

Positive control article: 8-Methoxypsoralen (8-MOP)

b(4)

Methods: Powdered _____ wetted in liquid paraffin, was applied to shaved backs of each guinea pig and the site covered for one hour with surgical tape. 8-MOP was applied to the contralateral site to serve as a positive control. The materials were removed 1 hr after application and the application sites exposed to 14 joules/cm² of UV light (320 – 420 nm, max. = 350 nm) at a distance of 10 cm. Skin irritation was scored by the method of Draize.

Dosing:

Species/strain: Guinea Pigs/Hartley

#/sex/group or time point (main study): 7 females/group (one group)

Age: 5 weeks

Weight: 293 – 317 g

Doses in administered units: 0.05 g of _____ or 0.02 ml of 0.02% (w/v) 8-MOP solution in ethanol applied to two 1.5 x 1.5 cm application sites.

Route, form, volume, and infusion rate: Topical application on the back of each guinea pig.

b(4)

Observations and times:

Clinical signs: Daily

Body weights: Day of application/irradiation and on the final day of observation

Skin reactions: 24, 48 and 72 hr after irradiation

Results:

Mortality: None was reported

Clinical signs: No abnormalities were observed.

Body weights: No treatment-related effects.

Skin reactions:

- _____ : No skin reactions in the UV(+) or UV(-) areas up to 72 hr post-irradiation.
- 8-MOP: Erythema of score 1 - 3 in all the animals and edema of score 1 or 2 in 5 animals at 24 hr after irradiation. The mean scores 24, 48 and 72 hr after irradiation were 3.7, 3.3, and 1.9, respectively. Skin reactions were not observed in the UV(-) area.

b(4)

Sponsor Conclusions: _____ had no skin phototoxicity potential in this animal model.

Reviewer Comments: The reviewer concurs with the conclusion that under the conditions of the study, _____ was not phototoxic. However, it is noteworthy that only UVA exposure was used in this study. It is preferable to conduct studies with exposure to UVA-UVB-Visible light spectrum to mimic solar conditions. This may not be necessary if the test material does not absorb in the UV-Visible region or the patch is opaque to light.

b(4)

Study title: A Skin Photosensitization Study of Alicyclic Saturated Hydrocarbon in Guinea Pigs

Objective: Evaluate the skin photosensitization potential of _____.

Key study findings:

- _____ did not induce skin photosensitization in guinea pigs.

b(4)

Study no: -1389

Volume #, and page #: 8, 1540

Conducting laboratory and location: _____

Date of study initiation: December 27, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: _____, lot # A92201, % purity was not specified

Formulation/vehicle:

Positive control article: 6-Methylcoumarin (6-MC)

b(4)

Methods: Powdered _____ wetted in liquid paraffin, was applied to shaved areas in the cervico-dorsal region of each guinea pig. The site had been prepared by intradermal injection of Freund's Complete Adjuvant and stripping of the skin surface with cellophane tape to produce abrasion of the epidermal surface. One hour after application, the materials were removed and the application sites exposed to 10 Joules/cm² of UV light (320-420 nm, max. = 350 nm) at a distance of 10 cm. This procedure was repeated for five consecutive days. Another group of animals received 6-MC as a positive control. A third group served as a control group and was not exposed to test materials during the induction phase. Two weeks after the fifth application, animals were shaved at the dorsal area, and received another one hour application of either _____ or the positive control material. On the control group, the animals received an application of _____ or 6-MC in separate areas. Sites were irradiated as described above.

b(4)

Dosing:

Species/strain: Guinea pigs/Hartley

#/sex/group or time point (main study): 10 females/group

Age: 7 weeks

Weight: 365 – 482 g

Doses in administered units: 0.1 g of _____ or 0.1 ml of 2% (w/v) 6-MC solution in ethanol were applied to a 2 x 4 cm application site.

Route, form, volume, and infusion rate: Topical application on the cervico-dorsal area

b(4)

Observations and times:

Clinical signs: Daily
Body weights: Starting day of photosensitization (Day 0), day of final photosensitization (Day 4), day of photochallenge (Day 21), and final day of observation (Day 23)
Skin reactions: 24 and 48 hr after irradiation for photochallenge exposure

Results:

Mortality: None was reported.
Clinical signs: No abnormalities were observed.
Body weights: No abnormalities were observed.
Skin reactions:

- No skin reactions in the UV(+) or UV(-) sites.
- 6-MC positive control: Erythema of grade 1 to 3 was observed at the UV(+) site in all animals and edema of grade 1 in 3 animals at 24 hr after irradiation for challenge exposure. The group mean scores 24 and 48 hr after irradiation for challenge exposure were 2.5 and 2.6, respectively. There were no skin reactions at the UV(-) site.
- Control group: No skin reactions following photochallenge exposure at the UV(+) or UV(-) sites.

b(4)

Sponsor Conclusions: _____ had no skin photosensitization potential under the conditions of the study.

b(4)

Reviewer Comments: The reviewer concurs with the conclusion that under the conditions of the study, _____ induced no photosensitization. However, it is noteworthy that only UVA exposure was used in this study. It is preferable to conduct studies with exposure to UVA-UVB-Visible light spectrum to mimic solar conditions. This may not be necessary if the drug product does not absorb in the UV-Visible region or the patch is opaque to light.

Study title: An Oral 4-Weeks Repeated Dose Toxicity Study of Alicyclic Saturated Hydrocarbon in Beagle Dogs with a 4-Weeks Recovery Period

Objective: Determine the dose-response for the occurrence of toxic signs and the nature of the toxicity.

Key study findings:

- The oral administration of _____ to dogs at doses up to 2000 mg/kg/day for 4-weeks did not produced any treatment-related toxicity.
- The Sponsor did not provide evidence of systemic exposure after oral administration of _____

Study no: 4468

Volume #, and page #: 8, 1593

Conducting laboratory and location: _____

Date of study initiation: January 27, 2000

b(4)

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: ——— lot # A98210, % purity was not specified

Methods: The requisite amount of the test article, calculated based on the most recent body weight of each animal on the day of administration, was pulverized using a mortar and was put into gelatin capsules of a ½ oz size. Five capsules/animal were used to administer the test-article dose. Additional animals were included in the control and highest dose group, and were subjected to a four-week recovery period. **b(4)**

Dosing:

Species/strain: Dogs/Beagle

#/sex/group or time point (main study): 3 dogs/sex/group for 4-weeks treatment group, and 2 dogs/sex/group for an additional control and highest dose group to serve as recovery animals

Satellite groups used for toxicokinetics or recovery: none

Age: 6 months

Weight: 6.1 – 9.1 kg

Doses in administered units: 0 (empty capsules), 80, 400 and 2000 mg/kg, once daily

Route, form, volume, and infusion rate: oral, capsules

Observations and times:

Clinical signs: Week -1: once daily in the morning; administration period: prior to, 1, and 3 hr after administration (prior to and 1 hr after administration only on weekends and holidays); recovery period: once daily in the morning

Body weights: Week -1: once daily in the morning; administration period: day 1 of administration, once a week during the administration period, day 28 of administration, day of necropsy; recovery period: day 1 of recovery, once a week during the recovery period, day 28 of recovery, day of necropsy.

Food consumption: Daily from week -1.

Ophthalmoscopy: Week -1: once; administration period: week 3 of administration (prior to administration); recovery period: once in week 4 of recovery. Macroscopical examination of external appearance including appendages of eye, observation of cornea, conjunctiva, iris and lens, observation and photographing of fundus oculi and vitreous body

EKG: Week -2: once; administration period: week 4 of administration (prior to administration); recovery period: once in week 4 of recovery

Hematology: once each in weeks -2, -1, 2 and 4 of administration and week 4 of recovery

Clinical chemistry: once each in weeks -2, -1, 2 and 4 of administration and week 4 of recovery

Urinalysis: once each in weeks -2, -1, 2 and 4 of administration and week 4 of recovery

Gross pathology: Termination

Organs weighed: Refer to Appendix

Histopathology: Termination, refer to Appendix for tissues examined

Results:

Mortality: No deaths occurred.

Clinical signs: **Administration Period:** Feces containing test article-like yellowish white granular substance were observed in 2 males and 2 females in the 400 mg/kg group and in all animals in the 2000 mg/kg group. Vomiting occurred in each experimental and control groups. Soft feces and ectropion of the third eye lid was observed in 1 male in the 80 mg/kg. **Recovery Period:** No noticeable changes except for fecal changes during week 1 and vomitus in weeks 2 and 4 in the 2000 mg/kg group.

Body weights: No noticeable changes.

Food consumption: No noticeable changes.

Ophthalmoscopy: No test-article related effects.

Electrocardiography: No noticeable changes.

Hematology: No test-article related effects.

Clinical chemistry: No test-article related effects.

Urinalysis: No test-article related effects.

Organ weights: No test-article related effects.

Gross pathology: Smallness of the prostate was observed in 2 animals in the 80 mg/kg group and in all 3 animals in the 400 mg/kg group but not in the 2000 mg/kg group. It was also observed in one animal in the control group during the recovery period. The Sponsor judged the variation to be incidental because of their feature of occurrence and pathological characteristics.

Histopathology: No test-article related effects.

Sponsor's Conclusions: The administration of _____ at doses up to 2000 mg/kg/day for 4-weeks failed to produce any treatment related systemic toxicity, and this dose is considered a no-effect level. The presence of material in feces is considered to be the unchanged polymeric test material, and is not considered an adverse effect.

b(4)

Reviewer's Comments: The reviewer agrees that _____ caused no systemic or local toxicity after oral administration to dogs at doses up to 2000 mg/kg for 4-weeks. However, the Sponsor did not conduct a PK analysis to evaluate whether _____ (or contaminating monomers) reached the systemic circulation. The size of the recovery group (n=2) was not appropriate to obtain statistically significant findings.

Study title: A 4-Week Oral Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Rats with a Recovery Period of 4-Weeks

Key study findings:

- The oral administration of _____ at doses up to 1000 mg/kg/day for 4-weeks did not produce any drug-related toxicity.
- The Sponsor did not provide evidence of systemic exposure after oral administration of _____

b(4)

Study no: 4568

Volume #, and page #: Toxicology Information Amendment N-011

b(4)

Conducting laboratory and location: _____

Date of study initiation: November 10, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: _____ A98210, b(4)

Formulation/vehicle: 2% w/v *Gummi arabicum pulveratum* (GAP)

Methods: Requisite amounts of the test article were weighed and suspended in 2% w/v GAP solution using a mortar to prepare test suspensions of each concentration. Three dose levels were set. Additional animals were included in the control, middle and high dose groups to be subjected to a 4-week recovery period.

Dosing:

Species/strain: Rats/Sprague-Dawley

#/sex/group or time point (main study): 12 rats/sex/group

Satellite groups used for recovery: 6 rats/sex/group for an additional control, 500 and 1000 mg/kg dose groups

Age: 6 weeks

Weight: males 179 – 229 g; females 143 – 176 g

Doses in administered units: 0, 250, 500, and 1000 mg/kg

Route, form, volume, and infusion rate: oral (gavage), 10 ml/kg body weight

Observations and times:

Clinical signs: administration period: prior to dosing, immediately after dosing and 2 hours after dosing; recovery period: once daily in the morning

Body weights: administration period: days 1 (prior to dosing), 4 and 7, thereafter twice a week, every 3 or 4 days, before dosing on these days, and on day of necropsy; recovery period: days 1, 3, and 7, then twice a week, every 3 or 4 days, thereafter, and on day of necropsy

Food consumption: administration period: day –1, thereafter one day's food consumption was calculated from 3 or 4 days' cumulative consumption every 3 or 4 days, before dosing on these days; recovery period: one day's food consumption was calculated for days 1 to 3 from 2 days' cumulative consumption and days 3 to 7 from 4 days' cumulative consumption. Thereafter, one day's consumption was calculated for each animal from 3 or 4 days' cumulative consumption at 3 or 4-day intervals.

Ophthalmoscopy: day 8 of quarantine/acclimatization period, before dosing on day 24 of administration and in week 4 of recovery. Macroscopic examination followed by ophthalmoscopic examination of the anterior portion of the eye, transparent body and fundus oculi, photographing of fundus oculi in the control and 1000 mg/kg groups.

