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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION
Labeling Review for NDA Re-Submission

Application number:	NDA 204803
Supporting document/s:	SDN 28, 31, 41, 42
Applicant's letter date:	6/27/2019, 8/30/2019, 2/28/2020, 3/13/2020
CDER stamp date:	6/27/2019, 8/30/2019, 2/28/2020, 3/13/2020
Product:	SABER-Bupivacaine (Posimir)
Indication:	Administration in to the surgical incision to produce post-surgical analgesia
Applicant:	DURECT Corporation
Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine
Reviewer:	Misol Ahn, PhD
Team Leader:	Jay H. Chang, PhD
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1 Executive Summary

1.1 Introduction

NDA 204803 was originally submitted by the Applicant, Durect Corporation, on 4/12/2013 to seek marketing approval of their product Posimir (referred to as SABER-bupivacaine during development) for post-surgical analgesia. The NDA was submitted as a 505(b)(2) application relying on the Agency's previous determination of safety and efficacy of the listed drug Marcaine (Bupivacaine Hydrochloride Injection, USP; NDA 16964; approved 10/3/1972). Posimir is a sterile nonpyrogenic, clear, light yellow to amber solution containing bupivacaine (12%, 132 mg/mL, 660 mg in 5 mL), benzyl alcohol (22%, 242 mg/mL, 1210 mg in 5 mL), and sucrose acetate isobutyrate (SAIB) (66%, 726 mg/mL, 3630 in 5 mL). Upon single instillation of the maximum proposed dose of 5 mL total (single dose (b) (4)), the biodegradable matrix (SAIB) serves as a depot for delayed release of bupivacaine over 24-72 hours. The SAIB forms this depot for bupivacaine as the solvent benzyl alcohol (BA) diffuses away from the dosing site thereby providing an extended release of bupivacaine over time compared to Marcaine (bupivacaine HCl).

The NDA was not approved during the first review cycle primarily due to deficiencies from the clinical review team. Notably, no nonclinical deficiencies were included in the complete response (CR) letter issued on 2/12/2014. The nonclinical secondary review noted that "Though not intended to be administered intravascularly, the risk of inadvertent administration was partially assessed in an in vitro hemolysis assay – no direct IV/IA administration of SABER-bupivacaine in an animal model was provided." In the primary review, Dr. Bond noted that "SABER-Bupivacaine and SABER placebo both caused hemolysis when added to human whole blood *in vitro*. In addition, a viscous (and, for the placebo, cloudy) macroscopic appearance, with globules resembling bubbles visible microscopically, were observed in tested human plasma." Further, he stated that "SABER-Bupivacaine is not intended for vascular injection, which should make the potential for hemolysis not a real concern but one to be addressed by the medical review team." Dr. Roca's Deputy Division Director Summary review on 2/12/2014 notes "There was no in vivo evaluation of the adverse effects of inadvertent intravenous or intra-arterial administration, as it was felt that, due to the physical properties of the drug product, such administration would result in profound toxicity due to occlusion of the vessels as the benzyl alcohol rapidly leaves the matrix."

NDA 204803 was resubmitted on 6/27/2019 with new clinical data to address the deficiencies outlined in the Division's CR letter. Although there were no nonclinical deficiencies, the Applicant included two non-GLP studies to evaluate possible effects of SABER-bupivacaine vehicle on local skin discoloration when administered post-surgically into a surgical wound. These studies were exploratory in nature to apparently follow-up observations of skin redness in clinical studies. The Applicant noted that transient skin discoloration was observed but that this had no impact on wound healing. These studies alone would not be adequate to address the impact on wound healing as they were not GLP, included too few animals, and did not include appropriate wound healing endpoints, including histopathology. However, adequate wound healing studies

were included with the original NDA submission, so these studies do not impact the overall nonclinical safety profile of the drug. In addition, a nonclinical literature review for bupivacaine for labeling was submitted (SDN 31; 8/30/2019) in accordance with the Pregnancy, Lactation, and Labeling Rule (PLLR) in response to an IR from the Division. Based upon our review of the literature, no additional labeling language is recommended.

The Applicant submitted a Pediatric Research Equity Act (PREA) waiver/deferral request (b) (4) (b) (4). The Division met with the Pediatric Review Committee (PeRC) on 2/4/2020 to discuss the proposed pediatric waiver request based in part on the potential safety concern of benzyl alcohol exposure and PeRC recommended waiving the studies in all pediatric populations due to its modest efficacy or because the product does not represent a meaningful therapeutic benefit over existing products and is unlikely to be used in these patients.

At the advisory committee meeting held on January 16, 2020, concern was raised by several advisory committee members regarding the potential risk of inadvertent intravascular administration of SABER-bupivacaine (Posimir). To ensure that this concern was adequately addressed, information requests regarding safety assessment of inadvertent administration into the intravascular space were sent to the Applicant on 2/7/2020 and 3/4/2020. Based on the risk assessments provided by the Applicant, there are still potential concerns of emboli and hemolysis via inadvertent intravascular injection from the nonclinical perspective (See Section 1.2. Brief Discussion of Nonclinical Findings below). Briefly, as per the Applicant, based on in vitro models, Posimir droplets are expected to form upon intravascular injection and the injected material may increase in viscosity over a period of minutes to hours. Since the intravascular degradation of Posimir and/or SABER vehicle has not been investigated, it is not clear whether it stays long enough in the systemic circulation to form viscous droplets or what the fate of the droplets may be, hence the potential for microvascular occlusion/ embolism cannot be dismissed. Additionally, the Applicant provided a risk assessment for the hemolytic potential based on a theoretical calculation of benzyl alcohol levels assuming steady-state is achieved with Posimir in the systemic circulation. However, this does not address the potential effects from the higher local concentration at the injection site when inadvertently injected into the intravascular space. The Applicant proposed to include a boxed warning of adverse embolic risks as part of the Posimir prescribing information and to conduct a post-approval nonclinical study to investigate the effects of intravascular injection of Posimir.

1.2 Brief Discussion of Nonclinical Findings

Appropriate nonclinical studies were submitted with the original NDA submission to support the safety of SABER-bupivacaine for the intended clinical use. Refer to the nonclinical pharmacology toxicology review by Dr. Gary Bond dated 1/8/2014 in DARRTS for detailed reviews of these data. In brief, there were no nonclinical deficiencies that would preclude approval; however, there were some concerns for local

toxicity with SABER-bupivacaine, specifically with local effects associated with the SAIB vehicle and the resulting formation of the depot along with its persistence in tissues. In single subcutaneous injection studies of SABER-bupivacaine in rodents and rabbits, there were local toxicity findings including swelling, discoloration, and mild-to-marked inflammation of the subcutaneous tissues associated with cyst formation in rats and a granulomatous inflammation around vacant spaces thought to represent the SAIB depot in rabbits. The SAIB depot vehicle was found to be essentially unchanged and still present 12 months after injection described as viscous materials. In wound healing studies in rats and minipigs, microscopic evidence of inflammation, cysts, and mild dermal gap were observed in rats and slightly less advanced re-epithelialization, more inflammation (moderate in severity), giant cells, and clear vacuoles thought to contain SAIB. These local effects were consistent with a depot-provoked foreign body reaction and the severity of which should be generally related to the volume of SABER-bupivacaine instilled to the area of a particular site. While bupivacaine in SABER-bupivacaine is released over 3 days, the SAIB depot is expected to remain at injection sites for a year or longer.

From a systemic safety perspective, an acceptable systemic safety profile was demonstrated from subcutaneous administration studies of SABER-bupivacaine in rats and rabbits that provided exposures that, in the absence of clear nonclinical systemic toxicity, exceed those achieved in the clinical trials. Studies that addressed inadvertent release of bupivacaine demonstrated that there was no dose-dumping and bupivacaine in SABER-bupivacaine is released over 3 days. However, none of these studies addressed the potential for dose dumping if the product were inadvertently exposed to the intravascular space as discussed below.

The original NDA submission also included a full battery of genetic toxicology studies, including the Ames test, in vitro chromosomal aberrations assay, and in vivo micronucleus rat study, that demonstrated bupivacaine is negative for genetic toxicity. The reference drug label for Marcaine lacks genetic toxicology information so summaries of these studies will be included in labeling for this product.

Drs. Bond's and Wasserman's original reviews raised concerns if the product were to gain access to or be injected into intra-articular space. As noted in Dr. Wasserman's secondary review:

> SABER-bupivacaine is not intended for intra-articular injection; however, evaluations of this route were conducted both rabbit and dog and were notable as described by Dr. Bond. Microscopic findings of minimal to moderate synovial hyperplasia, fatty degeneration, inflammation, fibrosis and osseous metaplasia were noted in rabbits two weeks after administration and were still present six weeks post-injection. Findings were slightly worse in SABER-bupivacaine animals than SABER-placebo and was not observed in saline treated joints or in the contralateral joint. Intra-articular administration of SABER-bupivacaine or SABER-placebo to dogs produced similar and additionally significant joint effects including subchondral bone fibrosis and necrosis of cartilage and at the highest volumes administered, the necrosis considered of marked severity. I concur with Dr. Bond that, if approved, this should be described in Animal Toxicology section of the label as a further method to discourage this route of administration.

The Applicant provided risk assessments for inadvertent intravascular injection on February 26, 2020, which include their assessment of the likelihood of inadvertent intravascular administration of Posimir, risks of intravascular administration of Posimir, and mitigation strategies to reduce the risk of inadvertent intravascular administration of Posimir. Part of this response included their assessment of the likelihood for IV injection. Briefly, the Applicant contended the likelihood of inadvertent intravascular administration of Posimir to be low based on the estimated incidence of local anesthetic systemic toxicity (LAST) to be 5.7 per 100,000, limited use of needles for the indication of Posimir, a larger-gauge needle used for Posimir compared to immediate-release local anesthetics, and physical properties of Posimir (color difference (light amber) from immediate-release bupivacaine HCl (colorless) and its viscosity). We defer to the clinical team to assess the likelihood of the IV injection in the clinical setting.

In their response, the Applicant also discussed the potential risks of intravascular administration of Posimir. They note that based on the release rate of bupivacaine following the subcutaneous injection of Posimir in healthy volunteers, there appears to be no dose dumping and the release rate of bupivacaine was found to be stable over 72 hours. However, it is not clear if the same release rate would occur in the intravascular space, particularly if the material forms small droplets with larger surface area. The Applicant stated that they would be willing to conduct an animal study to investigate intravascular injection of Posimir post approval and they proposed the use of lipid emulsion to treat any resulting bupivacaine-induced LAST. The Applicant failed to discuss the potential risk of the development of emboli and/or intravascular hemolysis, and mitigation strategy related to the systemic exposure to the SABER vehicle. In addition, risk assessments of the possibly longer duration of anticipated resuscitation using lipid emulsion as a rescue measure from LAST of Posimir were not addressed.

In response to a follow-up IR to address the risks from the SABER vehicle, the Applicant provided additional information on March 13, 2020 that included summaries of in vitro studies that investigated the potential for Posimir to cause emboli and hemolysis following inadvertent intravascular injection. Small Posimir droplets were formed when Posimir was injected into a tubing with both open ends immersed in a beaker containing bovine plasma at room temperature and these droplets remained fluid and distensible over approximately 1 hour. However, studies that added a (b) (4) filter to the in vitro bovine serum with tubing system to simulate passage through capillary beds demonstrated higher pressures were required to pass the droplets through the system with longer incubation times of Posimir. For example, injected Posimir, without any incubation, passed through the system with normal physiologic pressure of 4 to 12 mmHg. However, Posimir injected for 10 and up to 120 minutes required pressures of 50 and up to 10 times this, respectively to pass through the system, suggesting that Posimir droplets may be retained in the pulmonary capillary bed or other capillary systems though the Applicant contended that the pressure required to pass the injected drug would likely be lower if the system was run at (b) (4). Nevertheless, without data evaluating intravascular degradation of Posimir and/or SABER vehicle, it is not clear

whether Posimir stays long enough in the systemic circulation to form viscous droplets, which may cause a pulmonary embolism or other microvascular blockade.

The Applicant also discussed the risk for hemolysis. Although there was hemolysis observed through in vitro studies, reviewed with the original NDA submission, likely from benzyl alcohol, the Applicant concluded that Posimir would not cause hemolysis because the level of benzyl alcohol would not be sufficient to cause clinically relevant hemolysis. The Applicant estimated the concentration of benzyl alcohol to be (b) (4) less than the in vitro test, which showed hemolytic potential of Posimir and SABER vehicle, based on 5 mL of Posimir in a total blood volume of 5 L. However, this is a theoretical calculation of the concentration of benzyl alcohol when Posimir has reached steady state in systemic circulation and it would not address the potential effects from the higher local concentration of benzyl alcohol at the injection site if inadvertently injected into intravascular space.

1.3 Recommendations

1.3.1 Approvability

Although no approval issues were identified in the first review cycle, safety concerns regarding inadvertent intravascular injection of Posimir were raised during the 2020 advisory committee meeting. Based on the data to date, we cannot state that there is minimal risk should the product be injected intravascularly. If the potential risks of the inadvertent injection of Posimir and clinical mitigation strategies and risk assessments provided by the Applicant are not considered to be sufficient to dismiss potential safety concerns, the following nonclinical studies to investigate the potential toxicity of inadvertent intravascular injection can be conducted to further inform the benefit;risk profile for this product and inform labeling in case of overdose or Local Anesthetic Systemic Toxicity (LAST).

Deficiency 1
You have not adequately characterized the potential toxicity of your drug product if it is inadvertently administered into the intravascular space. To address this deficiency, conduct a nonclinical intravenous toxicity study that includes both an acute and delayed timepoint with full histopathology to determine the ultimate impact of the drug product and fate of the vehicle. The study should include collection of pharmacokinetic data to determine if intravascular injection results in more rapid release of the bupivacaine dose from the vehicle. We recommend a small pilot study prior to the pivotal study to confirm that IV administration does not result in catastrophic moribundity.

Deficiency 2
You have not provided adequate data to conclude that standard treatments with lipid infusions can be effective if LAST were to occur following use of this drug product. Conduct a nonclinical toxicology study to confirm that lipid infusions will counteract the adverse effects due to LAST or provide adequate justification that lipid infusions will be successful to treat LAST should this occur in the clinical setting. Ultimately, provide

recommendations on how prescribers should treat LAST should it occur following use of this drug product.

1.3.2 Additional Nonclinical Recommendations

N/A

1.3.3 Labeling

The table below provides the original approved labeling language, the proposed language (b) (4) by the Applicant, recommended changes by this reviewer, and rationale for the recommended changes. Note that the final label will be found on the Action letter after further internal discussion and negotiations with the Applicant.

Labeling Recommendation

Marcaine (listed drug) Labeling (2018)	Applicant's Proposed Labeling under SDN-031 (8/30/2019)	Reviewer's Recommended Changes in Labeling	Rationale for Changes/Comments
8 USE IN SPECIFIC POPULATIONS			
8.1 Pregnancy			
There are no adequate and well-controlled studies in pregnant women. MARCAINE should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Bupivacaine hydrochloride produced developmental toxicity when administered subcutaneously to pregnant rats and rabbits at clinically relevant doses. This does not exclude the use of MARCAINE at term for obstetrical anesthesia or analgesia (See Labor and Delivery).	Risk Summary (b) (4)	Risk Summary There are no studies conducted with POSIMIR in pregnant women. In animal studies, embryo-fetal lethality was noted when bupivacaine was administered subcutaneously to pregnant rabbits during organogenesis at 0.6 times the maximum recommended human dose of POSIMIR at 660 mg bupivacaine. Decreased pup survival was observed in a rat pre- and post-natal development study (dosing from implantation through weaning) at 0.6 times the maximum recommended human dose of POSIMIR at 660 mg bupivacaine. Based on animal data, advise pregnant women of the potential risks to a fetus. *[see Data]* The background risk of major birth defects and	We defer to the Maternal Health and clinical review teams on clinical labeling language. Wording was revised to be consistent with Marcaine label. (b) (4)

	(b) (4)	miscarriage for the indicated population is unknown. However, the background risk in the U.S. general population of major birth defects is 2-4% and of miscarriage is 15-20% of clinically recognized pregnancies.	(b) (4)
Bupivacaine hydrochloride was administered subcutaneously to rats at doses of 4.4, 13.3, & 40 mg/kg and to rabbits at doses of 1.3, 5.8, & 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). The high doses are comparable to the daily maximum recommended human dose (MRHD) of 400 mg/day on a mg/m2 body surface area (BSA) basis. No embryo-fetal effects were observed in rats at the high dose which caused increased maternal lethality. An increase in embryo-fetal deaths was observed in rabbits at the high dose in the absence of maternal toxicity with the fetal No Observed Adverse Effect Level representing approximately 1/5th the MRHD on a BSA basis.		Data <u>Animal Data</u> Bupivacaine hydrochloride was administered subcutaneously to rats at doses of 4.4, 13.3, & 40 mg/kg and to rabbits at doses of 1.3, 5.8, & 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). The high doses are approximately 0.6 times the daily maximum recommended human dose (MRHD) of 660 mg/day on a mg/m^2 body surface area (BSA) basis. No embryo-fetal effects were observed in rats at the high dose which caused increased maternal lethality. An increase in embryo-fetal deaths was observed in rabbits at the high dose in the absence of maternal toxicity with the fetal No Observed Adverse Effect Level representing approximately 0.2 times the MRHD on a BSA basis**.** In a rat pre- and post-natal developmental study	(b) (4)

In a rat pre- and post-natal development study (dosing from implantation through weaning) conducted at subcutaneous doses of 4.4, 13.3, & 40 mg/kg, decreased pup survival was observed at the high dose. The high dose is comparable to the daily MRHD of 400 mg/day on a BSA basis.	(b) (4)	(dosing from implantation through weaning) conducted at subcutaneous doses of 4.4, 13.3, & 40 mg/kg, decreased pup survival was observed at the high dose. The high dose is approximately 0.6 times the daily MRHD of 660 mg/day on a BSA basis.	(b) (4)
8.2 Lactation			
	Risk Summary POSIMIR has not been studied in nursing mothers. Bupivacaine can persist in plasma for up to (b) (4) hours [see Clinical Pharmacology (12)] and benzyl alcohol, a POSIMIR excipient, for up to 12 hours after POSIMIR administration. Both bupivacaine and benzyl alcohol have been reported to be excreted in human milk. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for POSIMIR and any potential adverse effects on the breastfed infant from POSIMIR or from the underlying maternal condition.	None	We defer to the Maternal Health and clinical review teams on clinical labeling language.

13 NONCLINICAL TOXICOLOGY			
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility			
Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine hydrochloride have not been conducted. The mutagenic potential and the effect on fertility of bupivacaine hydrochloride have not been determined.	(b) (4)	*Carcinogenesis* Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine hydrochloride have not been conducted. *Mutagenesis* (b) (4) *Impairment of Fertility* The effect of bupivacaine on fertility has not been determined.	Section 13 should have subheadings. A full battery of genetic toxicology studies was submitted with the NDA during the first cycle and are included in labeling.
13.2 Animal Toxicology			
		13.2 Animal Toxicology and/or Pharmacology Necrosis of the joint cartilage was observed following intra-articular injection of a single dose of Posimir or SABER vehicle in the dog model.	Language should also be included in warnings and precautions that directs the reader to this section.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)
2180-92-9; 38396-39-3

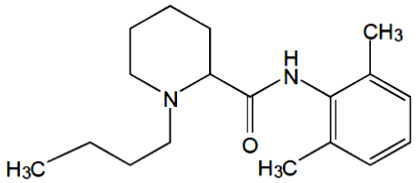
Generic Name
Bupivacaine or Bupivacaine base

Code Name
NA

Chemical Name
(±)-1-Butyl-2',6'-pipecoloxylidide-(2*RS*)-1-butyl-*N*-(2,6-dimethylphenyl)piperidine -2-carboxamide

Molecular Formula/Molecular Weight
$C_{18}H_{28}N_2O$/288.43

Structure or Biochemical Description



Pharmacologic Class
An amide-type local anesthetic

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 66086 (SABER-Bupivacaine)
NDA 16964 (Marcaine; bupivacaine HCl; referenced NDA), approved October 3, 1972
DMF (b) (4)
DMF

2.3 Drug Formulation

The composition of the drug product is in the following table. See the NDA 204803 nonclinical review dated January 08, 2014 in DARRTS.

Composition and Ingredient Functions of SABER-Bupivacaine

Ingredient	Composition % w/w	Composition mg/mL [a]	Amount (mg) Administered in a 5 mL Dose	Function	Specification
Bupivacaine	12	132	660	Active Pharmaceutical Ingredient	In-House Specification
Sucrose acetate isobutyrate	66	726	3630	Extended release agent	In-House Specification
Benzyl alcohol	22	242	1210	Solvent	NF, EP
(b) (4)					
Total	**100**	**1100**	**5500**	-	-

[a] The density of SABER-Bupivacaine is 1.1 g/mL at 25°C, therefore the concentration expressed as 12% w/w is equivalent to 13.2% w/v

2.4 Comments on Novel Excipients

See the NDA 204803 nonclinical review dated January 08, 2014 in DARRTS.

2.5 Comments on Impurities/Degradants of Concern

See the NDA 204803 nonclinical review dated January 08, 2014 in DARRTS.

2.6 Proposed Clinical Population and Dosing Regimen

NA

2.7 Regulatory Background

IND 66,086 (SABER-Bupivacaine)

• Original IND submitted October 23, 2002 by DURECT but inactivated December 17, 2002 as the preclinical data did not support the proposed clinical trial due to findings of injection site inflammation and necrosis for which no evidence of resolution has been submitted.

• IND reactivated January 10, 2006 with submission of additional nonclinical data and substitution of benzyl alcohol (b) (4) as drug product solvent.

