

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**21-717**

**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: 21-717

SERIAL NUMBERS: 000 000 BP 000 AZ 000 BZ

DATES RECEIVED BY CENTER: 14-Nov-03 7-Sep-04 30-Dec-05 26-May-06

DRUG NAME: S-Caine™ (Lidocaine and Tetracaine) Cream 7%/7%

INDICATION: S-Caine™ is indicated as a topical local anesthetic for use on normal intact skin for local dermal anesthesia

SPONSOR: Zars, Inc.

DOCUMENTS REVIEWED: All nonclinical information in the above submissions

REVIEW DIVISION: Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)

PHARM/TOX REVIEWER: R. Daniel Mellon, Ph.D. &  
Suzanne R. Thornton-Jones, Ph.D.

PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.

DIVISION DIRECTOR: Bob Rappaport, M.D.

PROJECT MANAGER: Pratibha Rana

Date of review submission to Division File System (DFS): June 14, 2006

## EXECUTIVE SUMMARY

### I. Recommendations

#### A. Recommendation on acceptability.

The NDA can be approved from a pharmacology/toxicology perspective.

#### B. Recommendation for nonclinical studies.

None

#### C. Recommendations on labeling.

The text below contains the recommendations for changes to the sponsor's proposed label of the nonclinical information. These recommendations have not been negotiated with the Sponsor at the time of this review, and therefore, the final label may not be identical to the suggestions below: Deleted text has been struck-through, and added text is in blue.

## PRECAUTIONS

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### Carcinogenesis, Mutagenesis, Impairment of Fertility:

**Carcinogenesis:** Long-term studies in animals have not been performed to evaluate the carcinogenic potential of either lidocaine or tetracaine.

**Mutagenesis:** The mutagenic potential of lidocaine base and tetracaine base has been determined in the in vitro Ames Bacterial Reverse Mutation Assay, the in vitro chromosome aberration assay using Chinese hamster ovary cells, and the in vivo mouse micronucleus assay. Lidocaine was negative in all three assays. Tetracaine was negative in the in vitro Ames assay and the in vivo mouse micronucleus assay. In the in vitro chromosome aberration assay, tetracaine was negative in the absence of metabolic activation, and equivocal in the presence of metabolic activation.

**Impairment of Fertility:** Lidocaine did not affect fertility in female rats when given via continuous subcutaneous infusion via osmotic minipumps up to doses of 250 mg/kg/day (1500 mg/m<sup>2</sup> or 243-fold higher than the single dermal administration [SDA]). Although lidocaine treatment of male rats increased the copulatory interval and led to a dose-related decreased homogenization resistant sperm head count, daily sperm production, and spermatogenic efficiency, the treatment did not affect overall fertility in male rats when given subcutaneous doses up to 60 mg/kg (360 mg/m<sup>2</sup> or <18-fold the SDA). Tetracaine did not affect fertility in male or female rats when given subcutaneous doses up to 7.5 mg/kg (45 mg/m<sup>2</sup> or <1-fold the SDA). Multiples of exposure are based on an SDA of 1 g of S-Caine to 10 cm<sup>2</sup> for 60 minutes to a 60 kg person (43645 mg/m<sup>2</sup>).

### Use in Pregnancy:

**Teratogenic Effects: Pregnancy Category B.** Lidocaine was not teratogenic in rats at doses up

**Nonteratogenic Effects:** Lidocaine containing 1:100,000 epinephrine at a dose of 6 mg/kg

Pre- and postnatal maturational, behavioral, or reproductive development was not affected by maternal subcutaneous administration of tetracaine during gestation and lactation up to doses of 7.5 mg/kg (45 mg/m<sup>2</sup> or <1-fold the SDA).

No adequate and well-controlled studies have been conducted in pregnant women. Because animal studies are not always predictive of human response, S-Caine should be used during pregnancy only if the potential benefit justifies risk to the fetus.

## II. Summary of nonclinical findings

### A. Brief overview of nonclinical findings

The non-clinical development program for S-Caine included an acute dermal irritation study of S-Caine Peel in rabbits, a dermal irritation and absorption study of the peel in neonatal pigs, Segment I and III reproduction and developmental toxicology of tetracaine in rats. Studies previously reviewed in NDA 21-623 included a dermal sensitization study in guinea pigs with the S-Caine Patch, a 28-day repeat-dose toxicology study in rabbits with the S-Caine Patch, genetic toxicology studies for lidocaine and tetracaine, Segment II reproductive toxicology studies on tetracaine in both rats and rabbits, a Segment III reproductive toxicology study on lidocaine in rats, and a phototoxicity study. Carcinogenicity studies are not required for this indication. All of the above studies were previously reviewed either during the first cycle of the S-Caine Peel NDA or during the review of the Sponsor's other NDA (Synera). The reviews of these studies are reproduced here, but do not differ from the original reviews.

Dermal Toxicology: The acute local tissue reaction was characterized in two species, the rabbit and the neonatal pig. The results from the rabbit studies indicated that a 1 hour exposure to the S-Caine™ patch produced only very slight erythema and no evidence of edema and the placebo patch produced no erythema or edema. The data suggested that the S-Caine™ patch was a mild irritant. There

was no clear suggesting for local tissue irritation, however, from studies conducted in the neonatal pig model, which is thought to be the best pre-clinical model for human skin. Collectively, the non-clinical studies suggest the potential for a mild local tissue reaction following acute exposure to non-abraded skin.

A 28-day repeat-dose toxicology study in rabbits was submitted to characterize the potential for the S-Caine™ patch to increase the severity of the local tissue reaction following repeated applications of the S-Caine™ patch. The study used 3 patches per animal, each applied for 2 hours once a day for a total of 28 days. This treatment regimen, which exceeded the maximum human daily exposure, did not produce any evidence of systemic toxicity. The repeated exposure to the S-Caine™ patch produced increased local tissue irritation compared to the placebo patch. This local tissue irritation was characterized microscopically as epidermal surface exudates, epidermal necrosis, acute dermatitis, trace to moderate epithelial hyperplasia and fibrosis of the dermis. These changes were not evident in the skin treated with the placebo patch. Under the conditions of the assay, there were no significant differences between plasma concentrations of lidocaine or tetracaine between males and females or between abraded or non-abraded skin.

The potential for the S-Caine™ patch to produce a dermal sensitization following repeated exposure was tested via the Buehler method in the guinea pig model. Animals were treated with either with the S-Caine™ patch or control patches for 6 hours of days 0, 7 and 14. Fourteen days after the third exposure, the guinea pigs were challenged with topical application of the test article and positive control patches on the previously unexposed flank. The application sites were scored for erythema and edema 24 and 48 hours later. The results indicated that the S-Caine™ patch induced sensitization in guinea pigs, although with less intensity than the positive control, dinitrochlorobenzene (DNCB).

Genetic Toxicology: The sponsor completed a standard genetic toxicology battery for both lidocaine and tetracaine. Lidocaine base tested negative in the *in vitro* bacterial reverse mutation assay (Ames assay), the *in vitro* chromosome aberrations assay in Chinese Hamster Ovary (CHO) cells and an *in vivo* mouse micronucleus assay. Tetracaine tested negative in the *in vitro* bacterial reverse mutation assay and the *in vivo* mouse micronucleus assay. Although tetracaine tested negative in the absence of metabolic activation in the *in vitro* chromosome aberrations assay, in the presence of metabolic activation, tetracaine was equivocal.

Fertility: The potential effects of lidocaine on female fertility were obtained from the published literature. Lidocaine administration via chronically implanted minipumps at doses of up to 250 mg/kg/day did not produce alterations in reproductive indices in the female (Fujinaga and Mazze, 1986). There are no reports of the effects of lidocaine on male fertility in the published literature. Therefore, the sponsor conducted a separate fertility study of lidocaine in male rats. Although there was an increased copulatory interval at a dose of 60 mg/kg, and a dose-related decreased homogenization resistant sperm head count, daily

sperm production, and spermatogenic efficiency, the treatment did not affect overall fertility in male rats when given subcutaneous doses up to 60 mg/kg.

The potential effects of tetracaine on male fertility in the rat were assessed in one study where both male and females were treated simultaneously. There were no effects of either tetracaine or lidocaine male or female fertility. The available literature suggests that lidocaine exposure may produce subtle alterations in post-natal behavior.

Embryonic Development: The sponsor is relying in part upon the published literature to characterize the effects of lidocaine on post-natal development. Smith et al. (1986) treated sperm-positive Long-Evans hooded rats with 6 mg/kg lidocaine (with epinephrine) on gestation day 11 via an inter masseter muscle of the jaw injection. There were no alterations in birth, growth, or litter composition. However, offspring had longer latencies on the first day of negative geotaxis training and were more sensitive to electric footshock. Lidocaine dosed offspring responded less to the correct cue in a visual discrimination task, were slower to develop righting reflexes, made more errors in acquiring a water maze, had longer suppression times in a conditioned suppression task and had longer latencies to tail-flick. These results suggest that lidocaine (with epinephrine) exposure during mid-gestation can produce significant behavioral changes in the offspring of rats (Smith, et al., 1986). This dose of lidocaine corresponds to a human equivalent dose of 58 mg dose based on a body surface area comparison.

The effects of lidocaine on peri- and post-natal development have been reported in the rat model by Smith et al. (1989). Smith treated Long-Evans hooded rats with 3, 6 or 9 mg/kg lidocaine injected into the gum on gestational day 4, 11 or 18. The offspring were evaluated via a variety of behavioral tests. The results indicate that pups treated with lidocaine on gestational day 4, showed greater sensitivity to footshock. Lidocaine administration on gestational day 11 was associated with a slight but significant alteration in sex ratios. Lidocaine administration on gestational day 18 was associated with significant alterations in behavior, including visual discrimination, shuttlebox avoidance, tail flick and water maze errors (Smith, et al., 1989). The authors conclude that lidocaine may be a behavioral teratogen and that exposure later in gestation in the rat may alter a broader range of behaviors than exposure earlier in gestation. In contrast to cocaine, neonatal exposure (birth to day 21) of rat pups to lidocaine (20 mg/kg, oral) did not significantly alter lymphocyte or total leukocyte levels or spleen weights.

Teratogenicity: The sponsor completed Segment II studies (embryofetal development) in the rat and rabbit models which included assessments for lidocaine alone, tetracaine alone and the eutectic mixture. Although signs of maternal toxicity were evident, there was no indication that lidocaine or tetracaine is teratogenic under the conditions of the assays.

Perinatal and Postnatal Development: There were no effects of tetracaine base on pre- and postnatal development.

Phototoxicity: S-Caine™ Peel (7% lidocaine, 7% tetracaine) was found to produce mild irritation in rabbits, but did not cause any irritation in neonatal piglets. In all animal species examined lidocaine > tetracaine for exposure and there was a delay in  $T_{max}$  due to a re-distribution from the skin to the systemic exposure after peel removal. Experimental shortcomings in the phototoxicity study make it difficult to interpret, but 1 out of 4 sites that were irradiated after peel application showed well-defined/moderately severe erythema and slight-moderate edema. The results of the test indicate a possibility of a phototoxic reaction if the treated skin is exposed to sun light as the absorption spectrum indicates S-Caine™ Peel absorbs light in the 312-314 nm range.

## **B. Pharmacologic activity**

Both lidocaine (amide-linked) and tetracaine (para-aminobenzoic acid ester) are local anesthetics which have similar pharmacological profiles and are about equipotent. Local anesthetics block nerve impulses by decreasing or preventing the large transient increase in the permeability of excitable membranes to  $Na^+$  that normally is produced by a slight depolarization of the membrane due to direct interaction with voltage-gated  $Na^+$  channels. Blockade of neuronal conduction prevents the action potential of sensory neurons and therefore blocks the transmission of pain signals to the CNS. Lidocaine and tetracaine blockade demonstrates both frequency and voltage-dependency. Both drugs block both open and inactivated  $Na^+$  channels.

## **C. Nonclinical safety issues relevant to clinical use**

In light of the problems with the current phototoxicity study regarding the lack of appropriate controls, the unknown affect of the peel on the stratum corneum, and the observation that human subjects in the clinical trials that lidocaine may be present in the body for up to 24 hrs (no data is available for tetracaine as it was not detected) there is a safety concern regarding the possible photo-irritation at the application site. It is unclear if the stratum corneum is removed when the peel is removed, although the Sponsor indicates that there is no evidence from the clinical trials that it is removed. It should be noted that a specific study was not conducted to address the removal of the stratum corneum when the peel is removed. In this light, if the stratum corneum is removed, either partially or completely, then it is probable that the skin will be sensitive to sun, not as a result of the drug's light absorption or photochemical properties, but as a result of physical disruption of the skin's integrity. This concern of photo-irritation can be adequately addressed in product labeling and will not require any further non-clinical testing. Eye irritation is also a concern and it can be handled in the label. The Sponsor \_\_\_\_\_ in their proposed label.

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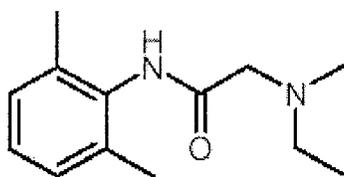
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## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

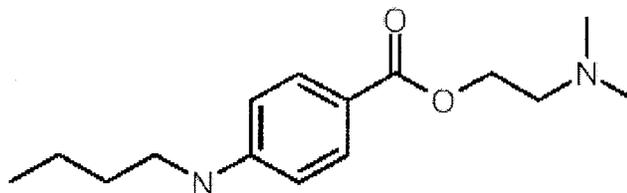
### 2.6.1 INTRODUCTION AND DRUG HISTORY

<b>NDA NUMBER:</b>	21-717
<b>REVIEW NUMBER:</b>	2
<b>SEQUENCE NUMBER/DATE/TYPE OF SUBMISSION:</b>	N000/17 November 2003/AZ
<b>INFORMATION TO SPONSOR:</b>	Yes ( ) No (X)
<b>SPONSOR:</b>	Zars, Inc. 1142 West 2320 South, Suite A Salt Lake City, UT 84119
<b>MANUFACTURER FOR DRUG SUBSTANCE:</b>	[lidocaine] [tetracaine]
<b>REVIEWER NAME:</b>	R. Daniel Mellon, Ph.D. & Suzanne R. Thornton-Jones, Ph.D.
<b>DIVISION NAME:</b>	DAARP
<b>HFD #:</b>	170
<b>REVIEW COMPLETION DATE:</b>	June 14, 2006
<b>DRUG:</b>	
<b>TRADE NAME:</b>	S-Caine™ Peel
<b>GENERIC NAME (LIST ALPHABETICALLY):</b>	lidocaine/tetracaine
<b>CODE NAME:</b>	NA
<b>CHEMICAL NAME:</b>	[lidocaine] 2-(Diethylamino)-N-(2,6-dimethylphenyl)-acetamide [tetracaine] 2-(Dimethylamino)ethyl p-(butylamino)benzoate
<b>CAS REGISTRY NUMBER:</b>	[lidocaine] 137-58-6 [tetracaine] 94-24-6
<b>MOLE FILE NUMBER:</b>	not specified
<b>MOLECULAR FORMULA/MOLECULAR WEIGHT:</b>	[lidocaine] C <sub>14</sub> H <sub>22</sub> N <sub>2</sub> O/234.3 [tetracaine] C <sub>15</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> /264.41

**STRUCTURE:**



Lidocaine



Tetracaine

<b>RELEVANT INDs/NDAs/DMFs:</b>	IND 58,823/NDA 21-623 (S-Caine Patch) IND 59,801 (S-Caine™ Peel)
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NDA 21-632

Zars, Inc. S-Caine Patch (Synera)

DMF  
DMF  
DMF

**DRUG CLASS:** Local anesthetics of the amide type (lidocaine) and ester type (tetracaine).  
**INTENDED CLINICAL POPULATION:** local dermal anesthesia on intact skin  
**ROUTE OF ADMINISTRATION:** topical  
**FORMULATION:**

Component	Function
Lidocaine, USP	Active, Anesthetic Agent
Tetracaine, USP	Active, Anesthetic Agent
Dibasic Calcium Phosphate, Anhydrous, USP	
Purified Water, USP	
Polyvinyl Alcohol, USP	
Petrolatum, USP	
Sorbitan Monopalmitate, NF	
Methylparaben, NF	
Propylparaben, NF	

The excipients in the above formulation can be found in approved drug products at equal or greater levels and therefore do not pose any unique toxicological concerns.

**BACKGROUND:** IND 59,801 was opened by ZARS, Inc. on February 2, 2000 to support clinical studies on a new formulation of local anesthetics called S-Caine Peel. S-Caine Peel contains a 1:1 (w:w) mixture of lidocaine base and tetracaine base. The S-Caine Peel IND was opened shortly after ZARS opened an IND for a similar product, the S-Caine Patch (IND 58,823). Due to the similarity in the formulations, the two products were developed in parallel and a majority of the preclinical studies were applicable to both products. Like the NDA for the S-Caine Patch (NDA 21-623), NDA 21-717 is filed as a 505(b)(2) application with the reference drug being EMLA Cream (NDA 19-941). EMLA Cream (lidocaine/prilocaine) serves as the reference for the lidocaine component of the S-Caine Peel.

The non-clinical development program included an acute dermal irritation study of S-Caine Peel in rabbits, a dermal irritation and absorption study of the peel in neonatal pigs, Segment I and III reproduction and developmental toxicology of tetracaine in rats. Studies previously reviewed in NDA 21-623 included a dermal sensitization study in guinea pigs with the S-Caine Patch, a 28-day repeat-dose toxicology study in rabbits with the S-Caine Patch, genetic toxicology studies for lidocaine and tetracaine, and Segment II reproductive toxicology studies on tetracaine in both rats and rabbits. Carcinogenicity studies are not required for this indication.

The majority of the nonclinical information to support of the S-Caine Peel NDA was also submitted in support of the Sponsor's S-Caine™ Patch NDA (21-623), specifically the genetic toxicology studies for lidocaine and tetracaine, reproduction and developmental toxicology studies, and dermal irritation and absorption studies for the S-Caine™ Peel. As previously agreed the Sponsor has referenced the 28-day dermal toxicity study with the S-Caine™ Patch in rabbits to support the S-Caine™ Peel. The 28-day study was previously reviewed under the S-Caine™ Patch NDA and findings included skin irritation, histological changes of epidermal surface exudates, epidermal necrosis, acute dermatitis, trace to moderate epithelial hyperplasia and fibrosis of the dermis, but no difference in gender, abraded or non-abraded sites for exposure.

Two degradation products are found in the S-Caine™ Peel, \_\_\_\_\_, \_\_\_\_\_ of \_\_\_\_\_ respectively. The Sponsor has established specifications of \_\_\_\_\_ respectively. Tetracaine *in vivo* is metabolized via hydrolysis by plasma esterases to both of these degradation products. The specifications established for these degradation products are below the levels normally found in the plasma when tetracaine is administered. These degradation products were also detected in the S-Caine™ Patch NDA. It should also be noted that the Sponsor decreased the specification for \_\_\_\_\_ There are no toxicology issues with the established specifications for these degradation products.

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

**Data reliance:** Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-717 are owned by Astra Zeneca or are data for which Zars, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 21-717 that Zars, 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 Zars, 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 21-717.

**Studies reviewed within this submission:** None.

**Studies not reviewed within this submission (previously reviewed):**

The following studies were reviewed for first cycle submission by Dr. Suzanne Thornton-Jones, Ph.D. and reproduced here:

Study Title	Study no.	Volume	Page
Report for _____ study: 30G-1PA final report, sample analysis of tetracaine and lidocaine in rabbit plasma by gas chromatography with nitrogen phosphorus detection	X91313G	9	9-3
Analysis of tetracaine and lidocaine in rabbit plasma	67GC	9	9-23

by LC/MS/MS			
Analysis of tetracaine and lidocaine in porcine plasma by LC/MS/MS	68G-1P	9	9-86
Final report for analysis of lidocaine in rat plasma by LC/MS/MS	67G-1P	9	9-190
Final report for analysis of lidocaine in rat plasma by LS/MS/MS	68GB	10	10-1
Final report for analysis of tetracaine and lidocaine in rabbit plasma by LC/MS/MS	67GB	10	10-81
Method validation report, analytical method GL-LID-01, lidocaine and tetracaine in human plasma by LC/MS/MS	---	10	10-181
A dermal irritation study of S-Caine™ Peel (lidocaine 7% and tetracaine 7% cream) in rabbits	925-018	14	14-1
Modified primary dermal irritation	X9L313G	14	14-64
Toxicokinetic report for modified primary dermal irritation test	X9L313G	14	14-80
Dermal absorption and dermal irritation study of S-Caine™ Peel (lidocaine 7% and tetracaine 7% cream) in neonatal piglets	925-005	14	14-89
Phototoxicity test in rabbits	0432LZ03.001	14	14-190
A study to assess the effects of fertility and early embryonic development to implantation in rats	925-014	17	17-1
Study for toxic effects on pre- and postnatal development, including maternal function, in rats	925-017	22	22-1

The following studies were reviewed in NDA 21-623 Synera:

Study Title	Study no.	NDA/IND
<b>Acute Toxicology/Dermal Irritation</b>		
Modified Primary Skin Irritation (Rabbits).	X9C009G	N21-623
A dermal irritation study of S-Caine™ Patch in rabbits	925-002	N21-623
Dermal Sensitization – Buehler Method	X9C010G	158,823
<b>Repeat Dose Toxicology</b>		
A 28 day dermal toxicity study of S-Caine™ Patch in rabbits	925-004	N21-623
<b>Genotoxicity</b>		
<i>Salmonella-Escherichia coli</i> mammalian-microsome reverse mutation assay with a confirmatory assay with lidocaine base	23840-0-409OECD	N21-623
<i>Salmonella-Escherichia coli</i> mammalian-microsome reverse mutation assay with a confirmatory assay with tetracaine base	23841-0-409OECD	N21-623
Chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with lidocaine base	23840-0-437OECD	N21-623
Chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with tetracaine base	23841-0-437OECD	N21-623
<i>In vivo</i> mouse micronucleus assay with lidocaine base	23840-0-455OECD	N21-623
<i>In vivo</i> mouse micronucleus assay with tetracaine base	23841-0-455OECD	N21-623
<b>Reproductive Toxicology</b>		

Pilot Study for effects on embryo-fetal development in rats	925-012	N21-623
Pilot prenatal development toxicity study in New Zealand white rabbits	925-013	N21-623
Final toxicology report for study 925-015, Study for effects on embryo-fetal development in rats	925-015	N21-623
Final toxicology report for study 925-016; Study for effects on embryo-fetal development in rabbits	925-016	N21-623
A study to evaluate functional effects on male fertility in rats	925-019	N21-623 N000 12/27/2004

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

No new pharmacology studies were performed by the sponsor for this application. A review of the pharmacology of local anesthetics in general, and lidocaine and tetracaine specifically, is provided in Goodman and Gilman's The Pharmacological Basis of Therapeutics. When applied locally to nerve tissue in appropriate concentrations, local anesthetics reversibly block the action potentials responsible for nerve conduction. A local anesthetic in contact with a nerve trunk can cause both sensory and motor paralysis in the area innervated. The action is reversible at clinically relevant concentrations; complete recovery in nerve function occurs with no evidence of damage to nerve cell fibers or cells.