EKG: not performed

Hematology: At necropsy

Clinical chemistry: At necropsy

Urinalysis: week 4 of administration (days 23 to 24) and in week 4 of recovery (days 23 to 24).

Gross pathology: At termination in all animals, organs in the cephalic, thoracic and abdominal cavities

Organs weighed: Refer to Appendix

Histopathology: At termination, all animals in the control and high dose groups from the administration period, gross lesions were examined microscopically regardless of dose levels, refer to Appendix for tissues examined

Results:

Mortality: No deaths occurred.

Clinical signs: No abnormalities were observed.

Body weights: No noticeable changes.

Food consumption: No noticeable changes.

Ophthalmoscopy: No abnormalities were observed.

Electrocardiography: Not measured.

Hematology: No toxicologically relevant differences.

Clinical chemistry: **At the end of the Administration Period:** In males, a significant increase in ALAT activity (19%) in the 500 and 1000 mg/kg groups. In females, a significant increase in urea nitrogen (29%) in the 250 mg/kg group but it was not dose-related; **At the end of the Recovery period:** In males: a significant decrease in γ -globulin ratio in the 1000 mg/kg group (17%). The Sponsor considered this change was not toxicologically significant because the change was not observed at the end of the administration period. A significant decrease in ASAT activity (30%) and significant increases in total cholesterol (24%) and phospholipids (17%) in the 500 mg/kg group but these changes were not dose-related.

Urinalysis: **Week 4 of Administration:** A significant increase in urine volume (24%) and a significant decrease in osmotic pressure (13%) were observed in males in the 500 mg/kg group but these changes were not dose-related; **Week 4 of Recovery:** In males, a significant decrease in pH (8.5 to 9.0) in 5 animals in the 500 and 1000 mg/kg groups. The Sponsor consider these changes were not of toxicological significance because no changes had been observed in week 4 of administration. In females, a significant decrease in osmotic pressure (24%) in the 500 mg/kg group but it was not a dose-related change.

Organ weights: **At the end of Administration:** No drug-related changes were observed in any treatment group; **At the end of Recovery:** In males, a significant increase in the relative weight of the thymus (23%) in the 1000 g/kg group. The Sponsor consider this change was not of toxicological significance because it had not been observed at the end of the administration period. In females, significant increases in the absolute and relative weights of the thyroids (30%) in the 500 mg/kg group, but these changes were not dose-related.

Gross pathology: The following lesions were consider to be incidental judging from the incidence: **At the end of Administration:** Thyroid: smallness (unilateral) was observed in 1 male in the 1000 mg/kg group. Lung: dark red focus was observed in 1 animal of each sex in the 250 mg/kg group. Stomach: dark red focus in the glandular stomach was observed in 1 female in the control and in 1 animal of each sex in the 250 mg/kg group. **At the end of Recovery:** white focus in the forestomach was observed in 1 male in the 1000 mg/kg group.

Histopathology: The following lesions were consider to be incidental judging from the incidence and pathological nature: **At the end of Administration: Changes in the control and 1000 mg/kg groups:** Lung: slight focal hemorrhage was observed in 1

animal of each sex in the 1000 mg/kg group. Prostate: slight or mild cell infiltration in the interstitium was observed in 1 animal of the control group and 2 animals in the 1000 mg/kg group. Stomach: slight erosion in the glandular stomach was observed in 1 female of the control group in which dark red focus in the glandular stomach was found at necropsy. Thyroid: Hypoplasia (unilateral) was observed in 1 male of the 1000 mg/kg group in which smallness (unilateral) was found at necropsy. Urinary bladder: mild thickening in the mucosa, mild dilatation in the lumina, slight cell infiltration in the serosal and slight arteritis were observed in 1 male of the 1000 mg/kg group. **Gross lesions in the 250 mg/kg group**: Lung: slight focal hemorrhage was observed in 1 female in the 250 mg/kg group in which dark red focus was found at necropsy. Slight focal cell infiltration was observed in 1 male in the 250 mg/kg group in which dark red focus was found at necropsy. Stomach: slight erosion in the glandular stomach was observed in 1 animal of each sex of the 250 mg/kg group in which dark red focus in the glandular stomach was found at necropsy. **At the end of Recovery**: Stomach: cyst in the forestomach was observed in 1 male of the 1000 mg/kg group in which white focus in the glandular stomach was found at necropsy.

Sponsor's Conclusion: The non-toxic level of alicyclic saturated hydrocarbon resin was 1000 mg/kg/day for both sexes in the present study.

Reviewer's Comments: The reviewer agrees that _____ caused no systemic or local toxicity after oral administration to rats at doses up to 1000 mg/kg for 4-weeks. However, the Sponsor did not conduct a PK analysis to evaluate whether _____ (or contaminating monomers) reached the systemic circulation.

b(4)

Toxicology summary: Studies were conducted to assess the dermal toxicity of FS 67A patch product. A single application of FS 67A to both abraded and intact sites in guinea pigs induced slight erythema of grade 1 and was classified as slightly irritant (Primary Irritation Index = 0.1). Similarly, slight erythema (grade 1) was observed from day 2 to end of a 14-day period with no cumulative skin irritability. The protocol for these studies did not specify if the investigators applied moisture to the test-substance to ensure good contact with the skin. Therefore, the skin irritation potential may be underestimated. FS 67A was neither phototoxic nor a photosensitizer following UVA light exposure to guinea pigs. However, the possibility for such a potential after exposure to also UVB and visible light irradiation was not evaluated. This might be of no concern if FS 67A patch is opaque to light or if the product does not absorb in the 280 – 700 nm region of the solar spectrum.

Studies were also conducted to assess the safety use of two excipients, styrene-isoprene-styrene (SIS) block copolymer and _____. In studies conducted to determine the dermal and systemic toxicity of SIS copolymer after a single dose in rats, no toxic effects were seen following application of SIS copolymer to 10% of the rat's body surface area. An oral single-dose toxicity study was also conducted in beagle dogs and SIS copolymer was not toxic at a dose of 2000 mg/kg. A percutaneous repeated study in rats and an oral study in dog both of 4-week duration with a 4-week recovery period were conducted. SIS copolymer was not toxic to rats when applied at levels of 2.5%, 5%, and 10% of the rat's body surface area/day. Physical

b(4)

irritation at the application site was observed but the Sponsor judged the irritation was due to treatment related-physical injuries and not test-article related. Similarly, the administration of SIS block copolymer to dogs at doses up to 2000 mg/kg/day for 4-weeks did not produce any treatment-related toxicity. The most common clinical signs were feces containing the test article and vomiting. Similar studies were conducted with _____ No test-article related effects were seen following single topical application or oral administration of _____ to rats at concentrations up to 2000 mg/kg. In dogs, a single oral administration of 2000 mg/kg. _____ was not associated with marked toxic changes. Vomiting and feces containing test-article were the major clinical signs. Similar findings were observed after repeated oral administration of _____ for 4-weeks to beagle dogs at doses up to 2000 mg/kg/day. In rats, no toxicologically significant effects were observed after repeated oral administration of _____ for 4-weeks at doses up to 1000 mg/kg/day. b(4)

The studies summarized above tend to support that both excipients are safe to use. However, the studies have the following deficiencies:

- The Sponsor did not conduct a PK analysis to determine whether the excipients (or contaminating monomers) were capable to reach systemic circulation.
- In several studies, the Sponsor did not conduct a full toxicological assessment.

A primary and 14-day cumulative skin irritation, skin sensitization, eye-irritation, skin phototoxicity and skin photosensitization studies were conducted with both SIS block copolymer and _____ SIS copolymer was negative in all assays and classified as a “nonirritant” and not phototoxic or a photosensitizer. _____ was also classified as a nonirritant in the primary skin irritation test and was not phototoxic or a photosensitizer. However, in the 3-week skin irritation cumulative study, slight to moderate to severe erythema and slight edema were observed. These changes were also observed with the vehicle (olive oil), which suggests the effects may be due to the olive oil in the _____ preparation and not _____ related. b(4) As mentioned above for FS 67 patch, the phototoxicity and photosensitization studies used only UVA light. Thus, the possibility for such a phototoxic or photosensitization potential after exposure to also UVB and visible light was not evaluated.

Toxicology conclusions: The preclinical studies suggest that FS 67A may cause “slight” skin irritation after a single exposure or a 14-day continuous exposure. FS 67A may not cause skin photoirritation and photosensitization after UV-A exposure. Whether exposure to the full UV-Visible light spectrum may cause skin photoirritation or photosensitization was not determined.

The two excipients analyzed, SIS block copolymer and _____ did not show evidence of having significant skin toxicity. Studies were also conducted to address the potential systemic toxicity of these excipients after dermal application or oral administration. Both compounds did not produce major systemic toxicity. However, the Sponsor did not measure whether these excipients (or contaminating monomers) reached systemic circulation. In Toxicology Information Amendment N-010, the Sponsor stated that TK analysis was not possible due to technical difficulties related to the nature of the polymers (for details refer to Section IX of current review). High molecular weight polymers themselves are considered inert, the monomers and impurities resulting from the manufacturing process comprise the biological b(4)

risk. This issue may not be of concern if the levels of contaminating monomers and impurities are negligible or kept within acceptable limits.

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Histopathology Inventory for IND #

Histopathology Inventory for IND # 62,735

Study	B4582 ^a	B4467	B-4468	B-4568
Species	Rats	Dogs	Dogs	Rats
Adrenals	X*	X*	X*	X*
Aorta	X	X	X	X
Bone Marrow smear	X	X	X	X
Bone (femur)	X	X	X	X
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix				
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X*	X*	X
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder		X	X*	
Gross lesions	X	X	X	X
Harderian gland	X			X
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland		X	X	
Larynx	X	X	X	X
Liver	X*	X*	X*	X*
Lungs	X*	X*	X*	X*
Lymph nodes, cervical		X	X	
Lymph nodes mandibular	X			X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity	X			
Optic nerves	X	X	X	X
Ovaries	X*	X*	X*	X*
Pancreas		X*	X*	X
Parathyroid	X*	X	X	X
Peripheral nerve				
Pharynx				
Pituitary	X*	X*	X*	X*
Prostate	X*	X*	X*	X*
Rectum	X	X	X	X
Salivary gland ^b	X*	X*	X	X*
Sciatic nerve	X	X	X	X
Seminal vesicles	X*			X*
Skeletal muscle	X	X		X
Skin ^c	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X*	X*
Sternum		X	X	
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X*	X*
Vagina	X	X	X	X
Zymbal gland				
Standard List				

X, histopathology performed

*, organ weight obtained

^aAlso examined the application site and auricle

^bSubmandibular and sublingual; submandibular weighed in study B-4468

^cAbdominal region

V. GENETIC TOXICOLOGY:**GENETOX STUDIES WITH STYRENE-ISOPRENE-STYRENE**

Study title: A Reverse Mutation Test of Styrene-Isoprene-Styrene Block Copolymer Using Bacteria

Key findings:

- SIS copolymer was negative in the Ames tests at concentrations up to 5000 µg/plate.

Study no: 1046

Volume #, and page #: 6, 1147

Conducting laboratory and location:

Date of study initiation: February 8, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS block copolymer, lot # 0902-2, % purity was not specified. SIS copolymer was extracted in methanol (1 g/10 ml), evaporated to dryness, and redissolved in DMSO.

Formulation/vehicle: Dimethyl sulfoxide (DMSO)

Methods:

Strains/species/cell line: *Salmonella typhimurium* TA100, TA98, TA1535 and TA1537, and *Escherichia coli* WP2uvrA

Dose selection criteria: Dose range finding study using the preincubation method ± S9. The dose levels tested were 0.305, 1.22, 4.88, 19.5, 78.1, 313, 1250 and 5000 µg/plate. Deposition of crystals occurred immediately after overlaying at concentrations of 313 µg/plate or above. After incubation, precipitation was also observed at 78 µg/plate. Inhibition of growth was not observed at any concentration irrespective of bacterial strain (the Sponsor did not show the data). Therefore, the highest concentration was set at 5000 µg/plate and a total of 6 concentrations were used for the main test using a common ratio of 2 (see below).