NDA 204803 (SABER-Bupivacaine)

- Original NDA submitted April 12, 2013
- A discipline response letter issued January 14, 2014 followed by a clinical response letter for the POSIMIR 5050(b)(2) NDA on Feb 12, 2014
- NDA resubmitted June 27, 2019

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study Number
An Investigational Study of Post-Surgical SABER-Bupivacaine Vehicle Instilled into a Surgical Wound with a 10-Day Recovery Period in Gottingen Minipigs	(b) (4)-434063
An Investigational Study of Post-Surgical SABER-Bupivacaine Vehicle Instilled into a Surgical Wound with a 10-Day Recovery Period in Beagle Dogs	(b) (4)-434060

3.2 Studies Not Reviewed

NA

3.3 Previous Reviews Referenced

- NDA 204803 nonclinical primary review (first cycle) dated January 08, 2014 in DARRTS
- NDA 204803 supervisory nonclinical review (first cycle) dated January 15, 2014 in DARRTS

4 Pharmacology

No new pharmacology studies were submitted with the resubmission of this NDA. See the NDA 204803 nonclinical review dated January 08, 2014 in DARRTS.

5 Pharmacokinetics/ADME/Toxicokinetics

No new pharmacokinetics/ADME/Toxicokinetics studies were submitted with the resubmission of this NDA. See the NDA 204803 nonclinical review dated January 08, 2014 in DARRTS.

6 General Toxicology

No new general toxicology studies were submitted with the resubmission of this NDA. See the NDA 204803 nonclinical review dated January 08, 2014 in DARRTS.

7 Genetic Toxicology

No new genetic toxicology studies were submitted with the resubmission of this NDA. A full battery of genetic toxicology studies was submitted with the NDA during the first cycle. See the NDA 204803 nonclinical review dated January 08, 2014 in DARRTS.

8 Carcinogenicity

No carcinogenicity studies were submitted and none were required for this NDA as the drug is intended for acute use.

9 Reproductive and Developmental Toxicology

No new reproductive and developmental toxicology studies were submitted with the resubmission of this NDA. As part of the requirement under the Pregnancy Lactation and Labeling Rule (PLLR), the Applicant conducted a review of the literatures in the original NDA application submitted on April 12, 2013. In addition to literature articles submitted by the Sponsor, a total of 20 literature articles were reviewed.

Bupivacaine and Pregnancy/Lactation – Animals literature review

Maternal-fetal distribution of bupivacaine in the rabbit (Carson and Reynolds 1988)

Objective: To evaluate whether the fetal:maternal (F:M) plasma concentration ratios of bupivacaine are relatively low due to extensive tissue uptake or slow placental transfer

Method: Bupivacaine (1.25 mg/mL) was infused ***intravenously*** into a neck vein of nine anesthetized pregnant rabbits (3.8 to 4.9 kg) at Gestation Day 30. Bupivacaine concentrations were measured in fetal plasma, brain, placenta, amniotic fluid, maternal plasma sampled synchronously and maternal brain by gas-liquid chromatography.

Key Findings:

- The mean maximum fetal:maternal (F:M) ratio was 0.31 (SD: 0.16, range: 0.18-0.64).
- The mean fetal brain:plasma ratios ranged from 2.04 to 5.09.
- No progressive increase in fetal brain bupivacaine concentration with time was observed.
- The maternal brain:plasma ratio was 1.62.
- The maximum fetal brain concentration was only 0.27 to 0.86 of maternal brain concentration.
- Concentrations increased with time in amniotic fluid but did not exceed those in maternal plasma.
- Taken together, tissue uptake could not account for low F:M ratios persisting beyond 80 min.

Reviewer's note: While the study data may be interesting, the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; therefore, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

Toxicity of bupivacaine and ropivacaine in relation to free plasma concentrations in pregnant rats: a comparative study (Danielsson, Danielson et al. 1997)

Objective: To compare the threshold for the induction of toxic effects in pregnant rats in relation to the plasma concentrations of bupivacaine and ropivacaine and relate plasma levels resulting in adverse effects in pregnant rats/offspring to plasma levels reported after epidural administration of therapeutic doses of bupivacaine or ropivacaine in Caesarean section

Method:

- Pilot Study: Pregnant female Sprague-Dawley rats (N=6-10 per dose) were ***subcutaneously*** injected with descending dose levels of bupivacaine (24, 22, 21, 20, 18, 16, and 14 mg/kg) and ropivacaine (26, 25, 16, and 5.3 mg/kg) from Day 15 of pregnancy, during delivery, and in postnatal days 3-21 after parturition.

- Main Study: Pregnant female Sprague-Dawley rats (n=15/group) were injected subcutaneously with saline, bupivacaine (5.5, 15, and 18 mg/kg), or ropivacaine (5.3, 14, and 25 mg/kg) in the back region once daily from day 15 of pregnancy to day 15 after parturition. Six satellite groups (n=8/group) were included in the study for evaluation of plasma concentration and protein binding of the test articles. The subcutaneous route was selected to mimic the plasma concentration profile obtained after different routes of administration in obstetric anesthesia (paracervical block or epidural routes).

Note that the authors stated that the experiment was conducted according to regulatory (OECD, FDA) and Good Laboratory Practice guidelines for peri- and postnatal studies for new drugs.

Key Findings:

- Pilot study:
  - Maternal – Chewing, spasm, dyspnea, drowsiness, salivation, and convulsion were observed in a dose-dependent manner in groups given 18 to 24 mg/kg of bupivacaine. In addition, there were dose-dependent increases in maternal death and a prolongation of the time for delivery (~30 min) in surviving mothers at the dose level of 20 mg/kg or higher. In contrast, no deaths or adverse symptoms were observed in groups treated with 25 mg/kg or lower ropivacaine. See the Table below.

Adverse effects observed on one or several occasions (number of animals showing symptoms/number of animals in the group) during the dosing period in the pilot study. Different subcutaneous doses of bupivacaine and a well-characterized dose of ropivacaine (26 mg/kg) after administration from day 15 of pregnancy to day 3 after parturition. Doses are expressed in mg/kg.

	Ropivacaine	Bupivacaine							Control
Adverse effects	26	24	22	21	20	18	16	14	0
Irregular breathing	2/7	7/7	4/6	4/5	5/5	2/5	5/6	5/6	0/7
Increased salivation	2/7	7/7	4/6	4/5	4/5	2/5	2/6	4/6	0/7
Chewing	2/7	7/7	2/6	4/5	3/5	1/5	0/6	0/5	0/7
Decreased activity	1/7	7/7	3/6	4/5	5/5	2/5	5/6	4/5	0/7
Clonic convulsions	0/7	7/7	2/6	4/5	4/5	1/5	0/6	1/5	0/7
Mortality	0/7	6/7	2/6	4/5	1/5	0/5	0/6	0/5	0/7
Impaired maternal care	1/7	3/3*	3/4*	1/1*	3/5*	1/5	1/6	1/5	

* Evaluated in surviving dams after parturition.

  - Litter – Increased incidence of postnatal death of the offspring in groups treated with bupivacaine were during the first few days after delivery. It appears that mortality of offspring was secondary to impaired maternal care of the neonates. Surviving neonates showed the same physical development compared to the control animals. No increased incidence of neonatal death was observed in groups treated with ropivacaine.
- Main study:
  - Maternal – Increased salivation, spasms, chewing, irregular breathing, drowsiness, and clonic convulsion were observed in a dose-dependent manner in groups treated with 15 mg/kg or higher of bupivacaine. See the Table below. The onset of signs was approximately 10 min after dosing and last 30 min to 5 hr. No adverse effects were observed at 5.5 mg/kg of bupivacaine. There was no shortening or prolongation of the gestation period and no disturbance of parturition, nursing and lactation related to bupivacaine or ropivacaine administration.

Adverse affects in the main comparative peri- and postnatal study (observed on one or several occasions (number of animals showing symptoms/number of animals in the group) during the dosing period) of bupivacaine and ropivacaine after subcutaneous administration during day 15 of pregnancy to day 15 after parturition. Doses are expressed af mg/kg (μmol/kg in brackets).

	Ropivacaine			Bupivacaine		
Adverse effects	5.3 (16)	14 (43)	25 (75)	5.5 (16)	15 (43)	18 (53)
Irregular breathing	0/13	0/15	0/13	0/15	15/15*	15/15**
Increased salivation	0/13	0/15	0/13	0/15	15/15*	15/15**
Piloerection	0/13	0/15	0/13	0/15	15/15*	15/15**
Chewing	0/13	0/15	0/13	0/15	0/15	15/15**
Clonic convulsions	0/13	0/15	0/13	0/15	1/15	13/15**
Mortality	0/13	0/15	0/13	0/15	1/15	3/15

* P<0.001 when compared to ropivacaine (14 mg/kg).
** P<0.001 when compared to ropivacaine (25 mg/kg).

There were no body weight gain and food consumption changes. Both bupivacaine and ropivacaine were rapidly absorbed and eliminated from the plasma. See the Table below.

Mean (±S.D.) pharmacokinetic parameters of ropivacaine and bupivacaine in rats on day 20 of pregnancy.

Dose mg/kg	Body weight, kg	C_{max} mg/l	t_{max} hr	AUC mg/l hr	$t_{1/2}$ hr
Ropivacaine					
5.3	0.354±0.030	0.30±0.10*	0.8±0.3	1.01±0.13	2.3±1.0*
14	0.361±0.026	0.49±0.13	1.0±0.9	4.12±1.03	6.5±2.9
25	0.344±0.020	1.33±0.47	0.6±0.2	4.94±0.62#	2.7±0.6
Bupivacaine					
5.5	0.353±0.030	0.19±0.05	0.6±0.2	2.52±1.48	15.0±12.3
15	0.359±0.026	0.63±0.12	0.8±0.3	3.15±0.25	5.2±0.8
18	0.351±0.021	1.14±0.41	0.5±0	3.24±0.47	2.7±2.2

* P<0.05 when compared to bupivacaine (5.5 mg/kg).
# P<0.05 when compared to bupivacaine (18 mg/kg).

Protein binding at 0.5 h after dosing was significantly lower for ropivacaine compared with bupivacaine in pregnant rats. See the Table below.

Mean (±S.D.) total and free plasma concentrations (mg/l) and protein binding (%) after administration of different doses (mg/kg) of ropivacaine and bupivacaine, 0.5 hr after dosing on day 21 of pregnancy.

	Ropivacaine				Bupivacaine		
Dose	Total	Free	Bound	Dose	Total	Free	Bound
5.3	0.20±0.05	0.11±0.04	47±7*	5.5	0.21±0.07	0.06±0.02	70±7
14	0.52±0.13	0.26±0.04	49±5*	15	0.50±0.18	0.16±0.07	67±6
25	1.26±0.07	0.64±0.02	50±8*	18	0.79±0.27	0.24±0.09	69±7

- Litter – There were no significant differences in litter size, offspring loss, or weight reduction at birth or on Days 7 and 21 after parturition. In addition, there were no significant changes in the timeline of the physical developmental milestones (i.e., completed development of pinna unfolding on Day 4, teeth eruption on Day 12, and completed eye opening on Day 17).

- Ropivacaine is less toxic in late pregnant rats than bupivacaine.

Reviewer's note: The NOAEL of bupivacaine appears to be the LD (5.5 mg/kg). In discussion, the author mentioned that potentiation of bupivacaine toxicity (i.e., increased sensitivity of myocardium and nerves to bupivacaine) may be associated with the increased levels of steroidal hormones (i.e., progesterone) occurring in late pregnancy, which was interesting. Nonetheless, the study data do not appear to provide additional

safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; therefore, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

Perinatal bupivacaine and infant behavior in Rhesus monkeys (Golub and Germann 1998)

Objective: To assess the effect of perinatal epidural bupivacaine analgesia on infant behavioral development

Method: Bupivacaine (1.2 mg/kg; an initial dose of 0.6 mg/kg (0.5% concentration) for the initial 2-min period then 0.6 mg/kg over the next 20-min period followed by a saline flush) was administered to term-pregnant rhesus monkeys (n=11, treated; n=8, procedural saline controls) via ***epidural catheter infusion*** on Gestation Day 165 and infant behavior was evaluated for 1 year using a test battery including infant neurobehavioral tests, observation of spontaneous behavior, and structured cognitive testing. Blood samples from dams and neonates were collected immediately following vaginal delivery and at 1, 12, and 60 h postnatal from the infant.

Key Findings:

- Mature and active behaviors was lower in bupivacaine-exposed infants than the controls during Week 8. Note that active behavior evaluation includes cage exploration (mouthing object, use of hands for exploration (manual explore)), visual surveillance (scan and fixate), locomotor activities (walking, climbing, swinging, jumping), and motor disturbance behaviors (flipping, circling, banging, climbing patterns, and rocking).

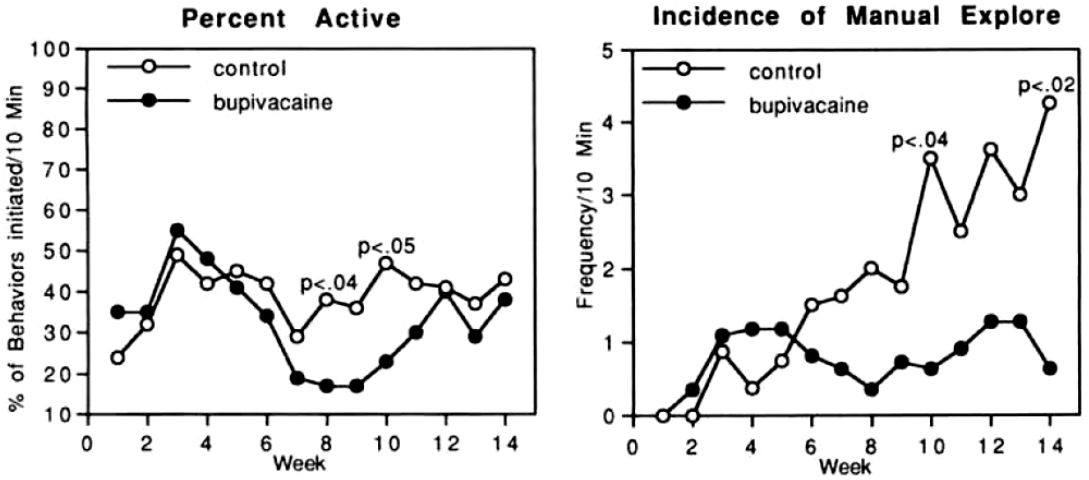


FIG. 1. Endpoints from observation of motor and postural maturation at 1 to 14 weeks of age that demonstrated group differences. Data are in terms of frequency of occurrence during the 10-min session.

- For the 6- to 12-month observation period, active behaviors were greater for the bupivacaine-exposed infants than controls during the last block of 6 session. Greater activities of bupivacaine treated infants were due to a greater proportion

of motor to total (motor and resting) disturbance behaviors. No group differences were observed for locomotor, quiet activities, or passive resting behaviors.

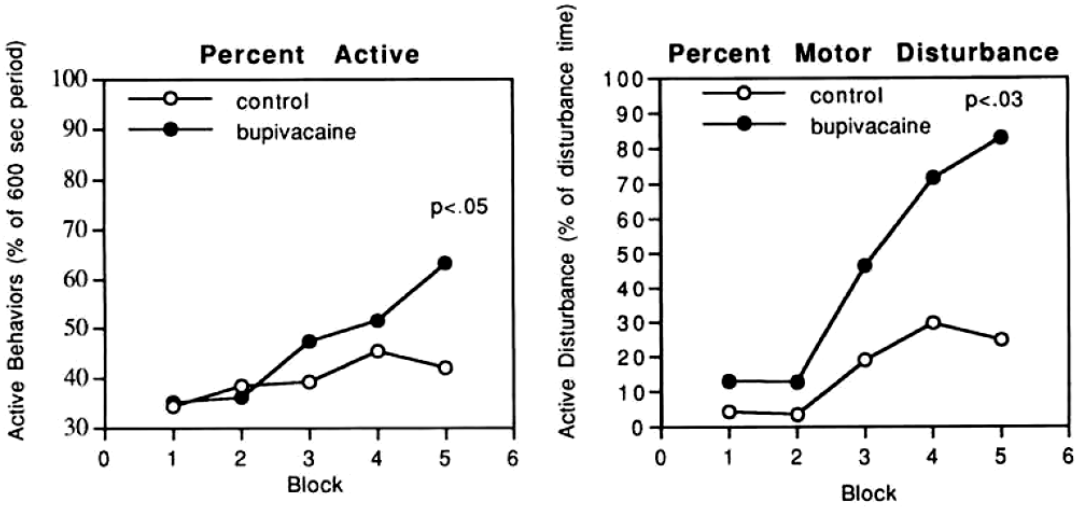


FIG. 2. Endpoints from observation of motor and postural maturation at 6 to 12 months of age that demonstrated group differences. Data are presented in terms of duration and are summarized as an initial block of four weekly sessions followed by four blocks of six weekly sessions for comparison with previous studies. For definition of behavior categories, see Table 3.

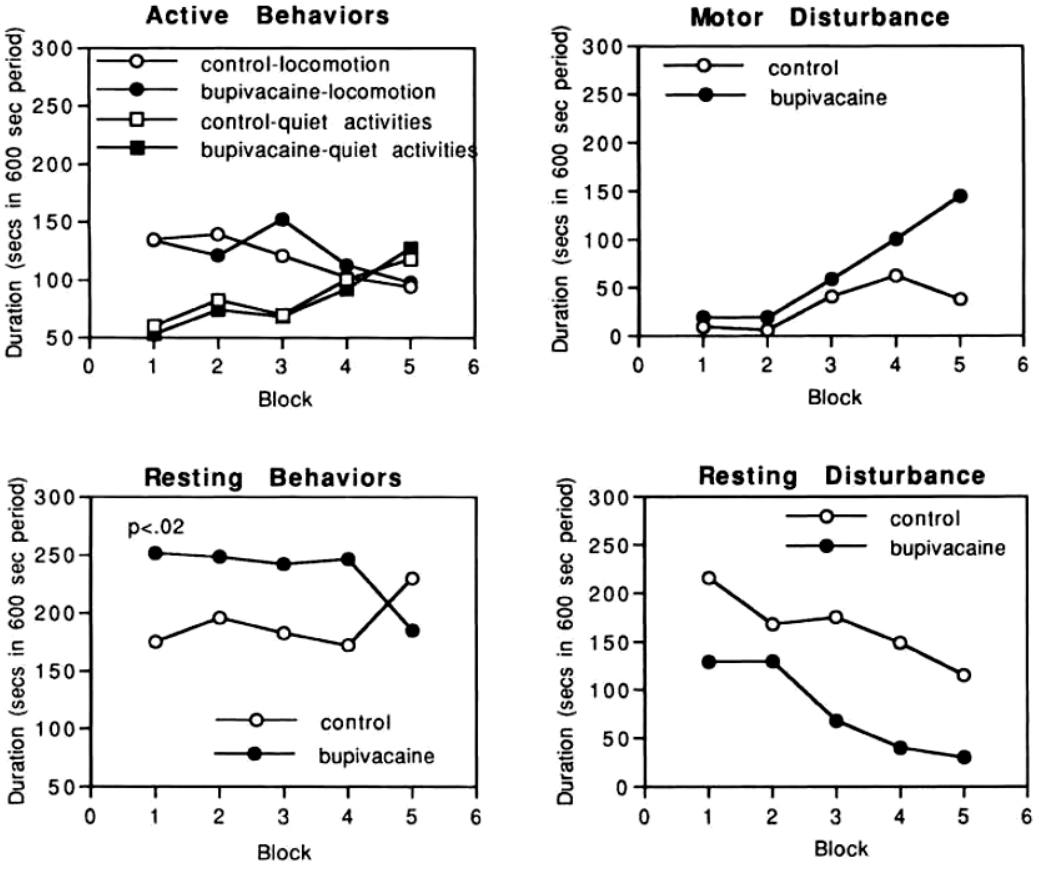


FIG. 3. Comparison of subcategories of active and resting behaviors observed from 6 to 12 months of age that made up the composite indices of Fig. 2. For definition of behavior categories, see Table 3.

- No differences were observed for behaviors reflecting fine motor maturation (i.e., voluntary grasp, reaching, and finger-thumb coordination).

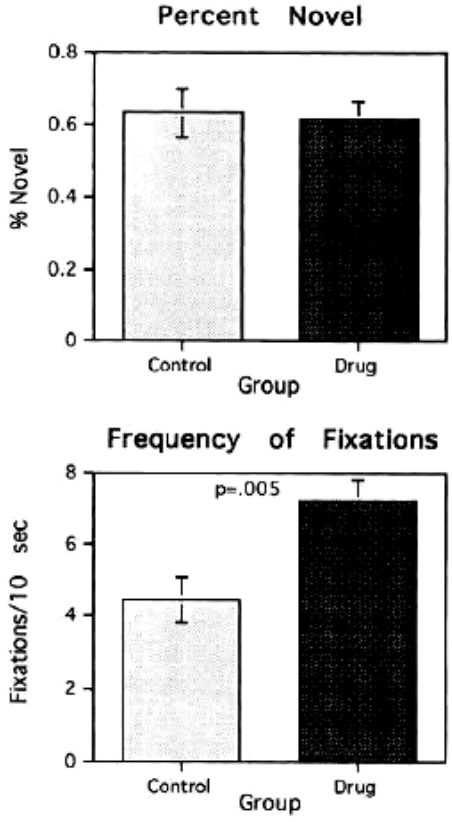
TABLE 4

MATURATION OF FINE MOTOR BEHAVIORS

	Voluntary Grasp	Reaching	Finger–thumb Coordination	Bimanual Coordination
Max sessions	15	40	44	44
No. Attaining criterion				
Control	8/8	8/8	8/8	4/8
Bupivacaine	9/11	11/11	10/11	5/11
Sessions to criterion				
Control	7.7 ± 0.6	8.7 ± 1.5	33.2 ± 2.2	34.6 ± 4.4
Bupivacaine	8.0 ± 1.1	11.9 ± 3.6	31.5 ± 2.6	37.2 ± 3.0

Mean ± SEM of sessions required to attain performance criterion are shown. If an infant did not attain criterion, the maximum number of sessions was used in computation of means.