Local anesthetics block conduction by decreasing or preventing the large transient increase in the permeability of excitable membranes to Na<sup>+</sup> that normally is produced by a slight depolarization of the membrane due to direct interaction with voltage-gated Na<sup>+</sup> channels. Local anesthetics can also bind to other membrane proteins such as K<sup>+</sup> channels. However, blockade of conduction is not accompanied by any large or consistent change in resting membrane potential due to block of K<sup>+</sup> channels since the interaction of local anesthetics with K<sup>+</sup> channels requires higher drug concentrations. Lidocaine and tetracaine have similar pharmacological profiles and are about equipotent. Lidocaine is considered to be the faster acting of the two components, although is shorter-acting compared to bupivacaine. Tetracaine is a para-aminobenzoic acid ester that has been used in a variety of formulations, including solutions and cream, gels, ointments, injectable, mouth spray, lozenge and even spinal block.

### 2.6.2.2 Primary pharmacodynamics

Lidocaine is an amide-linked local anesthetic, while tetracaine is an ester-linked local anesthetic. Local anesthetics prevent the generation and the conduction of nerve impulses.

Mechanism of action: Local anesthetics block the generation and conduction of nerve impulses in excitable tissues by decreasing or preventing the large transient increase in the permeability of the membrane to sodium ions. Local anesthetics bind directly to voltage-gated sodium channels from the inside of the membrane. The degree of block produced by

local anesthetics is dependent upon how the rate of nerve stimulation and on its resting membrane potential. Local anesthetics are only able to bind to sodium channels in their charged form and when the sodium channels are open. In this situation, the local anesthetic is able to bind more tightly to and stabilize the sodium channel. Differences in pKa, lipid solubility, and molecular size influence the binding of local anesthetics to sodium channels. The basic structure of a sodium channel subunit is depicted below:

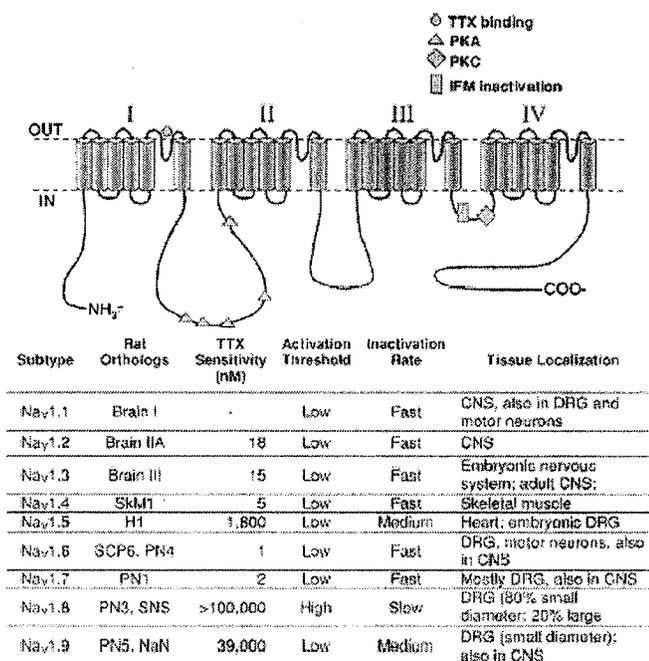


Figure 1 Schematic secondary structure of the family of VGSCs, their classification, tissue distribution, and functional characteristics.

the local anesthetic to bind. Clinically, the loss of nerve function proceeds as loss of pain, temperature, touch, proprioception, and then skeletal muscle.

**Drug activity related to proposed indication:** Blockade of neuronal conduction prevents the action potential of sensory neurons and therefore blocks the transmission of pain signals to the CNS. Lidocaine and tetracaine blockade demonstrates both frequency and voltage-dependency. Both drugs block both open and inactivated Na<sup>+</sup> channels. The frequency dependence of this blockade makes smaller unmyelinated nerve fibers more sensitive to blockade than larger heavily myelinated fibers. Therefore, Type C fibers (dorsal root and sympathetic nerves) and Type B (preganglionic autonomic nerves) are blocked at lower concentrations than heavily myelinated Type A (alpha, beta, gamma and delta) fibers. Of the type A fibers, pain and temperature sensitive neurons (delta) are more susceptible to local anesthetics than muscle spindles (gamma), touch and pressure sensitive neurons (beta) which are, in turn, more sensitive than proprioception and motor neurons (alpha). This sensitivity also correlates with the diameter of the nerve fiber, with smaller fibers being more sensitive to the local anesthetic action.

In general, small nerve fibers are more sensitive to local anesthetics than large nerve fibers. However, myelinated fibers are blocked before non-myelinated fibers of the same diameter. Autonomic fibers, small unmyelinated C fibers (mediating pain) and small myelinated Aδ fibers (mediating pain and temperature sensation) are blocked before larger myelinated Aγ, Aβ, or Aα fibers (mediating touch, pressure, muscle and postural inputs). Small, sensory fibers are preferentially blocked since nerve conduction is more easily blocked over shorter distances and these fibers have longer action potentials allowing more of

### 2.6.2.3 Secondary pharmacodynamics

In addition to Na<sup>+</sup> channels, local anesthetics can bind to other membrane proteins. Specifically, local anesthetics have been shown to bind to K<sup>+</sup> channels, at higher concentrations.

In addition to blockade of sensory nerves, local anesthetics also interfere with the functioning of all organs which require the conduction of electrical impulses for their activity. These organs include the CNS, autonomic ganglia, neuromuscular junction and all forms of muscle, including cardiac. The anti-arrhythmic effects of lidocaine are primarily due to action on the myocardium. Lidocaine leads to decreased electrical excitability, conduction rate and force of contraction. The ability for lidocaine to block cardiac conduction is the basis for its use as an anti-arrhythmic drug. This pharmacodynamic action occurs at systemic blood levels that range from 1-5 mg/ml.

### 2.6.2.4 Safety pharmacology

Safety pharmacology studies for either lidocaine or tetracaine were not conducted for this NDA, and are not required for drugs that have a long history of clinical use. However, extensive experience with local anesthetics has provided a clear understanding of the effects of these drugs on the critical systems of the body. Toxicity is due to an exaggerated pharmacological activity, primarily on the cardiovascular and central nervous system. Initial effects include mild hypertension and tachycardia, lightheadedness, mild agitation, and confusion. In severe cases this may progress to seizures, coma, respiratory depression, bradycardia, ventricular arrhythmias, and asystole. Toxicity may result from an excessive dose, mistaken drug identity, enhanced drug absorption, inadvertent intravascular injection, altered protein binding, slowed redistribution, and/or elimination.

**Neurological effects:** Sufficiently high plasma concentrations of lidocaine (5 µg/ml) can have toxic effects. However, “toxicity may occur with ‘therapeutic’ drug levels, particularly in patients with low serum protein concentrations who have increased levels of free (nonprotein-bound) drug (Micromedex Online Database).” Therapeutic plasma concentrations of lidocaine range from 1.5 to 5 µg/ml (Micromedex Online Database). CNS effects can include excitation and/or depression, light-headedness, nervousness, apprehension, euphoria, confusion, dizziness, drowsiness, tinnitus, blurred or double vision, vomiting, sensations of heat, cold or numbness, twitching, tremors, convulsions, unconsciousness, respiratory depression and arrest. In the peripheral nervous system, there is evidence that an excessively high concentration of local anesthetics can be toxic to nerve tissue.

High systemic plasma levels of lidocaine as well as other local anesthetics have been associated with convulsions and death. In the mouse model, the median convulsant dose was 111.0 mg/kg, intraperitoneal and the median lethal dose was 133.1 mg/kg, i.p. (de Jong and Bonin, 1980).

**Cardiovascular effects:** Toxic effects on the cardiovascular system may also occur with sufficiently high blood levels of lidocaine. These may include bradycardia, hypotension,

cardiac collapse and arrest. Lidocaine is used therapeutically to suppress cardiac arrhythmia with effective blood concentrations ranging from 1.2 to 5 µg/ml. These effects are mediated both via direct effects on the cardiac and smooth muscle and via effects on the autonomic nerves. Likewise, high concentrations of tetracaine may also lead to depression of the myocardium, hypotension or hypertension, bradycardia, ventricular arrhythmias and cardiac arrest.

**Pulmonary effects:** There are no known direct pulmonary effects associated with application of either lidocaine or tetracaine to the skin surface. With systemic dosing, apnea, anaphylaxis, and respiratory depression may occur in some patients.

**Renal effects:** There are no known renal effects following topical application of lidocaine or tetracaine to the oral mucosa.

**Gastrointestinal effects:** Local anesthetics can depress contraction of the smooth muscle in intact bowel and strips of isolated intestine. Nausea and vomiting may occur with tetracaine.

**Abuse liability:** The sponsor has not conducted specific studies to examine abuse liability. A review of the literature indicates that neither lidocaine nor tetracaine bind substantially to the dopamine transporter nor do they maintain self-administration in monkeys (Wilcox, et al., 2000). Overall, there is no indication that lidocaine or tetracaine would demonstrate any abuse liability concerns.

**Other: Hypersensitivity:** Local anesthetics can rarely cause a hypersensitivity reaction manifested as allergic dermatitis or an asthma attack. This is almost exclusively linked to anesthetics containing an ester linkage. Lidocaine contains an amide linkage, therefore, hypersensitivity and allergic reactions are rare, although have been reported. Tetracaine, however, contains an ester linkage, and may produce hypersensitivity reactions which may be characterized by cutaneous lesions, urticaria, bronchospasm, edema, shock or anaphylaxis depending on the route of exposure. It should be noted that anaphylaxis is a rare reaction.

#### 2.6.2.5 Pharmacodynamic drug interactions

The potential for local anesthetics to produce pharmacodynamic drug interactions results when coadministration of a drug would lead to altered effects. Coadministration of a vasoconstrictor, such as epinephrine, has been utilized to prolong the activity of injected local anesthetics by decreasing the diffuse of the anesthetic away from the intended site of action.

The effects of drugs that block other ionic currents in excitable membranes may be augmented in the presence of lidocaine. This is certainly true with other local anesthetics or sodium channel blockers such as amiodarone.

<b>Adverse Drug Interactions in Dentistry: Local Anesthetics</b>		
<b>Drugs</b>	<b>Interaction</b>	<b>Clinical Implications</b>
<i>Lidocaine/tetracaine with other local anesthetics (i.e., bupivacaine)</i>	Effects are additive	<b>Major Significance:</b> Local anesthetic toxicity is additive when these drugs are given in combination; although combination therapy with local anesthetics is acceptable; total dose should not exceed combined maximum recommended doses.
<i>lidocaine with antihistamines (i.e., cimetidine )</i>	Inhibition of local anesthetic metabolism	Inhibition of local anesthetic metabolism will have little effect on peak plasma levels of anesthetic when given as a single injection. Plasma clearance of lidocaine may be reduced in the presence of enzyme inducers.
<i>Local anesthetics and opioids (i.e., mepivacaine with meperidine)</i>	Increased sedation	Sedation with opioids may increase the risk of local anesthetic toxicity, particularly with children; local anesthetic dose should be reduced.
<i>Lidocaine with beta-blockers (i.e., propranolol)</i>	Inhibition of local anesthetic metabolism and perhaps reduced hepatic blood flow	Beta-Blockers and/or reduced hepatic blood flow can lead to elevated levels of lidocaine, possibly leading to lidocaine toxicity.
Sources: Drug Interaction Facts Online and (Moore, 1999).		

Pharmacodynamic interactions can occur with either lidocaine or tetracaine in the drug product. Briefly, lidocaine may interact with other antiarrhythmic drugs which also block sodium channels and increase the toxic effects of this class of drugs ultimately leading to seizures, heart failure or cardiac arrest. As listed in the EMLA® (lidocaine; prilocaine) label, the combination of lidocaine:prilocaine should be used with caution in patients receiving class I antiarrhythmics (e.g., disopyramide, encainide, flecainide, mexiletine, moricizine, phenytoin, procainamide, propafenone, quinidine, or tocainide) since the toxic effects are additive and potentially synergistic. Similarly, interaction with other local anesthetics would reduce the amount of lidocaine bound to  $\alpha$ -1-acid glycoprotein and thereby increase plasma levels of lidocaine. Similar caution should be taken with the combination of lidocaine and tetracaine.

### 2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not provided by Sponsor.

### 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

#### 2.6.4.1 Brief summary

The S-Caine™ Peel contains two active local anesthetic agents, lidocaine and tetracaine. Lidocaine is an amide linked local anesthetic while tetracaine is an ester-linked local anesthetic agent. Tetracaine is rapidly hydrolyzed by esterases in the plasma and tissues. The amide linkage in lidocaine is far less susceptible to hydrolysis than the ester linkage. Lidocaine and tetracaine bases diffuse through the lower membrane of the patch and into the underlying skin. The free bases are absorbed rapidly through mucous membranes, intact and damaged skin, and from the intestines and respiratory tract. The hydrochlorides are absorbed rapidly after parenteral administration, but absorption through intact skin or mucous membranes is poor. Following parenteral injection, lidocaine and tetracaine are widely distributed into highly perfused tissue, followed by redistribution into skeletal muscle and adipose tissue. Distribution is similar for both compounds. Lidocaine affinity for melanin has been demonstrated using labeled compound resulting in a longer elimination half-life in pigmented skin. Lidocaine readily crosses the placenta and blood brain barrier with plasma levels declining in parallel that of the mother animal. Lidocaine protein binding has been reported to be between 51 and 85% in humans. Protein binding of tetracaine in humans has been reported to be 76% (Micromedex Online Database). Local anesthetics are bound primarily by  $\alpha$ -1-acid glycoprotein.

In general, agents with an amide linkage are metabolized by hepatic microsomal enzymes and their elimination is prolonged by liver disease and decreased hepatic blood flow. Lidocaine is almost completely metabolized by the liver (95%) before excretion in the urine (< 10 % excreted unchanged). As such, any alteration in liver function or hepatic blood flow can have a significant effect on the pharmacokinetics and dosage requirements. Metabolism of lidocaine is qualitatively similar across species with quantitative variations. The three main types of metabolic reactions include aromatic hydroxylation, N-dealkylation and amide hydrolysis, followed by conjugation reactions. Major enzymes involved in lidocaine metabolism in human liver microsomes were CYP3A4 and CYP1A2. In a human liver slice system, MEGX and 2,6-xylidine were identified as major metabolites. In the urine of man and dogs, the major metabolite (4-hydroxy 2,6-xylidine) accounted for 70% and 35% of the dose, respectively. In rats, the urinary metabolites accounted for 70% of the administered dose and included 3-hydroxy lidocaine and its dealkylated product, 3-hydroxy-MEGX.

In general, agents with an ester type linkage are rapidly metabolized mainly by the plasma pseudocholinesterases yielding para-aminobenzoic acid derivatives. They are also metabolized by liver esterases to some degree. As such, tetracaine is hydrolyzed by plasma esterases to form aminobenzoic acid and diethylaminoethanol. Aminobenzoic acid is used by topical application as a sunscreen. The compound effectively absorbs light throughout the UVB range but not the UVA range. Aminobenzoate sunscreens, therefore can prevent sunburn but would not prevent photosensitivity reactions caused by UVA light. Adverse skin reactions have been reported following the topical administration of aminobenzoic acid sunscreens, including contact and photocontact allergic dermatitis and vitiligo.

#### 2.6.4.2 Methods of Analysis

[see under individual study reviews]

### 2.6.4.3 Absorption

Lidocaine is readily absorbed from the gastrointestinal tract, from mucous membranes and through damaged skin. Absorption through intact skin, however, is considered to be poor. Absorption from injection sites, including muscle is rapid. The oral bioavailability of lidocaine is about 35%, due to the extensive first-pass metabolism in the liver. Alterations in hepatic blood flow or liver function can have a pronounced effect on the pharmacokinetics and half-life of lidocaine.

Tetracaine is readily absorbed into open wounds. Tetracaine is reported to be about 15% bioavailable following application of a 4% gel to intact skin.

### 2.6.4.4 Distribution

Amide local anesthetics are widely distributed after intravenous administration. Intravenous lidocaine demonstrates a rapid distribution phase (into highly perfused tissues) following by slower distribution phases into muscle as well as fat tissues. Lidocaine can cross the blood brain barrier as well as the placenta and can be detected in breast milk. Systemic lidocaine has fairly high protein binding (33-80%) in humans, primarily to  $\alpha$ -1-acid glycoprotein. The volume of distribution has been reported to be 1.7 L/kg with a distributional half-life of 15-30 minutes (Rowland, et al., 1971; Thomson, et al., 1971). In distribution studies examining intravenous administration, there is a large first pass uptake of lidocaine by the lung. Lidocaine crosses the blood brain barrier by passive diffusion. Likewise, lidocaine rapidly crosses the placenta by passive diffusion and levels may be sufficient enough to reach the fetus. Lidocaine is detectable in the fetal circulation within minutes of administration to the mother.

Following intravenous infusion, tetracaine is initially distributed to the lung, and later redistributed to the liver, kidney and adrenals. The distribution of tetracaine has been examined following intravenous and spinal administration in the rabbit model. The table to the right presents the concentration of tetracaine and the metabolite para-butylaminobenzoic acid in selected rabbit tissues following intravenous administration (Hino, et al., 2001).

Table 3  
Concentrations of tetracaine and its metabolite in rabbit tissues following intravenous administration (ng/g)

	Tetracaine	Metabolite
Rabbit number	11	
Weight (kg)	3.02	
Dose (mg/kg)	1.08	
Survival time after injection (min)	26	
Cerebrum	268.3	4597.5
Cerebellum	199.5	2973.1
Brain stem	ND <sup>a</sup>	800.3
Spinal cord		
Cervical	ND	411.0
Thoracic	ND	332.4
Lumbar	ND	341.8
Heart blood	148.8	521.4
Peripheral blood	403.9	498.4

<sup>a</sup> Not detected.

### 2.6.4.5 Metabolism

About 90% of the administered lidocaine is metabolized in the liver (Elvin, et al., 1981; Zito and Reid, 1981). Lidocaine is not metabolized by plasma esterases. There is increasing evidence that lidocaine can be metabolized extrahepatically, including the intestines, lung and kidney. Both monoethylglycine xylidide (MGEX) and glycine xylidide (GX) exhibit some local anesthetic activity. In human beings, approximately 75% of the xylidide is excreted in the urine as 4-hydroxy-2,6-dimethylaniline. The 4-hydroxyxylidide is the predominant metabolite excreted in the urine after lidocaine administration. The conversion of lidocaine to MGEX *in vitro* appears to be mediated by CYP3A4 in humans and CYP2C11 and CYP2B1 in rats. Hydroxylation of lidocaine appears to be mediated by CYP2D and CYP1A2 isozymes. There is considerable interspecies variability in the metabolism of lidocaine. N-hydroxyxylidide has been shown to form hemoglobin adducts. Xylidide-hemoglobin adducts have been detected in the blood of tobacco smokers and non-smokers. The figure below details the metabolism of lidocaine to MEGX, GX and Xylidines (Alexson, et al., 2002).

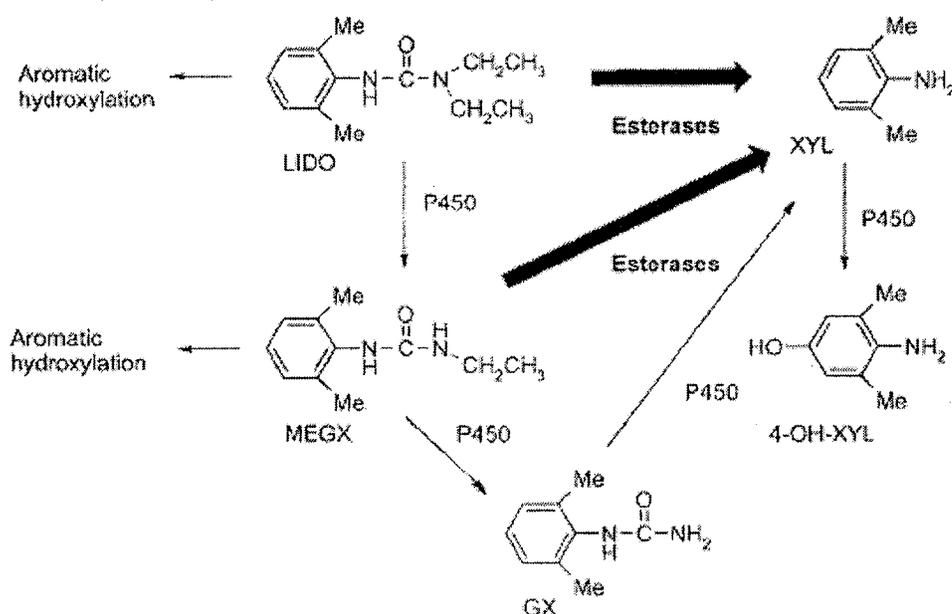
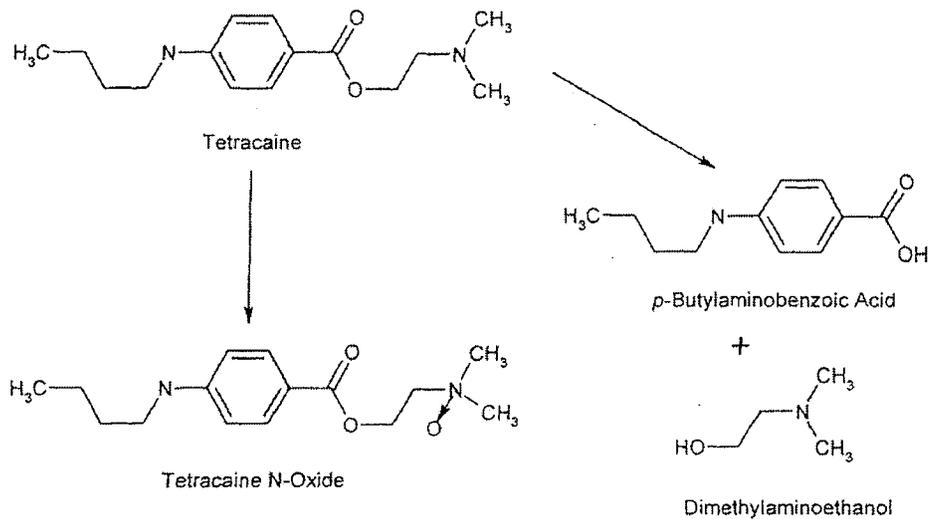


FIG. 5. Hypothetical scheme for the formation of 4-OH-XYL and other hydroxylated metabolites from LIDO, MEGX, and/or GX via the activity by esterases and/or P450.