Test agent stability: The Sponsor mentioned that the test article was confirmed to be stable during the mutation experiment period. The data was not submitted.

Metabolic activation system: S-9 fractions prepared from livers of Sprague-Dawley rats induced with phenobarbital (4 days, 30+60+60+60 mg/kg body weight) and 5,6-benzoflavone (1 day, 80 mg/kg body weight).

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: Furfurylamine (AF-2), sodium azide (SAZ), ICR-191, 2-aminoanthracene (2-AA), and benzo(a)pyrene (B[a]P) were selected based on the bacteria strain as indicated in the following table:

b(4)

Tester Strains	Positive Control Article (µg/plate)	
	-S9	+S9
TA100	AF-2 (0.01)	B[a]P (5.0)
TA98	AF-2 (0.1)	B[a]P (5.0)
TA1535	SAZ (0.5)	2-AA (2.0)
TA1537	ICR-191 (1.0)	B[a]P (5.0)
WP2 <i>uvrA</i>	AF-2 (0.01)	2-AA (10.0)

Comments: The OECD/EPA Guidelines state: "2-AA should not be used as the sole indicator of the efficacy of the S9 mix. If 2-AA is used, each batch of S9 should be characterized with a mutagen that requires metabolic activation by microsomal enzymes, e.g., B[a]P or dimethylbenzanthracene."

Exposure conditions: The preincubation method was used in the main study and the concentrations of test-article selected were 156, 313, 625, 1250, 2500 and 5000 µg/plate. The samples were preincubated at 37°C for 20 minutes in the presence or absence of S9 fraction, top agar was added and the mixture was used to inoculate a minimal glucose agar medium plate. After incubation for 48 hr, the bacteria were observed for growth inhibition and number of revertant colonies.

Analysis:

No. of replicates: triplicates

Counting method: Automated colony counter or counted microscopically when the colony counter could not be used due to precipitation.

Criteria for positive results: An increase in the number of revertant colonies more than twice that of the negative control plates, and if a dose-response and reproducibility was observed. Statistical analysis was not done.

Summary of individual study findings:

Study validity: The selection of the bacteria strain was adequate based upon the ICH S2A Guideline: Specific Aspects of Regulatory Genotoxicity Test for Pharmaceuticals. Dose selection was adequate based on maximum dose level of 5000 µg/plate specified in the guideline and the lack of toxicity in the preliminary test. The positive controls showed an adequate response, i.e., the number of revertant colonies was twice or more that of the solvent control for each bacterial strain.

Study outcome: Growth inhibition was not observed in any plate treated with SIS copolymer in the dose-range finding test or main test (the Sponsor did not show the growth inhibition data). The number of revertant colonies in the treatment groups was not more than twice that of the solvent control and no dose-dependent increase was observed irrespective of bacterial strains, concentration of test-article and the presence of metabolic activation. Deposition of crystals was observed immediately after overlaying at dose levels of 313 µg/plate or above. After incubation, deposition of crystals was also observed at 156 µg/plate.

Sponsor's Conclusion: SIS block copolymer had no mutagenic potential under the conditions of this test.

Reviewer's comments: The negative results at SIS copolymer concentrations of 78 µg/plate or above are questionable because of the solubility issue. In the main study, all concentrations

selected showed crystal deposition. It is possible that precipitation of the test-article may have contributed to the lack of toxicity/mutagenicity in this assay.

Study title: A Chromosomal Aberration Test of Styrene-Isoprene-Styrene Block Copolymer Using CHL Cells

Key finding:

- SIS block copolymer was negative in the chromosomal aberration assay.

Study no: 1047

Volume #, and page #: 6, 1181

Conducting laboratory and location:

Date of study initiation: February 8, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: SIS block copolymer, lot # 0902-2, % purity was not specified. SIS copolymer was extracted in methanol (1 g/10 ml), evaporated to dryness, and redissolved in DMSO.

Formulation/vehicle: Dimethyl sulfoxide (DMSO)

Methods:

Strains/species/cell line: Chinese hamster lung fibroblast cell line (CHL cells)

Dose selection criteria: Based on a preliminary study in which growth inhibition was determined following both a continuous and a short-term treatment method. The highest dose of the test-article was 5000 µg/ml and 8 dose levels were selected using a common ratio of 2. In the continuous method, samples were incubated for 24 or 48 hr in the absence of S9 fraction. In the short-term method, samples were incubated for 6 hr ± S9 fraction. Afterwards, the medium was changed and incubation continued for 18 hr. Deposition of crystals was observed at concentrations of 313 µg/ml and above. In the short-term treatment, the 50% growth inhibition concentrations were 133 µg/ml and 110 µg/ml with and without metabolic activation, respectively. The concentrations for the short-term treatment method of the definitive assay were then selected as 40, 60, 80, 100, 120, 140, and 160 µg/ml irrespective of the presence or absence of S9 fraction. In the continuous treatment, the 50% growth inhibition concentrations were 86 µg/ml and 95 µg/ml at 24 and 48 hr, respectively. The concentrations for the continuous treatment of the definitive assay were then selected as 20, 40, 60, 80, 100, 120 and 140 µg/ml.

Test agent stability: The Sponsor mentioned that the test article was confirmed to be stable during the chromosomal aberration experiment period. The data was not submitted.

Metabolic activation system: S-9 fractions prepared from livers of Sprague-Dawley rats induced with phenobarbital (4 days, 30+60+60+60 mg/kg body weight) and 5,6-benzoflavone (1 day, 80 mg/kg body weight).

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: +S9 = cyclophosphamide (CP), -S9 = mitomycin C (MMC)

b(4)

Exposure conditions:

Short-term Treatment Method: Cells were exposed to SIS copolymer at the concentrations indicated above, an incubated for 6 hr in the presence and absence of S9 fraction. Following the 6 hr incubation, the cells were washed and incubated in fresh medium for an additional 18 hr. Two plates for each dose level were prepared for observation. Colcemid (0.2 µg/ml) was added to cells 2 hr prior to chromosome preparation.

Continuous Treatment Method: Cells were exposed to SIS copolymer at the concentrations indicated above, an incubated for 24 or 48 hr (no S9 fraction). Two plates for each dose level were prepared for observation. Colcemid (0.2 µg/ml) was added to cells, 2 hr prior to chromosome preparation.

Analysis: For each dose level, 200 (100/plate) well-spread metaphases were observed microscopically, and the types of structural aberrations and the number of cells with chromosomal aberrations were recorded. The number of polyploid cells (cells with 37 or more chromosomes including triploid cells) was also recorded.

Criteria for positive results:

Judging criteria

Ratio of cells with chromosomal aberrations	Judgement
< 5%	Negative
5% to <10%	False positive
≥ 10%	Positive

The incidence of cells with chromosomal structural aberrations was calculated both including and excluding gaps. Judgement was made on the basis of the incidence excluding gaps. The test article was regarded as positive if the incidence of cells with chromosomal aberrations was dose dependent or reproducible.

Summary of individual study findings:

Study validity: The experimental design was adequate according to the OECD Guideline 473: In vitro Mammalian Chromosome Aberration Test. The highest SIS copolymer concentration was selected based on a greater than 50% growth inhibition. The positive controls exhibited the appropriate responses.

Study outcome: There was neither an increase exceeding 5% nor a concentration-dependent increase in the incidence of cells with chromosomal structural aberrations in any group treated with SIS copolymer in the short-term treatment (± S9 fraction) or continuous treatment method.

Sponsor’s Conclusion: SIS block copolymer does not have chromosomal aberration inducibility under the conditions of this test.

Reviewer’s Comments: The reviewer concurs.

GENETOX STUDIES WITH ALICYCLIC SATURATED HYDROCARBON RESIN

b(4)

Study title: Microbial Metabolic Activation Test to Assess the Potential Mutagenic Effect of Resin A: _____ b(4)

Key findings:

- _____ was negative in the Ames tests at concentrations up to 5000 µg/plate.

Study no: _____ 3B/84749/2

Volume #, and page #: 8, 1745 b(4)

Conducting laboratory and location _____

Date of study initiation: July 17, 1984

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: _____, lot # A-42048, % purity was not specified. _____

_____ was dissolved in toluene at a concentration ten times that required and diluted in dimethylsulfoxide (DMSO).

Formulation/vehicle: DMSO

Methods:

Strains/species/cell line: *Salmonella typhimurium* TA100, TA98, TA1535, TA1537 and TA1538, and *Escherichia coli* WP2uvrA

Dose selection criteria: Dose range finding study using poured plate method ± S9. The dose levels tested were 5, 50, 500 and 5000 µg/plate and incubation carried out for 72 hr. Toxicity was observed at the highest dose. As this could be due to small amount of toluene present, the concentration of _____ was increased to 1.0 g/ml, from this dilutions were made using DMSO. The top dose for the first mutation test was also reduced to 1000 µg/plate. No toxicity was observed in the first test, therefore 5000 µg/plate was chosen as the top dose for the second mutation test. b(4)

Test agent stability: not specified

Metabolic activation system: S9 fractions prepared from livers of Sprague-Dawley rats induced with Aroclor 1254.

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: N-ethyl-N-nitro-N-nitrosoguanidine (ENNG), 2-nitrofluorene (NF), 9-aminoacridine (9-AC), 2-aminoanthracene (2-AA), and 4-nitro-o-phenylenediamine (NOPD) were selected based on the bacteria strain as indicated in the following table:

Tester Strains	Positive Control Article (ug/plate)	
	-S9	+S9
TA100	ENNG (2.0)	2-AA (0.5)
TA98	NF (2.0)	2-AA (0.5)
TA1535	ENNG (10.0)	2-AA (1.0)
TA1537	9-AC (10)	2-AA (1.0)
TA1538	NOPD (10.0)	2-AA (0.5)
WP2uvrA	ENNG (5.0)	2-AA (40.0)

Comments: The OECD/EPA Guidelines states: "2-AA should not be used as the sole indicator of the efficacy of the S9 mix. If 2-AA is used, each batch of S9 should be characterized with a mutagen that requires metabolic activation by microsomal enzymes, e.g., B[a]P or dimethylbenzanthracene."

Exposure conditions: The poured plate method was used in the initial and confirmatory mutation tests. The concentrations selected of _____ were 5, 10, 50, 100 500 and 1000 µg/plate (initial test) and 10, 50, 100, 500, 1000, and 5000 µg/plate (confirmatory test). The samples were prepared in the presence or absence of S9 fraction, histidine or tryptophan deficient agar was added and the mixture was overlaid in minimal agar plates. After incubation for 72 hr at 37°C, the bacteria were observed for growth inhibition and number of revertant colonies.

Analysis:

No. of replicates: triplicates

Counting method: Automated colony counter

Criteria for positive results: (1) A statistically significant dose-related increase in the number of revertant colonies was obtained in two separate experiments and (2) the increase in the number of revertant colonies was at least twice the solvent control values

Summary of individual study findings:

Study validity: The selection of the bacteria strain was adequate based upon the ICH S2A Guideline: Specific Aspects of Regulatory Genotoxicity Test for Pharmaceuticals. Dose selection was adequate based on maximum dose level of 5000 µg/plate specified in the guideline. However, it is not clear why the Sponsor reduced the maximum concentration _____ to 1000 µg/plate in the initial mutation test unless it was to minimize the potential contribution of toluene to the observed toxicity. The positive controls showed an adequate response, i.e., the number of revertant colonies was twice or more that of the solvent control for each bacterial strain.

Study outcome: The number of revertant colonies in the treatment groups was not more than twice that of the solvent control and no dose-dependent increase was observed irrespective of bacterial strains, concentration of test-article and the presence of metabolic activation.

Sponsor's Conclusion: _____ is considered negative in the Ames test.

Reviewer's comments: The reviewer has reservations regarding the use of 2-AA as the sole indicator for the efficacy of the S9 mix. Otherwise, the reviewer concurs.