- No significant differences in novelty preference (1 to 1.5 months of age) were observed. Bupivacaine-exposed infants directed more fixations at the stimuli and their fixations were shorter than the control.



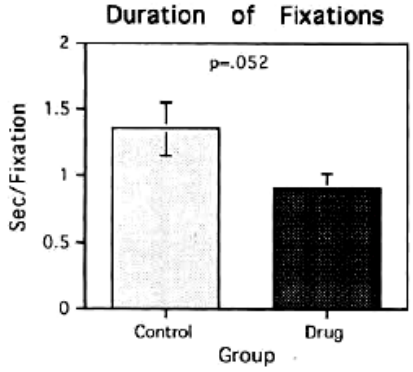


FIG. 4. Looking behavior during novelty preference testing. Data are summarized for the first two of the seven sets, because controls demonstrated novelty preference only for these more highly contrasting stimulus pairs.

- No treatment group differences were found on performance of cognitive tasks listed below. Note that object permanence, discrimination reversals, delayed nonmatch to sample, and continuous performance test were evaluated during 1.5

to 3.5 months, 3.8 to 7.4 months, 7.4 to 10.5 months, or 10.5 to 12 months of age, respectively.

TABLE 5

PERFORMENCE OF COGNITIVE TASKS

Cognitive Task	Control	Bupivacaine
Novelty preference (%)	52 ± 5*	60 ± 3
Object permanence (sessions to criterion)	12.2 ± 2.3	13.8 ± 2.0
Discrimination reversal (number of reversals)	4.0	3.4 ± 0.4
(sessions to criterion first reversal)	17.2 ± 2.3	19.6 ± 2.5
Delayed nonmatch to sample (sessions to criterion)	16.4 ± 2.4	13.1 ± 2.4
Continuous performance (% responses to target)	14 ± 3	18 ± 2
(% accuracy)	60 ± 6	68 ± 4

*Mean ± SEM.

Reviewer's note: This study was designed to administer bupivacaine at term via epidural injection but not during labor to avoid a possible confounding of labor length with the direct effects of bupivacaine on the fetus. It appears that bupivacaine-exposed infants showed less active behaviors (i.e., manual explore) from Week 7 to Week 14 but there appears to be no significant differences in cognitive performances over 12 months of age. While study data are interesting, it does not appear to be conclusive whether bupivacaine has significant effects on cognitive behaviors during the development; therefore, this reviewer does not consider the study data necessary to be included in the label at this time. The Division will continue to monitor the literature to determine if additional studies confirm and clarify these findings.

New born tissue concentrations of bupivacaine following maternal epidural administration during labor in guinea pigs (Golub, Kaaekuahiwi et al. 1998)

Objective: To quantitate the concentration of drug in newborn tissues after administration of epidural bupivacaine to the dam during parturition

Method: Bupivacaine (0.25%) was administered ***epidurally*** (0.12 mL/kg) 10 min prior to labor induction in 6 term-pregnant guinea pigs. Bupivacaine concentrations were measured in the plasma, brain, heart, and liver of newborns.

Key Findings:

- Bupivacaine concentrations were higher (2-3X) in the liver than in plasma and other tissues in newborns.

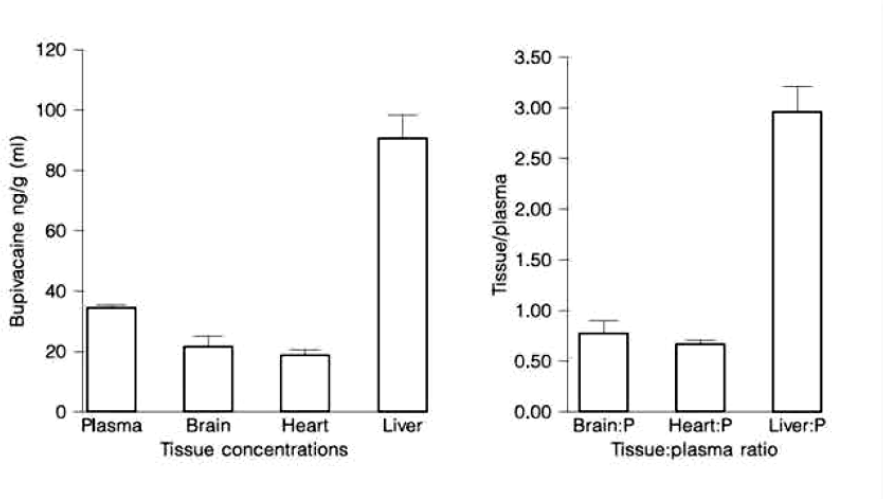


Fig. 1. Average tissue bupivacaine concentrations and tissue:plasma ratios in newborn guinea pigs whose dams received epidural bupivacaine prior to labor induction. Mean ± SEM are shown for the first newborn in each litter. Liver:plasma ratios were significantly greater ($p < 0.0001$) than brain:plasma or heart:plasma ratios for firstborns in each litter ($n = 6$) as well as for all newborns ($n = 22$).

- There were no significant relationships with drug-delivery interval and bupivacaine concentrations in newborn tissues except for the liver. It appears that bupivacaine is actively metabolized in the liver once administered by the epidural injection.

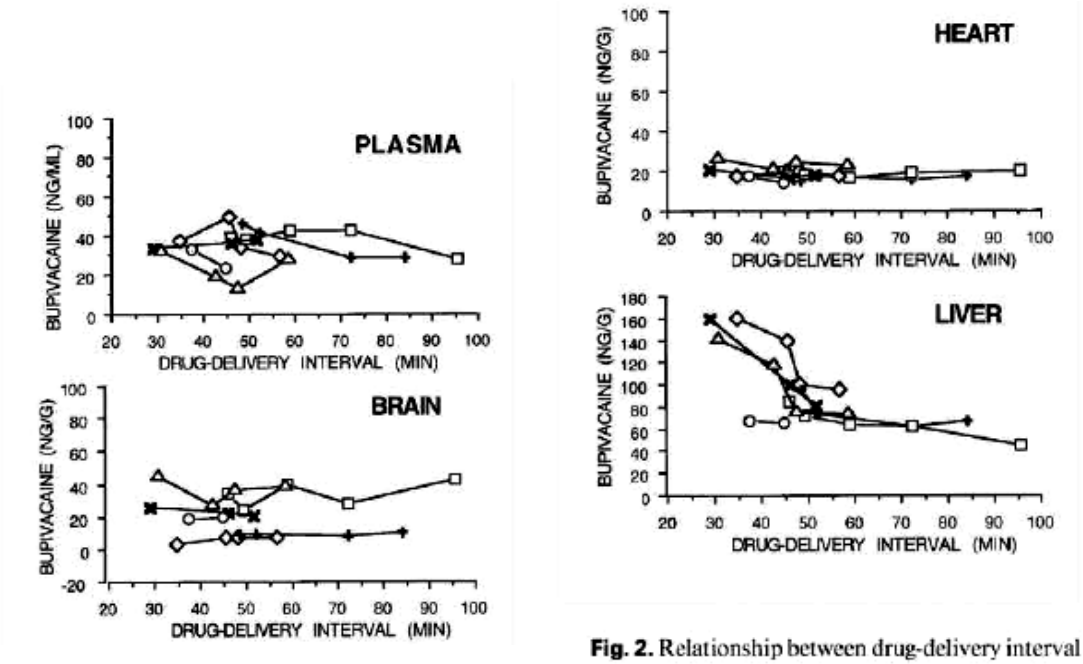


Fig. 2. Relationship between drug-delivery interval and tissue bupivacaine concentrations plotted for individual litters. The six litters used in the experiment are designated by different symbols. Each point represents 1 pup in the litter. Litter size ranged from 2 to 5 pups.

- Bupivacaine concentrations in the newborn brain was higher in animals with Cesarean section delivery compared to the vaginal delivery. Bupivacaine concentrations in the liver and plasma were slightly lower with Cesarean section. Similar bupivacaine concentrations were observed in the heart.

Table 2. Tissue bupivacaine concentrations in term guinea pig newborns delivered vaginally or by cesarean section after bupivacaine administration; only the firstborn of each litter is included

Delivery	n	Drug delivery min	Tissue bupivacaine concentration				
			brain, ng/g	liver, ng/g	heart, ng/g	lung, ng/g	plasma, ng/ml
Cesarean section	3	38 ± 5	53, 23[a]	176 ± 18	11 ± 1	70 ± 23	22 ± 4
Vaginal	6	38 ± 3	17 ± 5	119 ± 18	18 ± 2	no data	37 ± 2

[a] n = 2.

- There were no statistically significant differences between the anesthetized and unanesthetized groups for any acidosis parameter except pCO_2.

Table 3. Comparison of blood gas parameters from newborn guinea pigs that were anesthetized with CO_2 prior to blood sampling with those of unanesthetized newborns. No bupivacaine was administered to the pups that were vaginally delivered after induced labor. Blood gases for fetuses delivered by cesarean section after bupivacaine administration are shown for comparison

	Vaginal delivery[a]		Cesarean section delivery[b]
	anesthetized (CO_2) (n = 5)	unanesthetized (n = 6)	unanesthetized (n = 3)
pH	6.850 ± 0.18	7.018 ± 0.079	7.236 ± 0.052
pCO_2, mm Hg	146.1 ± 22.5	71.6 ± 14.5[c]	62.3 ± 8.4
pO_2, mm Hg	35.5 ± 9.8	26.1 ± 11.5	23.4 ± 7.7
HCO_3, mmol/l	25.3 ± 3.7	16.1 ± 0.8	27.7 ± 6.7
O_2 sat, %	34.2 ± 11.9	28.3 ± 14.2	33.0 ± 17.6
BE, mmol/l	−13.7 ± 3.0	−16.0 ± 2.3	−2.8 ± 1.6
Lactate, mmol/l	11.6 ± 1.2	13.3 ± 1.2	no data
Glucose, mmol/l	5.7 ± 0.4	4.7 ± 0.4	no data

[a] None of these fetuses were exposed to bupivacaine in utero.
[b] These fetuses were exposed to bupivacaine in utero.
[c] Anesthetized vs. unanesthetized, t-test, p = 0.014.

Reviewer's note: This study showed tissue distribution of bupivacaine in newborns after epidural injection during labor in guinea pigs. While the study data may provide information of potential adverse effects in newborns, reproductive toxicity endpoints were not evaluated in this study. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; therefore, this reviewer does not consider the study data necessary to be included in the label.

Disposition of bupivacaine and its metabolites in the maternal, placental, and fetal compartments in rats (Morishima, Ishizaki et al. 2000)

Objective: To determine the disposition of bupivacaine and its metabolites in the maternal, placental, and fetal compartments and evaluate if 1) the placenta plays a significant role in the uptake of bupivacaine, 2) the paraplacental sites contribute to the fetal transfer of bupivacaine, and 3) the metabolites of bupivacaine remain in the fetal tissues longer than in the parent compound

Method: Pregnant Sprague-Dawley rats (20-22 days of gestation) were ***intravenously infused*** with bupivacaine at a rate of 0.33 mg/kg/min over a period of 15 min. The fetuses were delivered either at the end of infusion or at 2 or 4 h after dosing. Maternal and fetal blood and tissue samples were obtained for the assays of bupivacaine and its metabolites using capillary gas chromatography-mass spectrometry. Animals were divided into two study groups: the pharmacokinetic group (n=8) and the transfer groups, which were divided into three subgroups: 0 h (N=7), 2 h (N=7), and 4 h (N=8).

Key Findings:

- No statistically significant changes in heart rate were observed and behavioral changes were not noticeable.
- The mean peak bupivacaine concentration in the maternal plasma was 3123 ± 370 ng/mL and the concentration decreased steadily and was undetectable by 240 min. The major metabolite, 3'-hydroxybupivacaine, was detected in the first plasma sample obtained at the end of infusion at a lower concentration of 80 ± 14 ng/mL. Its concentration decreased more slowly over time and was detectable at 15 ± 7 ng/mL at 240 min. In plasma, two other metabolites, 4'-hydroxybupivacaine and 2,6-pipecoloxylidide, were not detected. The elimination half-life of bupivacaine was 37.7 ± 2.4 min.

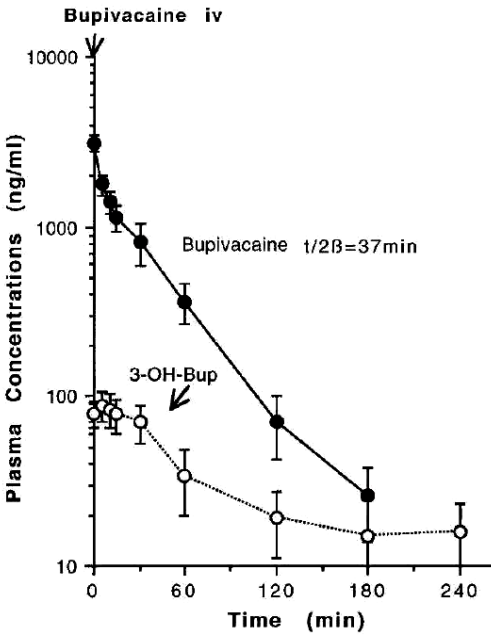


Fig. 1. Plasma concentrations of bupivacaine and 3′-hydroxy-bupivacaine (3′-OH-Bup) after an intravenous bolus dose of 1 mg/kg bupivacaine hydrochloride, followed by infusion at a rate of 0.33 mg · kg^{-1} · min^{-1} over a total period of 15 min to pregnant rats.

- The peak concentration of bupivacaine was measured in the plasma, brain, heart, and liver of dams and fetuses at the end of infusion (0 h). Bupivacaine was undetectable in both dams and fetuses 4 h post infusion. Also, bupivacaine was detected in the placenta, amnion, and myometrium. The fetal to maternal plasma concentration ratio of bupivacaine was 0.29 ± 0.4 and the fetal to maternal placental concentration ratio was 0.63 ± 0.06. The major metabolite, 3'-hydroxybupivacaine was present in all samples in lower concentrations than bupivacaine except for the maternal liver. The highest concentration of bupivacaine and 3'-hydroxybupivacaine were present in the amnion (the fetal membranes).

Table 1. Mean Values (± SD) for Concentrations of Bupivacaine and Metabolites (ng/ml or ng/g) in Pregnant Rats and Their Fetuses at 0-h, 2-h or 4-h Postmaternal Infusion of Bupivacaine (0.33 mg · kg^{-1} · min^{-1}) over 15 min

		End of Infusion (N = 7)			2-h Postinfusion (N = 7)		4-h Postinfusion (N = 8)	
Sample	Bupivacaine	3-OH Bup	4-OH Bup	PPX	Bupivacaine	3-OH Bup	Bupivacaine	3-OH Bup
Mother								
Plasma	1,217 ± 130	39 ± 4	T	ND	115 ± 32	24 ± 10	ND	54 ± 26
Brain	3,350 ± 686	111 ± 21	93 ± 63	ND	203 ± 77	87 ± 64	ND	71 ± 68
Heart	1,906 ± 372	109 ± 46	T	ND	289 ± 72	290 ± 70	ND	273 ± 64
Liver	2,684 ± 718	2,423 ± 322	184 ± 27	107 ± 55	147 ± 77	1,357 ± 138	ND	1,236 ± 564
Fetus								
Plasma	320 ± 38	21 ± 6	ND	ND	T	T	ND	ND
Brain	1,070 ± 227	70 ± 15	47 ± 7	ND	91 ± 60	38 ± 16	ND	31 ± 15
Heart	789 ± 114	T	ND	ND	T	T	ND	ND
Liver	1,041 ± 187	141 ± 28	T	T	38 ± 20	209 ± 132	ND	46 ± 18
Placenta								
Maternal	3,440 ± 413	191 ± 28	173 ± 73	ND	393 ± 126	227 ± 47	88 ± 55	223 ± 183
Fetal	2,163 ± 339	166 ± 42	90 ± 57	T	160 ± 85	162 ± 33	ND	166± 106
Amnion	4,817 ± 976	390 ± 114	593 ± 102	182 ± 70	760 ± 86	371 ± 177	303 ± 76	449 ± 63
Amniotic fluid	226 ± 32	T	T	ND	77 ± 57	37 ± 31	ND	23 ± 6
Myometrium	1,906 ± 243	564 ± 138	281 ± 89	T	413 ± 170	276 ± 61	96 ± 36	302 ± 187

3-OH Bup = 3'hydroxybupivacaine; 4-OH = 4'hydroxybupivacaine; PPX = 2,6-pipecolylxylidide; T = trace amount; ND = not detected.

Table 2. Mean (± SD) Concentrations (ng/g) of Bupivacaine and Its Metabolites in the Maternal and Fetal Liver and Placenta at Various Sampling Times

	Liver			Placenta		
Postinfusion Time (h)	0	2	4	0	2	4
Mother						
Bupivacaine	2,684 ± 718	147 ± 77	ND	3,440 ± 413	393 ± 126	88 ± 55
3-OH Bup	2,423 ± 322	1,357 ± 138	1,236 ± 564	191 ± 28	227 ± 47	223 ± 183
4-OH Bup	184 ± 27	51 ± 34	28 ± 18	173 ± 73	ND	61 ± 42
PPX-HCl	107 ± 55	ND	65 ± 34	ND	ND	32 ± 18
Fetus						
Bupivacaine	1,041 ± 187	38 ± 20	ND	2,163 ± 339	160 ± 85	ND
3-OH Bup	141 ± 28	209 ± 132	46 ± 18	166 ± 42	162 ± 33	166 ± 106
4-OH Bup	T	ND	ND	90 ± 57	ND	ND
PPX-HCl	T	ND	ND	T	ND	ND

ND = not detected; 3-OH Bup = 3'hydroxybupivacaine; 4-OH Bup = 4'hydroxybupivacaine; PPX-HCl = 2,6-pipecolylxylidine hydrochloride; T = trace amount.

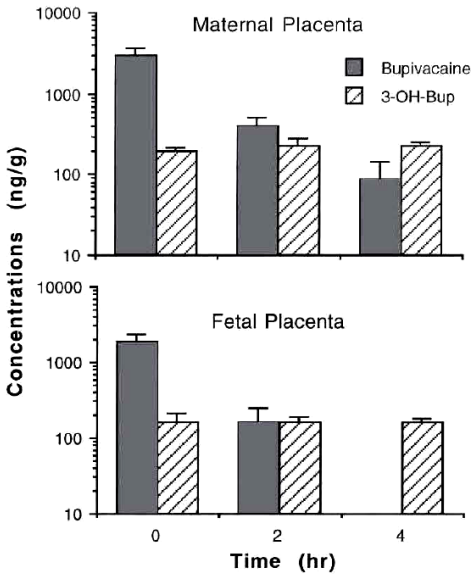


Fig. 2. Concentrations of bupivacaine and 3′-hydroxybupivacaine (3-OH-Bup) in the maternal and fetal placentae at the completion of bupivacaine infusion (0 h), and 2 and 4 h after dosing of bupivacaine to the pregnant rats.

Reviewer's note: The study data suggest that bupivacaine cross the placenta and the peak concentrations of bupivacaine are detected in the plasma, brain, heart, and liver of dam and fetus at the end infusion. It appears that bupivacaine slowly breaks down into its metabolites over time. The elimination half-life of bupivacaine was 37.7 ± 2.4 min. While these data may provide pharmacokinetic characteristics of bupivacaine and its metabolites, reproductive toxicity endpoints were not evaluated in this study. While the study data may be interesting, the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; therefore, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

Effects of oocyte exposure to local anesthetics on in vitro fertilization and embryo development in the mouse (Schnell, Sacco et al. 1992)

Objective: To evaluate the effect of exposure of mouse oocytes to increasing concentrations of local anesthetics such as lidocaine, bupivacaine, and chloroprocaine on subsequent in vitro fertilization rates, embryo cleavages, and embryo development

Method: Mouse oocytes were exposed in vitro to lidocaine, chloroprocaine, and bupivacaine at concentrations of 0, 0.01, 0.1, 1.0, 10.0, and 100.0 mcg/mL for 30 min and in vitro oocyte fertilization at 24 and 48 h and embryo development at 72 h were determined. Three separate experiments were conducted and analyzed.

Key Findings:

- Dose-dependent and statistically significant reduction of in vitro fertilization was observed in mouse oocytes exposed to lidocaine and chloroprocaine from 0.1 mcg/mL. At the highest dose (100 mcg/mL), in vitro fertilization in oocytes exposed to bupivacaine was reduced statistically significantly. Compared to lidocaine and chloroprocaine, bupivacaine showed significantly higher fertilization scores (See the Figure below)/

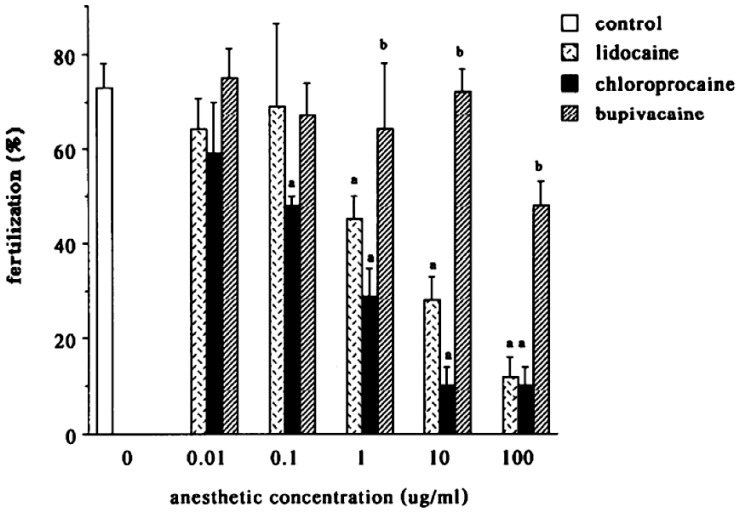


Fig. 1. Percent fertilization at 48 h (means ± standard deviation) for each anesthetic concentration exposure group. The control groups had percent fertilization rates of 66%, 74%, and 77% (mean = 72%) in each of the 3 experiments. For statistical analyses the actual fertilization score (mean ± standard deviation) and not percentages as show were compared using chi-squared analysis of independent samples with Bonferroni correction for multiple comparisons ($^aP < 0.05$ anesthetics compared with control, $^bP < 0.05$ lidocaine and chloroprocaine compared with bupivacaine).