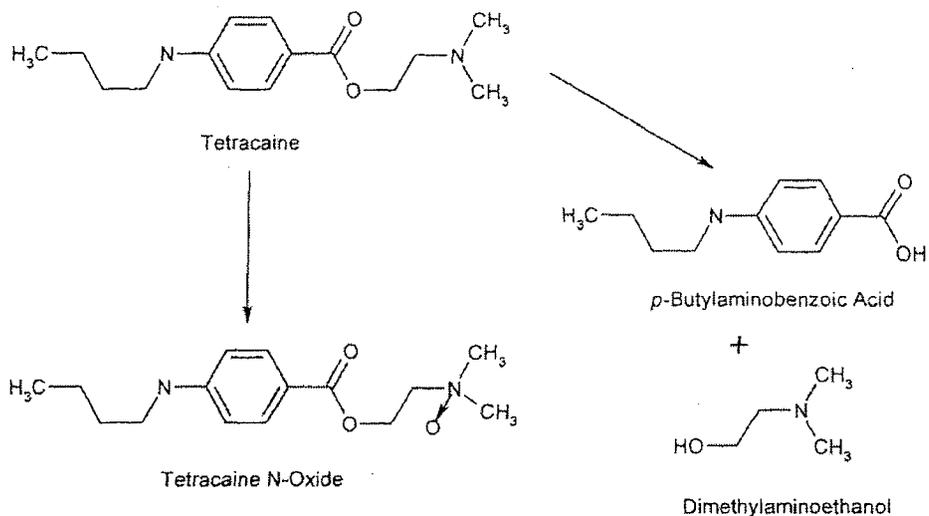
Tetracaine is metabolized by hydrolysis of the ester bond by plasma cholinesterases. Primary metabolites include para-butylaminobenzoic acid and diethylaminoethanol, both of which have an unspecified activity. In rats, rabbits and horses, the N-oxide of tetracaine is also formed.

Figure 2. Metabolic Pathways for Tetracaine



Information from Foldes, 1966 and Momose, 1976.

Figure 2. Metabolic Pathways for Tetracaine



Information from Foldes, 1966 and Momose, 1976.

#### 2.6.4.6 Excretion

Lidocaine and its metabolites are excreted in the urine. The elimination half-life of the parent compound is between 1.5 and 2 hours (Rowland, et al., 1971; Thomson, et al., 1971). MEGX has a half-life of 1-6 hours and GX has a half-life of about 1 hour.

The metabolite 2,6-xylidine has been detected in the breast milk of human females. Urinary excretion of local anesthetics is pH-dependent. Acidification of the urine results in increased concentrations of local anesthetics and some metabolites in urine. Alkalinization of the urine decreased levels of local anesthetics. Very little local anesthetic is eliminated in the feces of humans.

Tetracaine metabolites (aminobenzoic acid and diethylmanoethanol) are primarily excreted in the urine. Tetracaine has a long duration compared to other local anesthetics. The duration of local anesthesia following spinal anesthesia is 2-3 hours without epinephrine and 4-6 hours with epinephrine. It is not known if tetracaine can be excreted in breast milk.

#### **2.6.4.7 Pharmacokinetic drug interactions**

Local anesthetics such as lidocaine and tetracaine are largely metabolized in the liver and therefore any alteration in liver function or blood flow through the liver will alter the plasma levels of prilocaine.

The speed of onset of a local anesthetic may be increased by the addition of a vasoconstrictor. This prevents the drug from diffusing into the general circulation from the injection site. Epinephrine is commonly combined with a local anesthetic in a ratio of 1:100,000 or 1:200,000. The total amount of epinephrine injected should not be greater than 500 mg. Local anesthetics are generally administered in an acidic solution and alkalization of the solution with sodium carbonate is thought to increase the speed of onset. Alkalinization of the solution increases the proportion of the lipid soluble non-ionized free base allowing the active molecule to pass through the cell membrane. The buffering of the solution with sodium bicarbonate reduces the acidity and thereby also reduces the pain associated with their injection.

Amiodarone or one of its metabolites may inhibit lidocaine metabolism via CYP3A4 possibly leading to increased lidocaine plasma concentrations and lidocaine toxicity.

#### **2.6.4.8 Other Pharmacokinetic Studies**

#### **2.6.4.9 Discussion and Conclusions**

#### **2.6.4.10 Tables and figures to include comparative TK summary**

### **2.6.5 PHARMACOKINETICS TABULATED SUMMARY**

Not provided by Sponsor.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

General toxicology: No new studies were submitted for review.

Genetic toxicology: The genotoxic potential of lidocaine base and tetracaine base were determined in the *in vitro* Ames Bacterial Reverse Mutation Assay, the *in vitro* chromosome aberration assay using Chinese hamster ovary cells, and the *in vivo* mouse micronucleus assay. Lidocaine was negative in all three assays. Tetracaine was negative in the *in vitro* Ames assay and the *in vivo* mouse micronucleus assay. Tetracaine was negative in the absence of metabolic activation in the *in vitro* CHO chromosomal aberration assay, and equivocal in the presence of metabolic activation.

Carcinogenicity: The sponsor did not conduct carcinogenicity assessment for either lidocaine or tetracaine. There are no data in the public domain that indicates that either compound is carcinogenicity. The ICH M3 Guidance indicates that carcinogenicity assessment would not be required for a drug that would not be used either continuously for  $\geq 6$  months or intermittently over a lifetime such that total exposure would add up to approximately six months time. At this point in time, carcinogenicity assessment is not required for this drug product.

The metabolite of lidocaine, 2,6-xylylidine, has been tested for carcinogenic potential in Sprague-Dawley rats by the National Toxicology Program in 1990. Dietary administration of 2,6-xylylidine to the rat at 0, 300, 1000 or 3000 ppm for 102 weeks produced a significant increase in the incidence of nasal cavity adenomas, carcinomas and adenocarcinomas in both males and females. Rhabdomyosarcomas, previously unreported in this rat strain, were detected in two high-dose males and two high-dose females (none in the controls) (National Toxicology Program, 1990).

Reproductive toxicology: Tetracaine base did affect male or female fertility or pre- and postnatal development in rats when administered s.c. up to a dose of 2.5 mg/kg/day.

Special toxicology: S-Caine™ Peel was mildly irritating to rabbits. Experimental shortcomings in the phototoxicity study make it difficult to interpret, but 1 out of 4 sites that were irradiated after peel application showed well-defined/moderately severe erythema and slight-moderate edema. The results of the test indicate a possibility of a phototoxic reaction if the treated skin is exposed to sun light and the absorption spectrum indicates S-Caine™ Peel absorbs light in the 312-314 nm range.

### 2.6.6.2 Single-dose toxicity

**No new studies were submitted for review.** The results of acute toxicity studies of lidocaine hydrochloride have been reported in the Hazardous Substances Database (HSDB). The human equivalent dose conversion is based upon body surface area and expressed for a 60 kg individual.

Species	Route	LD <sub>50</sub> (mg/kg)	Human Equivalent Dose mg/60 kg person
Mouse	Oral	292	1,424
	i.p.	105	512
	s.c.	238	1,161
	i.v.	19.5	95
Rat	Oral	317	3,068
	i.p.	133	1,287
	s.c.	335	3,242
	i.v.	25	242

The acute toxicity of tetracaine is described in the RTECs database and is summarized below:

<b>TDLO/TCLO – Lowest Published Toxic Dose/Concentration</b>			
Species	Route	Dose	Toxic Effects
Human, Woman	Parenteral	4 mg/kg	<i>Behavioral – Excitation Behavioral – Coma Cardiac – Arrhythmias (including changes in conduction)</i>
<b>LDLO/LCLO – Lowest Published Lethal Dose/Concentration</b>			
Human, Man	Parenteral	1 mg/kg	<i>Behavioral – Muscle contraction or spasticity Behavioral – Coma Lung, Thorax, or Respiration – Cyanosis</i>
Rabbit	Intraspinal	4.9 mg/kg	
	Intravenous	6 mg/kg	<i>Lung, Thorax, or Respiration – Other changes</i>
	Subcutaneous	20 mg/kg	<i>Lung, Thorax, or Respiration – Other changes</i>
<b>LD50/LC50 – Lethal Dose/Concentration 50% Kill</b>			
Rat	intraperitoneal	33 mg/kg	
	Intratracheal	4 mg/kg	
	Intravenous	6 mg/kg	
Mouse	Intraperitoneal	20 mg/kg	
	Intravenous	6 mg/kg	
	Oral	300 mg/kg	
	Subcutaneous	41.5 mg/kg	
Rabbit	Intratracheal	6.5 mg/kg	
	Parenteral	33.5 mg/kg	

According to Martindale's online, tetracaine has high systemic toxicity and is absorbed rapidly from mucous membranes. As such, adverse reactions can occur abruptly without warning and fatalities have been reported. Topical application is frequently associated with mild erythema.

### 2.6.6.3 Repeat-dose toxicity

**No new studies were submitted for review.** The sponsor did not submit any systemic repeat dose toxicology studies with either tetracaine or lidocaine for this NDA application. The potential for systemic toxicity following repeated application of S-Caine™ Patches to the rabbit were characterized in the study described below:

#### **Study title: A 28-Day Dermal Toxicity Study of S-Caine™ Patch in Rabbits (with toxicokinetics)**

**Key study findings:** A repeat-dose dermal toxicology study was conducted in rabbits to evaluate the dermal toxicity of S-Caine™ patch following daily dermal application of 3 patches for 2 hours a day for a total of 28 days on abraded and non abraded skin. The key findings are as follows:

1. There was no evidence of systemic toxicity in any group under the conditions of this assay.
2. Repeated application of S-Caine™ patch (once every three days) produced a greater degree of skin irritation compared to the placebo patch for both abraded and non-abraded skin.
3. There were no differences in the blood levels of lidocaine or tetracaine between abraded and non-abraded animals or males and females.
4. Histological changes were limited to the test-article application sites and included epidermal surface exudates, epidermal necrosis, acute dermatitis, trace to moderate epithelial hyperplasia and fibrosis of the dermis.
5. The incidence and severity of histological changes were comparable between males and females and abraded and non-abraded sites.

<b>Study no.:</b>	925-004
<b>Volume #, and page #:</b>	Volume 11, Page 1
<b>Conducting laboratory and location:</b>	
<b>Date of study initiation:</b>	May 9, 2002
<b>GLP compliance:</b>	Yes
<b>QA report:</b>	yes ( X ) no ( )
<b>Drug, lot #, and % purity:</b>	Active S-Caine™ Patch, Lots 1262 and 1263 Placebo Patch, Lot 1264

#### **Methods**

**Doses:** 3 S-Caine™ Patch (70 mg lidocaine; 70 mg tetracaine) applied for 2 hours per day for 28 days.

**Species/strain:** New Zealand White Hra(NZW)SPF albino rabbits

**Number/sex/group or time point (main study):** The following groups were tested in this study:

Group Assignment				
Group	Treatment	Test Site	Number of Animals	
			Male	Female
1	S-Caine™ Placebo Patch	Non-Abraded	3	3
2	S-Caine™ Patch	Non-Abraded	3	3
3	S-Caine™ Placebo Patch	Abraded	3	3
4	S-Caine™ Patch	Abraded	2	4

The use of only 2 males and 4 females in group 4 animals was due to incorrect sexing that was noticed upon necropsy.

**Route, formulation, volume, and infusion rate:** Transdermal administration, via S-Caine™ Patch or Placebo S-Caine™ Patch. Groups 2 and 4 had patches applied to three of 9 sites and rotated such that each site was only exposed once every three days. Groups 1 and 3 had patches applied to two of six sites and rotated such that each site was only exposed once every three days. Patches were applied to the dorsal surface of each animal in a grid pattern of either three sites wide by three sites long or two sites wide by three sites long. The self adhesive patches were applied on Day 1 in a row across the two or three most cranial sites on the dorsal surface. The patches were applied for approximately 2 hours each day. They were held in contact with the skin with a gauze dressing and covered with a nonirritating tape. Elizabethan-type collars were applied and remained in place throughout the study.

**Satellite groups used for toxicokinetics or recovery:** None

**Age:** ~ 4 months

**Weight (nonrodents only):** Males 2.36 to 2.68 kg

Females 2.33 to 3.08 kg

**Unique study design or methodology (if any):** Patch sites were rotated such that each site received patch application once every three days.

### Observation times and results

**Mortality:** Observations for mortality and clinical signs were completed twice during each day, once prior to test article administration and once at the end of the exposure period.

One female rabbit in the abraded placebo patch group was sacrificed *in extremis* on Day 25. The animal had fluid in the thoracic cavity at necropsy and microscopically had mild pulmonary arterial hypertrophy. According to the sponsor, the lesions were considered to be incidental to treatment. There were no other mortalities noted.

**Clinical signs:** Detailed clinical examination was completed twice each day, once prior to test article administration and once at the end of the exposure period. Physical examination was conducted on all animals by a staff veterinarian both pretest and prior to terminal necropsy. There were no clear test article-related clinical findings observed. Minor skin changes noted included abrasions, hair absent or scabbed areas were noted in one female per group but do not appear to be clearly attributable to the treatment.

Summary of Clinical Findings Day (# times observed/# animals affected)								
Group	Males				Females			
	1	2	3	4	1	2	3	4
Treatment	Placebo Non-Abraded	Active Non-Abraded	Placebo Abraded	Active Abraded	Placebo Non-Abraded	Active Non-Abraded	Placebo Abraded	Active Abraded
N	3	3	3	2	3	3	3	4
<b>Skin</b>								
Abrasions	0/0	0/0	0/0	0/0	2/1	3/1	3/1	3/1
Hair absent	0/0	0/0	0/0	0/0	0/0	8/1	0/0	0/0
Scabbed area	0/0	0/0	0/0	0/0	5/1	0/0	0/0	0/0

Body weights: Body weights were recorded twice prior to randomization and weekly during the study.

All rabbits lost weight during the study. The weight loss was similar in all groups and was likely due to the wearing of the Elizabethan collars continuously during the entire study. There was no evidence that the S-Caine™ treated animals lost any more or less weight than the placebo groups.

Food consumption: Food consumption was measured and recorded daily during the pretest and study periods and reported on a weekly basis. Occasionally food consumption was excluded due to spillage or wet food. There was no evidence for a treatment-related alteration in food consumption between groups.

Ophthalmoscopy: Ophthalmologic examinations were conducted prior to exposure and prior to the scheduled sacrifice by a certified veterinary ophthalmologist. All findings were within normal limits.

EKG: Not examined.

Hematology: Blood samples were obtained from the central ear artery of all animals at pretest and study termination. Hematology parameters were evaluated for all animals at pretest and at study termination. There were no test article-related alterations in hematological parameters noted. An occasional value was significantly different from the placebo group, however, the sponsor did not consider these changes to be related to the test article. Of note, the number of samples examined for hematological changes were frequently limited to one per group, apparently due to technical errors. Therefore, statistical analysis was not possible to compare the values obtained. The hematological and clinical chemistry changes noted, therefore are of little utility. However, based upon comparison of the values obtained, there does not appear to be any strong signal for S-Caine™ patch induced alterations in hematological parameters.

Clinical chemistry: Blood samples were obtained from the central ear artery of all animals at pretest and study termination. Clinical chemistry parameters were evaluated for all animals at pretest and at study termination. There were no test article-related alterations in clinical chemistry parameters noted. An occasional value was significantly different from the placebo group, however, these were not considered to be related to the test article.

Urinalysis: Not completed.

Gross pathology: Complete necropsy was performed following euthanasia via overdose of sodium pentobarbital solution and exsanguinations. Animals were examined carefully for external abnormalities including palpable masses. The skin was reflected from a ventral midline incision and any gross lesions collected. The abdominal, thoracic and cranial cavities were examined for abnormalities. The organs were removed, examined, and placed in neutral buffered formalin (eye was placed Davidson’s fixative). Lungs were infused via the trachea with formalin.

Macroscopic changes noted were limited to the skin and noted in both male and female rabbits and in both abraded and non-abraded skin sites. When skin changes were noted in groups treated with the S-Caine™ patch non-abraded sites, the effect was not mimicked in the corresponding abraded sites. Overall, the lack of correlation is consistent with a mild inflammatory change induced by the S-Caine™ patch in some animals; however, the clinical significance of these changes appears minimal. These changes are summarized in the table below:

Incidence of Macroscopic Findings								
Group	Males				Females			
	1	2	3	4	1	2	3	4
Treatment	Placebo Non-Abraded	Active Non-Abraded	Placebo Abraded	Active Abraded	Placebo Non-Abraded	Active Non-Abraded	Placebo Abraded	Active Abraded
N	3	3	3	2	3	3	3	4
Skin								
Discolored, red, mild	0	1	0	0	0	0	1	2
Scab, mild	0	1	0	0	0	1	0	1
Abrasion, mild	0	0	0	0	0	1	0	0

Organ weights (specify organs weighed if not in histopath table): Organ weights were obtained at necropsy. Paired organs were weighed together. Organ weights were obtained for the following: adrenal gland, brain, epididymis, heart, kidney, liver, ovary, pituitary, spleen, testis and thyroid with parathyroid. There were no test-article related changes in organ weights detected.

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

Peer review: yes ( ), no ( X )

For the skin histology, the protocol indicated that 3 samples from each of the 4 exposure sites and 1 sample from an untreated site were to be examined. Test article microscopic observations were limited to the treated skin sites. These changes were noted in males and females in both abraded and non-abraded sites. Most non-abraded and abraded placebo treated sites were normal, with only trace hyperkeratosis, trace epithelial hyperplasia and trace chronic dermatitis noted in a few sites. The summary of the histological changes note are presented in the tables below:

Example of the Incidence of Microscopic Histological Findings in Treated Skin A Samples								
Group	Males				Females			
	1	2	3	4	1	2	3	4
Treatment	P	S	P	S	P	S	P	S
Skin Condition	NA	NA	A	A	NA	NA	A	A
<b>N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>4</b>
<b>Dermatitis, acute</b>	0	2	0	0	0	2	0	0
Trace	0	1	0	0	0	1	0	0
Moderate	0	1	0	0	0	1	0	0
<b>Exudate, epidermal, surface</b>	0	1	0	2	0	1	0	2
Trace	0	0	0	2	0	0	0	2
Mild	0	1	0	0	0	1	0	0
<b>Fibrosis, trace</b>	0	0	0	1	0	0	0	1
<b>Hyperkeratosis, trace</b>	0	1	0	1	0	1	0	1
<b>Hyperplasia, epithelial, trace</b>	0	1	0	2	0	1	0	2
<b>Necrosis, epidermal</b>	0	2	0	0	0	2	0	0
Mild	0	1	0	0	0	1	0	0
Severe	0	1	0	0	0	1	0	0

Key: P = Placebo Patch, S = S-Caine™ Active Patch, A=Abraded Tissue, NA = Non-Abraded tissue

Toxicokinetics: Blood samples were collected from the central ear artery of all animals in Groups 2 and 4 only for determination of the plasma concentrations of the test article. Samples were collected prior to exposure and at 1, 2, 4, 6 and 24 hours after application of patches on Days 1 and 26. Samples were placed in tubes containing potassium EDTA and neostigmine and stored on ice until centrifuged. Plasma was stored at -70°C until assayed.