Study title: Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *In Vitro* and Treated with Resin A: _____

Key finding:

- _____ induced a slight increase in chromosomal aberrations at the intermediate dose level in the presence of S9. This increase was statistically significant only when gaps were excluded.

Study no: — 4A/841081

Volume #, and page #: 8, 1768

Conducting laboratory and location: _____

Date of study initiation: September 17, 1984

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: _____, lot # A-42048, % purity was not specified. A test sample was prepared by eluting 750 mg _____ in 2 ml of each of three solvents: isopropyl alcohol, acetone and ethanol. The isopropyl alcohol and acetone eluates were removed and evaporated to dryness. These extracts were dissolved in the ethanol eluate.

Formulation/vehicle: Ethanol

Methods:

Strains/species/cell line: Chinese hamster ovary cell line (CHO-K₁BH₄)

Dose selection criteria: Based on a preliminary study in which growth inhibition was determined in cultures ± S9 fraction. Final concentration of test compound in both sets were 0.156, 0.313, 0.625, 1.25, 2.5, 5 and 10 µl eluate/ml. The cultures without S9 were incubated in the presence of the test compound for 20 hr. The cultures with S9 were incubated with the test compound for 2 hr, afterwards, the medium was replaced and cultures incubated for a further 18 hr. The mitotic index was used to measure cytotoxicity. No toxic effect was noted at the maximum dose (see Reviewer's Comments). Therefore, 10 µl eluate/ml was used as the highest concentration in the metaphase analysis.

Test agent stability: not specified

Metabolic activation system: S-9 fractions prepared from livers of Sprague-Dawley rats induced with Aroclor 1254 (5 days, 500 mg/kg body weight).

Controls:

Vehicle: Ethanol

Negative controls: sterile water

Positive controls: +S9 = 0.4 µg/ml cyclophosphamide (CP); -S9 = 290 µg/ml mitomycin C (MMC)

Exposure conditions: Cells were exposed to _____ at 1.0, 5.0, and 10.0 µl eluate/ml, and incubated as described above in the preliminary test. Colcemid (0.2 µg/ml) was added to cells, 2 hr prior to chromosome preparation.

Analysis:

No. of replicates:

Test article, positive control, and negative control = 2 cultures

Vehicle control = 4 cultures

Counting method: Metaphase spreads were identified using a magnification of x160 and examined at a magnification of x1000 using an oil immersion objective. Approximately 100 metaphase were examined and the number and type of chromosomal aberrations recorded.

b(4)

b(4)

b(4)

Criteria for positive results: The Sponsor did not specify the criteria used. The reviewer asked the Sponsor to provide this information. The Sponsor response was the following (Toxicology Information Amendment N-009):

“The following response was received from _____ center relative to the likely criteria for a positive response that would have been considered in 1985.

It is not possible to be definitive about the criteria to judge the results as positive in retrospect. However, some criteria for determining a positive result that were current in 1985 included:

- 1) A statistically significant dose-related increase in the number of structural chromosomal aberrations (OECD 473, 1983).
- 2) A reproducible and statistically significant positive result for at least one of the test points (OECD 473, 1983).
- 3) In the absence of a clear dose-response relationship, at least two consecutive doses giving a statistically significant increase above controls (Report of the UKEMS subcommittee on guidelines for mutagenicity testing, 1983).
- 4) In borderline situations, attaching greater significance to the observation of exchanges in treated cells than to a small numerical increase in gaps and breaks (Report of the UKEMS subcommittee on guidelines for mutagenicity testing, 1983).”

Summary of individual study findings:

Study validity: For the most part, the experimental design and dose selection were adequate according to the OECD Guideline 473: *In vitro* Mammalian Chromosome Aberration Test. The guideline recommends an incubation period of 3 - 6 hr both with and without metabolic activation. The Sponsor conducted the +S9 incubation for 2 hr. The positive control exhibited increases in chromosomal aberration number at least 8-fold higher than that in the solvent control.

Study outcome: _____ induced no increases in the proportion of metaphase spreads showing aberrant figures except for a slight increase at the intermediate dose level (5.0 µl eluate/ml) in the presence of S9 mix. This was statistically significant only when gap damage was excluded ($p < 0.05$, Fisher's Exact Test). In this case, the % mean number of aberrant cells were 1.25 and 4.25 for control and _____ respectively.

Sponsor's Conclusion: _____ showed no clear evidence of mutagenic potential in the chromosomal aberration assay.

Reviewer's Comments: The reviewer concurs with the Sponsor conclusion. In the dose ranging study, the Sponsor concluded that no toxicity was observed with _____. However, the mitotic index was lower in _____ treated cultures compared to the untreated control and, although to a lower extent, compared to the ethanol control. The decrease reached a plateau at low _____ concentrations and in some samples, it was close to 50% compared to the untreated control. The dose levels were given in µl eluate/plate, therefore the dose in weight units/ml was not provided.

Study title: A Micronucleus Test of Alicyclic Saturated Hydrocarbon Resin in Mice

b(4)

Key findings:

- _____ did not significantly increase the number of micronucleated PCE's at any dose.
- The Sponsor provided no evidence to support bone marrow exposure to _____.

Study no: 1068

b(4)

Volume #, and page #: 8, 1784

Conducting laboratory and location: _____

Date of study initiation: November 2, 2000

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: _____ lot # A98210, % purity was not specified

Formulation/vehicle: 2% (w/v) *Gummi arabicum pulveratum* (GAP)

Methods:

Strains/species/cell line: ddY/SPF male mice

Dose selection criteria:

Basis of dose selection: A dose-range finding study.

Range finding studies: Four oral doses of 250, 500, 1000 and 2000 mg/kg were selected and the proportion of micronuclei observed 24 and 48 hr after administration.

No toxic signs were observed and there was no apparent increase in the proportion of micronuclei. Therefore, the doses selected were 500, 1000 and 2000 mg/kg.

Test agent stability: Test article was suspended in 2% (w/v) GAP solution using a mortar. Suspensions of 10 and 100 mg/ml were stable for 10 days under refrigeration and protected from light.

Controls:

Vehicle: GAP (2%)

Negative controls: GAP (2%)

Positive controls: Mitomycin C (0.1 mg/ml)

Exposure conditions:

Incubation and sampling times: Bone marrow collection took place 24 hr after administration.

Doses used in definitive study: _____ = 0, 500, 1000 and 2000 mg/kg; mitomycin C = 1.0 mg/kg

Study design: Five groups (three _____ dose levels, one positive control, one vehicle control). Six males were used/group. Animals were administered a single oral dose of _____ or a single intraperitoneal injection of mitomycin C at the doses specified above. Animals were observed for clinical signs and body weight recorded.

b(4)

Analysis:

No. of replicates: 1 specimen from each animal

Counting method: The bone marrow smears were observed with a microscope at a magnification of x1000. The number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes and the number of polychromatic and normochromatic erythrocytes per 200 erythrocytes were counted.

Criteria for positive results: The Sponsor did not specify the criteria. The reviewer asked the Sponsor to provide the information. The Sponsor response was the following (Toxicology Information Amendment N-009):

“We analyzed the differences in the incidence of MNPCE between the treated group and the negative control group using Kastenbaum-Bowman’s table based on binomial distribution. In addition, we examine dose-dependency in the evidence of MNPCE using Cochran-Armitage’s trend test. Based on the results of these analyses, we judge that the test article is positive (1) if both analysis revealed statistical significance and the incidence of MNPCE was clearly higher than the negative control background data or (2) if Kastenbaum-Bowman’s test revealed statistical significance at the highest dose, the results were reproducible and the incidence of MNPCE was clearly higher than the negative control background data.

Summary of individual study findings:

Study validity: The Sponsor did not submit any evidence to support that _____ reached the target tissue (bone marrow) after oral administration. This compromise the validity of the negative results obtained, even though the positive control showed an increase in the proportion of MNPCE compared to the vehicle control group. b(4)

Study outcome: No abnormalities were observed in clinical signs and body weight. There was no significant difference in the proportion of PCE per 200 erythrocytes or in the proportion of MNPCE per 2000 PCE between the vehicle control group and any dosing group. The proportion of MCPCE in the vehicle and positive control groups were 0.13% and 2.23%, respectively.

Sponsor’s Conclusion: _____ was evaluated as negative in this micronucleus assay. b(4)

Reviewer’s Comments: The experimental design was deficient in that there is no evidence to show that _____ reached the target tissue (bone marrow). Therefore, it is questionable to conclude that _____ is negative in this test. The Sponsor has indicated that the assessment of systemic exposure to the excipients was technically difficult (Toxicology Information Amendment N-010). Refer to Section IX of current review for details.

Genetic toxicology summary: In the Ames test, SIS copolymer showed no mutagenic potential at concentrations up to 5000 µg/plate. However, the test article precipitated at all concentrations used in the final assay and therefore, the results are questionable. SIS copolymer was also evaluated in the chromosomal aberration assay using CHL cells. SIS copolymer did not induce chromosomal aberrations even at concentrations causing 50% cell growth inhibition. _____ was negative in the Ames test at concentrations up to 5000 µg/plate. No problems with precipitation of the test-article were reported in this case. _____ was evaluated in the chromosomal aberration test using CHO cells. An increase in chromosomal aberrations was observed at the 5.0 µl eluate/ml dose level. This was statistically significant only when gaps were excluded. A micronucleus test was also performed with _____. The results showed that _____ did not increase the number of PCE but the results are questionable due to the b(4)

lack of proof of bone marrow exposure. The Sponsor indicated that he could not perform a micronucleus assay with SIS block copolymer because it was not feasible to prepare a suspension for oral administration to mice (Toxicology Information Amendment N-010).

Genetic toxicology conclusions: Both excipients did not show a major potential to be genotoxic in the studies presented. For reasons described above, a negative judgement for the following two studies is questionable: (1) Ames test for SIS copolymer, and (2) Micronucleus assay for

b(4)

Labeling recommendations: Not applicable.

VI. CARCINOGENICITY:

Studies were not required at this time.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Studies were not required at this time.

VIII. SPECIAL TOXICOLOGY STUDIES:

None was submitted.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: The preclinical studies conducted with the FS 67A patch support that the Phase I protocols proposed may be safe. The maximum duration of application of the patch product in humans supported by the preclinical studies is 14 days. In spite of the deficiencies found in the studies conducted to evaluate the systemic toxicity of two excipients, the studies do support that both excipients may have low potential to cause dermal or systemic toxicity after topical application to humans. The Sponsor did not properly determine whether FS 67A may cause skin photoirritation or photosensitization. This issue might not be relevant if the patch is opaque to light or the product has no absorbance in the 280 – 700 nm region of the solar spectrum.

General Toxicology Issues: The safe use and potential risks associated with the use of methyl salicylate and menthol are well established due to the long history of use of both drugs. The preclinical studies conducted in this IND demonstrated that the new patch formulation may cause “slight” skin irritation after a single exposure or a 14-day continuous exposure. FS 67A did not cause skin photoirritation and photosensitization. However, the studies did not mimic the solar spectrum as they were conducted with only UVA light exposure. Whether FS 67A may cause skin photoirritation or photosensitization after exposure to UVB or Visible light is not known. This issue might not be relevant if the patch is opaque to light or the product has no absorbance in the 280 – 700 nm region of the solar spectrum.

The two excipients analyzed, SIS block copolymer and _____, did not show evidence of having significant skin toxicity. Studies were also conducted to address the potential systemic toxicity of these excipients after dermal application or oral administration. The findings were consistent with both excipients having low potential to produce systemic toxicity; the HEDs

b(4)

used were at least 100 fold higher than the expected dose if only one patch is used. However, the Sponsor did not provide any PK or TK data to demonstrate systemic exposure to these polymers (or contaminating monomers).