- Similar findings were observed in embryo development. Embryo development scores for bupivacaine treated samples (blastocyst formation) were significantly higher compared to samples treated with lidocaine or chloroprocaine.

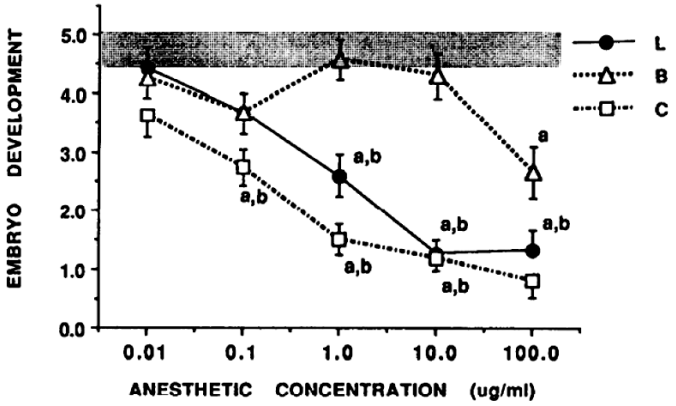


Fig. 2. Embryo development scores (mean ± standard deviation) at 72 h as a function of anesthetic and anesthetic concentration. Shaded area represents embryo development score 4.75 ± 0.28 for the control mouse embryos (non-anesthetic-exposed). Significant differences in groups were determined with ANOVA followed by Scheffé's procedure for multiple comparisons ($^aP < 0.01$ Lidocaine, Bupivacaine, Chloroprocaine compared with control, $^bP < 0.01$ Bupivacaine compared with Lidocaine, Chloroprocaine).

Reviewer's note: The authors conducted in vitro mouse oocyte fertilization and embryo development study suggesting bupivacaine may affect fertilization and embryo development less adversely compared to lidocaine and chloroprocaine. The authors also noted bupivacaine has different characteristics (high potency, slow onset, longer duration of action, and higher protein binding affinity) compared to lidocaine and chloroprocaine, it is not conclusive that bupivacaine is safer than lidocaine and/or chloroprocaine. While the study data may be interesting, this reviewer does not consider the study data necessary to be included in the label.

The effects of bupivacaine, ropivacaine and mepivacaine on the contractility of rat myometrium (Arici, Karsli et al. 2004)

Reviewer's note: This is a short publication (4 pg) of an in vitro study demonstrating bupivacaine, ropivacaine and mepivacaine's effects on rat myometrium contractility (See the following abstract). While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

SUMMARY. Local anesthetic agents are commonly used for obstetric anesthesia and analgesia. We determined the effects of bupivacaine, ropivacaine and mepivacaine on the contractility of isolated pregnant rat uterine muscle strips. Uterine specimens were obtained from 18- to 21-day pregnant Wistar rats ($n = 28$). Myometrial strips were obtained from the uterine horns after removing the fetuses and non-uterine tissue, incubated in organ baths and contractions stimulated with oxytocin. When contractions became regular, strips were exposed to increasing concentrations of the study drugs. Mepivacaine ($n = 8$), ropivacaine ($n = 10$) and bupivacaine ($n = 10$) were used at cumulative doses from 10^{-8} to 10^{-4} mol/L. Two of the local anesthetics, bupivacaine most, ropivacaine least, caused a dose-dependent inhibition of uterine contractility. In contrast, mepivacaine significantly increased uterine contractility. Bupivacaine, ropivacaine and mepivacaine were found to have no effect on frequency of uterine contractions. These results demonstrate that bupivacaine and ropivacaine may inhibit myometrium contractility.

Effect of epidural block on 24-hour urine protein in pregnant rat models with preeclampsia (Dong and Gao 2012)

Reviewer's note: This is a short paper (3 pg) investigating the effects of epidural block on 24-hour urine protein in rat with preeclampsia. From the 14th day of pregnancy, rats were subcutaneously injected with saline, 50 mg of L-nitroarginomethyl ester (L-NAME), or 50 mg L-NAME/epidural block with 25 mcL of 0.125% bupivacaine (BID) for a total of 7 days. It appears that epidural block reduced the 24-hour urine protein and systolic blood pressure on the 20th day of pregnancy, suggesting a potential strategy for treatment of preeclampsia. While the study data may be interesting, this reviewer does not consider the study data necessary to be included in the label.

A comparison of the inhibitory effects of bupivacaine and levobupivacaine on isolated human pregnant myometrium contractility (Fanning, Campion et al. 2008)

Reviewer's note: This is a short publication (5 pg) of an in vitro study demonstrating bupivacaine and levobupivacaine reduce the amplitude of contractions in human myometrium in a concentration-dependent manner. However, the concentrations required for significant inhibitory effects are about 33X higher (0.1 mM) than the clinically relevant plasma concentrations of these drugs after epidural administration and the authors concluded that these effects are unlikely to be significant in the setting of low-dose epidural analgesia in labor. Given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label. Note that potential effects of bupivacaine on contractility are discussed in the *Labor or Delivery* section in the label.

Prolonged local myometrial blockade prevents preterm labor after fetal surgery in a leporine model (Fauza, Berde et al. 1999)

Reviewer's note: This is a short publication (3 pg) investigating the effects of prolonged local anesthetic blockade of the myometrium on preterm delivery after open fetal surgery. Eighteen pregnant New Zealand rabbits at 23 days gestation (term 31 to 33 days) were divided into three groups: Group 1 (hysterotomy), Group 2 (0.5% bupivacaine injection into myometrium before hysterotomy plus treatment with 1.5 mL of polylactic-co-glycolic acid microspheres loaded with 75% bupivacaine and 0.05% dexamethasone before uterine closure), and Group 3 (saline injection into myometrium plus treatment with 1.5 mL of polylactic-co-glycolic acid microspheres before uterine closure). Microsphere suspension with bupivacaine and dexamethasone has been shown to provide peripheral nerve blockade for approximately 5 days. Abortion rates were significantly higher in Group 1 and 3 (83.3% and 71.4%, respectively) compared to Group 2 (0%) and fetal mortality rate was significantly higher in Group 2 (87.5%) than Group 1 and 3 (0% or 33.3%, respectively). Prolonged local blockade of the myometrium with 0.5% of bupivacaine inhibits preterm labor after fetal surgery in rabbits. While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

The effects of bupivacaine on the umbilical circulation and placental gas exchange in the fetal lamb (Fleming, Lambert et al. 1987)

Reviewer's note: In this study, bupivacaine (0.1 mg/kg/min for 2 hours) was administered directly to fetal sheep in utero (Gestation Day 125-140) to examine whether bupivacaine in fetal plasma impaired the umbilical circulation and placental gas exchange. Bupivacaine infusion (the plasma concentration ranges of 1.3 ± 0.3 mcg/mL) resulted in a significant depression of average fetal heart rate to 89% of control during the second hour of the infusion period but heart rate and blood flow subsequently returned to baseline over the 2-hour recovery period. There were no significant changes in umbilical arterial/venous pressures, blood gas/pH, or electrocorticogram. Nonetheless, the authors concluded that it is not conclusive whether depression of fetal heart rate relates to a direct effect of bupivacaine on the fetal myocardium or is mediated neurologically. While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

FLEMING JA, LAMBERT TF, WALKER AM. The effects of bupivacaine on the umbilical circulation and placental gas in the fetal lamb. Anesth Analg 1987;66:1121–6.

Seven chronically catheterized fetal sheep at 125–140 days gestation were studied in 12 experiments to determine the direct effects of the local anesthetic bupivacaine (infused intravenously to the fetus) on the umbilical circulation and placental gas exchange. Electrocortical activity, umbilical blood flow, heart rate, and umbilical arterial and venous pressures were continuously monitored in experiments comprising a baseline period, a drug infusion period and a recovery period, each of 2 hr duration. Samples of umbilical arterial and venous blood were taken for blood gas analysis, and for bupivacaine assay using high pressure liquid chromatography technique. Fetal plasma bupivacaine levels were 1.3 ± 0.3 μg/ml (mean ± SEM) between 60–120 min of infusion. Heart rate and umbilical blood flow decreased significantly to 89 and 94% of control, respectively, ($P < 0.05$) during the infusion and returned to control levels by 2 hr afterwards. Mean umbilical arterial and venous pressures were not significantly altered, and no significant rise in umbilical vascular resistance occurred. No changes occurred in umbilical arterial or venous pH, P_{O_2}, or P_{CO_2}. In summary, bupivacaine reversibly depressed fetal heart rate and umbilical blood flow without detrimental changes in fetal blood gas or acid-base status.

Key Words: ANESTHETICS, LOCAL—bupivacaine. ANESTHESIA—obstetric.

Effects of local anesthetics on pregnant uterine muscles (Karsli, Kayacan et al. 2003)

Reviewer's note: In this study, the effect of local anesthetic agents (prilocaine, bupivacaine, ultracaine) on myometrium in pregnant rats (18-21 days) were investigated. Exposure to prilocaine (0.1 – 1 mM), bupivacaine (0.01 – 0.1 mM), and ultracaine (0.1 -1 mM) decreased amplitude, duration, and integrated area under the concentration curve. Given the extensive clinical experience with this drug, this reviewer does not consider the study data to be included in the label. Note that potential effects of bupivacaine on contractility are discussed (b) (4) (b) (4) in the Label.

Effects of progesterone on the cardiac electrophysiologic action of bupivacaine and lidocaine (Moller, Datta et al. 1992)

Reviewer's note: This is a short publication (5 pg) investigating the relationship between increased progesterone concentrations and the electrophysiologic effects of bupivacaine and lidocaine in isolated Purkinje fiber-ventricular muscle preparations. The paper suggested that progesterone selectively increases the cardiac membrane depressant effects of bupivacaine but not lidocaine, which may contribute to the enhanced toxicity of bupivacaine in pregnant animals. Note that potential effects of bupivacaine on cardiac function are discussed in the Section 8.1 in the Label. While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

Bupivacaine toxicity in pregnant and nonpregnant ewes (Morishima, Pedersen et al. 1985)

Reviewer's note: This is a short publication investigating the relative central nervous system and cardiovascular toxicity of bupivacaine in pregnant and nonpregnant ewes during continuous infusion of bupivacaine into the jugular vein at the rate of 0.5

mg/kg/min. The paper suggested that the pregnant sheep may be more sensitive to the cardiotoxic effects of bupivacaine than the nonpregnant animals. Note that potential effects of bupivacaine on cardiac function are discussed in the Section 8.1 in the Label. While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

Effect of ropivacaine and bupivacaine on uterine blood flow in pregnant ewes (Santos, Arthur et al. 1992)

Reviewer's note: This is a short publication investigating the effects of ropivacaine (15 min IV infusion at 0.1 or 0.2 mg/kg/min followed by 45 min infusion at 0.05 or 0.075 mg/kg/min) and bupivacaine (15 min IV infusion at 0.077 or 0.1 mg/kg/min followed by 45 min of infusion at 0.039 or 0.058 mg/kg/min) on uterine blood flow and fetal well-being in 10 chronically instrumented pregnant ewes. The study showed that IV infusions of ropivacaine and bupivacaine, resulting in plasma concentrations relevant to clinical practice did not adversely affected the pregnant ewe and fetus (i.e., mean arterial blood pressure, heart rate, carbon dioxide tension, oxygen tension, and uterine blood flow). It appears that the data presented in this study are not conclusive. This reviewer does not consider the study data necessary to be included in the label.

Comparative pharmacokinetics of ropivacaine and bupivacaine in nonpregnant ewes (Santos, Arthur et al. 1997)

Reviewer's note: This is a publication determining the pharmacokinetics and protein binding of ropivacaine and bupivacaine (6 mcmol/kg or approximately 2 mg/kg) after IV administration over 15 min in nonpregnant and pregnant sheep (term 148 days). The study suggested that both drugs showed lower total body clearance and volume of distribution during the terminal phase of drug elimination and steady state in pregnant animals. Bupivacaine showed lower distribution half-life, elimination half-life, volume of central compartment, volume of distribution during the terminal phase of drug elimination and steady state than ropivacaine. While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

The effects of bupivacaine, L-Nitro-L-Arginine-Methyl Ester (L-NAME), and phenylephrine on cardiovascular adaptations to asphyxia in the preterm fetal lamb (Santos, Yun et al. 1997)

Reviewer's note: This is a publication determining whether the adverse effects of lidocaine in the preterm fetal lamb also occur with bupivacaine and whether the inhibition of nitric oxide results in effects similar to those of bupivacaine. Pregnant sheep (117-119 days of gestation) were treated with an IV infusion of bupivacaine (0.07 mg/kg/min for the first 15 min followed by 0.025 mg/kg/min for 165 min), L-NAME (25 mg/kg), or phenylephrine. Maternal and fetal blood pressure, heart rate, and acid-base

state were evaluated. Bupivacaine treatment during asphyxia did not affect the mean arterial blood pressure and acid base state but abolished the increases in blood flow to the myocardium and cerebral cortex, fetal cardiovascular adaptive responses to asphyxia induced by partial umbilical cord occlusion. While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

The placental transfer and fetal effects of levobupivacaine, racemic bupivacaine, and ropivacaine (Santos, Karpel et al. 1999)

Reviewer's note: This is a publication investigating the effects of levobupivacaine on uterine blood flow and fetal well-being and to compare its placental transfer with that of bupivacaine and ropivacaine in pregnant ewes (full term 148 days, N=10/group). Animals received a two-step IV infusion (0.014 mL/kg/min for the first 15 min followed by a 45 min infusion at 0.007 mL/kg/min) of levobupivacaine, bupivacaine, or ropivacaine (15.39 mcM/mL) for 1 hour. There were no significant changes in maternal blood pressure, central venous and intra-amniotic pressures, acid-base status, and uterine blood flow by any drugs. Unlike the other two drugs, bupivacaine reduced the maternal heart rate. No changes in fetal heart rate, mean arterial blood pressure, and arterial blood pH and gas tension were observed by these drugs over 60 min. There were no significant hemodynamic changes in the pregnant ewe and fetus and no significant differences in the fetal serum and tissue levels of these three drugs. Given the extensive clinical experience with this drug, this reviewer does not consider the study data to be included in the label.

Systemic toxicity of levobupivacaine, bupivacaine, and ropivacaine during continuous intravenous infusion to nonpregnant and pregnant ewes (Santos and DeArmas 2001)

Reviewer's note: This is a publication investigating whether pregnancy affects the systemic toxicity of levobupivacaine and comparing the systemic toxicity of levobupivacaine with that of bupivacaine and ropivacaine in nonpregnant and pregnant sheep. Animals were received an IV infusion of 0.52% levobupivacaine, 0.52% bupivacaine, or 0.50% ropivacaine at a constant rate of 0.1 mL/kg/min until circulatory collapse. Convulsion was observed at lower doses of all three drugs in pregnant animals compared to nonpregnant animals. There were no significant differences between pregnant and nonpregnant ewes in doses required to produce more advanced manifestations of systemic toxicity (i.e., hypotension, apnea, and circulatory collapse). The mean cumulative dose and serum concentration at each toxic manifestation was lowest for bupivacaine, intermediate for levobupivacaine, and highest for ropivacaine in both pregnant and nonpregnant animals. Note that potential effects of bupivacaine on cardiac function are discussed in the Section 8.1 in the Label. While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

The effects of epidural anesthesia on uterine vascular resistance, plasma arginine vasopressin concentrations, and plasma renin activity during hemorrhage in gravid ewes (Vincent, Chestnut et al. 1994)

Reviewer's note: This is a publication determining the effects of epidural anesthesia (0.5% bupivacaine) on the mean arterial pressure/uterine vascular resistance relationship and arginine vasopressin concentration and plasma renin activity during hemorrhage in gravid ewes (0.8 and 0.9 of timed gestation). The authors concluded that epidural anesthesia attenuated the increase in uterine vascular resistance during hemorrhage. While the study data may be interesting, given the extensive clinical experience with this drug, this reviewer does not consider the study data necessary to be included in the label.

10 Special Toxicology Studies

Study Title: An Investigational Study of Post-Surgical SABER-Bupivacaine Vehicle Instilled into a Surgical Wound with a 10-Day Recovery Period in Gottingen Minipigs

Study Number: (b) (4)-434063

The object of the study: To evaluate the possible effects of SABER-Bupivacaine vehicle when instilled into a surgical wound during an exploratory laparotomy to Gottingen minipigs during a 10-day post-surgical observation period.

This study is briefly reviewed and summarized below. This non-GLP study was determined to be inadequate due to the following reasons: an insufficient number of animals evaluated (N=3), inadequate local toxicity evaluation, and confusing labeling in data which raises some concerns whether data were adequately evaluated, etc. In addition, a 10-day recovery period appears to be insufficient to evaluate duration or quality of wound healing.

Summary: Three female minipigs (6-7 months old, 12.3 kg to 13.6 kg) had two incisions (approximately 4 inches long) through the skin and abdominal musculature, penetrating into the abdomen. Each incision was approximately 4 cm from the abdominal midline. Five mL of SABER-bupivacaine vehicle (right side incision) or 0.9% sterile saline control (left side incision) were administered vial instillation during surgical closure of an abdominal laparotomy.

- No significant clinical signs (mortality and weight changes) were found.
- Transient skin discoloration (red) was observed (mostly evident by 7-8 hours after surgery and last up to 4 days in the SABER-bupivacaine vehicle-treated side while skin discoloration in the control incisions was normal or less evident. The Sponsor concluded that there were no effects on the duration or quality of wound healing. Note that SABER-bupivacaine vehicle-treated right side incisions appear to have some local reactions (a stich abscess/inflammation) on Day 10 post treatment and the Sponsor stated that it was not considered to be related to treatment as abdominal incisions with skin sutures occasionally abscess when surgical adhesive

is not used. Overall, SABER-bupivacaine vehicle-treated right side incisions appear to show more local reactions (skin discoloration and stich abscess) compared to the control-treated left side incisions.

- No histopathology evaluations were conducted and photographic analysis was conducted, which appears that all labels in the photo were switched.

Reviewer's comment: Data presented in this study report do not appear to be qualified to evaluate local safety as stated above. Wound healing with the SABER-bupivacaine product was evaluated in rats and minipigs (See the nonclinical review by Dr. Gary Bond in DARRTS, Jan 08, 2014).

Study Title: An Investigational Study of Post-Surgical SABER-Bupivacaine Vehicle Instilled into a Surgical Wound with a 10-Day Recovery Period in Beagle Dogs

Study Number: (b) (4)-434060
The object of the study: To evaluate the possible effects of SABER-Bupivacaine vehicle when instilled into a surgical wound during an exploratory laparotomy to Beagle dogs during a 10-day post-surgical observation period.

This study is briefly reviewed and summarized below. This non-GLP study was determined to be inadequate due to the following reasons: an insufficient number of animals evaluated (N=3) and inadequate local toxicity evaluation (i.e., no histopathological evaluation). In addition, a 10-day recovery period appears to be insufficient to evaluate duration or quality of wound healing.

Summary: Three female minipigs (9-22 months old, 8.5 kg to 9.9 kg) had two incisions (approximately 4 inches long) through the skin and abdominal musculature, penetrating into the abdomen. Each incision was approximately 4 cm from the abdominal midline. Five mL of SABER-bupivacaine vehicle (right side incision) or 0.9% sterile saline control (left side incision) were administered vial instillation during surgical closure of an abdominal laparotomy.

- No significant clinical signs (mortality and weight changes) were found.
- Transient skin discoloration (light brown or dark red) and swelling were observed (mostly evident on Day 1 after surgery and last up to 6 days in the SABER-bupivacaine vehicle-treated side but had no effect on the duration or quality of wound healing. On Day 2, one animal showed red discoloration of skin extending up onto the rib cage and across the ventral abdomen to the left side just slightly over the midline and another animal showed light brown discoloration of skin in the same location. These discolorations became fainter on Day 3 and were no longer noted after Day 6. No further evaluation (i.e., histopathology) were conducted to provide wide skin discoloration in these animals. While these discolorations were not observed after Day 6, note that there were clinical concerns of bruise, hematoma, dehiscence, and bleeding in Clinical Response Letter sent to the Sponsor (April 12, 2013).
- No histopathology evaluations were conducted and photographic analysis was conducted, which appears that all labels in the photo were switched.

Reviewer's comment: Data presented in this study report do not appear to be qualified to evaluate local safety. Wound healing with the SABER-bupivacaine was evaluated in rats (See the nonclinical review by Dr. Gary Bond in DARRTS, Jan 08, 2014).

11 Integrated Summary and Safety Evaluation

See Executive Summary.

12 Appendix/Attachments

NA

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

Supervisory Pharmacologist Memorandum

NDA NUMBER:	**204803**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**4/12/2013**
PRODUCT:	
(Proposed) Trade Name:	**Posimir®**
Established Name:	**bupivacaine solution**
Code name:	**SABER-Bupivacaine**
INDICATION:	**Post-surgical analgesia**
SPONSOR:	**DURECT Corporation**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Addiction Products**
PHARM/TOX REVIEWER:	**Gary Bond, Ph.D., DABT**
PHARM/TOX SUPERVISOR:	**Adam Wasserman, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Ayanna Augustus, Ph.D.**

EXECUTIVE SUMMARY

I. BACKGROUND

General Background

SABER-bupivacaine (Posimir®) is an extended release depot formulation of the amide local anesthetic bupivacaine which was developed under IND 66,086 and submitted as NDA 204-803 by Durect Corporation for use in post-surgical analgesia. The product is intended to be instilled into surgical wound sites as a viscous solution which, upon diffusion of an excipient away, sets up as a depot, releasing bupivacaine over 72 hr.