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The figures below depict the mean plasma concentrations of lidocaine and tetracaine in male rabbits with intact skin:

Figure 1a. Mean Concentrations of Lidocaine in Male Rabbits, Non-Abraded

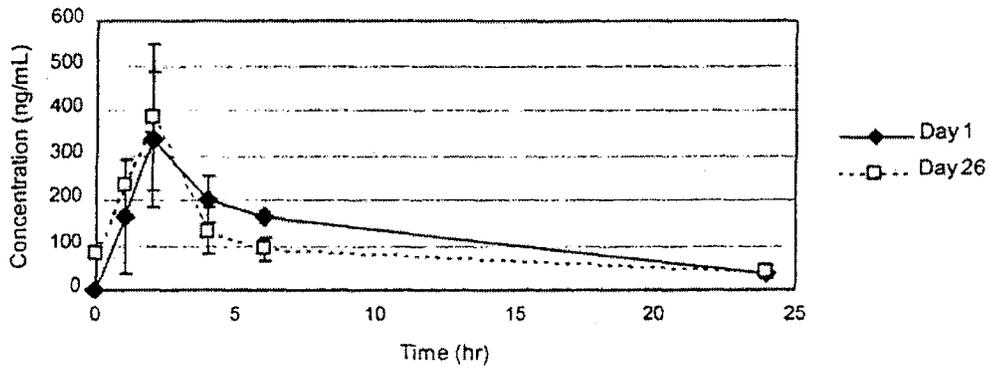


Figure 1b. Mean Concentrations of Tetracaine in Male Rabbits, Non-Abraded

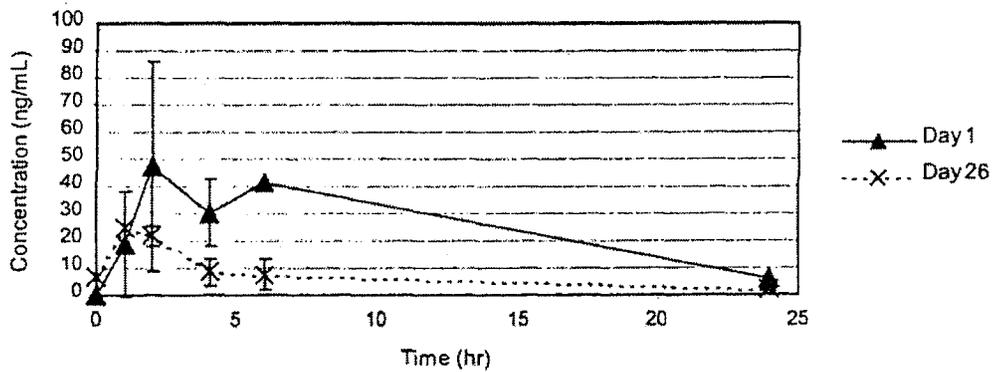
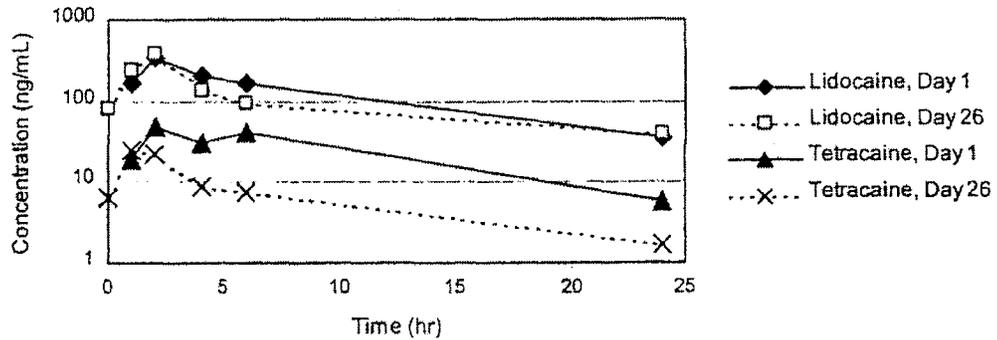


Figure 1c. Mean Concentrations of Lidocaine and Tetracaine in Male Rabbits, Non Abraded



The figures below depict the mean plasma concentration of lidocaine and tetracaine in female rats with intact skin:

Figure 2a. Mean Concentrations of Lidocaine in Female Rabbits, Non-Abraded

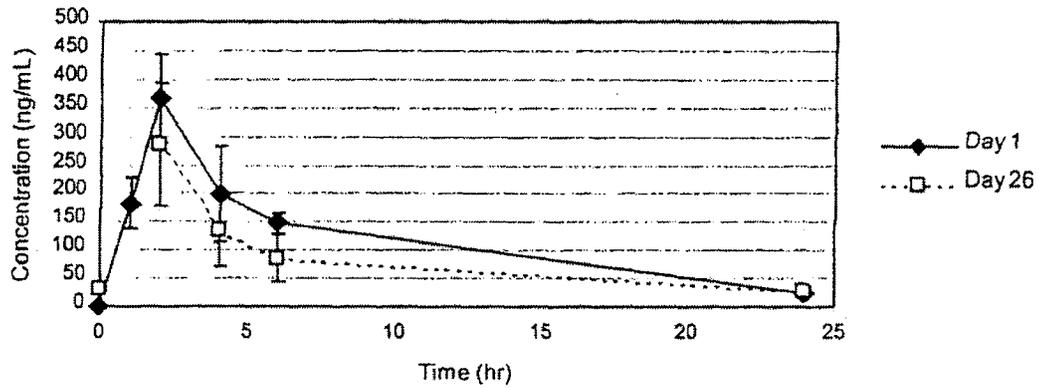


Figure 2b. Mean Concentrations of Tetracaine in Female Rabbits, Non-Abraded

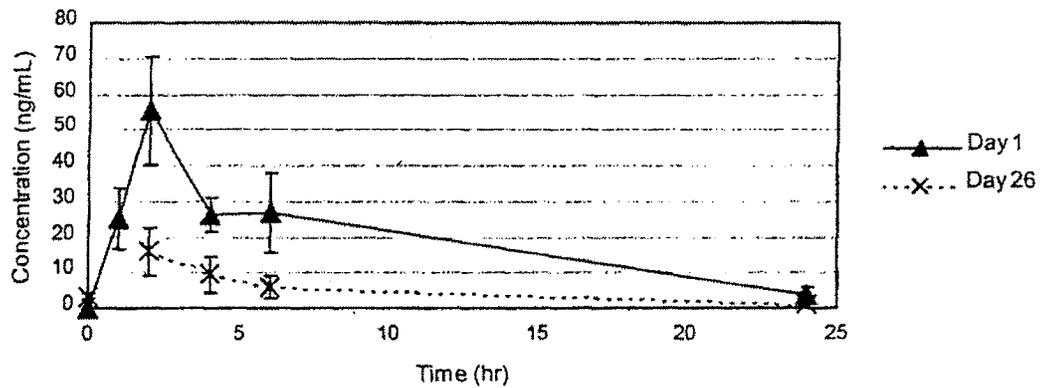
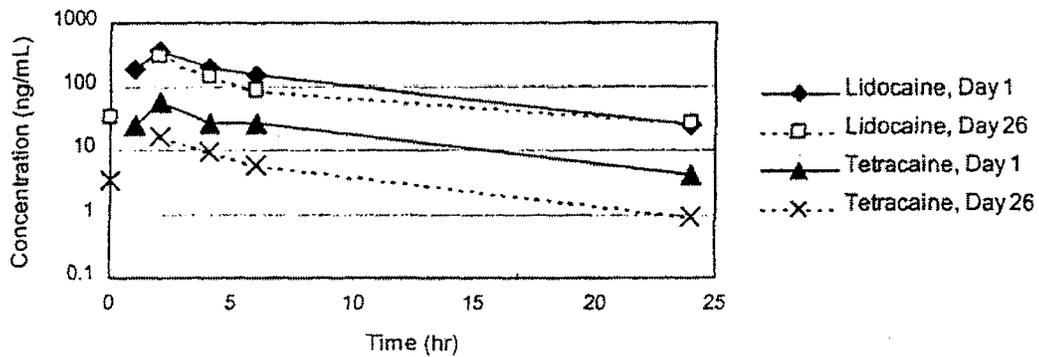


Figure 2c. Mean Concentrations of Lidocaine and Tetracaine in Female Rabbits, Non-Abraded



The figures below depict the mean plasma concentrations of lidocaine and tetracaine in male rats with abraded skin:

Figure 3a. Mean Concentrations of Lidocaine in Male Rabbits, Abraded

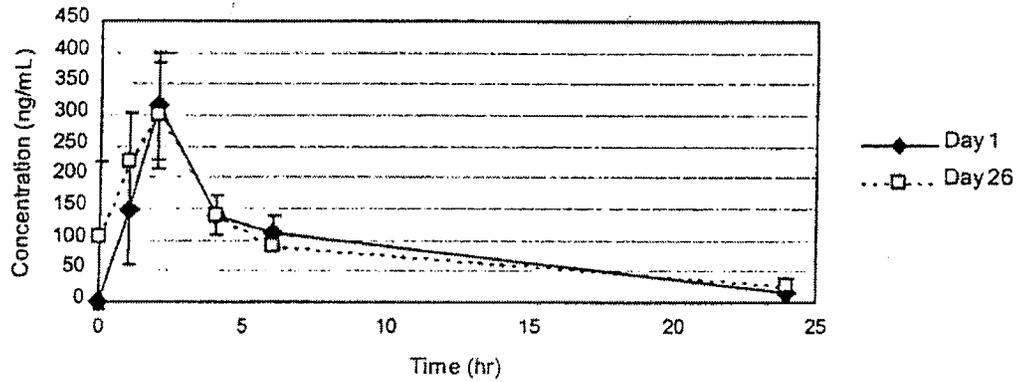


Figure 3b. Mean Concentrations of Tetracaine in Male Rabbits, Abraded

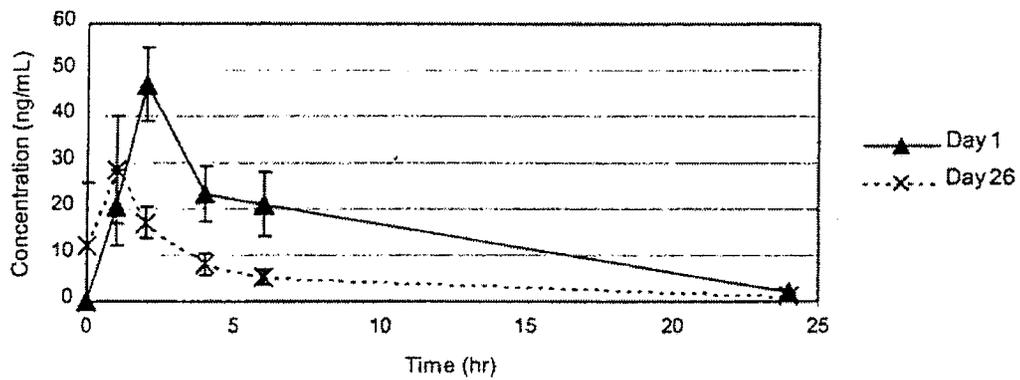
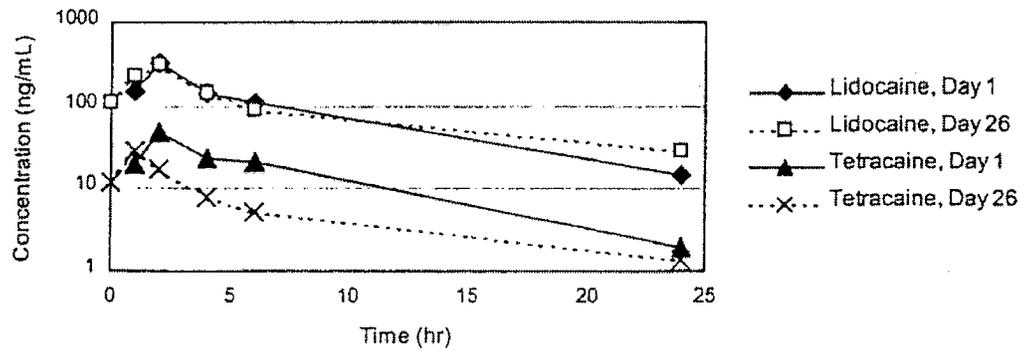


Figure 3c. Mean Concentrations of Lidocaine and Tetracaine in Male Rabbits, Abraded



The figures below depict the mean plasma concentrations of lidocaine and tetracaine in female rats with abraded skin.

Figure 4a. Mean Concentrations of Lidocaine in Female Rabbits, Abraded

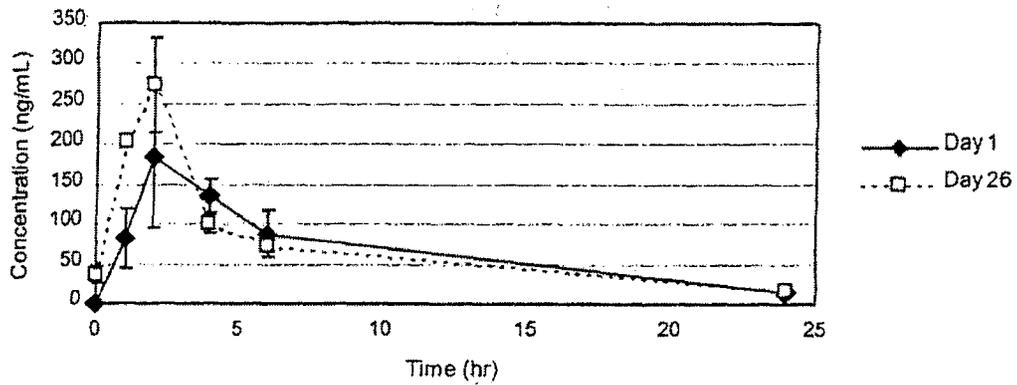


Figure 4b. Mean Concentrations of Tetracaine in Female Rabbits, Abraded

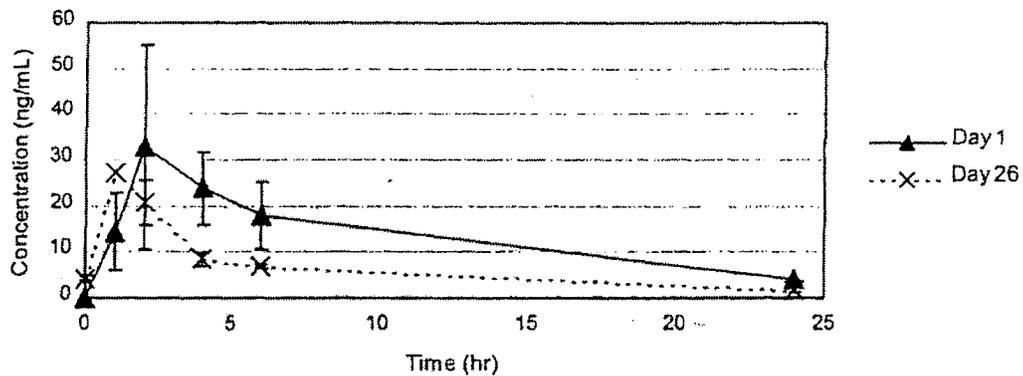
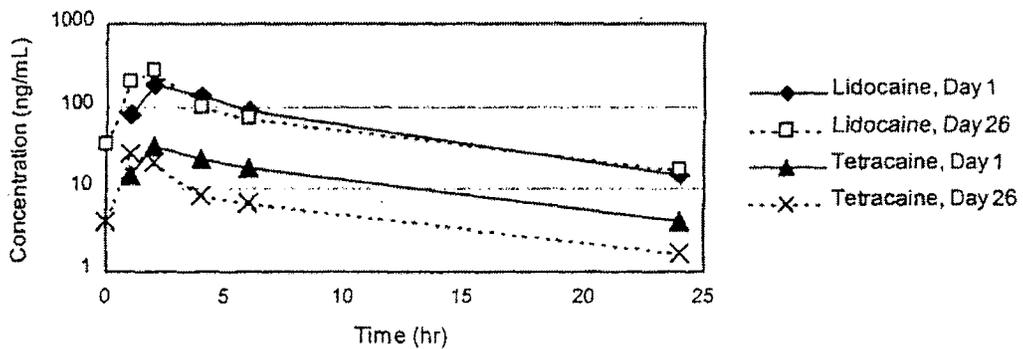


Figure 4c. Mean Concentrations of Lidocaine and Tetracaine in Female Rabbits, Abraded



The table below provides the pharmacokinetics parameters for lidocaine in rabbits treated with 3 S-Caine™ patches applied for 2 hours daily:

Table 3. Pharmacokinetic Parameters for Lidocaine for Rabbits with 3 S-Caine Patches Applied for 2 Hours Daily

Group	Skin	Sex	Day	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (ng/mL)	AUC <sub>0-24</sub> (ng•hr/mL)
				Mean ± SD	Mean ± SD	Mean ± SD
2	Non-abraded	M	1	335 ± 151	2.0 ± 0.0	3,076 *
			26	383 ± 163	2.0 ± 0.0	2,431 *
			Mean % Change	15%		-19% *
		F	1	366 ± 77	2.0 ± 0.0	2,813 ± 454
			26	286 ± 107	2.0 ± 0.0	1,962 ± 794
			Mean % Change	-21%		-32%
4	Abraded	M	1	314 ± 84	2.0 ± 0.0	2,143 ± 496
			26	299 ± 85	2.0 ± 0.0	2,156 ± 186
			Mean % Change	2%		6%
		F	1	185 ± 88	2.7 ± 1.2	1,452 *
			26	277 ± 55	1.7 ± 0.6	1,684 ± 162
			Mean % Change	75%		28% *

n = 3 unless otherwise noted.

\* n = 2

Standard deviation values were not calculated for n < 3.

The table below provides the pharmacokinetics parameters for tetracaine in rabbits treated with 3 S-Caine™ patches applied for 2 hours daily:

Table 4. Pharmacokinetic Parameters for Tetracaine for Rabbits with 3 S-Caine Patches Applied for 2 Hours Daily

Group	Skin	Sex	Day	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (ng/mL)	AUC <sub>0-24</sub> (ng•hr/mL)
				Mean ± SD	Mean ± SD	Mean ± SD
2	Non-abraded	M	1	48.6 ± 37.5	2.7 ± 1.2	640 *
			26	22.8 ± 4.0	1.7 ± 0.6	180 *
			Mean % Change	-38%		-70% *
		F	1	55.3 ± 15.4	2.0 ± 0.0	461 ± 111
			26	16.4 ± 6.5	2.7 ± 1.2	119 ± 52
			Mean % Change	-67% *		-73%
4	Abraded	M	1	46.7 ± 8.1	2.0 ± 0.0	363 ± 96
			26	28.4 ± 11.7	1.0 ± 0.0	140 ± 39
			Mean % Change	-39%		-61%
		F	1	33.9 ± 21.3	2.7 ± 1.2	263 *
			26	28.0 ± 9.9	1.7 ± 0.6	156 ± 7
			Mean % Change	-16%		-38% *

n = 3 unless otherwise noted.

\* n = 2

Standard deviation values were not calculated for n < 3.

As noted in the tables above, plasma levels of lidocaine were approximately 6 times higher than those of tetracaine. There were no differences noted between males and females or between abraded and intact skin.

Other: Dermal Scoring: Each exposure site on the back was evaluated for erythema and edema according to skin reaction scales based upon those of Draize and reproduced below:

Erythema and Eschar Formation	
Score	Observation
0	No erythema
1	Very slight erythema (barely perceptible)
2	Well-defined erythema
3	Moderate to severe erythema
4	Severe erythema (beet redness) to slight eschar formation (injuries in depth)
Maximum possible score = 4	

Edema Formation	
Score	Observation
0	No edema
1	Very slight edema (barely perceptible)
2	Slight edema (edges of area well-defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)
Maximum possible score = 4	

Mean primary irritation index (PII) is the average erythema score after patch removal plus the average edema score after patch removal. The descriptive range is as follows:

Mean Primary Irritation Index	
<u>Descriptive Rating</u>	<u>Range of Index Values</u>
Non-Irritating	0
Mildly Irritating	0.1 - 2
Moderately Irritating	2.1 - 5
Severely Irritating	5.1 - 8

In placebo patch groups, there was a very slight to slight erythema noted in the six application sites (both abraded and non-abraded) in both males and females. The incidence was generally similar between abraded and non-abraded and male and females.

Table 2 Cont. Summary of Dermal Irritation Scores (Incidence At All Sites Combined) - Erythema/Eschar<sup>+</sup> - MALE

Group	Severity	Study Interval (Day)													
		15	16	17	18	19	20	21	22	23	24	25	26	27	28
S-Caine™ Placebo Patch (Non-Abraded)	0-no erythema	11	13	14	18	17	15	12	12	10	12	18	13	12	14
	1-very slight	7	5	4	0	1	3	6	6	8	6	0	5	6	3
	2-slight	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S-Caine™ Patch (Non-Abraded)	0-no erythema	1	1	2	2	2	4	1	0	2	1	5	4	5	1
	1-very slight	5	4	9	10	2	8	7	4	3	4	10	8	5	2
	2-slight	10	8	8	4	9	7	11	15	14	14	6	9	12	13
	3-moderate	6	8	3	5	8	6	6	6	5	7	4	6	4	8
4-severe	5	6	5	6	6	2	2	2	3	1	2	2	1	3	
S-Caine™ Placebo Patch (Abraded)	0-no erythema	12	15	11	18	12	12	12	9	10	9	17	12	13	9
	1-very slight	6	3	7	0	6	6	6	9	8	9	0	6	4	8
	2-slight	0	0	0	0	0	0	0	0	0	0	1	0	1	1
S-Caine™ Patch (Abraded)	0-no erythema	3	3	2	8	1	5	1	0	3	1	5	4	1	1
	1-very slight	4	6	5	1	1	6	4	6	4	4	0	2	2	1
	2-slight	3	3	4	3	6	2	10	5	4	6	6	5	8	5
	3-moderate	4	4	4	4	6	5	3	3	4	2	3	5	5	8
4-severe	4	2	3	2	4	0	0	2	3	5	4	2	1	3	

+ - Number of times observed

Table 2 Summary of Dermal Irritation Scores (Incidence At All Sites Combined) - Erythema/Eschar<sup>+</sup> - MALE

Group	Severity	Study Interval (Day)													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
S-Caine™ Placebo Patch (Non-Abraded)	0-no erythema	6	12	18	18	17	18	18	9	13	17	10	18	10	9
	1-very slight	0	0	0	0	1	0	0	7	5	1	8	0	8	9
	2-slight	0	0	0	0	0	0	0	2	0	0	0	0	0	0
S-Caine™ Patch (Non-Abraded)	0-no erythema	7	15	17	19	21	24	19	8	5	13	6	6	4	3
	1-very slight	2	3	10	8	6	1	6	10	12	7	7	6	6	11
	2-slight	0	0	0	0	0	2	1	5	7	6	7	8	8	4
	3-moderate	0	0	0	0	0	0	1	4	3	1	5	6	7	3
4-severe	0	0	0	0	0	0	0	0	0	0	2	1	2	6	
S-Caine™ Placebo Patch (Abraded)	0-no erythema	6	11	18	18	18	18	18	9	7	18	13	16	9	11
	1-very slight	0	1	0	0	0	0	0	7	10	0	5	2	9	7
	2-slight	0	0	0	0	0	0	0	2	1	0	0	0	0	0
S-Caine™ Patch (Abraded)	0-no erythema	5	10	17	15	13	18	10	8	4	11	8	7	3	6
	1-very slight	1	2	1	3	5	0	6	5	6	3	3	1	7	3
	2-slight	0	0	0	0	0	0	2	2	6	3	3	4	3	2
	3-moderate	0	0	0	0	0	0	0	3	2	0	2	5	3	3
4-severe	0	0	0	0	0	0	0	0	0	1	2	1	2	4	