In Toxicology Information Amendment N-010, the Sponsor addressed the Agency request to provide evidence of systemic absorption of both excipients in the studies conducted to evaluate the potential for systemic toxicity after dermal or oral administration. The Sponsor stated that concomitant toxicokinetic studies could not be performed for reasons related to the chemical structures of these excipients as well as limitations associated with the bioanalytical methods. These polymers are composed of a wide range of molecules with weights ranging from 100,000 – 200,000 and 1200 – 1400 for SIS block copolymer and _____, respectively. The Sponsor determined gel permeation chromatography (GPC) was the only method suitable to measure these polymers because a specific analyte could not be identified. b(4)

The Sponsor developed a GPC method to analyze SIS and _____ polymers in tetrahydrofuran (not in biological matrix). However, they decided that GPC method was not appropriate to perform TK analysis due to the following reasons:

- The lower limits of quantitation (LOQ) were estimated to be around 0.25 and 0.13 mg/mL for SIS and _____, respectively.
 - The Sponsor concluded that the LOQs would not provide enough sensitivity for TK analysis because low absorbability of the excipients was expected in the animals (due to the high molecular weight of the excipients).
 - Endogenous proteins with similar molecular weights affected the specificity of the separation procedure.
- b(4)

Therefore, determination of systemic exposure appeared technically difficult. Due to the high molecular weight of the polymers and the fact that polymers are usually inert themselves, the use of these polymers topically may not pose a major toxicological risk. The risk could become significant if the monomers or impurities resulting during the manufacturing process are not kept within acceptable levels.

A series of genetic toxicology studies were also conducted with both excipients. In general, the assays did not show the excipients have a strong genetic toxicity risk. Limitations were found in two of the studies: Ames test for SIS copolymer, and *in vivo* micronucleus assay for _____. Technical difficulties were encountered with solubility or with measuring systemic exposure, respectively. Taking into consideration the topical route intended for administration into humans, and the high molecular weight of these polymers, it is not expected that these excipients *per se* may pose a genotoxic risk. As indicated above for systemic toxicity, levels of contaminating monomers above regulatory standards may pose a risk. b(4)

Recommendations:

Internal comments:

The Sponsor did not submit the individual animal listings (data) for several studies conducted with the two excipients, styrene-isoprene-styrene block copolymer and _____ . The reviewer wrote a Memorandum requesting this information as well as other information required to complete an adequate Pharm/Tox review (see Appendix). The Sponsor response was received on 9-24-01 (Toxicology Information Amendment N-009).

b(4)

The animal listings are no longer available for the following two studies (both were conducted in 1980):

- The acute Toxicity Test (_____)
- Allergenic Study (_____)

b(4)

The Sponsor submitted the signed Quality Assurance and GLP statements for all studies except for the following six studies:

- A Primary Skin Irritation Study of Styrene-Isoprene-Styrene Block Copolymer in Rabbits
- A Skin Phototoxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Guinea Pigs
- The acute Toxicity Test (_____)
- The Primary Skin Irritation Test (_____)
- The Cumulative Skin Irritation (3 Weeks) Test (_____)
- Allergenic Study (_____)

b(4)

The Sponsor indicated that these studies were completed in 1980, and predate GLP requirements in Japan.

External recommendations (to sponsor):

The Sponsor should ensure the completeness of the study reports before submitting the IND. The data should also be submitted accurately and in a clear format. Several tables were not easy to interpret, the data values were missing, or categories mislabeled.

The major issue with the safety of the excipients is the possible contamination with monomers and impurities resulting from the manufacturing procedure. The Sponsor should specify the purity of SIS copolymer and _____ used for the clinical studies. Data should be provided to confirm that the levels of potentially toxic monomers are kept within regulatory standards.

b(4)

Labeling with basis for findings: not applicable

Reviewer signature: _____

Maria I. Rivera, Ph.D.

Supervisor signature: **Concurrence -** _____
Robert E. Osterberg, Ph.D.

Non-Concurrence - _____
(see memo attached)

cc: list:

- IND 62,735 Division File
- Robert E. Osterberg/TL/HFD-550
- Jane Dean/PM/HFD-550
- Christina Fang/MO/HFD/550

X. APPENDIX/ATTACHMENTS:

Addendum to review: none

Other relevant materials (appended consults, etc.):

Draft letter content for sponsor (dated Aug-22-01):

The following information is needed for an adequate Pharm/Tox safety review. Please, provide the individual animal listings (data) for the following studies:

- a) A 4-Week Percutaneous Toxicity Study of Styrene-Isoprene-Styrene Block Copolymer in Rats with a Recovery Period of 4 Weeks
- b) The Acute Toxicity Test o. _____
- c) An Oral Single Dose Toxicity Study of Alicyclic Saturated Hydrocarbon Resin in Beagle Dogs
- d) Allergenic Study _____
- e) A Micronucleus Test of Alicyclic Saturated Hydrocarbon Resin in Mice

b(4)

Please, provide the information specified under each title for the following studies:

- a) A Primary Eye Irritation Study of Alicyclic Saturated Hydrocarbon Resin in Rabbits
Please, submit the photographs referred to in the study.
- b) A Skin Phototoxicity Study of Alicyclic Saturated Hydrocarbon Resin in Guinea Pigs
Please, submit the photograph referred to in the study.
- c) A Reverse Mutation Test of Styrene-Isoprene-Styrene Block Copolymer Using Bacteria, and Microbial Metabolic Activation Test to Assess the Potential Mutagenic Effect of Resin A-

b(4)

Specify the rationale for use of 2-aminoanthracene to indicate test efficacy in the presence of S9 fraction. A positive control should be characterized with a mutagen that requires metabolic activation by S9 microsomal enzymes.

- d) Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *In Vitro* and Treated with Resin A: _____

Submit criteria for positive results.

- e) A Micronucleus Test of Alicyclic Saturated Hydrocarbon Resin in Mice

Submit criteria for positive results.

There is also a concern regarding whether the test-article reached the bone marrow. Please, provide evidence to support exposure of the target tissue to _____

b(4)

In the studies conducted to evaluate the potential for systemic toxicity of SIS block copolymer or _____ after oral administration or dermal application, no pharmacokinetic data was submitted. Please, provide evidence to support whether there is systemic absorption of these excipients by the exposure routes used in the studies.

The Sponsor should also submit the signed Quality Assurance and Good Laboratory Practice Statement within 120 days from the submission date for all studies except for the following:

- a) Microbial Metabolic Activation Test to Assess the Potential Mutagenic Effect of Resin A- _____

- b) Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *In Vitro* and Treated with Resin A: _____

b(4)

Please call if you have any questions.

Any compliance issues: none

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/s/

Maria I. Rivera
1/17/02 09:17:13 AM
PHARMACOLOGIST

Robert Osterberg
1/23/02 02:59:42 PM
PHARMACOLOGIST

APPENDIX/ATTACHMENT 2:

Dr. María I. Rivera's Review of IND 62,735 (N038)

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PHARMACOLOGY/TOXICOLOGY REVIEW

IND number: 62735

Sequence number/date/type of submission: N-038/8-3-05/Toxicology Information Amendment

Information to Sponsor: Yes

Sponsor and/or agent: Hisamitsu Pharmaceutical Co., Inc.

Reviewer name: Maria I. Rivera, PhD

Division name: Anesthesia, Analgesia, and Rheumatology Drug Products

HFD #: 550

Review completion date: August 31, 2005

Drug: FS 67A topical patch (10% Methyl Salicylate/3% *l*-Menthol)

Background: The FS-67 patch contains _____ in the patch backing cloth not previously used in US marketed drug products.

b(4)

In the Pre-NDA meeting held on July 9, 2002, the Division recommended conducting genotoxicity studies _____. In response to Amendment # 21 (Oct 3, 2002), the Division further clarified that the full battery of genotoxicity studies should be performed. In the current amendment, Hisamitsu submitted the results of the genotoxicity studies. Hisamitsu is seeking the FDA's response to the following question:

Does the FDA agree that this mutagenicity program is adequate to support the NDA and that there is little, if any, risk to humans using FS-67, since the Ames test on the extract of FS-67 backing cloth showed to be negative and no _____ was detected in the DMSO extract of a FS-67 backing cloth?

b(4)

Studies submitted: The sponsor did not submit the study reports _____ under this IND. These study reports were submitted under IND _____ also being developed by Hisamitsu. The sponsor herein submitted the following final reports of genotoxicity studies conducted with _____ an additional Ames test where the mutagenic potential of a DMSO extract of the backing cloth was evaluated.

Study #	Title
19-37	A Bacterial Reverse Mutation Test
68-63	A Gene Mutation Assay in Mouse Lymphoma Cells
19-38	A Chromosomal Aberration Test in Cultured Mammalian Cells
19-39	A Micronucleus Test in Rat Bone Marrow Cells
68-62	A Bacterial Reverse Mutation Test of FS-67 Backing Cloth

b(4)

Among the studies listed above, those with negative results are briefly summarized below. All these studies were reviewed and found valid according to standard criteria.

Assay	Design	Main Findings
<p>Chromosomal aberration</p> <p>Drug:</p> <p>Lot #: 03Z4232246 Purity: 97.4%</p>	<p>Cells: CHL/IU fibroblastic cell line</p> <p>Incubations and sampling times: 6 hr (\pm S9), 24 hr (-S9), and 48 hr (-S9), duplicates/dose</p> <p>Doses (definitive study): 313, 625, 1250, 2500, and 5000 μg/ml</p>	<p>Negative</p> <p>A minimal increase in the number of chromosomal aberrations was noted at 6 hr (5000 μg/ml) and 48 hr (1250 μg/ml). The mean values (n=2) were:</p> <p>6 hr (-S9): 4 vs 0.5% SA^a in control 1.5 vs 0.5% NA^b in control</p> <p>6 hr (+S9): 2 vs 0.5% SA^a in control 2.5 vs 1% NA^b in control</p> <p>48 hr: 1.5 vs 0.5% SA^a in control 3.5 vs 0.5% NA^b in control</p> <p>At these doses, cytotoxicity (cell proliferation ratio) was decreased to 58.7% (6 hr, - S9), 69.6% (6 hr, + S9), and 66.1% (48 hr).</p> <p>These minimal increases (<5%) were not considered toxicologically significant.</p>
<p>Micronucleus test</p> <p>Drug:</p> <p>Lot #: 03Z4232246 Purity: 97.4%</p>	<p>Species/Strain: Rat/Crj:CD(SD)IGS</p> <p>n = 6 males/dose</p> <p>Doses: 0 (vehicle), 500, 1000, and 2000 mg/kg, single dose by ip injection in a volume of 10 ml/kg</p> <p>Sampling times: 24 hr postdose</p>	<p>Negative</p> <p>Purple urine and stool at all test-article doses; decreased body weight gain by 5.5 g and 6 g in the 500 and 1000 mg/kg compared to control; body weight loss of 4.2 g at 2000 mg/kg compared to a gain of 6 g in controls</p> <p>Mean IE%^c was 51% in the negative control, 50.6% at 500 mg/kg, 49.3% at 1000 mg/kg, and 47.6% at 2000 mg/kg; only the decrease at 2000 mg/kg was significant (p\leq 0.01).</p> <p>TK was not performed but the clinical signs support the systemic distribution of the test article and the slight decrease in the mean IE% support bone marrow exposure to the test-article at the high dose. However, it must be noted that the IE% was still within the negative control historical range (49.998 \pm 9.994%; mean \pm 3SD).</p>

b(4)

b(4)

Assay	Design	Main Findings
Ames test – backing cloth extract Drug: FS-67 backing cloth Lot #: 48062	<p>Cells: <i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537 and <i>E. coli</i> WP2 <i>uvrA</i></p> <p>Doses (definitive study): 3.13, 6.25, 12.5, 25, 50, and 100% extract/plate</p> <p>Incubations and sampling times: pre-incubation method ± S9 in both the dose-finding and main test, duplicate samples, 48 hr.</p>	<p>Negative</p> <p>No test article precipitation or bacteria growth inhibition was noted at any dose.</p> <p>Study flaw: The components of the extract were not quantitated. Therefore, it is not known whether any — was eluded into the extract.¹</p>

b(4)

^aSA = structural aberrations; ^bNA = numerical aberrations; ^cIE% = ratio of immature erythrocytes (IE) to total erythrocytes

The studies with positive results are summarized in more detail below.