The Applicant is utilizing a 505(b)(2) pathway to support approval referencing the Agency's prior finding of safety and efficacy for the listed drug Marcaine®, approved in 1972 under NDA 16-964. There are numerous bupivacaine-containing products on the market, principally immediate release injectable solutions with concentrations of 0.25-0.75% (Marcaine®, Sensorcaine®, generic products) with or without addition of epinephrine and with or without preservatives, the latter to allow for spinal (intrathecal) administration. Approved indications encompass the production of local or regional anesthesia or analgesia for various surgical procedures including local infiltration, peripheral nerve blocks, and epidural and spinal blocks. Notably, Exparel® (N22,496), an extended release bupivacaine liposomal suspension intended for infiltration into surgical site for postsurgical analgesia, was approved in 2011 but is not directly relied upon by the Applicant.

The product, Posimir®, is formulated as a 12% solution (132 mg bupivacaine/mL) with a maximum administration volume of 5 mL allowing for 660 mg of bupivacaine to be instilled within a site. For comparison, the listed drug Marcaine® as an IR solution has a label limit of 400 mg bupivacaine/day. Exparel® currently has a dosage limit of 266 mg bupivacaine (1.3% bupivacaine in 20 mL diluted into 30 mL) for hemorrhoidectomy procedures. Posimir also contains two principal excipients to provide for reduced viscosity on initial instillation with subsequent formation of the depot: benzyl alcohol (BA) 22% (b) (4) (b) (4) and sucrose acetate isobutyrate (SAIB) 66% (b) (4), respectively.

Regulatory Summary (Pharmacology/Toxicology)

Amide local anesthetics are used widely to reduce pain of surgical and dental procedures as well as, in the case of lidocaine, an acute anti-arrhythmic. Clinical toxicities commonly associated with all amide local anesthetics are well understood and include cardiovascular block and myocardial depression as well as central nervous system (CNS) sensorium changes and seizure. These toxicities are believed to directly relate to excessively high plasma concentrations from administration into highly vascularized regions, inadvertent intravascular administration, or inappropriately large doses.

As Posimir is formulated as a depot, the level of bupivacaine administered is high. Therefore, a regulated and extended release from the depot is paramount for the systemic safety of the drug product. Local evaluation is necessary to support the safety of prolonged release of bupivacaine from a specific area as well as justify the acceptability of the depot formulation for use in wound sites. The duration of depot residence in the tissue must also be addressed.

II. SUMMARY OF NONCLINICAL DATA SUPPORT

Please see the primary review of Dr. Gary Bond for a detailed listing and examination of the supporting nonclinical data. Briefly, the applicant conducted a number of nonclinical studies to address drug product ADME. Also supporting the NDA were single dose SC toxicity studies in Sprague Dawley rats and in New Zealand White Rabbits with examination out to 6 weeks post-dosing as well as a 4-week repeated-dose SC toxicity study in rat using a degraded drug product with examination out to 4 weeks post-administration. The effects of SAIB-based formulations on wound healing was examined in a rat linear incision model while SABER-bupivacaine was used in a minipig wound model. To address the duration of depot residence and examine local response a study involving 12-month follow up of a single subcutaneous injection of SABER-bupivacaine in the rabbit was provided. Though not intended to be administered intravascularly, the risk of inadvertent administration was partially assessed in an in vitro hemolysis assay – no direct IV/IA administration of SABER-bupivacaine in an animal model was provided. A significant number of reports and scientific papers from the public domain were provided by the Applicant to support pharmacology, SAIB metabolism and safety for the oral route, BA safety, and other aspects of the NDA submission but are largely secondary due to the available study data.

To support labeling, genetic toxicology testing of bupivacaine base was conducted in two in vitro assays: an Ames reverse mutation assay and a chromosomal aberration study in human peripheral blood lymphocytes.

Product quality was addressed by specifications and justifications and in particular support for long term storage and stability was evaluated in an Extractable/Leachable analysis and report containing risk assessment.

Also submitted, but considered less important for regulatory consideration at this time were single-dose toxicity studies examining intra-articular dosing in rabbit and in dog, oral toxicity study of Posimir in rat, and studies conducted with a previous drug product formulation using (b) (4) which was abandoned in favor of BA, and perineural administration of various bupivacaine formulations, including one which was similar to SABER-bupivacaine.

III. MAJOR NONCLINICAL ISSUES IDENTIFIED IN PRIMARY REVIEW

Systemic Exposure to Bupivacaine

The acute systemic toxicity of bupivacaine is well known and has been generally thought to arise with serum concentrations in excess of 1000 ng/mL, with reports considering 2000-4000 ng/mL to be the range of clinical toxicity (Tucker, 1986). Although published clinical studies have reported exposures near this level with typical use of immediate release bupivacaine for standard procedures (Vainionpaa et al., 1995), the relative BA study provided by the Applicant demonstrated lower Cmax levels than with use of SABER-bupivacaine and, as would be expected of a depot product, significantly lower exposure over time as reflected by AUC (see table below). Therefore, from the nonclinical perspective exposure coverage in animal studies is necessary for both Cmax, but perhaps more importantly AUC. The submitted studies of single dose subcutaneous administration of SABER-bupivacaine in both rat and rabbit models provided exposures that, *in the absence of clear nonclinical systemic toxicity* (i.e. systemic NOAEL), exceed those achieved in the clinical trials. Therefore, the nonclinical data support the systemic safety of SABER-bupivacaine for use as proposed, though we note that clinical levels achieved are variable and may range to levels associated with clinical signs of toxicity.

Comparison of Clinical Exposure to Bupivacaine to Nonclinical Exposure

Species	Product	Cmax (ng/mL)	$AUC_{0\text{-inf}}$ (ng*h/mL)
Human	Marcaine[1]	342	5,650
	SABER-B[1]	625	35,230
	SABER-B[2]	965	41,942
Rat	SABER-B	1,432	74,423
Rabbit	SABER-B	3,033	48,645

[1] BU-001-IM Hysterectomy study; 100 mg Marcaine®, 660 mg Saber-Bupivacaine
[2] CLIN005-0006 Subacromial decompression study; 660 mg Saber-Bupivacaine
[3] Study Report 11519.01.04
[4] Study Report A784.6.1

Local safety for use in wounds

Wound Healing Models: As a product which is administered to wounds, the risk of interference with wound repair was examined. The effects of instillation of a formulation equivalent to SABER-bupivacaine on wound healing was explored in both rat and pig full-depth incision models.

Rat. The rodent study involved a 2.5 cm full thickness incision in the dorsolateral flank within which test articles (0.125 mL, multiple formulations including a SABER-bupivacaine-equivalent) were administered which could be compared to an incision-only group (i.e. sham) 7 days after suturing wound. Biomechanical strength testing 7 days post-incision, a time-point which has been used to examine wound repair in published studies (Pickett et al., 1996; Sohn et al., 2001; Gal et al., 2006) revealed no difference in the pressure needed to produce wound failure.

Biomechanical Wound Strength Test Results in Rat

	mmHg disruption pressure		
	SABER-bupivacaine equivalent (15 mg)	**SABER-placebo equivalent**	**Incision only**
Wounds evaluated	*15*	*10*	*15*
Mean	201	207	190
S.D.	25	33	20

Microscopic evaluation of the wound site revealed some differences in response between SABER-bupivacaine and Incision only animals (see table below). In particular, there was more inflammation, granulation, angiogenesis and an incidence of 2/5 animals with minimal to mild gap observed in dermis. Both of these animals were observed to have a cyst at the site (and there was one additional SABER-bupivacaine rat with cyst in which a gap was not observed); however, this was not observed in sham animals. Findings were more enhanced in animals with cysts than those without. No evaluation of SABER-placebo was conducted and no comparator IR bupivacaine was tested to determine if any of these responses could be a result of bupivacaine administration though cystic formation is consistent with a foreign body response.

Microscopic Examination of Wound in Rat

Finding	**Average Score (0-4; higher number more apparent)**	
	SABER-bupivacaine equivalent (15 mg) n=5	**Incision only n=5**
Inflammation	2.4	1.4
Granulation	2.4	1.8
Angiogenesis	2.8	1
Epithelization	3	3
Neutrophils	1.6	1
Gap	0.5	0
Fibrillogenesis	2.8	3.6
	Incidence	
Cyst	3	0
Abcess	0	1

Minipig The wound healing study conducted in the minipig differed from the rodent study having a longer observation time (15 days post-incision) and being exclusively limited to histologic assessment of the wound site without biomechanical strength testing. Equivalent volumes (0.5 mL) of SABER-bupivacaine (66 mg bupivacaine), SABER-placebo, or a negative control solution of 5% carboxymethylcellulose (CMC) gel was instilled into one of multiple sites (each 20 mm diameter full-thickness wounds; again, no bupivacaine immediate release product was used for comparison for reasons not specified.

Macroscopically, there were no obvious differences between treatment groups. Inflammation, hemorrhage and exudates appeared in all groups and with similar grades of severity. There was no apparent necrosis. Histologic analysis of tissue revealed similar findings between the three groups as shown in the table below. As Dr. Bond notes, "Microscopically, a slight tendency towards less advanced re-epithelialisation and more inflammation and clear vacuoles occurred in the SABER-bupivacaine animals compared to the CMC animals." Table below taken from study report.

Microscopic findings

Observation	Treatment		
	Control wounds (Treatment 3, 5% CMC)	Vehicle (treatment 2)	SABER Bupivacaine injectable formulation (treatment 1)
Crust, focal			
Total number observed	6	7	6
Minimal	4	7	2
Slight	2	-	4
Re-epithelialisation			
Total number observed	8	8	8
Moderate	-	1	-
Marked	1	1	3
Massive	7	6	5
Thickness epithelium			
Total number observed	8	8	8
Moderate	4	4	4
Marked	4	4	4
Granulation tissue			
Total number observed	8	8	8
Marked	8	7	7
Massive	-	1	1
Inflammation superficial			
Total number observed	8	8	8
Minimal	3	2	1
Slight	4	4	6
Moderate	1	2	1
Inflammation deep			
Total number observed	8	8	8
Minimal	6	4	1
Slight	2	4	5
Moderate	-	-	2
Cystic spaces			
Total number observed	3	1	3
Minimal	2	-	2
Slight	1	1	1
Giant cells			
Total number observed	8	8	8
Minimal	5	1	1
Slight	3	6	4
Moderate	-	1	3
Clear vacuoles			
Total number observed	8	8	8
Minimal	5	1	1
Slight	3	6	5
Moderate	-	1	1
Marked	-	-	1
Collagen (Masson's)			
Total number observed	8	8	8
Moderate	-	1	-
Marked	8	7	8
Angiogen (immun.st.)			
Total number observed s	8	8	8
Moderate	8	8	8
Total number of wounds examined	8	8	8

Planimetric evaluation of wound closure identified no statistically significant differences between treatment groups in wound area, granulation tissue area, or epithelialized area when evaluated daily between post-incisional days 2-15 (D2-15); however, numerically it appears the wound area was slightly slower to re-epithelialize and contract as shown on D10 though by the end of the D15 measurement period this effect seemed less apparent (see table below), suggesting that any slowing of wound healing would tend to be transient. Histology as described above was obtained at D15 therefore there are no interim findings which might explain these early differences. I note there was no indication of cyst development in the report.

Assessment of Wound Healing in the Minipig

	Area observed (mm^2) N=8					
	Day 10			**Day 15**		
Measurement	**CMC**	**Veh**	**SAIB-B**	**CMC**	**Veh**	**SAIB-B**
Wound area (% contracted) †	155 (51.2%)	181 (43.2%)	186 (40.8%)	119 (62.6%)	129 (59.6%)	131 (58.2%)
Granulation tissue	78	101	106	10	16	15
Epithelialized tissue	78	81	80	109	113	116

†, Day 1 wound area values: 0.5% CMC 318 mm^2, SABER-placebo (i.e. vehicle) 319 mm^2, SABER-bupivacaine 314 mm^2

As no comparison was made against sham, saline, or immediate release bupivacaine, and as 5% CMC has not been previously established to be similar to saline or sham in this model it is not clear this is a true “negative” control and therefore it is possible the degree of effect may be underestimated.

Subcutaneous studies: Although perhaps of less relevance for support of clinical use in surgical settings, single-dose subcutaneous administration studies of SABER-bupivacaine were conducted in rat and rabbit with sacrifice and evaluation on SD15 or 43. The Applicant provided no justification that the initial evaluation at SD15 was representative of the maximum acute toxicity. As the solvent BA and the API bupivacaine are expected to be fully released by SD1 and SD3, respectively, the initial evaluation should have been not later than SD3/4. I note that for the Exparel nonclinical studies, sacrifice and evaluation was conducted on SD3 and SD15. I note as well that the Applicant’s initial SC studies with the earlier SAIB/ (b) (4) version of the product had sacrifices at SD4 and 15 and noted necrosis at the earlier time-point and other findings were reduced at by SD15. Also notable neither study in rat or rabbit included an immediate release bupivacaine control and the rabbit study also did not include a saline/negative control.

Rat. SABER-bupivacaine as well as SABER-placebo administration was associated with discoloration and swelling (severity not described) after administration in the rat which was maximally apparent by SD15 (observations

were taken weekly). These findings largely resolved in all SABER groups except swelling at the injection site remained apparent in males in the SABER-bupivacaine 240 mg/kg group at SD43. Saline injection was used as a negative control and provided no remarkable findings at either SD15 or SD43.
Macroscopic examination revealed skin changes at the injection site which included observation of crust, masses, and nodules, as well as similar subcutaneous tissue changes in 1-9/15 animals, generally increasing in incidence with volume/dose of SABER administered. Again, these findings were not observed in the saline control.
Microscopic examination of tissue revealed a number of findings at the injection site of SABER-treated animals (bupivacaine and controls) with severity and incidence largely volume-related though some enhancement of toxicity by bupivacaine may be in evidence. Findings on SD15 in both males and females included: mild to marked development of medium to large cysts in the majority of SABER-animals, minimal to marked panniculitis, minimal to marked epidermal hyperplasia (acanthosis), minimal to moderate dermatitis including, at the high dose, minimal to marked ulcerative dermatitis (26/80 SABER-animals), and at the larger volume sizes (0.6-2 mL) evidence of cutaneous necrosis which in females (SABER-placebo) was scored as mild to moderate while in males (≥ 72 mg/kg SABER-bupivacaine) ranged from moderate to marked. With the exception of one cyst in 20 saline-injected animals, no findings were noted in this control group.

On SD43, cutaneous necrosis was not observed, instead evidence of dermal fibrosis and fibrosis of the subcutis was observed in the minority of animals remaining for evaluation; cysts were still present in the majority of animals along with minimal to mild panniculitis. There was no clear difference between SABER-placebo and SABER-bupivacaine animals at the 2.0 mL volume but again, as expected, there was no similar findings observed in the saline control group.

Rabbit. As previously mentioned the single-dose subcutaneous toxicity study conducted in rabbit compared SABER-bupivacaine at two doses (30 or 120 mg/kg) differing by volume administered (0.25 and 1.0 mL/kg, respectively) with volume equivalent SABER-placebo. No evaluation of these against immediate release bupivacaine or saline negative control was included in the study. High volume administration of SABER-bupivacaine was associated discharge in several animals while both SABER-bupivacaine and SABER-placebo were observed with sporadic signs of scabbing and discoloration. Macroscopically the sites were characterized as having thick subcutis with crust with both SABER groups. Microscopic findings on SD15 revealed chronic inflammation (mild to marked) in both low and high volume SABER-placebo and SABER-bupivacaine as well as vacant spaces thought to represent SAIB, occasionally exudate was observed in these spaces. Findings that were only observed in SABER-bupivacaine groups included hemorrhage which could be marked at the high volume, acanthosis, hyperkeratosis, and in single animals granulomatous

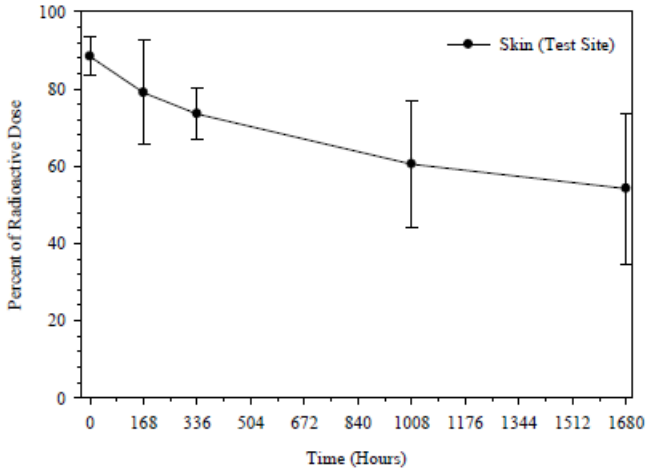
inflammation and ulcer. Microscopic findings on SD43 clearly suggest a reversal with generally minimal to moderate chronic inflammation in a minority of animals across all SABER groups and a reduced incidence and degree of vacant spaces as may be expected. Sporadic findings of hemorrhage and acanthosis was noted in all treatment groups when genders were combined and one incidence of erosion (tissue not specified) was observed in a high dose/volume SABER-bupivacaine animal.

Excipients

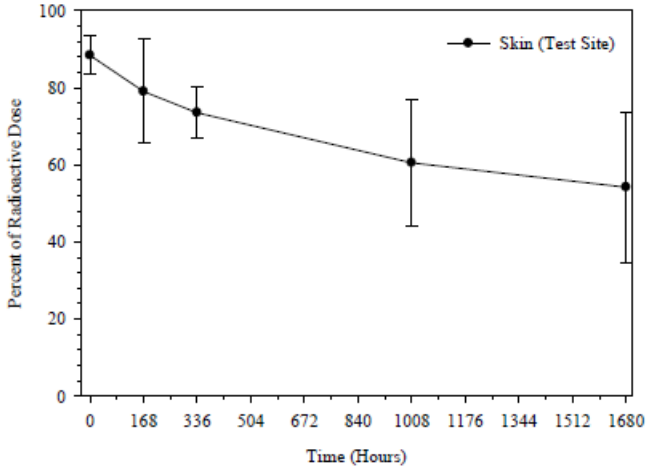
The excipient sucrose acetate isobutyrate (SAIB) has an extensive prior database investigating safety for oral use as described by Dr. Bond. The World Health Organization established an Acceptable Daily Intake of 20 mg/kg. This is exceeded in amount by the proposed maximum use of SABER-bupivacaine (3,630 mg in 5 mL), however as SAIB sets up a depot and degrades very slowly over months (see below), the systemic exposure to this compound is far smaller and the equivalent dose/day is well within the range of the WHO ADI. Additionally, I concur with Dr. Bond the 4-week repeated subcutaneous study with degraded SABER-bupivacaine product does not elicit any systemic safety concerns and provides a safety margin based on amount administered although I note 4-week observation cannot itself address long-term safety. The safety of its use as a local depot formulation can be determined from the included nonclinical studies. As noted previously, there is local toxicity associated with this product, principally appearing as a foreign body reaction. The long duration of the product in tissues, however, means this local effect persists.

Several mass balance studies were conducted to determine the rate of elimination of SAIB from animals after a single subcutaneous administration of ^{14}C-SAIB. Slow elimination of radiolabel was noted through all routes of elimination, with 61% of radiolabel still retained 6 weeks post-administration. The persistence of ^{14}C-SAIB after subcutaneous or wound instillation was determined in rodents. As can be seen from the Sponsor's graphs below (Study Report 8255730), a slow release of radiolabel from the site occurs which by 10 weeks is still above (b) (4) % of total radiolabel administered by the wound instillation and subcutaneous routes, respectively.

Mean percent radioactive dose in skin and subcutaneous tissues after surgical wound administration of ^{14}C-SAIB (400 mg/kg) to rats



Mean percent radioactive dose in skin and subcutaneous tissues after subcutaneous administration of ^{14}C-SAIB (400 mg/kg) to rats



The persistence of SAIB after a subcutaneous administration of a prior version of the product using [redacted] (b) (4) as part of the vehicle (in place of BA) was examined in a rabbit model. Approximately 2.5 mL (37.5 mg/kg) of SABER[redacted] (b) (4) -bupivacaine or vehicle was injected with observations at various time-points through SW52. Gross lesions including cysts were noted throughout 26 weeks of macroscopic evaluation. Injection sites obtained at SW39 and SW52 revealed spaces with “viscous contents” which was confirmed to be SAIB.

The excipient benzyl alcohol (BA) also is extensively used in industrial and cosmetic settings, and as a food additive. As noted by Dr. Bond there exists a large safety database for the compound and the ADI for BA based on WHO evaluation is 5 mg/kg. This is lower than the amount of BA liberated from use of SABER-bupivacaine at maximum levels (1,210 mg in 5 mL). Dr. Bond has identified an injectable product producing high levels of BA (~1,000 mg;

FASLODEX) which is administered every other week initially, then monthly. I concur the usage generally covers the usage in this product and provides some assurance of local as well as systemic safety. Additionally, high doses were administered in nonclinical studies, including the 4-week degraded product study, which in the absence of systemic toxicity provides an acceptable safety margin. The propensity of BA to evoke a serious metabolic acidosis in pre-term neonates with immature hepatic metabolism has been reported. The amount of BA in the present product is significant. It is not proposed for use in this population and it seems improbable that milk transfer through nursing would occur in the post-surgical setting unless used for procedures like caesarian section. Based on animal data, the level of BA administered to maternal rats which reached milk was approximately 0.4%. It is unknown if this would be expected in human; however, if similar, the levels an infant would be exposed to would be below those levels that have previously been associated with this syndrome. Nevertheless this should be described in labeling in Warnings and Precautions sections.