+ - Number of times observed

**Table 2 Cont. Summary of Dermal Irritation Scores (Incidence At All Sites Combined) - Erythema/Eschar<sup>+</sup> - FEMALE**

Group	Severity	Study Interval (Day)													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
S-Caine™ Placebo Patch (Non-Abraded)	0-no erythema	6	11	17	18	18	18	18	11	12	18	11	16	5	7
	1-very slight	0	1	1	0	0	0	0	5	6	0	7	2	13	11
	2-slight	0	0	0	0	0	0	0	2	0	0	0	0	0	0
S-Caine™ Patch (Non-Abraded)	0-no erythema	8	11	18	15	15	25	14	8	5	4	6	3	2	4
	1-very slight	1	6	8	11	11	1	11	5	8	17	4	5	2	1
	2-slight	0	1	1	1	1	0	1	13	12	4	9	14	14	8
	3-moderate	0	0	0	0	0	1	1	1	1	1	6	4	4	9
4-severe	0	0	0	0	0	0	0	0	1	1	2	1	5	5	
S-Caine™ Placebo Patch (Abraded)	0-no erythema	6	12	17	18	18	18	18	9	10	17	13	12	9	9
	1-very slight	0	0	1	0	0	0	0	7	5	1	4	4	9	9
	2-slight	0	0	0	0	0	0	0	2	3	0	1	2	0	0
S-Caine™ Patch (Abraded)	0-no erythema	7	15	25	26	28	31	16	10	6	14	13	9	6	7
	1-very slight	4	9	9	10	5	1	14	10	10	7	3	2	7	4
	2-slight	1	0	2	0	3	2	5	6	11	11	7	14	8	8
	3-moderate	0	0	0	0	0	2	1	10	8	3	7	9	10	11
4-severe	0	0	0	0	0	0	0	0	1	1	6	2	5	6	

+ - Number of times observed

**Table 2 Cont. Summary of Dermal Irritation Scores (Incidence At All Sites Combined) - Erythema/Eschar<sup>+</sup> - FEMALE**

Group	Severity	Study Interval (Day)													
		15	16	17	18	19	20	21	22	23	24	25	26	27	28
S-Caine™ Placebo Patch (Non-Abraded)	0-no erythema	13	13	10	18	14	11	13	7	11	11	16	14	14	16
	1-very slight	4	5	8	0	4	7	5	11	7	7	2	4	3	1
	2-slight	1	0	0	0	0	0	0	0	0	0	0	0	1	1
S-Caine™ Patch (Non-Abraded)	0-no erythema	2	4	5	7	5	6	2	1	3	0	11	11	10	10
	1-very slight	3	3	7	6	2	8	11	9	12	11	6	5	3	4
	2-slight	8	9	5	7	7	6	9	9	4	11	3	6	9	7
	3-moderate	10	4	4	1	6	3	1	3	3	1	1	2	2	3
4-severe	4	7	6	6	7	4	4	5	5	4	8	3	3	3	
S-Caine™ Placebo Patch (Abraded)	0-no erythema	10	14	9	18	14	8	12	10	10	10	12	9	11	10
	1-very slight	8	4	9	0	4	10	6	8	8	8	0	3	1	2
S-Caine™ Patch (Abraded)	0-no erythema	3	4	2	7	5	6	3	6	6	4	10	8	4	9
	1-very slight	6	7	9	12	1	10	10	3	6	10	7	7	8	7
	2-slight	14	9	7	10	12	7	10	13	10	11	7	13	16	14
	3-moderate	7	7	8	0	11	10	10	12	10	8	8	5	5	3
4-severe	6	9	10	7	7	3	3	2	4	3	4	3	3	3	

+ - Number of times observed

For the S-Caine™ patch treated groups, there was a very slight to slight erythema noted in male and female animals in both the non-abraded and abraded S-Caine™ Patch groups during the initial treatment days. Moderate erythema was noted at one site in one male (non-abraded) and one site in one female (non-abraded) and two sites in two females (abraded) during week 1. Very slight to severe erythema was seen in most application sites in male and female non-abraded and abraded S-Caine™ patch sites after the first week of the study. The incidence and severity was greater in females than in males.

No edema was observed in male and female non-abraded and abraded placebo patch sites under the conditions tested. There was a low incidence of very slight to slight edema in males and females in both the abraded and non-abraded groups.

The overall Mean Primary Irritation Index for Weeks 1 through 28 is given below.

Mean Primary Irritation Index		
	Males	Females
S-Caine™ Placebo Patch (Non-Abraded)	0.21	0.25
S-Caine™ Patch (Non-Abraded)	1.59	1.58
S-Caine™ Placebo Patch (Abraded)	0.26	0.26
S-Caine™ Patch (Abraded)	1.49	1.63

These data indicate that the severity of the irritation was greater in the S-Caine™ patch group compared to placebo regardless of the site of application being intact or abraded.

**Histopathology inventory (optional)**

<b>Study</b>	<b>925-004</b>
<b>Species</b>	<b>Rabbit</b>
Adrenals	*X
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	*X
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	*X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	X
Heart	*X
Ileum	X
Injection site	
Jejunum	X
Kidneys	*X
Lachrymal gland	
Larynx	
Liver	*X
Lungs	X
Lymph nodes, ileococcolic	X

Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	
Mammary Gland	X
Nasal cavity	
Optic nerves	
Ovaries	*X
Pancreas	X
Parathyroid	
Peripheral nerve	
Pharynx	
Pituitary	*X
Prostate	X
Rectum	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord (Cervical, lumbar, thoracic)	X
Spleen	*X
Sternum	
Stomach	X
Testes	*X
Thymus	X
Thyroid	*X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	

X, histopathology performed

\*, organ weight obtained

#### 2.6.6.4 Genetic toxicology:

No new studies were submitted for review. The studies below were previously reviewed by R. Daniel Mellon, for the first cycle of Zars' Synera submission and reproduced here.

#### Study title: Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with Lidocaine Base

**Key findings:** Under the test conditions, lidocaine base was not mutagenic in the bacterial reverse mutation assay in the tested species examined.

Study no.: 23840-0-409OECD  
Volume #, and page #: Volume 12, Page 1

**Conducting laboratory and location:**

**Date of study initiation:** May 13, 2002  
**GLP compliance:** Yes  
**QA reports:** yes ( X ) no ( )  
**Drug, lot #, and % purity:** Lidocaine base, Lot # 811D0013 (expiration date of June 15, 2005), purity was verified by the sponsor.

**Methods**

Tester strains were exposed to the test article via the plate incorporation method originally described by Ames. In the plate incorporation methodology, test article, tester strain and the S9 mix were combined in molten agar which was overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. All doses of the test article, vehicle control and positive controls were plated in triplicate. The condition of the bacterial lawn was evaluated macroscopically and microscopically (dissecting microscope) for indications of cytotoxicity and test article precipitate. Revertant colonies were counted by automated colony counter or by hand.

The acceptable ranges for spontaneous revertants (negative controls ranges) in protocol are listed in the table below:

Strain	Low-High
TA98	8-60
TA100	60-240
TA1535	4-45
TA1537	2-25
WP2uvrA	5-40

A positive result with tester strains TA98, TA100 and WP2uvrA was obtained when the test article produced at least a 2-fold increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control. This increase had to be accompanied by a dose response to increasing concentrations of test article. A positive response for

tester strains TA1535 and TA1537 was obtained when the test article produce at least a 3-fold increase in the mean revertants per plate over the mean revertants in the vehicle control. This increase had to show evidence of a dose response.

Strains/species/cell line: *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 (histidine auxotrophs) and *Escherichia coli* WP2uvrA (tryptophan auxotroph).

Doses used in definitive study: 33.3, 100, 333, 1000, 3330 and 5000 µg/plate with and without S9 for all 5 tester strains for both the initial mutagenicity assay and the independent confirmatory experiment.

Basis of dose selection: Dose range finding assay on lidocaine using tester strain TA100 (generally representative of the other strains) and WP2uvrA. The test article was freely soluble at all doses and conditions evaluated. Ten doses were tested, from 6.67 to 5000 µg/plate both with and without metabolic activation (S9). Evaluation of growth inhibition was completed by a decreased number of revertant colonies per plate and/or thinning or disappearance of the bacterial background lawn. Growth inhibition (number of revertants per plate) was observed in tester strain WP2uvrA at 5000 µg/plate with S9 (↓68%). The background lawn was considered to be reduced in tester strain TA100 at concentrations of ≥

3330 µg/plate in the absence of S9. This reduction appeared to be in relation to a slight stimulation over the 333 to 1000 µg/plate cultures while comparable to the appearance of the vehicle control lawns.

Negative controls: Vehicle control used a 50 µl aliquot of DMSO (the highest concentration used for the test article dilution).

Positive controls: The positive controls for the assay are summarized in the sponsor's table below:

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	-	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	-	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	-	ICR-191	2.0
WP2 <sub>uvrA</sub>	+	2-aminoanthracene	25.0
WP2 <sub>uvrA</sub>	-	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: Plates were incubated for 52 ± 4 hours at 37°C.

## Results

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

Criteria for a valid assay included validation of tester strain integrity and acceptable levels of spontaneous revertants. The positive controls must produce at least a 3-fold increase over the mean value of the vehicle control. Upon review, the study appears to be valid for the following reasons: 1) the methodology was consistent with currently acceptable protocols, 2) the positive controls produced a clear increase in the number of revertants per plate compared to the vehicle control group 3) the vehicle control group levels were within historical control ranges, and 4) although there were slight increases in lidocaine treated bacteria, there was no evidence of a dose-dependent alteration in the number of revertants per plate.

Study outcome: The results of the mutagenicity assay are summarized in sponsor's table 4 reproduced below. Briefly,

**Table 4 : Mutagenicity Assay Results – Summary**

Test Article ID: Lidocaine Base

Experiment ID: 23840-B1

Date Plated: 11-Jun-02

Vehicle: DMSO

Date Counted: 17-Jun-02

Plating Aliquot: 50 µL

Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn <sup>a</sup>	
	TA98		TA100		TA1535		TA1537		WP2uvrA			
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.		
Microsomes: Rat Liver												
Vehicle Control	18	5	67	3	19	2	8	2	22	2	N	
Test Article	33.3 µg	26	7	70	8	16	2	10	4	18	7	N
	100 µg	25	5	66	6	20	6	9	2	24	7	N
	333 µg	22	4	72	6	16	9	9	2	17	6	N
	1000 µg	26	11	79	16	17	1	7	4	14	4	N
	3330 µg	23	6	88	21	16	3	7	2	19	6	N
	5000 µg	26	3	88	8	11	1	5	4	13	5	N
Positive Control <sup>b</sup>		366	41	254	37	86	18	70	14	480	19	N
Microsomes: None												
Vehicle Control	12	6	99	12	12	4	8	3	18	1	N	
Test Article	33.3 µg	17	7	105	7	13	2	9	4	14	4	N
	100 µg	15	3	87	3	17	4	9	3	15	1	N
	333 µg	14	5	87	12	14	3	8	1	16	3	N
	1000 µg	15	2	105	2	19	1	9	3	15	1	N
	3330 µg	14	2	104	21	29	9	8	3	10	3	N
	5000 µg	16	2	95	1	20	4	7	4	12	4	N
Positive Control <sup>c</sup>		226	35	901	-	702	43	878	150	152	18	N

<sup>a</sup> Background Lawn Evaluation Codes:

N = normal R = reduced O = obscured A = absent P = precipitate

<sup>b</sup> TA98	benzo[a]pyrene	2.5 µg/plate	<sup>c</sup> TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2uvrA	2-aminoanthracene	25.0 µg/plate	WP2uvrA	4-nitroquinolone-N-oxide	1.0 µg/plate

The results of the confirmatory assay were in agreement with the mutagenicity assay, i.e., under the conditions of the assay, lidocaine was not mutagenic in the bacterial reverse mutation assay.

**Study title: Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with Tetracaine Base**

**Key findings:** Under the test conditions, tetracaine base was not mutagenic in the bacterial reverse mutation assay in the tested species examined.

**Study no.:** 23841-0-409OECD  
**Volume #, and page #:** Volume 12, Page 32  
**Conducting laboratory and location:** \_\_\_\_\_  
**Date of study initiation:** May 13, 2002  
**GLP compliance:** Yes  
**QA reports:** yes (X) no ( )  
**Drug, lot #, and % purity:** Tetracaine base, Lot # 721725 (expiration date of August 3, 2005), purity was verified by the sponsor.

### Methods

Tester strains were exposed to the test article via the plate incorporation method originally described by Ames. In the plate incorporation methodology, test article, tester strain and the S9 mix were combined in molten agar which was overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. All doses of the test article, vehicle control and positive controls were plated in triplicate. The condition of the bacterial lawn was evaluated macroscopically and microscopically (dissecting microscope) for indications of cytotoxicity and test article precipitate. Revertant colonies were counted by automated colony counter or by hand.

The acceptable ranges for spontaneous revertants (negative controls ranges) in \_\_\_\_\_ protocol are listed in the table below:

Strain	Low-High
TA98	8-60
TA100	60-240
TA1535	4-45
TA1537	2-25
WP2 <sub>uvrA</sub>	5-40

A positive result with tester strains TA98, TA100 and WP2<sub>uvrA</sub> was obtained when the test article produced at least a 2-fold increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control. This increase had to be accompanied by a dose response to increasing concentrations of test article. A positive response for

tester strains TA1535 and TA1537 was obtained when the test article produce at least a 3-fold increase in the mean revertants per plate over the mean revertants in the vehicle control. This increase had to show evidence of a dose response.

Strains/species/cell line: *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 (histidine auxotrophs) and *Escherichia coli* WP2<sub>uvrA</sub> (tryptophan auxotroph).

Doses used in definitive study: 33.3, 100, 333, 1000, 3330 and 5000 µg/plate tetracaine both with and without S9 for all 5 tester strains for both the initial mutagenicity assay and the independent confirmatory experiment.

Basis of dose selection: Dose range finding assay on lidocaine using tester strain TA100 (generally representative of the other strains) and WP2<sub>uvrA</sub>. The test article was freely soluble at all doses and conditions evaluated. Ten doses were tested, from 6.67 to 5000 µg/plate both with and without metabolic activation (S9). Evaluation of growth inhibition was completed by a decreased number of revertant colonies per plate and/or thinning or disappearance of the bacterial background lawn. The background lawn was considered to be

reduced in both tester strains at the one or two highest concentrations of tetracaine tested both with and without S9. Growth inhibition was noted in strain TA100 at concentrations of 3330 and 5000 µg/plate without S9 (↓52% and ↓93%, respectively) and at 5000 µg/plate (↓85%) with S9. Growth inhibition (number of revertants per plate) was observed in tester strain WP2uvrA at 5000 µg/plate with S9 (↓89%). Without S9, the number of revertants per plate was reduced at tetracaine concentrations of 3330 and 5000 µg/plate by 100% and 78%, respectively. The background lawn was considered to be reduced in tester strain WP2uvrA only at the concentration of 5000 µg/plate in the absence of S9. The apparent thinning of the background lawns observed in the absence of a decrease in revertant frequency toxicity is generally not considered to be an indication of toxicity.

Negative controls: Vehicle control used a 50 µl aliquot of DMSO (the highest concentration used for the test article dilution).

Positive controls: The positive controls for the assay are summarized in the sponsor's table below:

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	-	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	-	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	-	ICR-191	2.0
WP2uvrA	+	2-aminoanthracene	25.0
WP2uvrA	-	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: Plates were incubated for 52 ± 4 hours at 37°C.

## Results

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

— criteria for a valid assay included validation of tester strain integrity and acceptable levels of spontaneous revertants. The positive controls must produce at least a 3-fold increase over the mean value of the vehicle control. Upon review, the study appears to be valid for the following reasons: 1) the methodology was consistent with currently acceptable protocols, 2) the positive controls produced a clear increase in the number of revertants per plate compared to the vehicle control group 3) the vehicle control group levels used for evaluation purposes were within historical control ranges, and 4) there was no evidence of a dose-dependent alteration in the number of revertants per plate.

Study outcome: The revertant frequencies for all doses of tetracaine base, both with and without S9, were comparable to or less than the vehicle controls. The confirmatory assay

produced similar results. In the confirmatory study, the mean vehicle control value for tester strain TA1537 with S9 was above acceptable limits and therefore not-scored. The effects of tetracaine base were re-evaluated under these conditions with no evidence of mutagenicity. The results of the definitive study are reproduced below from sponsor's table 4.

**Table 4 : Mutagenicity Assay Results – Summary**

Test Article ID: Tetracaine Base

Experiment ID: 23841-B1

Date Plated: 11-Jun-02

Vehicle: DMSO

Date Counted: 17-Jun-02

Plating Aliquot: 50 µL

Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn <sup>a</sup>	
	TA98		TA100		TA1535		TA1537		WP2uvrA			
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.		
Microsomes: Rat Liver												
Vehicle Control		20	2	92	6	11	2	10	3	22	7	N
Test Article	33.3 µg	22	6	99	11	13	3	10	2	22	5	N
	100 µg	23	6	78	8	13	6	12	2	17	4	N
	333 µg	19	2	74	5	13	1	10	2	20	6	N
	1000 µg	20	5	84	5	12	4	8	1	12	6	N
	3330 µg	15	3	42	4	8	3	5	2	4	2	N/R <sup>d</sup>
	5000 µg	5	2	8	2	7	5	6	4	4	1	N/R <sup>d</sup>
Positive Control <sup>b</sup>		404	56	381	68	114	10	109	16	638	44	N
Microsomes: None												
Vehicle Control		11	2	77	6	38	8	7	2	16	7	N
Test Article	33.3 µg	10	1	90	18	47	6	7	3	17	4	N
	100 µg	9	2	108	5	37	8	12	2	12	2	N/R <sup>e</sup>
	333 µg	14	4	98	6	28	1	6	3	13	4	N/R <sup>e</sup>
	1000 µg	9	1	73	21	36	14	5	1	9	3	N/R <sup>f</sup>
	3330 µg	9	7	0	0	0	0	0	0	5	2	R
	5000 µg	0	1	0	0	0	0	0	0	1	1	R
Positive Control <sup>f</sup>		174	31	906	35	769	64	753	22	98	21	N

<sup>a</sup> Background Lawn Evaluation Codes:

N = normal    R = reduced    O = obscured    A = absent    P = precipitate

<sup>b</sup> TA98	benzo[a]pyrene	2.5 µg/plate	<sup>c</sup> TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2uvrA	2-aminoanthracene	25.0 µg/plate	WP2uvrA	4-nitroquinolone-N-oxide	1.0 µg/plate

<sup>d</sup> The first entry is the lawn evaluation for tester strains TA1535 and WP2uvrA.

The second entry is the lawn evaluation for tester strains TA98, TA100, and TA1537.

<sup>e</sup> The first entry is the lawn evaluation for tester strains TA98, TA100, and WP2uvrA.

The second entry is the lawn evaluation for tester strains TA1535 and TA1537.

<sup>f</sup> The first entry is the lawn evaluation for tester strain WP2uvrA.

The second entry is the lawn evaluation for tester strains TA98, TA100, TA1535, and TA1537.

**Study title: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Lidocaine Base**

**Key findings:** Under the assay conditions, lidocaine base did not produce alterations in either chromosome structure or number in CHO cells.

**Study no.:** 23840-0-437OECD  
**Volume #, and page #:** Volume 12, Page 63  
**Conducting laboratory and location:** \_\_\_\_\_  
**Date of study initiation:** May 10, 2002  
**GLP compliance:** Yes  
**QA reports:** yes ( X ) no ( )  
**Drug, lot #, and % purity:** Lidocaine base, Lot # 811D0013

**Methods**

The effect of lidocaine base to induce chromosome aberrations in cultured Chinese hamster ovary cells was examined in vitro both with and without metabolic activation. The initial assay examined a treatment period of 3 hours with and without metabolic activation. The confirmatory assay examined the effect of lidocaine for 20 hours without metabolic activation and 3 hours with metabolic activation. Prior to harvest of the cultures, visual observations of cytotoxicity were made, including an assessment of the percent confluence of the cell monolayer and determination of the mitotic index. Following harvest of cells, only cells with good morphology with the number of centromeres equal to the modal number  $21 \pm 2$  (range 19-23) were analyzed. One hundred cells, if possible, from each replicate culture from 4 concentrations of test article, the vehicle, negative and one dose from the positive control cultures were analyzed for chromosomal aberrations. At least 25 cells were analyzed from cultures that had greater than 25% of cells with one or more aberrations. The mitotic index was evaluated from the vehicle control and a range of concentrations by analyzing the number of mitotic cells in 1000 cells and the ratio expressed as a percentage of mitotic cells. All slides were coded prior to analysis.

The assay was considered to be acceptable by the laboratory if: 1) then negative control contains less than ~5% cells with aberrations, 2) the positive control must be significantly higher ( $p \leq 0.01$ ) than the vehicle control, 3) there is an acceptable high concentration tested ( $\geq 50\%$  reduction in mitotic index) or concentration limit reached (10 mM or 5 mg/ml, whichever is lower), or the concentration exceeds the solubility and 4) there are at least 3 concentrations evaluated.

A test article was considered positive for inducing chromosome aberrations if a significant increase ( $p \leq 0.01$ ) in the number of cells with chromosome aberrations is observed at one or more concentrations. A dose response should be observed if a significant increase was noted at one or more concentrations. The linear trend test evaluated the dose responsiveness.

Strains/species/cell line: Chinese Hamster Ovary (CHO) cells (CHO-WBL)

**Doses used in definitive study:** The confirmatory assay tested concentrations of 63.5, 127, 253, 505, 758, 1010, 1260, 1510 and 2010 µg/ml without metabolic activation and 253, 505, 1010, 1260, 1510, 1760, and 2010 µg/ml with metabolic activation.