Study title: A Bacterial Reverse Mutation Test

Key findings: Under the conditions of this study, — induced gene mutation in incubations performed with and without metabolic activation; the response was considered as weak but this may reflect little intracellular access after test-article precipitation.

b(4)

Study no.: — 19-37

Volume: 1

Conducting laboratory and location: —

Date of study initiation: Nov. 8, 2004

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: —

—, lot # 03Z4232246, % pure = 97.4%

b(4)

Methods

Strains/species/cell line: *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvrA*

Doses used in definitive study:

A) Main Study

¹ In the amendment summary, under the tab “Overview of Genotoxicity Studies”, the sponsor stated that — was not detected in the DMSO extract (See Evaluation Section at the end of this review).

b(4)

Strain	Doses	
	+S9	-S9
TA98	4.88, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate	2.44, 4.88, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250, and 2500 µg/plate
TA 100	39.1, 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate	156, 313, 625, 1250, 2500, and 5000 µg/plate
TA1535	156, 313, 625, 1250, 2500, and 5000 µg/plate	156, 313, 625, 1250, 2500, and 5000 µg/plate
TA1537	1.22, 2.44, 4.88, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, and 1250 µg/plate	1.22, 2.44, 4.88, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, and 1250 µg/plate
WP2uvrA	156, 313, 625, 1250, 2500, and 5000 µg/plate	156, 313, 625, 1250, 2500, and 5000 µg/plate

B) Confirmation Study (no S9)

Strain	Doses
TA 100	156, 313, 625, 1250, 2500, and 5000 µg/plate
WP2uvrA	156, 313, 625, 1250, 2500, and 5000 µg/plate

Basis of dose selection: It was based on the results of a dose-finding study; doses of 5, 15, 50, 150, 1500, and 5000 µg/ml were used in all 5 strains. The number of revertant colonies was increased at least 2x that of the negative control in TA98, TA100, TA1537, and WP2uvrA with metabolic activation and in TA98 and TA1537 without metabolic activation.

Negative controls: DMSO vehicle

Positive controls:

Tester strain	S9 mix	Positive Control	µg/plate
TA98	+	2-Aminoanthracene	0.5
	-	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.1
TA100	+	2-Aminoanthracene	1
	-	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.1
TA1535	+	2-Aminoanthracene	2
	-	Sodium azide	0.5
TA1537	+	2-Aminoanthracene	2
	-	2-Aminoacridine HCl hydrate	80
WP2uvrA	+	2-Aminoanthracene	10
	-	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.02

Incubation and sampling times: The pre-incubation method ± S9 was used in both the dose-finding and main test. The confirmation test was performed without S9 using the pre-incubation method. Duplicate samples were incubated for 48 hr.

Results

Study validity: The study was found valid according to standard criteria. However, the criteria for a positive result was considered as ≥ 2x that of the vehicle control for all strains; usually an increase of ≥ 3x the vehicle control defines a positive control in TA1535 and TA1537.

Study outcome:

Main study: Test article precipitation was observed at ≥ 78.1 µg/plate with metabolic activation and at 9.77 µg/plate without metabolic activation. Growth inhibition was not observed at any

dose in any strain. The number of revertant colonies was increased at least 2x in TA98, TA100, TA1537, and WP2uvrA with S9 and TA98, TA1537, and WP2uvrA without S9. The positive controls showed increases of 5-63x.

Considering the historical control range for the number of revertant colonies, the response was considered positive at the following doses:

Strain	Control range	Actual control	Number of revertants at the dose a which a positive response occurred	Control range	Actual control	Number of revertants at the dose a which a positive response occurred
	+S9			-S9		
TA100	73-140	93	180 at 5000 µg/plate	71-152	107	174-245 at 39.1-5000 µg/plate
WP2uvrA	11-40	21	49 at 5000 µg/plate	11-40	21	101 at 5000 µg/plate
TA98	10-30	23	51 at 2500 µg/plate	13-42	30	43-90 at 9.77-5000 µg/plate
TA1537	2-16	8	22-79 at 9.77-1250 µg/plate	3-19	11	37-62 at 19.5-1250 µg/plate

Confirmation study: Test article precipitation was observed at ≥ 156 µg/plate and growth inhibition was not observed at any dose in any strain. The number of revertant colonies was increased at least 2x in WP2uvrA. In TA100, the number of revertant colonies was increased but only up to ~1.75x.

Sponsor's Conclusions: Under the conditions of this study, [redacted] induces gene mutation in tests performed with and without metabolic activation. b(4)

Reviewer's Comments: The sponsor noted that the weak increase in the number of revertant colonies could be attributed to the fact that [redacted] is an anthraquinone. On the other hand, the sponsor also noted that it was difficult to judge if the [redacted] induced gene mutation in bacteria because an accurate dose-response curve was not obtained, other than that for a slow increase in the number or revertant colonies in regions of precipitation at the higher doses. The reviewer agreed that the response was weak and that there was not a clear dose-response. The precipitation of the test-article, observed even at low concentrations, may explain the lack of the dose response. Also, due to precipitation of the [redacted] it is not known how much [redacted] reached the inside of the cell. Therefore, the [redacted] was considered mutagenic in this assay. b(4)

Study title: A Gene Mutation Assay of [redacted] in Mouse Lymphoma Cells

Key findings: The [redacted] was positive in this assay. The maximum total mutation frequency (TMF) was 871.7×10^{-6} , 724.5×10^{-6} , and 495.7×10^{-6} in the 3-hr treatment without S9, 3-hr treatment with S9, and 24-hr treatment without S9, respectively.

Study no.: [redacted] 68-63

Volume: 1 b(4)

Conducting laboratory and location: [redacted]

Date of study initiation: April 18, 2005

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: _____
lot # 03Z4232246, % pure = 97.4%

b(4)

Methods

Strains/species/cell line: L5178Y (tk^{+/−} 3.7.2c) mouse lymphoma cells

Doses used in definitive study:

3 hr treatment (±S9): 0, 0.152, 0.457, 1.37, 4.12, 12.3, 37.0, 111, 333, 1000, and 3000 µg/ml

24 hr treatment (-S9): 0, 0.00188, 0.00565, 0.0169, 0.0508, 0.152, 0.457, 1.37, 4.12, 12.3, and 37.0 µg/ml

Basis of dose selection: Based on the results of a dose-finding test. The dose at which 80% cytotoxicity (RS=20%) was estimated to be ≥ 3000 µg/ml in the 3-hr treatment without S9 and 2873 µg/ml in the 3-hr treatment with S9. In the 24-hr treatment, ≥ 80% cytotoxicity was observed at all doses. Test-article precipitation was noted at ≥ 23.7 µg/ml.

Negative controls: DMSO vehicle

Positive controls: Methyl methanesulfonate (MMS) and cyclophosphamide monohydrate (CP)

3-hr treatment (-S9): 0.1 mg/ml MMS

3-hr treatment (+S9): 0.03 mg/ml CP

24-hr treatment (-S9): 0.1 mg/ml MMS

Incubation and sampling times: Samples were incubated for 3 (± S9) or 24 hr (-S9) with test-article. After removal of the control or test-article, the plates were incubated for 10 or 11 days in the 24-hr continuous treatment or 3-hr treatment, respectively. One plate was used at each dose.

Results

Study validity: The study was found valid according to standard criteria. The positive and negative controls showed the expected responses.

Study outcome: The _____ was positive in this assay. The maximum total mutation frequency (TMF) of the _____ in the 3-hr treatment without S9 was 871.7×10^{-6} in comparison to 82.7×10^{-6} in the negative control. The maximum TMF in the 3-hr treatment with S9 was 724.5×10^{-6} in comparison to 120.3×10^{-6} in the negative control. The maximum TMF in the 24-hour continuous treatment without S9 was 495.7×10^{-6} in comparison to 78.7×10^{-6} in the negative control. The data is shown in the table below. Test article precipitation was noted at ≥ 37.0 µg/ml. Cytotoxicity was apparent as dose-dependent reduction in plating efficiency and relative total growth. In general, a decrease close to or ≥ 50 % in both parameters was observed at ≥ 37 µg/ml in the 3 hr treatments (± S9) and at 4.12 µg/ml in the 24-hr continuous treatment.

b(4)

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Treatment	Test and Control Articles	Dose (µg/ml)	Total Positive Well No.				Total Well No.	Negative Well No.	PE2 (%)	Mutation Frequency(×10 ⁶)			%SC
			L	S	L/S	Total				L-MF	S-MF	T-MF	
b(4) -S9 mix 3 h	Vehicle	0	59	14	0	73	384	311	127	65.5	14.6	82.7	17.6
		0.152	26	6	0	32	192	160	120	60.5	13.2	75.8	17.4
		0.457	33	11	1	45	192	147	155	62.7	20.8	86.0	24.2
		1.37	29	17	2	48	192	144	120	73.2	43.3	119.5	36.2
		4.12	45	29	4	78	192	114	130	113.4	72.6	200.6	36.2
		12.3	70	37	4	111	192	81	112	217.4	107.3	385.3	27.8
		37.0	63	39	12	134	192	58	69	360.7	336.2	871.7	38.6
		111	6	5	1	12	192	180	1	1411.3	1206.4	2452.4	49.2
		333	0	1	0	1	192	191	1	0.0	399.0	399.0	100.0
		1000	0	0	0	0	192	192	1	0.0	0.0	0.0	0.0
		3000	25	29	3	57	192	135	8	1036.2	1198.5	2315.4	51.8
	PC (MMS)	10	51	43	4	98	192	94	130	129.8	108.0	274.8	39.3
b(4) +S9 mix 3 h	Vehicle	0	71	23	1	95	384	289	118	87.9	27.3	120.3	22.7
		0.152	28	6	3	37	192	155	105	84.2	22.9	102.3	22.4
		0.457	26	12	1	39	192	153	112	67.7	31.3	101.4	30.9
		1.37	16	15	1	32	192	160	135	34.2	32.1	67.3	47.7
		4.12	15	23	3	41	192	151	242	20.3	30.1	49.6	60.6
		12.3	47	49	4	100	192	92	116	133.1	139.2	317.1	43.9
		37.0	51	59	9	119	192	73	67	280.7	327.5	724.5	45.2
		111	6	8	0	14	192	178	2	800.0	1072.4	1907.8	56.2
		333	2	1	0	3	192	189	1	800.0	399.0	1203.2	33.2
		1000	0	0	0	0	192	192	2	0.0	0.0	0.0	0.0
		3000	14	34	1	49	192	143	5	747.9	1850.3	2709.1	68.3
	PC (CP)	3	34	75	6	115	192	77	58	201.7	473.0	788.7	60.0
b(4) -S9 mix 24 h	Vehicle	0	44	25	2	71	384	313	130	49.1	28.0	78.7	35.7
		0.00188	21	11	2	34	192	158	135	47.1	25.9	72.0	36.0
		0.00565	33	15	0	48	192	144	135	69.6	30.0	106.2	28.3
		0.0169	35	7	0	42	192	150	108	93.0	17.2	114.1	15.0
		0.0508	28	8	1	37	192	155	135	60.5	17.7	79.0	22.4
		0.152	39	13	1	53	192	139	155	75.2	24.4	104.0	23.4
		0.457	30	17	0	47	192	145	141	60.1	32.8	99.3	33.0
		1.37	45	9	1	55	192	137	120	113.8	22.2	140.2	15.8
		4.12	26	21	0	47	192	145	36	202.3	161.1	390.4	41.3
		12.3	24	25	1	50	192	142	30	229.2	239.1	495.7	48.2
		37.0	12	13	0	25	192	167	5	593.4	644.6	1282.6	50.3
	PC (MMS)	10	65	66	11	142	192	50	84	299.6	304.8	800.0	38.1

Vehicle: Dimethyl sulfoxide

PC: Positive Control, MMS: Methyl methanesulfonate, CP: Cyclophosphamide monohydrate

L: Well nos. containing ≥ 1 large colonies, S: Well nos. containing ≥ 1 small colonies, L/S: Well nos. containing ≥ 1 both large and small colonies

PE: Plating Efficiency

%SC: Mutation Frequency in small colonies

Sponsor's Conclusions: Under the conditions of this study, [redacted] induced gene mutation in mammalian cells, regardless of the presence or absence of a metabolic activation system or treatment length.

b(4)

Reviewer's Comments: Concur.