Drug Specifications

There are no impurities in the drug substance which require qualification. Four degradants required safety qualification as discussed in Dr. Bond's review. (b) (4) (b) (4) (b) (4). Levels are quite high in the drug product, reflected in the requested specifications of NMT (b) (4) respectively. Safety was demonstrated with identification of a systemic NOAEL in the 4-week repeated dose (weekly) SC toxicity study using an aged drug product which provides a >3.5X systemic safety margin over the maximum human intake. Local safety qualification is supported by the observation that the degraded material, which provoked a strenuous inflammation at the site of application, did not differ in quality of findings from a (low degradant) SABER-placebo. Additional information provided by the Applicant, including a negative clastogenicity assay in human peripheral blood lymphocytes for (b) (4), as well as a World Health Organization review adequately addressed genetic toxicity. The same 4-week repeated dose study also qualified the degradant (b) (4) at the NMT (b) (4) level (recently agreed to by Applicant) for both local and systemic safety, and the provided Bacterial Reverse Mutation Assay demonstrated an absence of mutagenic potential. Finally, (b) (4), has been identified as being genotoxic and, in rodents, a carcinogenic degradant of many local anesthetics. The maximum human intake at the proposed specification level of (b) (4) falls just slightly above our threshold for toxicologic concern (b) (4) This is a single dose product as Dr. Bond points out, and has been allowed in many products at higher daily intakes (Exparel is at (b) (4) for example). Prior Advisory Committee (ALSDAC August 23, 1993) considered the carcinogenicity finding not relevant for humans. Therefore, I believe the specification is acceptable and represents the recommendation to control the levels to "As Low As Reasonably Possible"

(ALARP principle). Therefore I concur with Dr. Bond the degradants are appropriately qualified and the specifications acceptable.

Dr. Bond has evaluated the Applicant's extractable and leachable studies and has concluded that all compounds are within our general limits without need for additional qualification. (b) (4) some of which are known carcinogens are limited to (b) (4) which is well below our threshold for toxicologic concern (1.5 mcg) even building in an extra (b) (4) safety factor. I concur with his assessment.

"Dose Dumping"

As with all depot and ER formulations, evidence of inadvertent release of the API ("dose dumping") was sought in the nonclinical program. This was not observed which is reassuring as the generally high plasma levels reached clinically at the maximum intended dose would not likely stand further increases without signs of CNS or CV toxicity.

Alternative routes

SABER-bupivacaine is not intended for intra-articular injection; however, evaluations of this route were conducted both rabbit and dog and were notable as described by Dr. Bond. Microscopic findings of minimal to moderate synovial hyperplasia, fatty degeneration, inflammation, fibrosis and osseous metaplasia were noted in rabbits two weeks after administration and were still present six weeks post-injection. Findings were slightly worse in SABER-bupivacaine animals than SABER-placebo and was not observed in saline treated joints or in the contralateral joint. Intra-articular administration of SABER-bupivacaine or SABER-placebo to dogs produced similar and additionally significant joint effects including subchondral bone fibrosis and necrosis of cartilage and at the highest volumes administered, the necrosis considered of marked severity. I concur with Dr. Bond that, if approved, this should be described in Animal Toxicology section of the label as a further method to discourage this route of administration.

Perineural administration of SABER-bupivacaine was evaluated against SABER-placebo, saline and IR bupivacaine in a rabbit model. Microscopic examination of the perineural area revealed increased neuronal inflammation and axonal degeneration in SABER-treated animals which was somewhat worse with the additional bupivacaine in SABER-bupivacaine and was absent from the bupivacaine IR and saline-treated animals. This was considered to be the result of foreign body reaction in the vicinity of the nerve, consistent with findings from other routes and placement though some additional effect of prolonged bupivacaine at the site may be augmenting the observed findings.

SABER-bupivacaine was not tested in vivo in an IV or intra-arterial toxicity study. It seems quite reasonable to conclude that administration of a drug product of this nature would result in profound toxicity due to occlusion of vessels as the BA

rapidly leaves the injectae. An in vitro hemolysis study was performed which demonstrated the vehicle itself has hemolytic potential.

Overall Risk Assessment and Nonclinical Support

The nonclinical data submitted in support of SABER-bupivacaine reveal this drug product has an acceptable systemic safety profile. Clinical bupivacaine exposures, though high, are within that supported by animal studies and appear to fall within the range of prior approved uses for immediate release bupivacaine products. There is no evidence of dose dumping from the nonclinical data provided and bupivacaine release appears to occur in controlled fashion primarily over the first 24 hr with continued release tapering over the following 48 hr. The quality of the drug product has also been adequately supported from the nonclinical perspective. The risks of the product identified in the nonclinical program principally relate to local toxicity associated with the vehicle and the resulting formation of the depot along with its persistence in tissues. This is noted in the single-dose subcutaneous administration studies in rodent and rabbit in which administration produces (by Study Day 14) swelling, discoloration, and a significant mild-to-marked inflammation of the subcutaneous tissue associated with, in rats, cyst formation while in rabbits this appeared as a granulomatous inflammation around vacant spaces thought to represent the SAIB depot. Other findings included dermal evidence of damage which may or may not be secondary to scratching of the administration site by the animals. Inflammation was slowly resolving over 6 weeks post-administration. Notably, the acute effects of the drug product at the site were not evaluated and therefore there may be significant toxicity, such as the necrosis observed with an earlier SAIB/ (b) (4) version of the product, which would not be observed with the delayed initial histologic assessment. Furthermore, appropriate negative (saline) and immediate-release bupivacaine control groups were not consistently included for distinguishing the effects of bupivacaine from vehicle and to a certain extent the vehicle itself from the injection procedure.

More pertinent to the proposed indication of surgical site instillation were studies in wound healing models conducted with a near-final version of SABER-bupivacaine in rat and minipig. Microscopic evidence of inflammation, cysts, and mild dermal gap was noted in rats with instillation of SABER-bupivacaine. Cysts were not apparent in an incision only (sham surgery) control group 7 days post-wounding and there was no gap in the dermal layer. Nevertheless, there was no evidence of reduced wound repair strength in the SABER-bupivacaine animals compared to sham surgery animals when tested at this single time-point. A more complete time-course with longer follow-up (to at least 14 days) to observe the full course of wound repair would have been ideal but was not conducted. A study in the minipig in which wounds were treated on SD1 and evaluated on SD15 identified slightly less advanced re-epithelialisation, more inflammation (moderate in severity), giant cells, and clear vacuoles thought to contain SAIB when compared to a control group administered a viscous

carboxymethylcellulose solution. Additionally, visual inspection of the wounds suggested a transient delay in healing in SABER-bupivacaine animals which appears no different than carboxymethylcellulose control by 15-days post wounding. Again it is notable that acute evaluation of the wound site was not incorporated into the study design and the carboxymethylcellulose solution was not previously established to be equivalent to a negative (saline) control; therefore, the short-term impact of SABER-bupivacaine on wound healing in this animal model may be underestimated.

The extrapolation of findings from the local toxicity studies conducted in these nonclinical models to the clinical application is made cautiously. The effects in the animal studies were principally due to vehicle provoking a foreign body reaction, the severity of which was related to the volume of vehicle instilled to the site. It is not apparent that the presence or specific dose of bupivacaine was a significant factor. The local "concentration" of vehicle introduced subcutaneously or into wounds in the nonclinical program is, however, of uncertain relationship to that achieved in the surgical setting due to uncertainties comparing the area of application. If it is assumed that for the human hernia repair usage the incision is approximately 12-15 cm, the maximum SABER-bupivacaine administration per cm of linear incision 0.4 mL/cm (equivalent to 55 mg bupivacaine/ (b) (4) SAIB/ (b) (4) BA per cm), while the rodent wound study model was .05 mL/cm (equivalent to 6.5 mg bupivacaine/ (b) (4) SAIB/ (b) (4) BA per cm). In minipig the administered volume is 0.16 mL/cm (as it was applied to a circular wound). In either case it would appear more is instilled per unit area with clinical usage though I suspect the full depth of incision and method of application may, depending on the type of procedure, spread the volume across a greater total area. Deposition of SABER-bupivacaine into smaller spaces may be expected to provoke a larger and/or longer local response.

Nonclinical data provided by the Applicant indicate that while the bupivacaine in SABER-bupivacaine is released over 3 days, evidence of intact SAIB depot remains at injected sites for many months, perhaps a year or more, and being associated with local irritation and fibrosis consistent with a foreign body reaction against a slowly eroding material.

When the nonclinical program provided to support SABER-bupivacaine is compared with the findings of the recently approved Exparel (NDA 22-496), a liposomal bupivacaine product for infiltration to surgical wound sites, the present studies appear to identify a greater degree of toxicity even in the absence of the missing acute evaluations. The Exparel program tested concentrations (15 and 25 mg/mL) which met and exceeded the clinical concentration (15 mg/mL) in wound healing, subcutaneous, and repeat-dose studies. In wound healing models, acute findings of minimal to mild inflammation gave way to granulomatous inflammation by two weeks post-administration. Subcutaneous administration in rat and dog models revealed little findings by two weeks post-administration of Exparel formulations, whereas with SABER-bupivacaine there

was significant local toxicity that was still observed for at least four additional weeks (last time point evaluated) in rats and rabbits. It is possible this relates to differences in volumes administered between the two programs but qualitatively the data, combined with the shorter duration of liposomal presence in tissues compared with SAIB, suggests that a greater degree of local toxicity may be expected with SABER-bupivacaine.

Intra-articular administration of SABER-bupivacaine is not supported by the nonclinical data showing significant local tissue toxicity which is mostly attributable to the vehicle. Chondrolysis, a concern described with prolonged bupivacaine administration in the approved label, was observed with equal incidence – though with greater severity (marked vs. moderate) – in SABER-bupivacaine compared to SABER-placebo group animals with equivalent injected volume.

Overall, the systemic safety and drug product quality issues appear to be addressed and the nonclinical program appears broadly sufficient. Based on nonclinical data, SABER-bupivacaine with clinical use is expected to produce local toxicity consistent with a depot-provoked foreign body reaction, the severity of which should be generally related to the volume of SABER-bupivacaine instilled to the area of a particular site. As the depot material is long lived, this reaction may be expected to remain present for an extended period of time, well past the time at which the product is producing any clinical benefit. Though reassuringly no nonclinical evidence of wound strength abnormalities were observed, the examination was conducted in a single animal model and limited to a single time-point which may not be fully reflective of the complicated and overlapping processes that occur in wound repair over time nor account for differences in wound healing between nonclinical species and the human. I agree with Dr. Bond that the nonclinical findings do not suggest SABER-bupivacaine carries significant risk with intended usage – and I understand Dr. Bond's rationale for recommending approval. However, the product itself is not toxicologically neutral with instillation to wound sites and this effect appears to be maintained for weeks at minimum and perhaps months, a situation which is not observed with immediate release bupivacaine and which far exceeds the intended pharmacologic benefit of SABER-bupivacaine. For this reason I do not recommend approval based solely on the nonclinical information provided. Should clinical efficacy present a positive risk:benefit for this expected local response and/or clinical safety data indicate that the analog or sequalae of these nonclinical findings are not apparent in the surgical population this would be reassuring and a sound basis for approval of the drug product.

Despite my concerns with some aspects of the design of the nonclinical studies submitted to support the NDA, should this application receive a Complete Response I do not believe additional nonclinical studies are warranted as the data obtained would be unlikely to alter my recommendation or greatly inform the clinical program beyond our current understanding.

IV. RECOMMENDATIONS

Recommendation on approvability
I do not recommend approval based solely on the nonclinical information provided. Should clinical efficacy present a positive risk:benefit when considering the expected local toxicity response and/or clinical safety data indicate that the analog or sequalae of these nonclinical findings are not apparent in the surgical population this would be reassuring and a sound basis for approval of the drug product.

Recommendation for nonclinical studies
I do not believe additional nonclinical studies are warranted as the data obtained would be unlikely to alter my recommendation or greatly inform the clinical program.

Recommendations on labeling
Labeling recommendations will be contained in a separate memo if this application moves towards approval. However, even though Warnings and Precautions section will warn against the intra-articular route I do concur with Dr. Bond that results of the intra-articular toxicity study conducted in dogs be included as part of the Animal Toxicology section in order to discourage use through this route.

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/s/

ADAM M WASSERMAN
01/15/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	204803
Supporting document/s:	eCTD in DARRTS
Applicant's letter date:	April 12, 2013
CDER stamp date:	April 12, 2013
Product:	SABER®-Bupivacaine (Posimir®)
Indication:	administration into the surgical incision to produce post-surgical analgesia
Applicant:	DURECT Corporation
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products
Reviewer:	Gary P. Bond, Ph.D., DABT
Supervisor/Team Leader:	Adam M. Wasserman, Ph.D.
Division Director:	Bob Rappaport, M.D.
Project Manager:	Matthew Sullivan, M.S.

Template Version: September 1, 2010

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Any information or data necessary for approval of NDA 204803 that DURECT Corporation does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 204803. In addition, some reviews, as noted, were conducted by Timothy J. McGovern, Ph.D. as part of his review of IND 66,086.

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1 Executive Summary

1.1 Background and Regulatory Issues

SABER®-Bupivacaine (POSIMIR™ - NDA 204803), is a proposed extended-release bupivacaine depot drug product. It has been submitted by DURECT Corporation and is pursuant to Section 505(b)(2) of the FD&C Act, relying on FDA's general findings of safety and efficacy for Marcaine (bupivacaine hydrochloride – NDA 16964) injection, the reference NDA approved October 3, 1972. Marcaine is a fast acting local anesthetic consisting of a bupivacaine hydrochloride solution. Exparel® (NDA 22496), another extended-release bupivacaine depot drug product which has been recently approved (October 28, 2011) using Marcaine as its reference NDA, is also referred to by the applicant, but it is not the reference NDA for support of safety, efficacy, or labeling. The excipients in SABER-Bupivacaine are sucrose acetate isobutyrate (SAIB) and benzyl alcohol (BA). They have been used in other approved drug products but not by the proposed parenteral, and more specifically, wound instillation route (SAIB) or at as high a dose level as proposed (BA).

The bupivacaine in SABER®-Bupivacaine is an amide-type local anesthetic indicated for administration into the surgical incision at a total volume of 5 mL to produce prolonged post-surgical analgesia. SABER-Bupivacaine is a sterile nonpyrogenic, clear, light yellow to amber solution that contains bupivacaine (12%, 132 mg/mL, 660 mg in 5 mL), BA (22%, 242 mg/mL, 1210 mg in 5 mL), and SAIB (66%, 726 mg/mL, 3630 in 5 mL). Upon the single instillation of the maximum proposed dose of 5 mL total (single dose (b) (4)), the biodegradable matrix (SAIB) serves as a depot for delayed release of bupivacaine over 24-72 hours. The SAIB forms this depot for bupivacaine as the solvent BA diffuses away from the dosing site thereby providing an extended release of bupivacaine over time compared to Marcaine (bupivacaine HCl).

1.2 Brief Discussion of Nonclinical Findings

The nonclinical program was designed to support the single administration of SABER-Bupivacaine by instillation around a surgical incision. Nonclinical batches of SABER-Bupivacaine (BA as solvent) used in testing were comparable to the proposed drug product. Nonclinical findings address the issues of systemic toxicity related to exposure to SABER-Bupivacaine and SABER placebo (no bupivacaine), their local toxicity, and a comparison of nonclinical exposure levels to proposed clinical exposure for the drug product (risk assessment – dose ratios). Nonclinical levels of bupivacaine exposure are important for drug approval in supporting human safety as human bupivacaine exposure from SABER-Bupivacaine is greater than the reference NDA bupivacaine exposure as part of this 505(b)(2) submission. Excipients, degradants, and extractables/leachables (E/Ls) and the effects on nerves, wound healing, and potential hemolysis were also evaluated to support human safety under the proposed use conditions.

Systemic Toxicity

The systemic safety of SABER-Bupivacaine is acceptable because the proposed bupivacaine exposure is supported by nonclinical:clinical safety margins, acceptable product quality specifications and stability, and valid nonclinical studies with an acceptable compositional comparability between the nonclinical test product and the proposed drug product. Apparently, only labeling is supported as a 505(b)(2) submission using the approved, referenced Marcaine as the proposed human exposure to bupivacaine is greater than that demonstrated for Marcaine. The nonclinical support also includes submitted pivotal nonclinical studies, most notably single dose studies and a repeat dose study with prolonged observation periods and toxicokinetic (TK) measurements. In addition, genotoxicity testing results for bupivacaine, SABER-Bupivacaine, SABER placebo, and select degradants support human safety. Potential carcinogenicity and reproductive toxicity of bupivacaine are addressed using a 505(b)(2) reference to the approved Marcaine (NDA 16964) label.

The systemic safety of inactive ingredients SAIB and BA for clinical use in SABER-bupivacaine is generally supported by their recognition as Generally Recognized as Safe (GRAS) by the FDA when used by oral routes, their use in approved and marketed products (oral only for SAIB), and extensive nonclinical databases on both ingredients as included in World Health Organization (WHO) data reports. However, for purposes of this application, as the amount of BA and SAIB in the proposed drug product exceeds that as listed for the above references and only the oral route of SAIB has been previously tested, the primary support for human safety is provided by the submitted nonclinical studies, most notably a repeat dose study for the proposed single dose indication. Nonclinical testing with SABER-Bupivacaine and/or SABER placebo includes that as listed in an earlier paragraph plus embryo-fetal testing of SABER placebo. This submitted nonclinical testing is consistent with the FDA *Guidance for Industry: Nonclinical Safety Studies for the Safety Evaluations of Pharmaceutical Excipients* (May 2005) noting that a full reproductive toxicology test battery data exists for both ingredients in the literature. See section 2.4 (Comments on Novel Excipients) for a detailed evaluation.

Human systemic safety is generally supported for the degradation products of bupivacaine (b) (4) and SABER-Bupivacaine (b) (4) and for the extractables/leachables (E/Ls) from the container/closure system (E/Ls and (b) (4) from the rubber stopper). This support is based on the use of the source drug substance (i.e., specification limits allowable or qualified in testing), inactive ingredients in FDA-approved and marketed products, and risk assessments of observed extractable levels in the drug product in excess of known safe levels (see section 2.5). But, again, the primary nonclinical support is the submitted nonclinical studies. Most notable is a repeat dose toxicity study with aged drug product containing increased levels of degradants that supports or identifies human safety levels at proposed human dosing levels for the proposed drug product. In addition, (b) (4) is controlled by an appropriate, stringent specification. (b) (4) were tested for genotoxicity and were not genotoxic. (b) (4) and E/Ls also have

literature data supporting the safety of levels in the proposed drug product. No (b) (4) were detected in the rubber stopper after attempted extraction. The submitted information is consistent with FDA/ICH *Guidances for Industry: Q3A Impurities in New Drug Substances* (February 2003) and *Q3B(R2) Impurities in New Drug Products* (August 2006)), *Safety Practices and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products*. Product Quality Research Institute, (September 8, 2006), and current FDA thinking supporting human exposures (b) (4) µg/day for genotoxins and (b) (4) µg/day for nongenotoxins. See section 2.5 (Comments on Impurities/Degradants and Extractables/Leachables of Concern) for a detailed evaluation.

Local Toxicity

The local toxicity associated with SABER-Bupivacaine and SABER placebo (SABER depot – SAIB and BA) were not unexpected as only the anticipated effects comprising of local inflammation and a foreign body reaction were observed in the absence of systemic toxicity. Literature data was also submitted to support the human safety of SAIB and BA. The submitted nonclinical testing satisfies testing needs as listed in the FDA *Guidance for Industry and Review Staff: Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route* (March 2008).

The local reaction to SABER-Bupivacaine was similar to the local reactions (i.e., foreign body reaction) in other approved drugs with different injectable depots such as Risperdal Consta (NDA 21346, polylactide-co-glycolide microspheres, chronic biweekly intramuscular dosing) , Eligard (multiple NDAs, polylactide-co-glycolide polymer, subcutaneous dosing q 1 to 6 months depending on dose level), Lupron Depot (multiple NDAs, microspheres containing carboxymethylcellulose and mannitol, intramuscular dosing q 1 and 3 months), and Zoladex (NDAs 19726 and 20578, polylactide-co-glycolide copolymer, subcutaneous implant q 3 or 6 months).

Of note is the persistence of the depot material SAIB for at least 12 months after a single injection of SABER-Bupivacaine in rabbits. At 12 months (last time point examined), the SAIB also demonstrates comparable local toxicity between SABER-Bupivacaine and SABER placebo and is compositionally the same material as injected 12 months earlier (i.e., unchanged). In ^{14}C-SAIB distribution studies in rats, ~40-60% of the SAIB radiolabel was shown to persist at the injection site at 10 weeks post-dosing (last time point evaluated) whether administered SC or into a surgical wound. The radiolabel of ^{14}C-SAIB administered in solvent (BA or (b) (4) (b) (4)) persisted at the injection site at a level (b) (4) at 6 week after dosing (BA solvent, last time point evaluated) and (b) (4) at 10 weeks (b) (4), last time point evaluated). Local toxicity is observed in animals immediately after dosing (e.g., marked inflammation) and decreases over time. The biological or toxicological significance of this persistent depot material to humans is unknown. The relevance of this nonclinical data to human safety is to be determined by the medical review team in conjunction with consideration of the overall human benefit of the proposed drug.