**Basis of dose selection:** Dose selection was based on initial chromosome aberrations assay both with and without metabolic activation. Solubility of lidocaine in McCoy's 5a culture medium limited the concentrations to be tested. Lidocaine was dissolved in DMSO at a concentration of 10.0 µl/ml. At a dosed concentration of 3930 µg/ml, a precipitate formed. At 1970 µg/ml a precipitate formed but went back into solution with repeated gentle agitation for about 5 minutes and the pH was 7.5 (same as culture medium). At a concentration of 985 µg/ml, a precipitate formed that went back into solution was formed (pH 7.5). For the initial chromosome aberrations assay, concentrations of 20.4, 29.1, 41.5, 59.3, 84.7, 121, 173, 246, 351, 500, 714, 1020, 1460, 2090 and 2990 µg/ml. Reductions of 0%, 0%, 19% and 42% were observed in the mitotic indices of the cultures treated with 500, 714, 1020 and 1460 µg/ml. Chromosome aberrations were analyzed from cultures without metabolic activation treated with 500, 714, 1020 and 1460 µg/ml. At 1460 µg/ml, there was a 42% reduction in mitotic index, indicating inadequate toxicity for a valid assay. In an assay with metabolic activation, precipitation was observed with culture concentrations of 2090 µg/ml and above. Reductions in mitotic indices of 0%, 0%, 0%, 25%, 51% and 57% were observed in cultures treated with 246, 351, 500, 714, 1020 and 1460 µg/ml. Chromosome aberrations were analyzed from cultures treated with 500, 714, 1020 and 1460 µg/ml lidocaine.

**Negative controls:** In non-activation assay, the negative controls were cultures containing only cells and culture medium. Vehicle controls were cultures containing the vehicle for lidocaine, DMSO at 10.0 µL/ml (the highest concentration used in test cultures).

**Positive controls:** The positive control conditions are presented in the table below:

Assay Conditions	Treatment Duration	Positive Control	Concentration (µg/ml)
+ S9	3 hr	Cyclophosphamide (CP)	5 and 10
- S9	3 hr	Mitomycin C (MMC)	0.75 and 1.5
	20 hr		0.2 and 0.4

**Incubation and sampling times:** CHO cells were grown in McCoy's 5a culture medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin G (100 units/ml) and streptomycin (100 µg/ml) in a humidified incubator at 37°C in an atmosphere of 5% CO<sub>2</sub> in air. For the assay without S9, cultures were incubated in the presence of test article for 3 hours and 20 hours. For the assay with S9, cultures were incubated for 3 hours with test article, then washed with buffered saline, re-fed with new medium, and incubated for the remainder of the culture period. For the last 2 ± 0.5 hours of incubation, Colcemid® (0.1 µg/ml). Prior to harvest, cells were examined visually for evidence of cytotoxicity, percent confluence of the cell monolayer, presence of mitotic (large rounded cells) or dead cells floating in the medium. Cultures were then trypsonized for collection, swollen with 75 mM KCl, fixed in methanol:glacial acetic acid (3:1, v/v) fixative, stained with Giemsa solution for analysis of mitotic index and chromosomal aberrations.

**Results**

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

The study appears valid for the following reasons: 1) The positive controls cyclophosphamide and mitomycin C produced clear increases in chromosome aberrations; 2) the assay was conducted according to standard protocols, 3) the concentrations tested were adequate to produce a valid assay, and 4) the negative controls produced an acceptable level of chromosomal aberrations.

Study outcome: The effects of a 3 hour treatment of CHO cells lidocaine in the presence of metabolic activation are presented in the sponsor's Table 4 depicted below. As noted in Table 4, 6 and 8 on the following pages, there were no significant increases in chromosome abnormalities following a 3 or 20 hour exposure to lidocaine in the absence of metabolic activation or 3 hour exposure to lidocaine in the presence of metabolic activation, respectively.

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**Table 8:**  
**Chromosome Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation -**  
**3.0 Hour Treatment, 19.8 Hour Harvest**

Assay No.: 23840	Trial No.: C1	Date: 07/02/02	Lab No.: CY8032	Test Article: Lidocaine Base	#	MITOTIC INDEX	ENDO-REDUPLICATION	# POLY-PLOID CELLS	JUDGE-MENT (+/-)	NUMBERS AND PERCENTAGES (% OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS)							JUDGE-MENT (+/-)
										CELLS SCORED	CELLS	CELLS	Simple Breaks	chic	chre	mab	
CONTROLS																	
NEGATIVE: McCoy's 5a																	
					A 100	1	0	0	0	2	1	1	1	3			
					B 100	0	0	0	0	2	1	1	1	0			
					TOTAL 200					4	2	2	2	3			
					AVERAGE %	0.5	0.0	0.0	0.0	1.0	0.5	0.5	0.5	1.5			
VEHICLE: DMSO																	
					A 100	0	0	0	0	3	1	1	1	1			
					B 100	0	0	0	0	3	1	1	1	3			
					TOTAL 200					6	2	2	2	4			
					AVERAGE %	0.0	0.0	0.0	0.0	1.5	0.5	0.5	0.5	2.0			
POSITIVE: CP																	
					A 50	1	0	0	0	2	17	4	2	20			
					B 50	0	1	1	1	4	14	8	1	22			
					TOTAL 100					6	31	12	2	42			
					AVERAGE %	0.5	0.5	0.5	0.5	6.0	31.0	12.0	2.0	42.0	43.0		
TEST ARTICLE																	
					A 100	0	0	0	0	3	1	1	1	1			
					B 100	0	0	0	0	3	1	1	1	4			
					TOTAL 200					6	2	2	2	5			
					AVERAGE %	0.0	0.0	0.0	0.0	1.5	1.0	1.0	1.0	2.5			
1010 µg/mL																	
					A 100	0	0	0	0	1	1	1	1	2			
					B 100	1	0	0	0	1	1	1	1	1			
					TOTAL 200					2	2	2	2	3			
					AVERAGE %	0.5	0.0	0.0	0.0	1.0	0.5	0.5	0.5	1.5			
1260 µg/mL																	
					A 100	1	0	0	0	2	1	1	1	2			
					B 100	2	0	0	0	3	2	2	1	3			
					TOTAL 200					5	3	3	2	5			
					AVERAGE %	1.5	0.0	0.0	0.0	2.5	1.5	1.5	0.5	2.0	4.0		
1510 µg/mL																	
					A 100	2	0	0	0	3	3	3	1	5			
					B 100	1	0	0	0	3	1	1	1	4			
					TOTAL 200					6	4	4	2	9			
					AVERAGE %	1.5	0.0	0.0	0.0	3.0	2.0	2.0	1.0	4.5	5.5		

chre: chromatid exchange  
 chrc: chromosome exchange  
 mab: multiple aberrations, greater than 4 aberrations  
 % Mitotic index reduction as compared to the vehicle control.  
 Significantly greater in % polyploidy and % endoreduplication than the vehicle control, p ≤ 0.01.  
 -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.  
 Significantly greater in -g than the vehicle control, p ≤ 0.01. McCoy's 5a = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

**Study title: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Tetracaine Base**

**Key findings:** The potential clastogenicity of tetracaine base was examined in an *in vitro* chromosome aberrations assay in CHO cells with the following findings.

1. In the absence of metabolic activation, tetracaine did not produce an increase in chromosomal aberrations.
2. In the presence of metabolic activation, tetracaine base produced a significant increase in chromosomal aberrations at a concentration of 300 µg/ml, which was associated with ~85% reduction in cell monolayer confluence. At concentrations of 250 and 300 µg/ml, tetracaine produced a significant increase in endoreduplication compared to controls.
3. The equivocal findings potentially related to excessive cytotoxicity at the 300 µg/ml concentration should be further explored.

**Study no.:** 23841-0-4370ECD  
**Volume #, and page #:** Volume 12, Page 97  
**Conducting laboratory and location:** \_\_\_\_\_  
**Date of study initiation:** May 10, 2002  
**GLP compliance:** Yes  
**QA reports:** yes ( X ) no ( )  
**Drug, lot #, and % purity:** Tetracaine base, Lot No. 721725

**Methods**

The effect of tetracaine base to induce chromosome aberrations in cultured Chinese hamster ovary cells was examined *in vitro*. The initial assay employed a treatment period of 3 hours with and without metabolic activation. The confirmatory assay examined the effect of tetracaine for 20 hours without metabolic activation and 3 hours with metabolic activation. Prior to harvest of the cultures, visual observations of cytotoxicity were made, including an assessment of the percent confluence of the cell monolayer and determination of the mitotic index. Following harvest of cells, only cells with good morphology with the number of centromeres equal to the modal number  $21 \pm 2$  (range 19-23) were analyzed. One hundred cells, if possible, from each replicate culture from 4 concentrations of test article, the vehicle, negative and one dose from the positive control cultures were analyzed for chromosome aberrations. At least 25 cells were analyzed from cultures that had greater than 25% of cells with one or more aberrations. The mitotic index was evaluated from the vehicle control and a range of concentrations by analyzing the number of mitotic cells in 1000 cells and the ratio expressed as a percentage of mitotic cells. All slides were coded prior to analysis.

The assay was considered to be acceptable by the laboratory if: 1) then negative control contains less than ~5% cells with aberrations, 2) the positive control must be significantly higher ( $p \leq 0.01$ ) than the vehicle control, 3) there is an acceptable high concentration tested ( $\geq 50\%$  reduction in mitotic index) or concentration limit reached (10 mM or 5 mg/ml, whichever is lower), or the concentration exceeds the solubility and 4) there are at least 3 concentrations evaluated.

A test article was considered positive for inducing chromosome aberrations if a significant increase ( $p \leq 0.01$ ) in the number of cells with chromosomal aberrations is observed at one or more concentrations. A dose response should be observed if a significant increase was noted at one or more concentrations. The linear trend test evaluated the dose responsiveness.

Strains/species/cell line: Chinese Hamster Ovary (CHO) cells (CHO-WBL).

Doses used in definitive study: The confirmatory assay tested concentrations of 9.4, 18.8, 37.5, 75.0, 150, 200, 250, 300 and 350  $\mu\text{g/ml}$  tetracaine in the absence of metabolic activation. Dosing for studies incorporating metabolic activation were 37.5, 75.0, 150, 200, 250, 300 and 350  $\mu\text{g/ml}$  tetracaine with metabolic activation.

Basis of dose selection: Dose selection was based on initial chromosome aberrations assay both with and without metabolic activation. Tetracaine base is insoluble in water at  $\geq 50$   $\text{mg/ml}$ . The solubility of tetracaine in McCoy's 5a culture medium limited the concentrations to be tested. Tetracaine was dissolved in DMSO at a concentration of 10.0  $\mu\text{l/ml}$ . At a dosed concentration of 3700  $\mu\text{g/ml}$ , a precipitate formed. At 1850  $\mu\text{g/ml}$  a fine precipitate formed that settled down slowly in the dilution tube and was pH was 7.5 (same as culture medium). At a concentration of 925  $\mu\text{g/ml}$ , a precipitate formed that went back into solution was formed (pH 7.5). For the initial chromosomal aberrations assay, concentrations of 13.7, 19.5, 27.9, 39.8, 56.8, 81.2, 116, 165, 235, 336, 480, 686, 980, 1400 and 2000  $\mu\text{g/ml}$ . Under these conditions, no cells were noted in cultures treated with 980  $\mu\text{g/ml}$  and above. Cultures treated with 336, 480 and 686  $\mu\text{g/ml}$  demonstrated an  $>85\%$  reduction in the cell monolayer confluence. A reduction of dividing cells and  $\sim 55\%$  reduction in the cell monolayer confluence were observed in the cultures treated with 235  $\mu\text{g/ml}$ . A slight reduction in dividing cells and a  $\sim 30\%$  reduction in the cell monolayer confluence were observed in the cultures treated with 165  $\mu\text{g/ml}$ . There was an approximate 15% reduction in the cell monolayer confluence in cultures treated with 116  $\mu\text{g/ml}$  tetracaine. The results of the sponsor's assessment of cytotoxicity are presented in the table below:

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**Table 1:  
Assessment of Toxicity for Chromosome Aberrations Assay - Without Metabolic Activation -  
3.0 Hour Treatment, 20.1 Hour Harvest**

Assay No.: 23841      Trial No.: B1      Date: 06/13/02      Lab No.: CY7052  
Test Article: Tetracaine Base

Treatment		Confluence <sup>a</sup> % Vehicle Control	% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	McCoy's 5a	100	10.8	11.4	11.1	---
Vehicle Control	DMSO	100	13.1	13.9	13.5	0
Test Article	81.2 µg/mL	100	15.3	15.6	15.5	0
	116 µg/mL	86	13.6	14.8	14.2	0
	165 µg/mL	71	13.2	13.9	13.6	0
	235 µg/mL	43	12.0	12.6	12.3	9

<sup>a</sup> This endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.  
McCoy's 5a = culture medium      DMSO = Dimethylsulfoxide

As noted above, reductions of 0%, 0%, 0% and 9% were observed in the mitotic indices of the cultures treated with 81.2, 116, 165 and 235 µg/ml. Chromosome aberrations were analyzed from cultures without metabolic activation treated with 81.2, 116, 165 and 235 µg/ml tetracaine. The concentration of 235 µg/ml tetracaine resulted in a 55% reduction in the cell monolayer confluence, and the concentration of 165 µg/ml produced a 49% reduction in mitotic index. The sponsor indicates that these observations demonstrate adequate toxicity for a valid high concentration for analysis. As these concentrations, there were no significant increases in cells with chromosome aberrations, polyploidy or endoreduplication in the cultures analyzed. The results are reproduced in sponsor's table 2 below:

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cell monolayer confluence were observed in the cultures treated with 336 µg/ml. A reduction of the cell monolayer of ~55% was observed in cultures treated with 235 µg/ml. Reductions in mitotic indices of 18%, 41%, 49% and 36% were observed in cultures treated with 81.2, 116, 165 and 235 µg/ml. The results of the cytotoxicity assessment are presented in sponsor's table 3 below:

**Table 3:  
Assessment of Toxicity for Chromosome Aberrations Assay - With Metabolic Activation -  
3.0 Hour Treatment, 20.1 Hour Harvest**

Assay No.: 23841      Trial No.: B1      Date: 06/13/02      Lab No.: CY7052  
Test Article: Tetracaine Base

Treatment		Confluence* % Vehicle Control	% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	McCoy's 5a	100	8.3	8.9	8.6	---
Vehicle Control	DMSO	100	8.4	8.6	8.5	0
Test Article	81.2 µg/mL	71	7.1	6.8	7.0	18
	116 µg/mL	86	3.9	6.0	5.0	41
	165 µg/mL	71	4.3	4.3	4.3	49
	235 µg/mL	43	4.8	5.9	5.4	36

\* This endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.  
McCoy's 5a = culture medium      DMSO = Dimethylsulfoxide

Based upon these results, chromosome aberrations were analyzed from cultures treated with 81.2, 116, 165 and 235 µg/ml tetracaine.

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**Table 4:**  
**Chromosome Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation -**  
**3.0 Hour Treatment, 20.1 Hour Harvest**

Assay No.: 23841	Trial No.: B1	Date: 06/13/02	Lab No.: CY7052	Test Article: Tetracaine Base	MITOTIC INDEX REDUCTION*	ENDO-REDUCED CELLS	# POLY-PLIOD CELLS	JUDGE-MENT (+/-) <sup>b</sup>	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS					JUDGE-MENT (+/-) <sup>d</sup>
									Gaps	Simple Breaks	chic	chr	mab	
CONTROLS														
NEGATIVE: McCoy's 5a														
			A 100	0	0	0	0					0	0	
			B 100	3	0	0	0					1	1	
			TOTAL 200									1	1	
			AVERAGE %		1.5	0.0	0.0				0.5	0.5	0.5	
VEHICLE: DMSO														
			A 100	0	0	0	0					0	0	
			B 100	0	0	0	0					1	1	
			TOTAL 200									1	1	
			AVERAGE %	0	0.0	0.0	0.0				0.5	0.5	1.0	
POSITIVE: CP														
			A 50	3	0	0	0					12	8	
			B 50	2	0	0	0					11	6	
			TOTAL 100									23	14	
			AVERAGE %		2.5	0.0	0.0				11.0	23.0	14.0	2.0
TEST ARTICLE														
			A 100	0	0	0	0					1	1	
			B 100	0	0	0	0					2	2	
			TOTAL 200									3	3	
			AVERAGE %	18	0.0	0.0	0.0				1.5	1.5	2.0	
116 µg/mL														
			A 100	0	0	0	0					1	1	
			B 100	2	1	1	1					1	1	
			TOTAL 200									2	2	
			AVERAGE %	41	1.0	0.5	0.5				1.0	0.5	0.5	1.5
165 µg/mL														
			A 100	1	0	0	0					2	2	
			B 100	1	0	0	0					3	3	
			TOTAL 200									5	5	
			AVERAGE %	49	1.0	0.0	0.0				2.5	0.5	0.5	3.0
235 µg/mL														
			A 100	2	0	0	0					3	3	
			B 100	3	1	1	1					2	2	
			TOTAL 200									5	5	
			AVERAGE %	36	2.5	0.5	0.5				2.5	1.0	0.5	3.5

chic: chromatid exchange  
 \*% Mitotic index reduction as compared to the vehicle control.  
<sup>b</sup> Significantly greater in % polyploidy and % endoreduplication than the vehicle control, p ≤ 0.01.  
<sup>c</sup> -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.  
<sup>d</sup> Significantly greater in -g than the vehicle control, p ≤ 0.01. McCoy's 5a = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

Negative controls: In non-activation assay, the negative controls were cultures containing only cells and culture medium. Vehicle controls were cultures containing the vehicle for

tetracaine, DMSO at 10.0  $\mu\text{L/ml}$  (the highest concentration of DMSO that was used in any test culture).

Positive controls: The positive control conditions are presented in the table below:

Assay Conditions	Treatment Duration	Positive Control	Concentration ( $\mu\text{g/ml}$ )
+ S9	3 hr	Cyclophosphamide (CP)	5 and 10
- S9	3 hr 20 hr	Mitomycin C (MMC)	0.75 and 1.5 0.2 and 0.4

Incubation and sampling times: CHO cells were grown in McCoy's 5a culture medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin G (100 units/ml) and streptomycin (100  $\mu\text{g/ml}$ ) in a humidified incubator at 37°C in an atmosphere of 5%  $\text{CO}_2$  in air. For the assay without S9, cultures were incubated in the presence of test article for 3 hours and 20 hours. For the assay with S9, cultures were incubated for 3 hours with test article, then washed with buffered saline, re-fed with new medium, and incubated for the remainder of the culture period. For the last  $2 \pm 0.5$  hours of incubation, Colcemid® (0.1  $\mu\text{g/ml}$ ). Prior to harvest, cells were examined visually for evidence of cytotoxicity, percent confluence of the cell monolayer, presence of mitotic (large rounded cells) or dead cells floating in the medium. Cultures were then trypsonized for collection, swollen with 75 mM KCl, fixed in methanol:glacial acetic acid (3:1, v/v) fixative, stained with Giemsa solution for analysis of mitotic index and chromosomal aberrations.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears valid for the following reasons: 1) The positive controls cyclophosphamide and mitomycin C produced clear increases in chromosomal aberrations; 2) the assay was conducted according to standard protocols, 3) the concentrations tested were adequate to produce a valid assay, and 4) the negative controls produced an acceptable level of chromosomal aberrations.

Study outcome: In the confirmatory assay without metabolic activation, tetracaine concentrations of 300 and 350  $\mu\text{g/ml}$  produced > 85% reduction in the cell culture monolayer confluence, dead cells, debris and no dividing cells. Concentration of 250  $\mu\text{g/ml}$  resulted in ~70% reduction in the cell monolayer confluence, dead cells and a reduction in dividing cells. The concentration of 200  $\mu\text{g/ml}$  tetracaine produced unhealthy cell monolayers with ~45% reduction in dividing cells. sponsor's Table 5, reproduced below, indicates that concentrations of tetracaine of 75 mg/ml and above produced significant toxicity (> 50%) as measured by a reduction in the mitotic index.

**Table 5:**  
**Assessment of Toxicity for Chromosome Aberrations Assay - Without Metabolic Activation -**  
**19.8 Hour Treatment, 19.8 Hour Harvest**

Assay No.: 23841      Trial No.: C1      Date: 07/02/02      Lab No.: CY8052  
 Test Article: Tetracaine Base

	Treatment		Confluence* % Vehicle Control	% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	McCoy's 5a		100	9.7	10.6	10.2	---
Vehicle Control	DMSO	10.0 µL/mL	100	9.9	9.8	9.9	0
Test Article		9.40 µg/mL	100	8.3	8.8	8.6	13
		18.8 µg/mL	100	6.3	7.4	6.9	30
		37.5 µg/mL	100	6.2	6.7	6.5	34
		75.0 µg/mL	100	3.9	5.0	4.5	55
		150 µg/mL	100	3.7	4.1	3.9	61
		200 µg/mL	57	1.8	2.4	2.1	79
		250 µg/mL	29	0.0	0.2	0.1	99
		300 µg/mL	14	0.0	0.0	0.0	100

\* This endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.  
 McCoy's 5a = culture medium      DMSO = Dimethylsulfoxide

Based upon the cytotoxicity results above, chromosomal aberrations from cultures treated with 9.4, 18.8, 37.5 and 75 µg/ml tetracaine were scored. As shown in sponsor's table 8 below, there were no significant increases in chromosomal aberrations, polyploidy or endoreduplication noted in these cultures.

Additional cytotoxicity assessments were made for the definitive assay in the presence of metabolic activation. As depicted in sponsor's table 7 below, a concentration of 300 µg/ml tetracaine produced a 43% reduction in mitotic index but an ~ 85% reduction in cell monolayer confluence. In contrast, only slight evidence of cytotoxicity was noted at the next highest concentration tested (250 µg/ml). In the definitive 3 hour treatment of CHO cells in the presence of metabolic activation are presented in the sponsor's Table 8 depicted below. The results indicate that a significant increase in cells with chromosomal aberrations was observed in the cultures treated with 300 mg/ml. There was also a significant increase in endoreduplication observed in cultures treated with 250 and 300 µg/ml tetracaine.