Evaluation of Study Reports submitted under [redacted] In the current amendment, the sponsor stated that based on the results of the Ames test, *in vitro* chromosomal aberration assay in CHL cells, and micronucleus assay in rats, there was no genotoxic potential for [redacted]. The [redacted] was negative in the full battery. The [redacted] showed ambiguous results in the chromosomal aberration assay; although the [redacted] did not induce structural aberrations, it increased the frequency of numerical aberrations (polyploid cells). This study is presented in more detail below. The negative studies are briefly summarized in the following table.

b(4)

Assay	Design	Main Findings
<p>Ames test Study #: [redacted] 9-29</p> <p>Drug: [redacted]</p> <p>Lot #: 205334 Purity: 99.97%</p>	<p>Cells: <i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537, and <i>E. coli</i> WP2uvrA</p> <p>Doses (definitive study): 156, 313, 625, 1250, and 5000 µg/plate</p> <p>Incubations and sampling times: pre-incubation method ± S9 in both the dose-finding and main test, duplicate samples, 48 hr.</p>	<p>Negative</p> <p>Test article precipitation was observed at all doses (even at ≥ 5 µg/plate in the dose-finding test).</p> <p>Bacterial growth inhibition was not noted at doses up to 5000 µg/plate.</p>
<p>Micronucleus test Study #: [redacted] 9-31</p> <p>Drug: [redacted]</p> <p>Lot #: 205334 Purity: 99.97%</p>	<p>Species/Strain: Rat/Crj:CD(SD)IGS</p> <p>n = 6 males/dose</p> <p>Doses: 0 (vehicle), 500, 1000, and 2000 mg/kg/day, ip 2x at 24 hr intervals, 10 ml/kg</p> <p>Sampling times: 48 hr after the administration of first dose</p>	<p>Negative</p> <p>Fluorescent yellow urine and stool at all test-article doses; orange and mucous stool at ≥ 1000 mg/kg; decreased body weight gain by 8-9.7, 12-15, and 13 g at 500, 1000, and 2000 mg/kg compared to a control gain of 2.3-12 g during days 1 and 2</p> <p>Mean IE%^a was 51% in the negative control, 48% at 500 mg/kg, 46% at 1000 mg/kg, and 42% at 2000 mg/kg; the decrease at ≥ 1000 mg/kg/day was significant (p ≤ 0.01).</p> <p>TK was not performed but the clinical signs support the systemic distribution of the test article and the slight decrease in the mean IE% support bone marrow exposure to the test-article. However, it must be noted that the IE% was still within the negative control historical range (49.853 ± 9.330%; mean ± 3 SD%).</p>

b(4)

b(4)

Assay	Design	Main Findings
		and close to background values, they were not considered toxicologically significant.
Micronucleus test Study #: — 19-35 Drug ————— Lot #: 211806 Purity: 99.88%	Species/Strain: Rat/Crj:CD(SD)IGS n = 3 males/dose Doses: 0 (vehicle), 500, 1000, and 2000 mg/kg/day, ip 2x at 24 hr intervals, 10 ml/kg Sampling times: 48 hr after the administration of first dose	Negative Watery peach to peach urine at all test-article doses; decreased body weight gain by 2.5-2.8, 0.5-8.2, and 3.8-11 g at 500, 1000, and 2000 mg/kg compared to a control gain of 5.8-9.5 g during days 1 and 2 Mean IE% ^a was 50.8% in the negative control, 49.3% at 500 mg/kg, 49.1 % at 1000 mg/kg, and 47.6% at 2000 mg/kg; no value was significantly different compared to control. TK was not performed but the clinical signs support the systemic distribution of the test article.

b(4)

^aIE% = ratio of immature erythrocytes (IE) to total erythrocytes; ^bSA = structural aberrations; ^cNA = numerical aberrations

Study title: A Chromosomal Aberration Test of _____ in Cultured Mammalian Cells

b(4)

Key findings: _____ did not induce structural aberrations but increased the frequency of numerical aberrations (polyploid cells) in CHL/IU cells.

Study no.: — 19-30

Volume #: _____

Conducting laboratory and location: _____

Date of study initiation: August 4, 2003

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: _____ lot # 205334, 99.97% pure

b(4)

Methods

Strains/species/cell line: Chinese lung hamster fibroblastic cell line (CHL/IU)

Doses used in definitive study:

6 hr (-S9) and 48 hr (-S9) = 90, 181, 362, 723, and 1447 µg/ml

6 hr (+S9) and 24 hr (-S9) = 181, 362, 723, 1447, and 2893 µg/ml

Basis of dose selection: It was based on the results of a dose-finding test at doses of 3.97, 11.9, 35.7, 107, 321, 964, and 2893 µg/ml for all treatment conditions. Test article precipitation was

observed at ≥ 35.7 $\mu\text{g/ml}$. The 50% growth inhibition was estimated to be 1405 $\mu\text{g/ml}$ for 6 hr (-S9) treatment, ≥ 2893 $\mu\text{g/ml}$ for 6 hr (+S9) and 24-hr continuous treatment, and 1143 $\mu\text{g/ml}$ for 48-hr continuous treatment.

Negative controls: Vehicle (DMSO)

Positive controls: 6 hr (-S9) = mitomycin C, 1.65 $\mu\text{g/ml}$; 6 hr (+S9) = benzo(a)pyrene, 4 mg/ml; 24 or 48 hr = mitomycin C, 0.55 $\mu\text{g/ml}$

Incubation and sampling times: 6 hr (\pm S9) and 24 and 48 hr continuous treatment without S9

Results

Study validity: The study was found valid according to regulatory criteria. However, there were some deviations from the standard protocol:

- (1) The pH was checked but not the osmolality.
- (2) The results were not analyzed statistically but judged using Ishidate's method. The judgment criteria was:
 - Negative: frequency $< 5\%$
 - Equivocal: frequency between 5 and $<10\%$
 - Positive: frequency $\geq 10\%$

The positive and negative control values were within the conducting laboratory historical range.

Study outcome: There was no increase in the frequency of structural aberrations at any dose or treatment condition. The frequency of numerical aberrations (polyploidy) was increased as indicated below. The values are the mean frequency (n=2) at 0, 90, 181, 362, 723, and 1447 $\mu\text{g/ml}$ for 6 hr (-S9) and 48 hr (-S9) and 0, 181, 362, 723, 1447, and 2893 $\mu\text{g/ml}$ for 6 hr (+S9) and 24 hr (-S9), respectively.

6 hr (-S9) = 0.5, 2.5, 5.0, 7.5, and 10.5% (not determined at 1447 $\mu\text{g/ml}$ due to toxicity)

6 hr (+S9) = 1.0, 2.5, 4.5, 7.5, 9.5, and 12.5%

24 hr (-S9) = 0.5, 1.0, 6.0, and 13.0% (not determined at ≥ 1447 $\mu\text{g/ml}$ due to toxicity)

48 hr (-S9) = 0.5, 14.0, 9.0, 7.5 and 7.5% (not determined at 1447 $\mu\text{g/ml}$ due to toxicity)

In the dose-finding test, dose-dependent cytotoxicity was noted at ≥ 964 $\mu\text{g/ml}$ in the 6 hr (\pm S9) and 24 hr treatments (cell proliferation ratios of 63-75.5% at 964 $\mu\text{g/ml}$) and at ≥ 107 $\mu\text{g/ml}$ in the 48-hr continuous treatment (cell proliferation ratio of 87% at 107 $\mu\text{g/ml}$). Cell proliferation ratios ranged from 27-55.5% at 2893 $\mu\text{g/ml}$. In the definitive study no metaphase cells were noted at ≥ 1447 $\mu\text{g/ml}$ due to cytotoxicity. Test-article precipitation was noted at all doses.

Sponsor's conclusion: Under the conditions of this study, _____ did not induce structural aberrations but increased the frequency of numerical aberrations (polyploid cells) in CHL/IU cells.

b(4)

Reviewer's comments: The sponsor made the following comment: "It was considered that the increase in polyploid cells was of little genotoxic significance, because the cells were expressed only at a high-dose level (including the precipitation level), and the frequency was comparatively low. It was also considered that the cause of polyploid cell induction was due to test article precipitation or mitotic inhibition."

Because there was not an increase in structural aberrations, it is possible that the increase in polyploidy was due to an effect on the mitotic apparatus or its proteins and not to a direct interaction with DNA. However, the genotoxic significance of the increase in polyploidy is uncertain at this point and should not be ignored. Regarding the level at which polyploidy was noted, the laboratory control values (mean \pm 2 S.D.) range from $0.71 \pm 0.83\%$ to $0.79 \pm 0.80\%$. Considering the laboratory control values, polyploidy outside the control range was observed at $\geq 90 \mu\text{g/ml}$. Therefore, in contrast to the sponsor assessment, polyploidy was not only observed at the high-dose levels.

Evaluation

The results of the Ames test and MLA suggest that the [redacted] may have potential to be mutagenic in humans. Two other assays, the chromosomal aberration in CHL/IU cells and micronucleus test in rats, were negative. Given the difference in sensitivity between assays, the two negative assays do not negate a potential risk to humans.

b(4)

The [redacted] increased the frequency of polyploid cells without inducing structural aberrations in CHL/IU cells. Although the toxicological relevance of this finding is not clear (given the absence of structural aberrations and the negative results in the *in vivo* micronucleus test), an increase in polyploidy may indicate a potential to induce aneuploidy². Aneuploidy is a frequent cause of mental retardation, congenital malformations, and abortions in humans and has been recognized as involved in the process of carcinogenesis^{2,3}.

The [redacted] was negative in all assays. However, as stated below, the NTP and CCRIS databases contain positive genotoxic results for this [redacted]

In all *in vitro* assays submitted in the current amendment, all [redacted] precipitated at very low concentrations. Therefore, except for the [redacted] which showed positive results in 2 *in vitro* assays, it is not known if a sufficient amount of [redacted] accumulated intracellularly and subsequently, was able to reach the DNA. In other words, the negative results may reflect low intracellular exposure.

b(4)

In order to prove that no mutagens are eluded from the backing cloth, the sponsor conducted an Ames Test using a DMSO extract of the FS-67 backing cloth. The DMSO extract

² Aadema M.J, Albertin S., Arni P., et al. Aneuploidy: a report of an ECETOC task force. *Mutat Res* 410 (1998) 3-79.

³ Kirsch-Volders M., Vanhauwaert A., De Boeck M., and Decordier I. Importance of detecting numerical versus structural chromosomal aberrations. *Mutat Res* 504 (2002) 137-148.

was negative in the Ames test. The sponsor did not quantitate the content of _____ in the extract used in the Ames test. However, this information was provided for the _____ under the tab "Overview of Genotoxicity Studies" and was copied below.

b(4)

Hisamitsu has determined the content of the pure blue dye in a FS-67 backing cloth. A FS-67 backing cloth (approx. 70 cm²) contained approx. _____ of the pure blue dye. Hisamitsu has also determined the content of it in a DMSO extract of a FS-67 backing cloth. Accordingly, no pure blue dye was detected. These results are summarized in the following table:

Contents of the _____	
A backing cloth (approx. 70 cm ²)	A DMSO extract of a FS-67 backing cloth
Approx. _____	Not detected (<0.23 µg/patch) ²

b(4)

- 1) A backing cloth was extracted several times for about 1 hr at 90°C in tetrahydrofuran (THF) in order to extract almost all of _____ contained in a FS-67 backing cloth. The _____ backing cloth changed _____
- 2) A backing cloth was extracted in the ratio of 70 cm²:23.3 ml (3 cm²/ml) for 72 hr at 37°C in DMSO in accordance with the prescribed method in ISO 10993. The _____ backing cloth did not change. The DMSO extract was condensed 100 times by evaporation to dryness and the concentrate was determined (detection limit = 1 µg/ml).

The sponsor did not submit any report on the validation of this extraction method.