Human safety related to potential effects on wound healing and hemolysis is generally supported for this proposed drug to be administered by instillation. SABER-Bupivacaine and SABER placebo caused no significant effects on the wound healing process in rats over 7 days (mechanical strength measured after linear incision) or in minipigs over 15 days (planimetric measured contraction after full-thickness wound). SABER-Bupivacaine and SABER placebo both caused hemolysis when added to human whole blood *in vitro*. In addition, a viscous (and, for the placebo, cloudy) macroscopic appearance, with globules resembling bubbles visible microscopically, were observed in tested human plasma. The lack of significant effects on wound healing appears to support human safety of the proposed drug product use. SABER-Bupivacaine is not intended for vascular injection, which should make the potential for hemolysis not a real concern but one to be addressed by the medial review team.

Injection site necrosis was observed in dogs after intra-articular (IA) dosing with SABER-Bupivacaine and SABER placebo. While the proposed dose route for this NDA submission is instillation, this observed local effect should be referenced in the event of any proposed IA dosing in the future. Notation of this potential adverse event after IA dosing is noted in the product label precaution section, referencing potential chrondrolysis.

In summary, potential systemic toxicity regarding human safety appears to have been supported in this submission. Local toxicity occurs related to SABER-Bupivacaine at a degree no greater than for SABER placebo (no bupivacaine, only excipients SAIB and BA). In addition, the depot SAIB in SABER-Bupivacaine is present for a prolonged period of time. The importance of local toxicity and the persistence of SAIB for at least 12 months after instillation need to be addressed regarding human safety.

1.3 Recommendations

1.3.1 Approvability

NDA approval is recommended from the nonclinical perspective noting that local toxicity persists after a single instillation of SABER-Bupivacaine which requires clinical review team acceptance of the observed, anticipated local toxicity (foreign body reaction). While systemic exposure to bupivacaine after dosing with SABER-Bupivacaine exceeds levels reported for the approved, referenced drug Marcaine, the submitted nonclinical data supports human safety at the proposed dosing for SABER-Bupivacaine.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Pharm/Tox-related labeling for the reference NDA 16-964 (Marcaine), initially approved in 1972 and amended in 2012.

Not complete pending NDA label review by review team and will not be complete until after the due date of this review. What follows is what was current as of the last labeling meeting.

Pharm/Tox-related Labeling Sections for NDA 204803		
Label Proposed by Applicant (09-25-13)	FDA-Proposed label - from current Marcaine label (07-30-13) and adjusted for applicant's proposal with new data and for PLLR (Pregnancy and Lactation Labeling Rule) as of 12-18-2013	Rationale for Difference
1. Indications and Usage	**1. Indications and Usage**	
(b) (4)		FDA established Pharmacologic class (7-1-10)
8. Use in Specific Populations **8.1. Pregnancy**	**8. Use in Specific Populations** **8.1. Pregnancy**	
(b) (4)		

2 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

2 Drug Information

2.1 Drug

CAS Registry Number - 2180-92-9; 38396-39-3

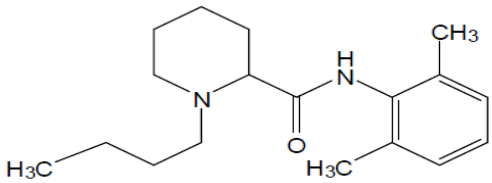
Generic Name – Bupivacaine or Bupivacaine base

Code Name – none reported

Chemical Name - (±)-1-Butyl-2',6'-pipecoloxylidide
- (2*RS*)-1-Butyl-*N*-(2,6-dimethylphenyl)piperidine -2-carboxamide

Molecular Formula/Molecular Weight – $C_{18}H_{28}N_{2O}$/288.43

Structure



Pharmacologic Class - an amide-type local anesthetic

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 66,086 (SABER-Bupivacaine)
NDA 16964 (Marcaine; bupivacaine HCl; reference NDA)
DMF (b) (4)
DMF
NDA 21344 (Faslodex – contains benzyl alcohol)

2.3 Drug Formulation

Drug Substance

The drug substance will be supplied from two manufacturers both of which use specifications used in another approved injectable bupivacaine with their respective DMFs (b) (4) At a proposed active ingredient dose level of 660 mg/day, the qualification threshold is 0.15% set by ICH Q3A, which is not exceeded for any impurities. In addition (b) (4) specifications are also acceptable for both drug substance suppliers as they are from confidential DMFs in another approved injectable bupivacaine drug product. (b) (4) at NMT (b) (4) is also acceptable for the same reason. Also see below and Section 2.5 Comments of Impurities/Degradants of Concern).

Specification for the (b) (4) (b) (4), which is positive in the Ames mutagenicity assay, is set at NMT (b) (4) in one of the supplied bupivacaine drug substances (b) (4) resulting in a total dose of (b) (4) less than the 1.5 µg/day total daily intake allowed as listed in the draft CDER Guidance for Industry: *Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (Dec 2008)*. Refer to the ONDQA review for more detail on this matter. The (b) (4) in the (b) (4) material (NMT (b) (4)) is at the acceptable ICH Q3 value of (b) (4).

The metabolite (b) (4) is genotoxic and has been shown to produce tumors in rodents. Consistent with previous NDA evaluations, the carcinogenicity finding was not relevant to humans [e.g., FDA OND Anesthetic and Life Support Drugs Advisory Committee Meeting of August 23, 1993 which included input from the OND Pharm/Tox Executive Carcinogenicity Assessment Committee]. The current generally acceptable level (b) (4) is to reduce levels to as low as reasonably possible (ALARP) as determined by ONDQA. In the past this has been in

the (b) (4) ppm range for drug product, but improved analysis technique has reduced the limit of quantitation (LOQ) to near 1 ppm. Therefore, the proposed (b) (4) ppm considered to be as low as reasonably possible (ALARP), meaning also as low as technically feasible under current conditions of production and analysis.

(b) (4), which are not structural alerts and would not be genotoxic in the Ames bacterial mutation assay according to CompTox analysis **(**FDA CDER Informational and Computational Safety Analysis Staff (ICSAS)**.** As both are currently within ICH specifications of NMT 0.15% for both drug substances (b) (4) no further action is indicated in regard to human safety.

Specifications for Bupivacaine Base (b) (4)

Test	Acceptance Criteria	Regulatory Analytical Procedure
Description	Crystalline white powder	Visual
Identification IR	Conforms to reference standard	USP <197A>, Ph. Eur.2.2.24
Identification GC retention time	Complies with reference standard	(b) (4)
Color of Solution	(b) (4)	
Melting Point	105 – 110°C	USP <741>
(b) (4)		
Impurities (b) (4)	(b) (4)	
Assay (b) (4)		
Aerobic Microbial Count	NMT (b) (4)	USP <61>
Total Combined Yeast and Mold Count	NMT	USP <61>
Bacterial Endotoxin	NMT	USP <85>

NMT = Not more than

[a] (b) (4) (non-USP) analytical procedures are provided in Section 3.2.S.4.2.

[b] Not tested by (b) (4) taken from Supplier COA.

Specifications for Bupivacaine Base (b) (4)

Test	Acceptance Criteria	Regulatory Analytical Procedure
Identity by IR	Conforms with Reference Spectrum	USP <197A>, Ph. Eur.2.2.24
Melting Point	105 – 108°C	USP <741>
(b) (4)		
Appearance Color Form	 White or Off White Crystalline powder or Crystals or Granules	 Visual
Identity by TLC	Complies with Reference	Hospira, Inc. AP # X-1083 [a]
Color		
(b) (4)		
Chromatographic Purity by HPLC		(b) (4)
(b) (4)		
(b) (4)		
Assay		(b) (4)
Bacterial Endotoxins	NMT (b) (4)	USP <85>
Aerobic Microbial Count	NMT	USP <61>
Total Combined Yeast & Mold	NMT	USP <61>

NMT = Not more than

[a] (b) (4) (non-USP) analytical procedures are provided in Section 3.2.S.4.2 for Bupivacaine from (b) (4)

Drug Product

The composition of the drug product is in the following table with the specifications listed in the subsequent table. The components of the Drug Product are considered qualified and acceptable/safe for human use based on conducted nonclinical studies (notably the repeat dose study using the aged product with increased levels of impurities and degradants – see sections 2.4 and 2.5 for more detail) and ICH guidance. According to ICH Q3B, at 660 mg/day the qualification threshold is 0.2%. Note that the specification level of (b) (4) has been changed by the Applicant to NMT (b) (4)% from NMT (b) (4)% (see section 2.5 for more detail).

Composition and Ingredient Functions of SABER-Bupivacaine

Ingredient	Composition % w/w	Composition mg/mL [a]	Amount (mg) Administered in a 5 mL Dose	Function	Specification
Bupivacaine	12	132	660	Active Pharmaceutical Ingredient	In-House Specification
Sucrose acetate isobutyrate	66	726	3630	Extended release agent	In-House Specification
Benzyl alcohol	22	242	1210	Solvent	NF, EP
(b) (4)					
Total	**100**	**1100**	**5500**	-	-

[a] The density of SABER-Bupivacaine is 1.1 g/mL at 25°C, therefore the concentration expressed as 12% w/w is equivalent to 13.2% w/v

Commercial Specifications for SABER-Bupivacaine

Attribute	Acceptance Criteria	Analytical Procedure [a]
Appearance [b]	Clear, light yellow to amber solution; essentially free of particulate matter	PR-1436
Identity: A) HPLC B) UV	Retention time matches reference standard for bupivacaine UV spectrum matches reference standard for bupivacaine	PR-1457
Assay [b]	(b) (4) label strength	PR-1457
Uniformity of Dosage Units	Meets USP<905> criteria for content uniformity of liquid dosage forms	PR-1457
Degradation Products [b]	(b) (4)	PR-1457 PR-1536 PR-1457 PR-1457
Dissolution [b]	USP <711> Extended-Release Dosage Form Conforms to USP Acceptance Table 2 Time Point — % Label Strength 1 hour — (b) (4) 18 hours 72 hours	PR-1461
Degree of Coloration of Liquids [b]	NMT (b) (4)	PR 1562
Sucrose Acetate Isobutyrate [b]	(b) (4)	PR-1702
Volume	NLT 5.0 mL	USP <1>
Particulate Matter [b]	NMT NMT (b) (4)	USP <788> Method 2
Sterility [b]	No microbial growth	USP <71>
Bacterial Endotoxins [b]	NMT (b) (4) EU/mL	USP <85>

NMT: Not more than NLT: Not less than
[a] Non-compendia analytical procedures are provided in Section 3.2.P.5.2.
[b] Attributes tested on stability for the commercial product.

NOTE: specification for (b) (4) has been reduced to NMT (b) (4) %

SABER-Bupivacaine and SABER-placebo were not genotoxic *in vivo* in a rat micronucleus test. The absolute value of this information is unknown as the rats received a single SC dose and sacrificed 3 days after dosing. As noted in the next section for SAIB, it persists for at least six months resulting in prolonged exposure and these time periods were not evaluated for micronuclei.

2.4 Comments on Novel Excipients

Sucrose Acetate Isobutyrate (SAIB)

SAIB is an approved GRAS substance for direct food additive use (Rulis AM. FDA/CFSAN: Agency Response Letter: GRAS Notice No. GRN000104. August 16, 2002). The public literature safety database for SAIB although essentially restricted to oral exposure, is extensive and includes acute, subchronic, and chronic toxicity studies, carcinogenicity bioassays, genotoxicity assays, and reproductive/ developmental toxicity studies. The No Observed Effect Level (NOEL) for SAIB was 2000 mg/kg/day (12,000 mg/m^2/day) in male and female rats (10 animals/sex/dose for 52 weeks in the diet) and 2400 mg/kg/day (29,000 mg/m^2/day) in male and female monkeys (4 animals/sex/dose) by the World Health Organization (WHO. Sucrose acetate isobutyrate. WHO Food Additive Series 32. 1993). Based on the NOELs from these studies, the WHO established an Acceptable Daily Intake (ADI) of 20 mg/kg for SAIB (oral dose). The proposed single injected instillation dose for SABER-Bupivacaine contains 726 mg/mL or 3630 mg SAIB in the proposed 5 mL dose. This

SAIB dose is ~60-70 mg/kg for a 50-60 kg human body weight range with an assumed increased bioavailability after injection compared to oral administration.

Available nonclinical data on SAIB support its human safety relative to genotoxicity, carcinogenicity, and reproductive/developmental toxicity, but the proposed single instillation dose needs safety qualification as it is administered by a novel route. Based on extensive nonclinical literature data for acute, subchronic, and chronic toxicity in multiple species, qualification testing is required in only one species, consistent with the FDA *Guidance for Industry: Nonclinical Safety Studies for the Safety Evaluations of Pharmaceutical Excipients* (May 2005).

This qualification was done using the 4-week repeat dose study in rats (study BR1265) using a 54-month aged drug product that met proposed drug product specifications at release. SABER-Bupivacaine contained (b) (4)% of the release level of SAIB or (b) (4) mg/mL. Safety Margins (SM) calculations are based on the systemic effects No Observed Adverse Effects Level (NOAEL), body surface area (BSA) comparisons between rat (human equivalent dose – HED) and human doses (mg/kg) were calculated with adequate SMs considered being (b) (4). At the systemic NOAEL of (b) (4) mL/kg, the total dose was (b) (4) mg/kg adjusted to an HED of (b) (4) mg/kg using a rat adjustment factor of (b) (4). The proposed human dose of SAIB is (b) (4) mg or (b) (4) mg/kg for a (b) (4) kg person. On this basis, the animal:human dose ratio or safety margin as the ratio is based on a NOAEL is (b) (4), indicating human safety based on the nonclinical data. Therefore, the SM for systemic toxicity of SAIB is adequate based on the 4-week repeated dose study in rats using aged drug product.

<u>Systemic SM</u> (based on high dose systemic NOAEL)

(b) (4) mg/kg SAIB in rat high dose group x (b) (4) (rat BSA factor)
= (b) (4) mg/kg HED
(b) (4) mg/kg HED in rat for SAIB ÷ (b) (4) mg/kg maximum human dose
= SM (b) (4)

Local toxicity was no different between SABER-Bupivacaine and SABER placebo. See discussion in section 11 as to the relevance of this to human safety from dosing with SABER-Bupivacaine.

Of note is the persistence of the depot material SAIB for up to 12 months after a single injection also demonstrating local toxicity consistent with SABER-Bupivacaine and SABER placebo. In ^{14}C-SAIB studies in rats and rabbits, 40-60% of the SAIB radiolabel was shown to persist at the injection site at 6 week post-dosing but was not present at 10 weeks post-dosing. This doesn't mean the SAIB was not present, just that the radiolabel was not measureable. SAIB was evidenced in rabbits after a single subcutaneous injection for at least up to1 year (last time point measured). The biological or toxicological significance of this unknown and its relevance to human safety is to be determined by the medical review team.

<u>Benzyl Alcohol</u> (BA)

BA is an aromatic alcohol used in a variety of cosmetic formulations as a fragrance component, preservative, solvent, and viscosity-reducing agent. BA is also approved as a food additive. An ADI was established by the WHO at up to 5 mg/kg (WHO. (b) (4). Food Additive Series 37. 1996.). As is the case for SAIB, the safety database for BA is extensive and includes acute, subchronic, and chronic toxicity studies, carcinogenicity bioassays, genotoxicity assays, and reproductive/developmental toxicity studies. No adverse effects of BA were seen in chronic (up to 2 years) exposure animal studies using rats and mice (b) (4), (b) (4). *International Journal of Toxicology.* 2001; 20(3):23-50.).

The proposed single subcutaneous dose for SABER-Bupivacaine is ~20-25 mg/kg for a 50-60 kg human body weight range at 22% BA. At the Maximum Recommended Human Dose (MRHD) for SABER-Bupivacaine of 5 mL/person, the dose of BA would be 1.21 grams (5.5 grams times 22% or 242 mg/mL x 5 mL). An approved parenteral product marketed as FASLODEX (NDA 21344 – 50 mg/mL) contains 10% BA (w/v) and is approved for intramuscular (IM) injection at a dose volume of 10 mL/person. Therefore, the approved dose of BA for FASLODEX is 1.0 gram/person, which is only slightly lower than the dose of BA from SABER-Bupivacaine. Considering that, approved, recommended FASLODEX dosing is every 2 weeks for three doses then monthly, the level of BA in SABER-Bupivacaine is considered acceptable/supported for human safety for a single dose by the reviewer.

Available nonclinical data on BA support its human safety relative to genotoxicity, carcinogenicity, and reproductive/developmental toxicity. Based on extensive nonclinical literature data for acute, subchronic and chronic toxicity in multiple species, qualification required testing in only one species, consistent with the FDA *Guidance for Industry: Nonclinical Safety Studies for the Safety Evaluations of Pharmaceutical Excipients* (May 2005).

In addition to the literature data, the 4-week repeat dose study in rats (study BR1265) using a 54-month aged drug product that met proposed drug product specifications at release also supports proposed human dosing. SABER-Bupivacaine was assumed to contain (b) (4)% of the release level for BA, the same as for SAIB or (b) (4). At the systemic NOAEL o (b) (4)/kg, the total BA dose was (b) (4) adjusted to an HED of (b) (4) using a rat adjustment factor of (b) (4). The proposed human dose of BA is (b) (4) for a (b) (4) person. On this basis, the animal:human dose ratio or safety margin as the ratio is based on a NOAEL is (b) (4), supporting human safety based on the nonclinical data. Therefore, the SM for systemic toxicity of BA is adequate based on the 4-week repeated dose study in rats using aged drug product.

Systemic SM (based on high dose systemic NOAEL)

(b) (4) BA in rat high dose group x (b) (4) (rat BSA factor)
= (b) (4) HED

(b) (4) HED in rat for BA ÷ (b) (4) maximum human dose = SM of (b) (4)

Something to note is that it appears that the BA component of SABER-Bupivacaine and SABER placebo causes some hemolysis while bupivacaine and SAIB alone did not cause hemolysis with the only difference being the component BA. The toxicological significance of this is unknown. BA was also shown in rats to pass into mother's milk and to the neonate, an occurrence already well known for humans.

2.5 Comments on Impurities/Degradants and Extractables/Leachables of Concern

Impurities/Degradants

(b) (4) are degradation products formed by transesterification of benzyl alcohol with SAIB. The proposed specification in SABER-Bupivacaine for (b) (4) is NMT (b) (4) mg/mL and for (b) (4) is NMT (b) (4) mg/mL. The level of these degradation products in the aged SABER-Bupivacaine used in a 4 weekly dosing study in rats (study BR1265 reported in section 6.2) was (b) (4) mg/mL (b) (4) and (b) (4) mg/mL (b) (4). Considering a single dose is proposed in humans, this 4 weekly dose study in rats at the highest dose tested which was a NOAEL, is considered adequate to qualify these degradation products along with the negative *in vitro* genotoxicity data submitted by the applicant for (b) (4) and the previously cited WHO review document and safety assessment for (b) (4) See nonclinical:human safety margins below and additional information in section 11 for specific calculations/assessment and considerations of human safety for the proposed formulation degradants.

The intended human use for this product is administration of a single-dose of SABER-Bupivacaine around the wound at the time of surgery. At the maximum proposed human dose of 5 mL SABER-Bupivacaine and at the proposed specification of (b) (4) mg/mL (b) (4) and (b) (4) mg/mL (b) (4) degradant dose levels will be (b) (4) mg (b) (4) ((b) (4) mg/kg) and (b) (4) mg (b) (4) ((b) (4) mg/kg) for a (b) (4) person.

Safety Margins (SM) calculations are based on the systemic effects NOAEL (high dose at (b) (4) mL/kg) and BSA comparisons between rat HEDs and human doses (mg/kg) with adequate SMs considered being (b) (4) SMs for systemic toxicity for (b) (4) are adequate based on the 4-week repeated dose study in rats using aged drug product.

Systemic SM (based on high dose systemic NOAEL)

(b) (4) mg/kg (b) (4) in rat high dose group x (b) (4) (rat BSA factor) = (b) (4) mg/kg HED

(b) (4) mg/kg HED in rat for (b) (4) ÷ (b) (4) mg/kg maximum human dose = SM of (b) (4)

(b) (4) mg/kg (b) (4) in rat high dose group x (b) (4) (rat BSA factor) = (b) (4) mg/kg HED

(b) (4) mg/kg HED in rat for (b) (4) ÷ (b) (4) mg/kg maximum human dose = SM of (b) (4)

Local toxicity was essentially no different between SABER-Bupivacaine and SABER placebo indicating that the degradants had no specific effects on human safety as levels were 10-20-fold less in SABER placebo.

(b) (4) is one of the degradation products of bupivacaine that has been observed in the stability studies of SABER-Bupivacaine. The proposed specification for (b) (4) was NMT (b) (4)% but is now NMT (b) (4)%. In the 4 week dosing study in rats using aged drug product containing (b) (4), no toxicity was observed at the highest dose tested. Considering negative *in vitro* genotoxicity results with (b) (4) and that dosing with SABER-Bupivacaine will be a single dose, NMT (b) (4)% is considered an adequate specification for potential systemic and local toxicity.

(b) (4) - In addition to the degradation products mentioned above, the bupivacaine of SABER-Bupivacaine contains a potentially genotoxic/carcinogenic degradant. DURECT has attempted to reduce the concentration of this impurity to the lowest feasible level, with a proposed specification of (b) (4) ppm. At this level and at the anticipated MRHD of 5 mL/person of SABER-Bupivacaine, the dose of (b) (4) would slightly exceed the maximum dose of 1.5 µg/person/day specified in the FDA Draft Guidance document for Genotoxic Impurities (December, 2008). The maximum anticipated human dose would be (b) (4) µg/person/day vs. the recommended maximum dose of 1.5 µg/person/day for one day only. Because of the limited human exposure (single-use administration) to this product, the applicant proposes that the specification is reasonable and fulfills the intent of the FDA Guidance, which is to minimize human exposure to genotoxic substances. For a single dose, the reviewer agrees. This specification is also consistent with previous NDA evaluations that the carcinogenicity finding was not relevant to humans (FDA OND Anesthetic and Life Support Drugs Advisory Committee Meeting of August 23, 1993 which included input from the OND Pharm/Tox Executive Carcinogenicity Assessment Committee). The current generally acceptable level of (b) (4) is to reduce levels to as low as reasonably possible (ALARP) as determined by ONDQA. In the past this has been in the (b) (4) ppm range, but now is in the (b) (4) ppm range as proposed by the applicant.