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**Table 7:**  
**Assessment of Toxicity for Chromosome Aberrations Assay - With Metabolic Activation -**  
**3.0 Hour Treatment, 19.8 Hour Harvest**

Assay No.: 23841      Trial No.: C1      Date: 07/02/02      Lab No.: CY8052  
 Test Article: Tetracaine Base

Treatment			Confluence*	%	%	Average	%
			% Vehicle	Mitotic	Mitotic	%	%
			Control	Index A	Index B	Mitotic	Mitotic
				Culture	Culture	Index	Reduction
Negative Control	McCoy's 5a		100	11.1	11.5	11.3	---
Vehicle Control	DMSO	10.0 µL/mL	100	10.2	11.5	10.9	0
Test Article		150 µg/mL	100	11.6	12.8	12.2	0
		200 µg/mL	100	9.6	10.2	9.9	9
		250 µg/mL	86	7.2	8.5	7.9	28
		300 µg/mL	14	5.9	6.4	6.2	43

\* This endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.  
 McCoy's 5a = culture medium      DMSO = Dimethylsulfoxide

As noted in sponsor's Table 6 and 8 below, there were no significant increases in chromosomal abnormalities following a 3 or 20 hour exposure to lidocaine in the absence of metabolic activation or 3 hour exposure to lidocaine in the presence of metabolic activation, respectively.

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**Table 6:**  
**Chromosome Aberrations in Chinese Hamster Ovary Cells - Without Metabolic Activation -**  
**19.8 Hour Treatment, 19.8 Hour Harvest**

Assay No.: 23841	Trial No.: C1	Date: 07/02/02	#	Lab No.: CY8052	Test Article: Tetracaine Base	MITOTIC INDEX REDUC-TION*	ENDO-CATED CELLS	# POLY-PLOID CELLS	JUDGE-MENT (+/-) <sup>d</sup>	NUMBERS AND PERCENTAGES (% OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS)							JUDGE-MENT (+/-) <sup>d</sup>	
										CELLS SCORED	Gaps	Simple Breaks	chrc	chrc	mab	-g		+g
CONTROLS																		
NEGATIVE: McCoy's 5a																		
			A 100	0	2		5						0	5				
			B 100	0	3		6						3	9				
			TOTAL 200				11						3	14				
			AVERAGE %	0.0	2.5		5.5						1.5	7.0				
VEHICLE: DMSO		10.0 µL/mL	A 100	0	3		10						4	12				
			B 100	0	2		13						6	18				
			TOTAL 200	0.0	2.5		23						10	30				
			AVERAGE %	0.0	2.5		11.5						5.0	15.0				
POSITIVE: MMC		0.200 µg/mL	A 50	0	3		10						3	23				
			B 50	2	3		9						3	27				
			TOTAL 100				19						6	50				
			AVERAGE %	1.0	3.0		19.0						6.0	50.0		+		
TEST ARTICLE		9.40 µg/mL	A 100	0	4		7						2	9				
			B 100	0	2		12						0	12				
			TOTAL 200				19						2	21				
			AVERAGE %	1.3	3.0		9.5						1.0	10.5				
		18.8 µg/mL	A 100	0	2		6						2	8				
			B 100	0	2		4						2	6				
			TOTAL 200				10						4	14				
			AVERAGE %	3.0	2.0		5.0						2.0	7.0				
		37.5 µg/mL	A 100	0	2		8						3	10				
			B 100	0	3		5						3	8				
			TOTAL 200				13						6	18				
			AVERAGE %	3.4	2.5		6.5						3.0	9.0				
		75.0 µg/mL	A 100	0	2		6						0	6				
			B 100	0	3		4						0	4				
			TOTAL 200				10						0	10				
			AVERAGE %	5.5	2.5		5.0						0.0	5.0				

chrc: chromatid exchange  
 \*% Mitotic index reduction as compared to the vehicle control.  
<sup>b</sup> Significantly greater in % polyploidy and % endoreduplication than the vehicle control, p ≤ 0.01.  
<sup>c</sup> -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.  
<sup>d</sup> Significantly greater in -g than the vehicle control, p ≤ 0.01.  
 McCoy's 5a = culture medium  
 DMSO = Dimethylsulfoxide  
 MMC = Mitomycin C

**Table 8:**  
**Chromosome Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation -**  
**3.0 Hour Treatment, 19.8 Hour Harvest**

Assay No.: 23841	Trial No.: C1	Date: 07/02/02	Lab No.: CY8052	Test Article: Tetracaine Base	MITOTIC INDEX REDUCTION %	ENDO-REDUCED CELLS	# POLY-PLOID CELLS	JUDGE-MENT (+/-) <sup>b</sup>	NUMBERS AND PERCENTAGES (% OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS)					JUDGE-MENT (+/-) <sup>d</sup>	
									Gaps	Simple Breaks	chrc	chrc	mab		TOTALS <sup>c</sup>
CONTROLS															
NEGATIVE: McCoy's 5a															
		A 100	0	2					2				0	2	
		B 100	0	4					6				1	7	
		TOTAL 200							8				1	9	
		AVERAGE %	0.0	3.0					4.0				0.5	4.5	
VEHICLE: DMSO	10.0 µL/mL	A 100	1	3					3				0	3	
		B 100	0	2					5				1	5	
		TOTAL 200							8				1	8	
		AVERAGE %	0	2.5					4.0				0.5	4.0	
POSITIVE: CP	5.00 µg/mL	A 50	0	2					5		8	2	23	26	
		B 50	0	3					3		8	2	27	29	
		TOTAL 100							8		16	4	50	55	
		AVERAGE %	0	2.5					8.0		16.0	4.0	50.0	55.0	+
TEST ARTICLE	150 µg/mL	A 100	2	4					5				1	6	
		B 100	2	3					7		2	2	4	10	
		TOTAL 200							12		2	2	5	16	
		AVERAGE %	0	3.5					6.0		1.5	1.0	2.5	8.0	
		A 100	1	2					7		1		1	8	
		B 100	1	4					4		1	1	1	5	
		TOTAL 200							11		1	1	2	13	
		AVERAGE %	9	3.0					5.5		0.5	0.5	1.0	6.5	
		A 100	8	4					9		3	1	4	13	
		B 100	7	3					3		2	1	2	5	
		TOTAL 200							12		5	1	6	18	
		AVERAGE %	28	3.5					6.0		2.5	0.5	3.0	9.0	
		A 100	13	5					17		13	4	16	31	
		B 100	15	5					9		8	4	12	20	
		TOTAL 200							26		21	8	28	51	
		AVERAGE %	43	5.0					13.0		10.5	4.0	14.0	25.5	+

chrc: chromatid exchange  
 chrc: chromosome exchange  
 mab: multiple aberrations, greater than 4 aberrations  
<sup>a</sup> % Mitotic index reduction as compared to the vehicle control.  
<sup>b</sup> Significantly greater in % endoreduplication than the vehicle control, p ≤ 0.01.  
<sup>c</sup> -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.  
<sup>d</sup> Significantly greater in -g than the vehicle control, p ≤ 0.01. McCoy's 5a = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

**Study title: *In Vivo* Mouse Micronucleus Assay with Lidocaine Base**

**Key findings:** Under the conditions of the assay, lidocaine base was negative in the *in vivo* mouse bone micronucleus assay. However, the dose concentration samples could not be analyzed due to technical difficulties and therefore not verified. Given the toxicity noted, this does not jeopardize the conclusions of the study.

**Study no.:** 23840-0-455OECD  
**Volume #, and page #:** Volume 12, Page 133  
**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** May 13, 2002  
**GLP compliance:** Yes  
**QA reports:** yes ( X ) no ( ). NOTE. QA report indicates that a critical portion of the study was not audited by the Quality Assurance auditor. This deviation was determined to have no impact on the integrity of the study or the interpretation of the results.  
**Drug, lot #, and % purity:** Lidocaine base, Lot No. 811D0013, not indicated. Test article samples were sent to \_\_\_\_\_ for analysis; however, they were not able to be analyzed due to "technical difficulties."

**Methods**

**Strains/species/cell line:** ~~\_\_\_\_\_~~ CD-1 (ICR) BR Mouse.

**Doses used in definitive study:** 0, 50, 100 and 200 mg/kg, i.p. as summarized in the following table (sponsor's):

**Dosing Scheme for the Micronucleus Assay with Lidocaine Base**

Target Treatment (mg/kg)	Stock Concentration (mg/mL)	Route of Administration	Dosing Volume (mL/kg)	Animals/Harvest Timepoint <sup>a</sup>		Replacement Animals <sup>b</sup>
				24 Hour Male	48 Hour Male	
50	2.5	intraperitoneal injection	20	6	-	-
100	5.0	intraperitoneal injection	20	6	-	-
200	10.0	intraperitoneal injection	20	6	6	6
Vehicle Control, cell culture grade water	0	intraperitoneal injection	20	6	6	-
Positive Control, Cyclophosphamide, 80	8	oral gavage	10	6	-	-

<sup>a</sup> Six animals were dosed to ensure the availability of five animals for analysis.

<sup>b</sup> Animals were dosed as potential replacements for the original high-dose groups.

Note: Animals not used as replacements were euthanized at the completion of the trial.

**Basis of dose selection:** A dose range finding study was conducted with both males and females. No doses higher than 200 mg/kg were tested due to solubility constraints. The maximum tolerated dose was determined from daily observations of toxic signs and/or mortality. There were no relevant differences in toxicity noted between males and females,

therefore only males were used in the micronucleus assay (this is acceptable based upon current methodology).

In the dose range-finding study, lidocaine doses of 100 mg/kg and 200 mg/kg (i.p.) were administered to 3 mice/sex in a dosing volume of 20 mg/kg. Lidocaine doses were prepared prior to dosing. Animals were observed immediately after dosing, approximately 1 hour after dosing and daily for the duration of the assay for toxic signs and/or mortality. There were no mortalities in this study. The toxicities noted all occurred immediately post dosing and are summarized in the table below:

<b>Incidence of Clinical Signs in Dose Range Finding Study Immediately Post-Dosing</b>				
<b>Toxicity (N=3/sex)</b>	<b>100 mg/kg</b>		<b>200 mg/kg</b>	
	<b>Males</b>	<b>Females</b>	<b>Males</b>	<b>Females</b>
Flattened posture	1	2	3	1
Labored respiration	1	2	2	0
Ataxia	0	2	3	3
Convulsions	0	2	0	0

From the above results, the sponsor concluded that a dose of 200 mg/kg was determined to be the maximum tolerated dose. The above table indicates that lidocaine treatment produced convulsions in 2 of 3 females following administration of 100 mg/kg but not 200 mg/kg. Convulsions are clearly a sign of a maximum tolerated dose and they can frequently lead to death, however the lack of a dose-dependency of this finding and differential effects between males and females makes the sponsor's conclusions difficult to defend based exclusively on these findings (see definitive study results below).

Negative controls: The vehicle control article was cell culture grade water.

Positive controls: Cyclophosphamide (CP from — 30 mg/kg via oral gavage. CP was dissolved in cell culture grade water.

Incubation and sampling times: Following treatment, animals were sacrificed at 24 hours. Animal observations were conducted as described for the dose range finding study. Bone marrow was extracted at either 24 or 48 hours post dosing via flushing with fetal bovine serum. Slides were prepared and the cells were fixed in methanol, stained with May-Grünwald solution followed by Geimsa for analysis. Slides from the first 5 animals per group were scored for micronuclei and the polychromatic and normochromatic erythrocyte ratio was determined. Micronuclei frequency was determined by analyzing the number of micronucleated PCE format least 200 PCEs per animal. The PCE:NCE ratio was determined by scoring PCEs and NCEs observed while scoring at least the first 500 erythrocytes per animal. The historical background for the frequency of micronuclei in the CD-1 strain at — laboratory is about 0.0 to 0.4%.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):  
The sponsor described their acceptance criteria as follows:

**Acceptable Controls:** The vehicle control group had less than approximately 0.4% micronucleated PCEs and the group mean was within the historical control range. The positive control group had a statistically significantly higher ( $p < 0.01$ ) number of micronucleated PCEs than the vehicle control group and was consistent with historical positive control data.

**Acceptable High Dose:** The high dose produced clinical signs of toxicity and mortality in the animals.

The study appears to valid for the following reasons: 1) the positive control, cyclophosphamide, produced a clear response, 2) the number of cells scored per animal is within acceptable protocols, 3) the dosing reached an MTD as defined by mortality and convulsions, 4) the background data is within the historical control range of <0.4%.

Study outcome: The doses used in the definitive study produced acceptable toxicity to establish a maximum tolerated dose. The clinical signs are summarized in the sponsor's table below:

**APPEARS THIS WAY  
ON ORIGINAL**

**Animal Observations for Toxicity for Micronucleus Assay with Lidocaine Base**

Target Dose Level (mg/kg)	Harvest Timepoint	Animal ID	Time After Dosing			
			IPD	1 hour PD	1 day	2 days
50	24	3394	1,2,3	0	0	NA
		3403	1,2,3	0	0	NA
		3409	1,2,3	0	0	NA
		3420	1,2,3	0	0	NA
		3422	1,2,3	0	0	NA
		3429	1,2,3	0	0	NA
100	24	3389	1,2,3	0	0	NA
		3392	1,2,3	0	0	NA
		3396	1,2,3	0	0	NA
		3401	1,2,3	0	0	NA
		3419	1,2,3	0	0	NA
		3426	1,2,3	0	0	NA
200	24	3386	1,2,3	9	-	-
		3406	1,2,3	4,10,11	0	NA
		3412	1,2,3	4,10,11	0	NA
		3416	2,4	0	0	NA
		3428	2,4	4,10,11	0	NA
		3432	2,4	9	-	-
	48	3387	1,2,4,8	4,10	0	0
		3388	6→5	-	-	-
		3397	2,4,8	4,10	0	0
		3400	2,4,8	4,10	0	0
		3417	2,4,7	4,10	0	0
		3427	6→5	-	-	-
	Secondary	3393	1,2,3	0	0	NA
		3414	2,4,8	1,3	0	0
		3423	1,2,3	0	0	0
		3425	2,4,8	1,3	0	0
		3430	2,4,5	-	-	-
		3431	2,4,8	9	-	-

Key: 0 = Normal, 1 = Ataxic, 2 = flattened posture, 3 = slightly hypoactive, 4 = hypoactive, 5 = died approximately 5 minutes postdose, 6 = convulsions, 7 = gasping, 8 = labored breathing, 9 = found dead, 10 = hunched posture, 11 = squinted eyes; →followed by  
 IPD = immediately post dosing last animal, PD = post dosing last animal, NA = not applicable, animal harvested at the 24-hour harvest timepoint.

As indicated in the table above, one of 6 animals in the high dose group were found dead approximately 1 hour post dosing. Two animals in the 48 hour group had convulsions which preceded death, indicating that **the maximum tolerated dose was achieved** in this study.

The results of the micronucleus assay are reproduced in sponsor's table 1 below. As indicated in the table, cyclophosphamide produced a significant increase in the % micronucleated PCEs. In contrast, lidocaine doses of up to 200 mg/kg did not produce an increase in % micronucleated PCEs at either 24 or 48 hour time points. The lack of an effect on the PCE:NCE ratio indicates a lack of bone marrow toxicity in this study.

**Table 1: Micronucleus Data Summary Table**

ASSAY NO.: 23840

TEST ARTICLE: Lidocaine Base

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E. MALES	RATIO PCE:NCE MEAN ± S.E. MALES	
CONTROLS					
VEHICLE	CCGW	24 hr	0.05 ± 0.00	0.59 ± 0.02	
		48 hr	0.10 ± 0.02	0.50 ± 0.05	
POSITIVE	CP 80 mg/kg	24 hr	3.63 ± 0.37*	0.52 ± 0.03	
TEST ARTICLE	50 mg/kg	24 hr	0.04 ± 0.04	0.51 ± 0.05	
		100 mg/kg	24 hr	0.07 ± 0.03	0.57 ± 0.06
		200 mg/kg	24 hr	0.06 ± 0.01	0.58 ± 0.09
		48 hr	0.05 ± 0.00	0.65 ± 0.05	

\* Significantly greater than the corresponding vehicle control, p&lt;0.01.

CCGW = Cell culture grade water

CP = Cyclophosphamide

PCE = Polychromatic erythrocyte

NCE = Normochromatic erythrocyte

**Study title: *In Vivo* Mouse Micronucleus Assay with Tetracaine Base**

**Key findings:** Under the conditions tested, tetracaine base was negative in the *in vivo* mouse bone marrow micronucleus assay.

**Study no.:** 23841-0-455OECD  
**Volume #, and page #:** Volume 12, Page 154  
**Conducting laboratory and location:** \_\_\_\_\_  
**Date of study initiation:** \_\_\_\_\_  
**GLP compliance:** Yes  
**QA reports:** yes ( X ) no ( ) NOTE: there is no statement as to any portions of the study that were not subject to quality assurance inspections.  
**Drug, lot #, and % purity:** Tetracaine base, Lot No. 721725, Samples were shipped to \_\_\_\_\_ for analysis. Upon receipt at \_\_\_\_\_ it was determined that the samples could not be analyzed due to technical difficulties.

**Methods**

**Strains/species/cell line:** \_\_\_\_\_ CD-1 (ICR) BR mouse model.

Doses used in definitive study: 0, 125, 250 and 500 mg/kg tetracaine.

Basis of dose selection: A dose-range-finding study was conducted in both males and females (n=up to 3/sex/group). Doses higher than 2000 mg/kg were not tested based on information provided by the sponsor. Daily observations of toxicity or mortality were used to estimate the maximum tolerated dose. There were no relevant differences in toxicity noted between males and females, therefore only males were used in the micronucleus assay (this is acceptable based upon current methodology).

In the dose range-finding study, tetracaine doses of 1000 mg/kg, 1500 mg/kg and 2000 mg/kg (i.p.) were administered to up to 3 mice/sex/group in a dosing volume of 20 mg/kg. Tetracaine doses were prepared prior to dosing. Animals were observed immediately after dosing, approximately 1 hour after dosing and daily for the duration of the assay for toxic signs and/or mortality. The toxicities noted all occurred immediately post dosing and are summarized in the tables below:

#### Summary of Mortalities for Dose Rangefinding Assay with Tetracaine Base

Target Treatment (mg/kg)	Number of Males (Died/Total Dosed)	Number of Females (Died/Total Dosed)
1000	0/3 <sup>a</sup>	0/3
1500	2/3	1/3
2000	1 <sup>b</sup> /1	1/1

<sup>a</sup>One animal's death may have been due to a possible dosing complication; the death is therefore deemed to have been accidental.

<sup>b</sup>The male was humanely sacrificed per Study Director.

As noted above, 1 of 3 females and 2 of 3 males treated with 1500 mg/kg died following dosing. In addition 1 of 1 females dosed treated with 2000 mg/kg tetracaine died following clinical signs of tremors and recumbency (see table below for clinical signs). The single male treated with 2000 mg/kg demonstrated clinical signs of ataxia, labored respiration and flattened posture and was sacrificed within 1 hour of treatment *in extremis*.

**Animal Observations for Toxicity for Dose Rangefinding Assay with Tetracaine Base**

Target Dose Level (mg/kg)	Sex	Animal ID	Time After Dosing			
			IPD	1 hour PD	1 day	2 days
1000	M	2984	0	0	0	0
		2987	1 <sup>a</sup>	-	-	-
		2990	2,3	4	0	0
	F	2994	0→2,8	0	0	0
		2996	3	0	0	0
		2998	3	0	0	0
1500	M	2985	2,3	0	0	0
		3003	1	-	-	-
		3004	1	-	-	-
	F	2999	5,6,7	0	0	0
		3000	0	0	0	0
		3001	1	-	-	-
2000	M	2986	0→3	5,6,7→9	-	-
	F	2993	2,3→1	-	-	-

Key: 0 = Normal, 1 = found dead, 2 = tremors, 3 = recumbent, 4 = slightly hypoactive, 5 = ataxic, 6 = labored respiration, 7 = flattened posture, 8 = animal on its back, 9 = humanely sacrificed per Study Director  
IPD = immediately post dosing last animal, PD = post dosing last animal, → = followed by

<sup>a</sup>Death may have been due to a possible dosing complication; the death is therefore deemed to have been accidental.

From the above results, the sponsor concluded that a dose of 1000 mg/kg tetracaine was determined to be the maximum tolerated dose. Initial dosing for the micronucleus assay was to be 250, 500 and 1000 mg/kg, however, due to excessive mortality in the 1000 mg/kg group as well as a 750 mg/kg preliminary dosing group; the high dose was set at 500 mg/kg. The final dosing design is presented below:

**Dosing Scheme for the Micronucleus Assay with Tetracaine Base**

Target Treatment (mg/kg)	Stock Concentration (mg/mL)	Route of Administration	Dosing Volume (mL/kg)	Animals/Harvest Timepoint <sup>a</sup>	
				24 Hour Male	48 Hour Male
125	6.25	intraperitoneal injection	20	7	-
250	12.5	intraperitoneal injection	20	6	-
500	25.0	intraperitoneal injection	20	6	6
Vehicle Control, cell culture grade water	0	intraperitoneal injection	20	6	6
Positive Control, Cyclophosphamide, 80	8	oral gavage	10	6	-

<sup>a</sup> Six animals were dosed to ensure the availability of five animals for analysis.

<sup>b</sup> Animals were dosed as potential replacements for the original high-dose groups.

Note: Animals not used as replacements were euthanized at the completion of the trial.

Negative controls: The vehicle control article was cell culture grade water.

Positive controls: Cyclophosphamide (CP from — served as the positive control (80 mg/kg oral gavage) and was dissolved in cell culture grade water.

Incubation and sampling times: Following treatment, animals were sacrificed at 24 hours. Animal observations were conducted as described for the dose range finding study. Bone marrow was extracted at either 24 or 48 hours post dosing via flushing with fetal bovine serum. Slides were prepared and the cells were fixed in methanol, stained with May-Grünwald solution followed by Geimsa for analysis. Slides from the first 5 animals per group were scored for micronuclei and the polychromatic and normochromatic erythrocyte ratio was determined. Micronuclei frequency was determined by analyzing the number of micronucleated PCE format least 200 PCEs per animal. The PCE:NCE ratio was determined by scoring PCEs and NCEs observed while scoring at least the first 500 erythrocytes per animal. The historical background for the frequency of micronuclei in the CD-1 strain at — laboratory is about 0.0 to 0.4%.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The sponsor described their acceptance criteria as follows:

**Acceptable Controls:** The vehicle control group had less than approximately 0.4% micronucleated PCEs and the group mean was within the historical control range. The positive control group had a statistically significantly higher ( $p < 0.01$ ) number of micronucleated PCEs than the vehicle control group and was consistent with historical positive control data.