Internal recommendations: In overall, the studies submitted herein in conjunction with literature findings suggest that all _____ may have the potential to be genotoxic. Nevertheless, given the **route and method** of administration, it is considered that there will be little human exposure and therefore, little genotoxic risk. As indicated in the discussion below, the sponsor needs to confirm that the human exposure _____ is indeed negligible.

b(4)

An internet search revealed that all _____ are commonly used _____. An occupational exposure survey shows that a large number of people have been exposed to these agents in the industrial setting, particularly textile sewing machine operators⁴. Therefore, there is evidence of human exposure _____. However, **to ensure that the human exposure through the FS-67 patch is minimal and does not pose a genotoxic concern**, the sponsor will be asked to demonstrate that the total exposure _____ from the backing cloth amounts to _____ a threshold at which the carcinogenic risk is considered negligible for a genotoxic impurity assuming, in this case, that all _____ are absorbed through the skin.

If the dyes are proposed to be used by routes of administration other than dermal topical, based on the results submitted herein, a concern still exists for all _____ because of the following reasons:

b(4)

- A. The _____ was positive in the Ames test and mouse lymphoma assay submitted herein.

⁴National Occupational Exposure Survey (1981-1983): www.cdc.gov/noes/noes2/occs747.html

- B. The [redacted] induced polyploidy in the chromosomal aberration assay in CHL/IU cells.
- C. Results obtained from the NTP and CCRIS databases showed that [redacted] was mutagenic in *S. typhimurium* TA 98 (100-10000 µg/plate in the presence of rat liver S9; 333-10000 µg/plate without S9 and in the presence of hamster liver S9). The [redacted] has an [redacted]
- D. Results obtained from the NTP database also showed that [redacted] was genotoxic in the mouse lymphoma assay (270-1000 µg/ml without S9).
- E. The fact that all [redacted] precipitated at very low doses in all *in vitro* assays, minimizes the confidence in the negative results obtained.

b(4)

The sponsor made the following comment: "For the genotoxicity studies [redacted] Hisamitsu purchased a marketed product [redacted] and purified it in order to test only the [redacted] component. The marketed product includes several dispersants. Therefore, only [redacted] of the marketed product is considered to be [redacted] in its high purity forms. A [redacted] concentration of the marketed product containing [redacted] is used to [redacted] backing cloth of FS-67. During manufacture, the [redacted] is added to the total [redacted] mixture. Therefore, the actual concentration of the [redacted] that is used on [redacted] process of FS-67 backing cloth is even lower than [redacted]"

b(4)

An e-mail was sent to the chemist reviewer to find out whether the sponsor has submitted the composition of the marketed product and if so, whether the components are acceptable according to regulatory standards.

External (sponsor) recommendations: The answer to the sponsor question "Does the FDA agree that this mutagenicity program is adequate to support the NDA and that there is little, if any, risk to humans using FS-67, since the Ames test on the extract of FS-67 backing cloth showed to be negative and [redacted] was detected in the DMSO extract of a FS-67 backing cloth?" is:

b(4)

The reviewer considered that the increase of polyploid cells observed with the [redacted] in the chromosomal aberration test should not be ignored. The reviewer also recognized that there was a common problem with all *in vitro* assays; all [redacted] precipitated at very low concentrations. Therefore, except for the [redacted] which showed positive results in 2 *in vitro* assays, it is not known if a sufficient amount of [redacted] accumulated intracellularly and subsequently, was able to reach the DNA. In other words, the negative results may reflect low intracellular exposure.

b(4)

The reviewer agrees that because [redacted] are in the backing cloth and no [redacted] was detected in the DMSO extract, [redacted] it could be assumed that humans would not be exposed to significant levels of these [redacted] by the dermal route. However, to confirm this assumption, it is recommended that the sponsor (1) submit the analytical reports for the THF and DMSO extracts of the [redacted] (2) submit the validation tests used to demonstrate that DMSO is able to extract the [redacted] (3) demonstrate that the total exposure to [redacted] from the backing cloth amounts to [redacted] (a threshold at which the [redacted])

b(4)

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/s/

Maria I. Rivera
9/9/2005 03:44:31 PM
PHARMACOLOGIST

Josie Yang
9/9/2005 04:26:10 PM
PHARMACOLOGIST

APPENDIX/ATTACHMENT 3:

Dr. María I. Rivera's Review of IND 62,735 (N041)

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**MEMO TO FILE
PHARMACOLOGY/TOXICOLOGY**

IND number: 62735

Sequence number/date/type of submission: N-041/11-14-05/General Correspondence

Information to Sponsor: Yes

Sponsor and/or agent: Hisamitsu Pharmaceutical Co., Inc.

Reviewer name: María I. Rivera, PhD

Division name: Anesthesia, Analgesia, and Rheumatology Drug Products

Review completion date: December 13, 2005

Background: Following review of a series of genotoxicity studies for the [redacted] found in the backing cloth of the FS-67 patch (refer to SN-038), Hisamitsu was asked to (1) submit the analytical reports for the THF and DMSO extracts of the [redacted] (2) submit the validation tests used to demonstrate that DMSO is able to extract [redacted] (3) demonstrate that the total exposure to [redacted] from the backing cloth amounts to [redacted] (a threshold at which the carcinogenic risk is considered negligible for a genotoxic impurity assuming that al. [redacted] are absorbed through the skin).

b(4)

Hisamitsu's response to the Division's comments and requests were the following:

Hisamitsu's Response to FDA Comment 1A: An increase in polyploid cells was observed with the [redacted] in the chromosomal observation test with CHL cells. However, this phenomenon was only observed at the high dose levels and the frequency was relatively low (up to 13.0%). Hisamitsu searched the literature and found no data associating this [redacted] with genetic disturbances. Hisamitsu provided a photo of the [redacted] (as a precipitate in the form of [redacted]). It also included a reference that supports that [redacted] have been shown to induce polyploidy.

b(4)

Reviewer's response: It is possible that the increase in polyploidy was due to an effect of the [redacted] formed upon precipitation of the [redacted]. Regarding the level at which polyploidy was noted, the laboratory control values (mean \pm 2 S.D.) range from $0.71 \pm 0.83\%$ to $0.79 \pm 0.80\%$. Considering the laboratory control values, polyploidy outside the control range was observed at $\geq 90 \mu\text{g/ml}$. Therefore, in contrast to the sponsor assessment, polyploidy was not only observed at the high-dose levels.

b(4)

Hisamitsu's Response to FDA Comment 1B: In summary, Hisamitsu concluded that because there was dose-dependent cytotoxicity, this proved the intracellular accumulation of [redacted]. Also, it used the argument that because the [redacted] was clearly able to accumulate intracellularly, based on the positive results of the Ames and MLA studies, it is likely that the [redacted] were also similarly transported across the cell.

b(4)

Reviewer's response: The argument that accumulation of [redacted] was demonstrated by the observed dose-dependent cytotoxicity is considered weak because precipitation of the [redacted] could also induce dose-dependent cytotoxicity. It is also not considered a good argument to assume that enough [redacted] were also transported inside the cells based on the positive

results obtained with the — Unless the amount of soluble form for each — was quantitated, this comparison is not appropriate.

Hisamitsu's Response to FDA Comment # 2: In summary, Hisamitsu emphasized that the — are contained in the backing cloth only and do not come in contact with the patient's skin. They further reevaluated the extract to quantify the exact amount — in the DMSO product as summarized below (excerpted from the submission):

As recommended by FDA on October 20, 2005, this DMSO extract has been further reevaluated to quantify the exact amount — in the DMSO extract. Hisamitsu extracted the backing cloths from 42 month old FS-67 patch products. In order to maximize the extraction conditions, the backing cloths were extracted for 72 hours at 37°C using DMSO and quantification was performed using UV visible spectrophotometer (400-800 nm) which allowed quantification of all related — peaks. Hisamitsu also physically separated the adhesive mass containing the drug products from the backing cloths, and then quantified the — content. The employed conditions for the adhesive mass included extraction with THF and detection by HPLC (625 nm).

Hisamitsu recovered only small — quantities from both the backing cloth — and the separated adhesive mass component —. These data confirm that the quantities — in the backing cloth and in the adhesive mass are substantially less than the genotoxic threshold permitted by EMEA and stated by FDA.

Reviewer's response: Hisamitsu did not actually comply with the Division's requests. The sponsor did not submit the analytical reports for the THF and DMSO extracts of — and the validation tests used to demonstrate that DMSO is able to extract — and did not demonstrate that the total exposure — from the backing cloth amounts to —. As summarized above, Hisamitsu only quantitated the amount of extractable — from the patch. Based on this information, the total amount — in the patch was actually — in the part of the patch (adhesive mass) in contact with the skin. The reviewer considers that by using DMSO and a 42 month old patch, the physiological conditions in the skin, where the solvent will be sweat, were maximized. Therefore, it is not expected that significant amounts — will leach out of the patch when in contact with the skin.

Conclusions and Recommendations: As stated in the review of SN-038, the reviewer agrees that because the dyes are in the backing cloth (which is not in contact with the skin) and little amount is expected to leach out of the patch, it could be assumed that humans would not be exposed to significant levels of these dyes by the dermal route. **Therefore, the reviewer agrees with the sponsor's assessment that there is little genotoxic concern with the dyes employed in the FS-67 backing cloth and no further nonclinical studies are needed.** However, given the issue of dye precipitation plus the other reasons given in the review of SN-038, the genotoxic studies may have not appropriately qualified the dyes if these are to be used by other routes of administration.

The chemist reviewer (Ali Al Hakim, PhD) input was sought regarding whether validation

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/s/

Maria I. Rivera
1/11/2006 05:28:39 PM
PHARMACOLOGIST

Josie Yang
1/12/2006 08:48:22 AM
PHARMACOLOGIST

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/s/

Belinda Hayes
12/13/2006 07:43:42 PM
PHARMACOLOGIST

R. Daniel Mellon
12/13/2006 07:57:41 PM
PHARMACOLOGIST
I concur with the recommendations made by Dr. Hayes.

PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST

NDA Number: 22-029

Applicant: Hisamitsu Pharm

Stamp Date: March 1, 2006

Drug Name: Salonpas

(10% Methyl salicylate & 3% l-menthol topical patch)

b(4)

IS THE PHARM/TOX SECTION OF THE APPLICATION FILEABLE? Yes [X] 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?	X		
2	Is the Pharmacology/Toxicology section of the NDA indexed and paginated in a manner to allow substantive review to begin?	X		
3	On its face, is the Pharmacology/Toxicology section of the NDA legible so that substantive review can begin?	X		
4	Are 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)?	X		Pharmacology, Pharmacokinetics, Toxicology, and Carcinogenicity studies for methyl salicylate and l-menthol are from the published literature. The adequacy of these studies to support this NDA application can only be determined upon formal review of the studies.
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?	X		Most literature studies to address the toxicity of each active ingredient used oral administration. The sponsor conducted skin irritation and local toxicity studies with the FS-67 patch. The adequacy of the sponsor's safety justification will be a review issue.
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?			Not applicable for OTC products.
7	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions?	X		
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?	X		See number 5 above.
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?	X		
10	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?		X	The Sponsor did not submit a statement but the protocols reflect that animals were treated humanely.
11	From a pharmacology perspective, is this NDA fileable?	X		

Note: Although originally submitted as a 505(b)(1) NDA application, the Sponsor has been informed that due to reliance upon data in the public domain makes this submission a 505(b)(2) submission. Although both methyl salicylate and menthol are to be evaluated for monograph

status, the final monograph for external analgesics has not yet been finalized. The NDA is filable; however, the adequacy of the referenced information will be determined upon review.

Reviewing Pharmacologist:

María I. Rivera, Ph.D.

Date:

Supervisor:

Dan Mellon, Ph.D.

Date

cc:

Original NDA 22-029

HFD-170/Division File

/PM/K. Olin

/MO/C. Fang

/Pharm-ToxTL/D. Mellon

/Pharm-Tox/M. Rivera

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/s/

Maria I. Rivera
5/5/2006 11:03:30 AM
PHARMACOLOGIST

R. Daniel Mellon
5/5/2006 06:47:44 PM
PHARMACOLOGIST
I Concur