**Acceptable High Dose:** The high dose produced clinical signs of toxicity and mortality in the animals.

The study appears to valid for the following reasons: 1) the positive control, cyclophosphamide, produced a clear response, 2) the number of cells scored per animal is within acceptable protocols, 3) the dosing reached an MTD as defined by mortality and convulsions, 4) the background data is within the historical control range of <0.4%.

Study outcome: There were no mortalities noted following treatment with 125, 250 or 500 mg/kg tetracaine. As noted in the table below, most animals in the 250 and 500 mg/kg groups demonstrated clinical signs of ataxia, irregular respiration, flattened posture, hypoactivity and head tremors immediately post dosing.

**Animal Observations for Toxicity for Micronucleus Assay with Tetracaine Base**

Target Dose Level (mg/kg)	Harvest Timepoint	Animal ID	Time After Dosing				
			IPD	1 hour PD	1 day	2 days	
125	24	3458	2,5,6	0	0	NA	
		3460	0	0	0	NA	
		3461	0	0	0	NA	
		3462	0	0	0	NA	
		3463	0	0	0	NA	
		3464	0	0	0	NA	
		3465	0→1	0	0	NA	
250	24	3341	1,2,3,4	0	0	NA	
		3351	0	0	0	NA	
		3352	1,2,3,4	0	0	NA	
		3356	1,2,3,4	0	0	NA	
		3379	1,2,3,4	0	0	NA	
		3381	1,2,3,4	0	0	NA	
500	24	3340	1,2,3,4,5	0	0	NA	
		3346	1,2,3,4,5	0	0	NA	
		3347	1,2,3,4,5	0	0	NA	
		3354	1,2,3,4,5	0	0	NA	
		3366	1,2,3,4,5	0	0	NA	
		3374	0	0	0	NA	
	48		3350	0	0	0	0
			3353	0	0	0	0
			3358	0	0	0	0
			3378	0	0	0	0
			3382	0	0	0	0
		3383	0	0	0	0	

Key: 0 = Normal, 1 = ataxic, 2 = irregular respiration, 3 = flattened posture, 4 = hypoactive, 5 = head tremors, 6 = recumbent, IPD = immediately post dosing last animal, PD = post dosing last animal, NA = not applicable, animal harvested at the 24-hour harvest timepoint.

The summary of the micronucleus data is reproduced in the sponsor's table below. Although cyclophosphamide produced a clear increase in the % micronucleated PCE, tetracaine base tested negative under the conditions of this assay.

**APPEARS THIS WAY  
ON ORIGINAL**

**Table 1: Micronucleus Data Summary Table**

ASSAY NO.: 23841

TEST ARTICLE: Tetracaine Base

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL $\pm$ S.E. MALES	RATIO PCE:NCE MEAN $\pm$ S.E. MALES
CONTROLS				
VEHICLE	Water	24 hr	0.10 $\pm$ 0.02	0.40 $\pm$ 0.04
		48 hr	0.02 $\pm$ 0.01	0.58 $\pm$ 0.06
POSITIVE	CP 80 mg/kg	24 hr	3.38 $\pm$ 0.48*	0.50 $\pm$ 0.04
TEST ARTICLE	125 mg/kg	24 hr	0.09 $\pm$ 0.05	0.53 $\pm$ 0.04
		24 hr	0.10 $\pm$ 0.02	0.68 $\pm$ 0.07
	500 mg/kg	24 hr	0.03 $\pm$ 0.01	0.57 $\pm$ 0.05
		48 hr	0.05 $\pm$ 0.03	0.48 $\pm$ 0.04

\* Significantly greater than the corresponding vehicle control,  $p < 0.01$ .

CP = Cyclophosphamide

PCE = Polychromatic erythrocyte

NCE = Normochromatic erythrocyte

**2.6.6.5 Carcinogenicity:**

Studies examining the carcinogenic potential of either lidocaine or tetracaine have not been completed. These studies are not required for drug products which are not used on a chronic basis, as described in ICH M3 Guidance.

The metabolite of lidocaine 2,6-xylylidine, has been tested for carcinogenic potential in Sprague-Dawley rats by the National Toxicology Program in 1990. Dietary administration of 2,6-xylylidine at 0, 300, 1000 or 3000 ppm for 102 weeks produced a significant increase in the incidence of nasal cavity adenomas, carcinomas and adenocarcinomas in both males and females. Rhabdomyosarcomas, previously unreported in this rat strain, were detected in two high-dose males and two high-dose females (none in the controls) (National Toxicology Program, 1990).

As previously detailed by Dr. McGovern in the first cycle NDA review of NDA 21-431, The major lidocaine metabolite 2,6-xylylidine is "carcinogenic in mice and/or rats (see the reviews for NDA 19-941 dated June 19, 1992 and September 28, 1992 by Dr. Dou Lucy Jean for details). The compound 2,6-xylylidine produced carcinomas and adenomas in the nasal cavity and a rhabdomyosarcoma (rare) in rats at an oral dose of 150 mg/kg. An increased incidence of subcutaneous fibromas and/or fibrosarcomas was also noted in males while neoplastic nodules of the liver were observed in females. In May 1996, the relevancy of the rat tumor findings to humans was discussed by CDER's Executive Carcinogenicity Assessment Committee (see minutes dated May 14, 1996 under NDA 19-941).

The committee concluded that the tumor findings are not relevant to humans and should not be included in the labeling nor should the labeling of other anesthetic products containing lidocaine be revised.”

#### 2.6.6.6 Reproductive and developmental toxicology:

##### Fertility and early embryonic development

**Study Title:** A study to assess the effects of fertility and early embryonic development to implantation in rats.

**Key study findings:** Tetracaine base administration to both the male and female rat resulted in the following key findings:

- **Clinical observations:** decreased activity, prostration, rapid breathing, and scabs at injection site in male and female rats at a dose of 7.5 mg/kg
- **Body weight gains:** decreased in male rats at a dose of 7.5 mg/kg during the entire treatment period; decreased in female rats in all treated groups during pre-mating, and at a dose of 7.5 mg/kg during GD 0-7
- **Organ weights:** decrease in prostate weights and an increase in ovary weights at a dose of 7.5 mg/kg
- No effect on male or female fertility when tetracaine base was given s.c.
- NOAEL (general)= 2.5 mg/kg/day for male and female rats (based on observations and body weight gains)
- NOAEL (fertility)=7.5 mg/kg/day for male and female rats

**Study no:** 925-014

**Volume #, and page #:** 17, pp. 17-1

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 28 March 2003

**GLP compliance/QA report:** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** tetracaine base/Z-02-003/purity not specified on CoA

**Formulation/vehicle:** sterile water containing NaH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub>

#### **Methods:**

**Species/strain:** Sprague Dawley rats, CD(SD)IGS BR, \_\_\_\_\_

**Doses employed:** 0.75, 2.5, 7.5 mg/kg/day @ 1 mL/kg

**Route of administration:** s.c. (injections alternated between right and left shoulder and lumbar regions)

**Study design:** daily dosing, [males] 28 days pre-mating, 14-21 days mating, through GD7; [females] 14 days pre-mating, 14-21 days mating, through GD7

**Number/sex/group:** 25/sex/group

**Parameters and endpoints evaluated:** [male rats] clinical observations twice daily; body weights were recorded every 3-4 days, and food consumption was recorded weekly; gross pathology, terminal body weights, testes, epididymis, seminal vesicle, and prostate organs were weighted, sperm analysis was conducted; [female rats] clinical observations

twice daily, body weights and food consumption were recorded every 4 days during the pre-mating and mating periods and on GD 0, 4, 7, 10, 13; cesarean section on GD13 with standard parameters collected, gravid uterine and ovaries/cervix were weighed.

Observation times and results:

**Observations**

**Results**

**Male rats**

Mortality

All animals survived to scheduled euthanasia.

Clinical signs

Decreased activity, ataxia, prostration, rapid breathing, hair absent or sparse and scabs at the injection sites were observed at a dose of 7.5 mg/kg during the pre-mating, mating, and postmating periods.

Body weights

Body weights were statistically significantly decreased (5-9%) beginning on SD22 (pre-mating period) and continuing through postmating at a dose of 7.5 mg/kg. Also at 7.5 mg/kg body weight gains were decreased for the pre-mating, mating, and postmating periods (14%, 30%, and 17%, respectively).

Food consumption

Statistically significantly decreased at a dose of 7.5 mg/kg during the pre-mating period (SW3-9, 9-10%), and during the postmating period (SW9-10, 12%).

Terminal/necroscopic evaluations

Unremarkable.

Organ weights

Terminal body weight was statistically significantly decreased (9%) and prostate weight was decreased (13%) at a dose of 7.5 mg/kg.

Reproductive/fertility Indices

Unremarkable.

Sperm analysis

Unremarkable.

**Female rats**

Mortality

One died at a dose of 7.5 mg/kg on SD16 30 mins after dosing. Cause of death was not determined. All other animals survived to scheduled euthanasia.

**Female rats**

Clinical signs

Decreased activity, prostration, rapid breathing, and scabs at the injection sites were observed at a dose of 7.5 mg/kg during the pre-mating, mating, and gestation periods.

Body weights

Body weights were statistically significantly decreased on SD15 (pre-mating, 4%), and on GD7-13 (3-6%) at a dose of 7.5 mg/kg.

Body weight gains were decreased during the pre-mating period on SD4-8 in all doses (18-31%), SD11-15 at doses  $\geq 2.5$  mg/kg (18-29%), and SD1-15 for all doses (11-21%, statistically significant at doses  $\geq 2.5$  mg/kg). Body weight gains were decreased at a dose of 7.5 mg/kg during GD0-4 (19%, statistically significant), and GD4-7 (24%), GD0-7 (21%, statistically significant).

Food consumption	Unremarkable during pre-mating and gestation.
Terminal/necroscopic evaluations	Unremarkable.
Organ weights	Ovary weight was increased (43%) at a dose of 7.5 mg/kg. Uterus/cervix weight was increased (16%) at a dose of 2.5 mg/kg.
Reproductive/fertility Indices	Estrous cyclicity was normal for the length and number of cycles. Unremarkable for fertility indices.
Cesarean section data	Unremarkable.

[Note: GD = gestation day; SD=study day; SW=study week]

**Study Title: A study to evaluate functional effects on male fertility in rats (Lidocaine).**

Key study findings:

- Clinical observations: Treatment-related scabbing and hair loss at the injection site
- Body weight gains: Decreased in male rats at a dose of 60 mg/kg during the entire study
- Organ weights: Decrease in terminal body weight and prostate weights at a dose of 60 mg/kg
- Increased copulatory interval at a dose of 60 mg/kg, and a dose-related decreased homogenization resistant sperm head count, daily sperm production, and spermatogenic efficiency, the treatment did not affect overall fertility in male rats when given subcutaneous doses up to 60 mg/kg
- NOAEL (general) = 15 mg/kg/day for male (based on observations and body weight gains)
- NOAEL (male fertility) = 60 mg/kg/day

Study no: 925-019

Volume #, and page #: 4, pp. 5-2

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 16 March 2004

GLP compliance/QA report: Yes (X) No ( )

Drug, lot #, radiolabel, and % purity: lidocaine base/Z-02-002/purity not specified on CoA

Formulation/vehicle: sterile water containing  $\text{NaH}_2\text{PO}_4$  or  $\text{Na}_2\text{HPO}_4$  and hydrochloric acid for pH adjustment to 6.0

Methods:

Species/strain: Sprague Dawley rats/ — CD(SD)IGS BR, —————

Doses employed: 5, 15, 60 mg/kg/day @ 1 mL/kg

Route of administration: s.c. (injections alternated between right and left scapular and lumbar regions)

Study design: only male rats were dosed, daily dosing 28 days pre mating, 14-21 days mating, through GD 7

Number/sex/group: 25/sex/group

Parameters and endpoints evaluated: [treated male rats] clinical observations twice daily; body weights were recorded twice weekly; food consumption was recorded weekly during pre mating and after mating; gross pathology, terminal body weights, testes, epididymis, seminal vesicle, and prostate organs were weighed and histopathology evaluated; sperm analysis was conducted; [untreated female rats] clinical observations twice daily; body weights and food consumption were recorded twice weekly prior to mating and GD 0, 4, 7, 10, and 13; uterine data were collected on GD 13.

Observation times and results:

**Observations**

**Results**

**Male rats**

Mortality	One control rat was humanely euthanized on SD 43 and one rat was found dead on SD 19. No gross pathology findings were unremarkable. All other animals survived to scheduled euthanasia.
Clinical signs	Treatment-related findings associated with the s.c. administration were observed at a dose of 60 mg/kg and including abrasions, hair sparse, and scabbed area on the dorsal surface, and hair sparse and scabbed area on the lumbar region.
Body weights	Body weights were unremarkable. Body weight gains were statistically significantly decreased on SD 18-22 (pre mating, 28%), SD 43-46 (mating, 32%), SD 60-64 (postmating, 79%), and overall (SD 1-64, 12%) at a dose 60 mg/kg. Body weight gains were decreased in all treated groups on SD 50-53 (postmating, 44-78%).
Food consumption	Unremarkable.
Terminal/necroscopic evaluations	Scabs were observed at the injection site at doses of 15 mg/kg (11/25, 10/11 mild, 1/11 minimal) and 60 mg/kg (24/25, 5/24 mild, 19/24 moderate).
Organ weights	Terminal body weight was statistically significantly decreased (7%) and prostate weight were decreased (13%) at a dose of 60 mg/kg.
Reproductive/fertility Indices	Fertility and fecundity indices were unremarkable. The copulatory interval was statistically significantly increased by 1-2 days (4.3 days vs. 2.6 days in the control) at a dose of 60 mg/kg. The

significance of the increase in the copulatory interval (number of days it takes the male to impregnate the female rat) is unknown and did not have an adverse effect on female fertility.

Sperm analysis Sperm analyses showed a dose-related decrease (26%, 33%, and 36% for doses of 5, 15, and 60 mg/kg, respectively) in homogenization resistant sperm head count, daily sperm production, and spermatogenic efficiency. The significance of the sperm analysis findings is unknown because there were no effects of treatment on the testes weight which determine the number of sperm produced and female fertility was unaffected.

### **Female rats**

Mortality	All animals survived to scheduled euthanasia.
Clinical signs	Unremarkable.
Body weights	Unremarkable.
Food consumption	Unremarkable.
Terminal/necroscopic evaluations	Unremarkable.
Reproductive/fertility Indices	Unremarkable for fertility indices.
Cesarean section data	Uterine data revealed a decrease, although not statistically significant, in the pre-implantation loss at doses of 15 mg/kg (6.09% per animal) and 60 mg/kg (6.49% per animal) compare to the control (7.87% per animal). There were no corresponding decreases in the number of viable embryos.

[Note: GD = gestation day; SD=study day]

## **Embryofetal development**

### **Study title: Pilot Study for Effects on Embryo-Fetal Development in Rats (with Toxicokinetics)**

**Key study findings:** A pilot study to determine dose levels for the definitive rat segment II study was conducted with a toxicokinetic portion was included. The key findings are as follows:

1. Under the conditions of the assay, no external variations or malformations were observed at any dose of Lidocaine or tetracaine tested.

**Study no.:** 925-012  
**Volume #, and page #:** Volume 3, Page 1  
**Conducting laboratory and location:** \_\_\_\_\_  
**Date of study initiation:** January 8, 2003  
**GLP compliance:** Yes  
**QA reports:** yes ( X ) no ( )  
**Drug, lot #, and % purity:** Lidocaine base, Lot 811D0013, —  
 Tetracaine base, Batch 72172<sup>d</sup> —

### Methods

**Doses:** Lidocaine doses were 15, 30, 60 and 75 mg/kg, s.c.  
 Tetracaine doses were 1, 2, 5 and 10 mg/kg, s.c.  
**Species/strain:** Sprague-Dawley female rats [— CD(SD)IGS BR]  
**Number/sex/group:** 7 group (females)  
**Route, formulation, volume, and infusion rate:** subcutaneous, vehicle was sodium phosphate buffer, volume was 1 ml/kg.

**Satellite groups used for toxicokinetics:** 7 rats/group

**Study design:** Pregnant rats were treated on gestation days 6-17 of gestation. Test article was administered to the main study animals and TK animals once a day. Injections were made on the back of the animal in the scapular and lumbar regions, alternating left and right sides per day.

Group Assignment		
Group Number	Dose Level (mg/kg/day)	Number of Time-mated Female Rats
1	0 (Vehicle Control)	5
2	15 (Lidocaine)	5
3	30 (Lidocaine)	5
4	60 (Lidocaine)	5
5	75 (Lidocaine)	5
6	1 (Tetracaine)	5
7	2 (Tetracaine)	5
8	5 (Tetracaine)	5
9	10 (Tetracaine)	5
10 <sup>a</sup>	15 (Lidocaine)	7
11 <sup>a</sup>	30 (Lidocaine)	7
12 <sup>a</sup>	60 (Lidocaine)	7
13 <sup>a</sup>	75 (Lidocaine)	7
14 <sup>a</sup>	1 (Tetracaine)	7
15 <sup>a</sup>	2 (Tetracaine)	7
16 <sup>a</sup>	5 (Tetracaine)	7
17 <sup>a</sup>	10 (Tetracaine)	7

<sup>a</sup>Animals in Groups 10 through 17 were used for toxicokinetic evaluations

**Parameters and endpoints evaluated:**

**Mortality and Cage-side Observations:** twice daily. Detailed clinical examinations were conducted from Day 6 through 20 of gestation.

**Body Weight and Body Weight Changes:** Individual body weights for main study animals were recorded on Days 0, 6, 9, 12, 15, 18 and 20 of gestation. Individual body weight change for main study animals was calculated for the following gestation day intervals: 0-6, 6-9, 9-12, 12-15, 15-18, 18-20, 6-18 and 0-20. Adjusted body weight (Day 20 gestation body weight minus the gravid uterine weight) and adjusted body weight change (Days 0-20 of gestation) for main study animals were also calculated.

**Food Consumption:** Main study animal food consumption was recorded on corresponding body weight days and calculated for the following intervals: 0-6, 6-9, 9-12, 12-15, 15-18, 18-20, 6-18 and 0-20.

**Toxicokinetics:** Blood samples were collected from the orbital sinus after carbon dioxide/oxygen inhalation of six rats/TK group on Day 17 of gestation. Samples were collected at 0.5, 1, 2, 4, 8 and 24 hours post dose. Samples were placed in tubes containing potassium EDTA and neostigmine and stored on ice until centrifuged to collect the plasma. Plasma samples were stored at -70C until shipped for analysis. Blood samples collected for determination of plasma concentration of Tetracaine were not analyzed.

**Maternal Necropsy:** Complete necropsy was performed on all main study dams. Emphasis was placed on structural abnormalities or pathologic changes that may have been influenced by the pregnancy. Gross lesions were saved in 10% neutral buffered formalin for possible microscopic examination. Following determination of the pregnancy status of the animal, the carcass was discarded without further analysis.

**Ovarian and Uterine Examinations:** On Day 20, main study dams were euthanized by carbon dioxide inhalation and the uterus and ovaries were exposed by a mid-abdominal incision. The location of viable and nonviable fetuses, early and late resorptions for each uterine horn, position of the cervix, and the total number of implantations were recorded. The number of corpora lutea on each ovary was recorded. The uterus was excised and gravid uterine weight recorded. Fetuses were removed, and the placenta was grossly examined. Fetuses were individually weighed, sexed externally, and examined for external malformations and variations. No malformations or variations were noted on external examination and all fetuses were euthanized and discarded.

## Results

**Mortality (dams):** One pregnant rat in the 75 mg/kg/day lidocaine treatment group was found dead on Day 14 of gestation. There were no clinical signs leading to the death of the animal. There were no other deaths.

**Clinical signs (dams):** Clinical signs in dams during gestation are reviewed in the table below (extracted from the sponsor's table with only representative skin description noted). Overall, the highest dose of lidocaine and tetracaine increased the incidence of decreased activity and prostration. All other doses did not demonstrate this response. The skin changes were primarily described as regions showing only sparse hair or scabbed areas at the sites of injection. These were noted in lidocaine treated rats treated with 30 mg/kg lidocaine and above but not in the tetracaine treated rats at any dose tested. The observation of rapid breathing rate was observed in one rat treated with the 75 mg/kg dose of lidocaine or 5

animals treated with the 10 mg/kg dose of tetracaine demonstrated a rapid rate of respiration on several occasions.

Summary of Clinical Observations in the Dams (# times observed/total number of animals affected)									
Group	Vehicle	Lidocaine				Tetracaine			
N	5	5	5	5	5	5	5	5	5
Dose (mg/kg)	0	15	30	60	75	1	2	5	10
<b>Behavior</b>									
Activity Decreased	0	1/1	0	0	10/1	0	0	0	67/5
Prostration	0	1/1	0	0	5/1	0	0	0	38/5
<b>Skin</b>									
Hair sparse, lumbar	0	0	0	13/1	6/1	0	0	0	0
Scabbed area, lumbar	0	0	12/1	54/5	40/5	0	0	0	0
Scabbed area, shoulder right	0	0	6/2	18/2	18/4	0	0	0	0
Scabbed area, shoulder left	0	0	8/2	29/4	41/5	0	0	0	0
<b>Respiration</b>									
Rapid breathing	0	2/1	0	0	6/1	0	0	0	55/5

**Body weight (dams):** There were no statistically significant changes in body weight noted following either lidocaine or tetracaine at any dose tested. There were no statistically significant differences in body weight change values between groups.

**Food consumption (dams):** Food consumption was not significantly altered by treatment with either lidocaine or tetracaine under the conditions of this assay.

**Toxicokinetics:** Figure 1 below depicts the mean lidocaine concentration for female rats on Day 17 of gestation. sponsor's table 1 below provides the mean plasma concentrations of lidocaine in female rats on Day 17 of gestation. The lower limit of quantitation of the assay was 0.9 ng/ml. The greatest concentration of lidocaine was noted at the first time point examined, 0.5 h.  $C_{max}$  increased with dose; however this increase was not proportional to the dose given. The concentration following 60 mg/kg was only slightly below that following 75 mg/kg. The  $t_{1/2}$  of the 15 mg/kg dose of lidocaine was 0.8 h, while the doses of 30, 60 and 75 mg/kg were 2.0, 2.4 and 2.4 respectively.