Extractables/Leachables

Potential human exposure is supported at the level of < 1.5 µg/day for structural alert chemicals and (b) (4) µg/day for nonstructural alert chemicals otherwise a risk assessment is required (*Safety Practices and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products*. Product Quality Research Institute, September 8, 2006). This is consistent with current FDA/Division thinking on this matter in that, for the toxicological risk assessment, any leachable that contains a structural alert for mutagenicity should not exceed 1.5 µg/day total daily exposure or be adequately qualified for safety. A toxicological risk assessment should be provided for any non-genotoxic leachable that exceeds (b) (4) µg/day. The risk assessment should

be based on the levels of leachables detected in long-term stability samples that include any intended secondary container closure system(s) unless otherwise justified.

In the original NDA submission, extractables and leachables studies with risk assessment reports were submitted. FDA Product Quality determined that the extractables study methodology was not adequate to fully determine potential extractables (see Product Quality review for specifics). Another extractables study was requested and submitted. Risk assessments for potential human exposure to the extractables and leachables were conducted by the Applicant based on the updated Extractables study (13-2890) and the original Leachables study (09-2370). The levels of (b) (4) in the stoppers were also evaluated (study 13-2884).

The overall risk assessment by this reviewer is based on the updated extractables risk assessment (report 13-04122-N1), the original leachables risk assessment (report 10-4948-N1), and the Applicant's response to an information request by Product Quality related to extractable compounds listed as unknown (reference ID 3339952, July 12, 2013) not being present in the leachables study. Note that for this single dose SABER-Bupivacaine, all of the extractables/leachables are assumed to be absorbed in 1 day (i.e., single dose).

The largest extractable value for each compound by the differing extraction techniques is listed in the following summary table from study 13-2890. The largest leachable value for each compound over the 36-month stability study is listed in the second table.

Extractables Summary

Analytical Method	Analyte	Maximum Extractable Concentration (µg/g)	Maximum Extractable Concentration (µg/stopper)
HPLC-DAD/MS	(b) (4)		
GC/MS			
Der-HPLC-DAD			
GC-NCD			
HS-GC/MS			
ICP/MS			

*At wavelength (b) (4)
**At wavelengt

Leachables Summary

Compound	Maximum Concentration	Tolerable Intake Level	Tolerable Exposure Level	Maximum Exposure per Administration
	µg/mL	mg/kg/day	µg/day	µg/5 mL
(b) (4)				

The potential human exposure risk assessment for extractables was based on these maximum values for each compound as listed in study 13-04122-N1. Of note is the total mg dose (mg/5mL) listed in the extractable table below is based on 5 mL of a (b) (4) mL total volume of drug product. The total 5 mL dose is (b) (4)% less than the total extractable level/compound as listed above. With the status of total drug product volume to be contained in the drug product vial is still an on-going discussion between Product Quality and the Applicant, the most conservative risk assessment requires reduction of the Safety Margin by (b) (4)%. This is not the case for the leachable values as no adjustment was made as for the extractables.

Product Quality reviewer Dr. Edwin Jao has determined that, other than (b) (4), none of the compounds are structural alerts, therefore, any values (b) (4) µg are considered acceptable regarding human safety (PQRI Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled Products, September 8, 2006).

For extractables, four compounds do not require risk assessment based on their total potential dose being (b) (4) – see extractables table above). For the other identified compounds, risk assessments were conducted based on ISO 10993012 (Establishment of allowable limits for leachable substances). This risk assessment technique utilizes animal or human data NOAELs/LOAELs divided by uncertainty factors (e.g., factors of 10 for animal to human data extrapolation and for inter-individual differences between humans), and exposure factors (e.g., bioavailability for an oral drug compared to the subcutaneous dose to be used for SABER-Bupivacaine) to determine the Tolerable Intake Level (TI – mg/kg/day) and Tolerable Exposure Level (TE - mg/day), the maximum predicted dose at which no adverse effects are predicted. This risk assessment technique is very similar to what the US EPA's Integrated Risk Information System (IRIS) uses and is widely accepted and considered appropriate by this reviewer.

Animal and human pharmacology and toxicology data is assessed using the ISO-based risk assessment method for the following compounds/groups of compounds. Based on my detailed review of the data and risk assessments, these extractables are at acceptable levels in regard to potential human exposure even if the Safety Margins are reduced by (b) (4)% as noted previously. As this is the case, the applicant's original numbers, unadjusted, will be reported.

The unknowns listed in the extractables table, except for the unknown (b) (4) (to be discussed next), are not considered a human risk issue even though they did not show up in the leachable study analysis per Quality Product determination as the a retrospective comparison of method controls and stability samples indicated the absence of any peaks that could be associated with the unknowns.

The unknown (b) (4) identified in the extractables study could not be detected in the leachables study as the sensitivity of the leachables assay could not established since reference material was not available. At a potential maximum dose of (b) (4) µg/5 mL, the Applicant considers this relatively low exposure of low hazard potential. I concur by also considering that acceptable levels of up to (b) (4) µg are considered safe for other (b) (4) as noted in some of the additionally submitted toxicology data.

ExtractablesTable

Exposure threshold and safety margin summary

Analytical Method	Compound	CAS ID	mg/5mL dose	Tolerable Exposure Level (mg/day)	Safety Margin
(b) (4)					

a = maximal exposure used in calculation of safety margin
b = additive exposures used in calculation of safety margin
N/A = not applicable
NC = not calculated

For leachables, four of the six compounds do not require risk assessment based on their total levels at (b) (4) – leachables table below). (b) (4) at (b) (4) µg is also acceptable based on the risk assessment for it as an extractable for which (b) (4) µg is considered acceptable. (b) (4) at (b) (4) µg is also acceptable based on the accepted extractable level of (b) (4) µg.

Leachables Table

Compound	Maximum Concentration µg/mL	Tolerable Intake Level mg/kg/day	Tolerable Exposure Level µg/day	Maximum Exposure per Administration µg/5 mL
(b) (4)				

For (b) (4) in the container-closure system rubber stopper, the level of any (b) (4) was (b) (4) ng/g of stopper or (b) (4) ng per stopper ((b) (4) g/stopper - study 13-2884). The largest amount of any (b) (4) per stopper was (b) (4) ng or (b) (4) µg/stopper, less than the 1.5 µg human safety value for structural alerts. However, an additional (b) (4) safety margin was included because some (b) (4) are carcinogenic results in an acceptable level of (b) (4) µg. For a single dose drug product, a (b) (4) µg potential (b) (4) exposure level is considered acceptable by this reviewer.

In summary, extractables and leachables that may be present in SABER-Bupivacaine are at acceptable levels for human exposure.

2.6 Proposed Clinical Population and Dosing Regimen

The current (proposed commercial) formulation of SABER-Bupivacaine was subsequently developed (b) (4) (b) (4) and (b) (4) was replaced with BA, a well-established pharmaceutical excipient in which bupivacaine base is highly soluble. The formulation of SABER-Bupivacaine consists of 12% bupivacaine base in 22% benzyl alcohol (BA) and 66% SAIB on a weight/weight (w/w) basis. After the solution is instilled directly into a surgical incision, (b) (4) the formulation rapidly diffuses into the surrounding tissue and is cleared from the circulation over a period of 12–24 hours (BU-001-IM Clinical Study report [CSR]), leaving a viscous subcutaneous depot of bupivacaine in SAIB. The SABER-Bupivacaine formulation was used for all clinical trials (except SABER01-01) in this NDA, and is the proposed formulation for marketing. The proposed indication for SABER-Bupivacaine (solution for instillation) is post-surgical analgesia at a total dose volume of 5 mL as a single

dose around the incision (b) (4) (b) (4).

2.7 Regulatory Background

IND 66,086 (SABER-Bupivacaine)

- Original IND submitted October 23, 2002 by DURECT but inactivated December 17, 2002 as the preclinical data did not support the proposed clinical trial due to findings of injection site inflammation and necrosis for which no evidence of resolution has been submitted.
- IND reactivated January 10, 2006 with submission of additional nonclinical data and substitution of Benzyl Alcohol (b) (4) as drug product solvent.

NDA 204803 (SABER-Bupivacaine) – submitted April 12, 2013

NDA 16964 (Marcaine; bupivacaine HCl; reference NDA) – approved October 3, 1972 with current owner Hospira

3 Studies Submitted

3.1 Studies Reviewed

Listed according to the eCTD Module 4 (Nonclinical Study Reports) categories

- Unless note, studies used Benzyl Alcohol (BA) versus (b) (4) (b) (4) excipient

Reference	Detailed Information
4.2 Study Reports	
4.2.2 Pharmacokinetics	
4.2.2.1 Analytical Methods and Validation Reports	
BAS-0100 Plasma Drug Level Validation Report	Study # BAS-0100 Title: Amended Summary Validation Report for the Analytical Method for Determination of Bupivacaine, in Rat, Rabbit, Dog, Minipig, and Mouse Plasma Using HPLC/MS/MS (BACG 3596 F)
04-1885	Study 04-1885 Analysis of Sucrose Acetate lsobutyrate (SAIB) for Molecular Weight Comparison to a Provided Reference Material (in Appendix F of study (b) (4)-434007 – section 4.2.3.6)
04-1200	Study 04-1200 Analysis of Sucrose Acetate Isobutyrate (SAIB) (b) (4) (in Appendix F of study (b) (4)-434007 – section 4.2.3.6)
4.2.2.2 Absorption	
11519.01.08 Rat PK SC	Study # 11519.01.08 Title: Pharmacokinetic Comparison Study in Rats of SABER-Bupivacaine Formulations Administered Subcutaneously vs. Intra-Wound Administration
7116-109 Rat SAIB absorption	Study # 7116-109 Title: Metabolic Disposition of ^{14}C-Sucrose Acetate Isobutyrate (SAIB) Following Oral and Subcutaneous Administration to Male Rats (SAIB (b) (4) control)

Reference	Detailed Information
4.2.2.3 Distribution	
7116-109 Rat SAIB distribution	Study # 7116-109 Title: Metabolic Disposition of ^{14}C-Sucrose Acetate Isobutyrate (SAIB) Following Oral and Subcutaneous Administration to Male Rats (SAIB (b) (4) control)
4.2.2.4 Metabolism	
7116-109 Rat SAIB metabolism	Study # 7116-109 Title: Metabolic Disposition of ^{14}C-Sucrose Acetate Isobutyrate (SAIB) Following Oral and Subcutaneous Administration to Male Rats (SAIB (b) (4) control)
4.2.2.5 Excretion	
7116-109 Rat SAIB excretion	Study # 7116-109 Title: Metabolic Disposition of ^{14}C-Sucrose Acetate Isobutyrate (SAIB) Following Oral and Subcutaneous Administration to Male Rats (SAIB (b) (4) control)
RPOS/FKM/204 Rat ADME SC	Study # RPOS/FKM/204 Title: Tissue Distribution and Excretion into the Milk Following a Single S.C. Dose of 0.2 or 0.6 mL of Posidur/kg to Rats
4.2.2.7 Other Pharmacokinetic Studies	
B167-05 Rat ADME SAIB	Study # B167-05 Title: Two, Six and Ten Week Subcutaneous Distribution Study of ^{14}C-SAIB in Male Sprague-Dawley Rats ((b) (4)- and BA-based test articles)
8255730 Final Report (12-11-803-R-SC-D)	Study # 8255730 Title: Determination of 14C-SAIB in the Skin and Subcutaneous Tissues of Male Sprague-Dawley Rats Following Application to a Surgical Wound or Subcutaneous Injection
4.2.3 Toxicology	
4.2.3.1 Single-Dose Toxicity	
11519.01.04 Rat Tox SC	Study # 11519.01.04 Title: Six-Week Toxicity Study of SABER™-Bupivacaine Injectable Formulation in Sprague Dawley Rats
04-07-803-R-PO-ATX Rat Tox Oral	Study # 04-07-803-R-PO-ATX Title: Acute Oral Toxicity Study of POSIDUR™ (SABER-Bupivacaine) in Albino Rats
A624.1.1 Rat Tox SC	Study # A624.1.1 Title: A Two-Week Toxicity Study of Bupivacaine in Sprague-Dawley Rats ((b) (4)-based test article)
A784.6.1 Rabbit Tox SC	Study # A784.6.1 Title: A Six-Week Toxicity Study of Bupivacaine in SAIB in New Zealand White Rabbits
02-06-803-B-IJ-TXR Rabbit Tox Intra-articular Pilot	Study # 02-06-803-B-IJ-TXR Title: Two-Week Pilot Toxicity Study of SABER-Bupivacaine Injectable Formulation in New Zealand White Rabbits
02-07-803-B-IJ-TX Rabbit Tox Intra-articular	Study # 02-07-803-B-IJ-TX Title: Six-Week Intra-Articular Toxicity Study of SABER-Bupivacaine Injectable Formulation in New Zealand White Rabbits
A624.1.2 Rabbit Tox SC	Study # A624.1.2 Title: A Two-Week Toxicity Study of Bupivacaine in New Zealand White Rabbits ((b) (4)-based test article)
A624.1.2 Protocol Amendments	Study # A624.1.2 Title: A Two-Week Toxicity Study of Bupivacaine in New Zealand White Rabbits ((b) (4)-based test article)
03-07-803-D-IJ-TX Dog Tox Intra-articular	Study # 03-07-803-D-IJ-TX Title: Six-Week Intra-Articular Toxicity Study of SABER-Bupivacaine Injectable Formulation in Beagle Dogs

Reference	Detailed Information
4.2.3.2 Repeat-Dose Toxicity	
BR1265 Rat Tox SC Repeated	Study # BR1265 Title: 4-Week Toxicity and Toxicokinetic Study of SABER-Bupivacaine After Subcutaneous Administration in the Rat
4.2.3.3 Genotoxicity	
4.2.3.3.1 In Vitro	
7116-116 AMES Assay	Study # 7116-116 Title: *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay (Bupivacaine free base))
7116-117 Chromosomal Aberrations	Study # 7116-117 Title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes (Bupivacaine free base)
4.2.3.3.2 In Vivo	
(b) (4)-434056 Final Report	Study # 08-11-803-R-SC-MN Title: An In Vivo Bone Marrow Micronucleus Test of SABER-Bupivacaine in Sprague Dawley Rats
4.2.3.5 Reproductive and developmental toxicity	
4.2.3.5.2 Embryo-fetal Development	
11-11-803-R-SC-TT Rat SC Developmental	Study # 11-11-803-R-SC-TT Title: Developmental Toxicity Study of SABER placebo in Rats
4.2.3.6 Local Tolerance	
022-010 Rat Nerve Tox	Study # 022-010 Title: A Single-Dose Toxicity Study With Peri-Sciatic Nerve Administration of Bupivacaine Solutions, Pastes, or Solids in the Sprague Dawley Rat Followed by a 7-Day Recovery Period
(b) (4)-434007 Rabbit Tox SC	Study # (b) (4)-434007 Title: A Repeated Histopathological Injection Site Evaluation up to 12 Months Following a Single Subcutaneous Administration of SABER Bupivacaine in the Rabbit
4.2.3.7 Other Toxicity Studies	
4.2.3.7.6 Impurities	(b) (4)
4.2.3.7.7 Other	
DUR 1 Rat Wound Healing	Study # DUR1 Title: A Study of the Effects of Sucrose Acetate Isobutyrate (SAIB)-based Formulations on Wound Healing Using the Rat Linear Incision Model ((b) (4)-based test article)
DUR 2 Rat Wound Healing	Study # DUR2 Title: A Study of the Effects of Sucrose Acetate Isobutyrate (SAIB)-based Formulations on Wound Healing Using the Rat Linear Incision Model

Reference	Detailed Information
60111 Minipig Wound Healing	Study # 60111 Title: SABER-Bupivacaine Injectable Formulation - A Single Dose Wound Healing Study in Full-Thickness Wounds in Minipigs
01-11-803-X-VO-ATX Human Blood Compatability	Study # 01-11-803-X-VO-ATX Title: Hemolytic Potential and Plasma Compatibility Study with Five Test Articles in Human Blood

4.3 Literature References	
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Rulis 2002	Rulis AM. FDA/CFSAN: Agency Response Letter: GRAS Notice No. GRN000104. August 16, 2002.
Who 1993	WHO. Sucrose acetate isobutyrate. WHO Food Additives Series 32. 1993.
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3.2 Studies Not Reviewed

4.3 Literature References	
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Black 2006	Black HE, Montgomery SB. Sucrose acetate isobutyrate (SAIB). An evaluation of the safety of SAIB for use as an inactive pharmaceutical ingredient in the DURECT ORADUR oral drug delivery system. September 6, 2006.
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Brau 1995	Brau ME, Nau C, Hempelmann G, et al. Local anesthetics potently block a potential insensitive potassium channel in myelinated nerve. *Journal of General Physiology.* 1995; 105:485-505.
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3.3 Previous Reviews Referenced

- IND 66,086

4 Pharmacology

No pharmacology studies were conducted for this NDA. Included are brief summaries for information purposes that are modified from the Applicant's submission. Bupivacaine is an approved local anesthetic used for the treatment of postsurgical pain with over 40 years of clinical experience. Pharmacological studies have described both the molecular and tissues/organ-system mechanisms for both desired analgesic and side effects (CNS, CVS). The applicant considers SABER-Bupivacaine as a next generation, extended release formulation of bupivacaine base, designed to provide post-surgical analgesia for up to 72 hours. SABER-Bupivacaine is expected to have similar pharmacology as aqueous bupivacaine-HCl.

4.1 Primary Pharmacology

The pharmacology/pharmacodynamics of bupivacaine has been extensively described in the literature and is well conserved across species. Bupivacaine is a locally acting, amide-type anesthetic, acting primarily through inhibition of neuronal voltage-gated sodium (Na+) channels. Local anesthetics block the generation and the conduction of nerve impulses, presumably by increasing the threshold for electrical excitation in the nerve, by slowing the propagation of the nerve impulse, and by reducing the rate of rise of the action potential.

4.2 Secondary Pharmacology

SABER-Bupivacaine is intended for direct instillation into surgical wounds and has been shown to have minimal impact on surgical wound healing in non-clinical models (see reviews in section 10.1) and in clinical studies (see medical review). In agreement with these studies, the effect of bupivacaine-HCl on wound healing in the published nonclinical literature supports wound instillation as a safe route of administration with few consequences. The public literature also appears to support the minimal effects on wound healing after bupivacaine use.

4.3 Safety Pharmacology

No safety pharmacology studies were conducted. Potential CNS and cardiac effects are well documented in the public literature.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Analytical method validation (study BAS-0100) – An HPLC/MS/MS method was validated for the determination of bupivacaine in mouse, rat, rabbit, dog, and minipig plasma. The validated concentration range identified a lower limit of quantitation (LLOQ) of 0.5 ng/mL for the rat and of 1 ng/mL for the other species. The upper limit of the concentration was identified as 500 ng/mL. The accuracy and precision of analyte recovery was 85-115%. Stability of bupivacaine in plasma was identified as at least 3 hours at room temperature and at least one month stored frozen at -70^{o}C. Lidocaine was the internal standard. In addition, there was no evidence of matrix suppression in the analyses.

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Tissue distribution and excretion into the milk following a single SC dose of 0.2 or 0.6 ml Posidur/kg to rats (**study RPOS/FKM/204; 89/2009 - non-GLP) - Tissue distribution and excretion into the milk following a single SC dose of 0.2 or 0.6 ml Posidur/kg to rats** (**study RPOS/FKM/204; 89/2009 - non-GLP) -** The purpose of this study was to determine the distribution of benzyl alcohol (BA) in selected tissues of the rat over time and to quantify the excretion of radioactivity into the milk of lactating rats after subcutaneous (SC) administration of Posidur (SABER-Bupivacaine, batch KG02/252; 541913F) containing [^{14}C]-labeled benzyl alcohol (BA, batch KG 02/252).

Single SC doses were 0.2 ml/kg and 0.6 ml/kg in 24 female Wistar rats (n = 3/ group) and 144 pups (n = 18/group). Body weight range of dams was 198-282g. At different time points post dose (1h, 4h, 8h, 24h), the pups were allowed suckle, the milk from each pup was removed and the radioactivity content was determined in the milk of the pups. In addition, radioactivity was determined in pooled plasma from each time point and in liver of the pups and in plasma of the dams at those time points.

Peak concentrations of benzyl alcohol-associated radioactivity (^{14}C BA) in plasma of dams were attained 1h after dosing. Following SC administration of 0.2 ml/kg, concentration of radioactivity in plasma and liver of pups were generally below the lower limit of quantitation (LLOQ). Excretion of radioactivity into the milk was highest 4 h after suckling with concentrations ranging from 4.026 to 12.95 µg equiv./g. Following SC administration of 0.6 ml/kg, concentrations of radioactivity in plasma and liver of pups were highest 1h post-suckling and amounted to a maximum of 0.61 µg equiv./g in plasma and to a maximum of 1.043 µg equiv./g in liver. Excretion of radioactivity into the milk showed a maximum 4h after suckling, with concentrations

ranging from 51.75 µg equiv./g to 65.83 µg equiv./g. At 24h, concentrations of radioactivity ranged from 2.15 µg equiv./g to 2.80 µg equiv./g.

Concentrations of radioactivity in plasma of dams and pups increased with increasing dose, with peaks being generally attained 1 h after subcutaneous dosing in milk. Concentrations of radioactivity were lower in pup plasma than in dam plasma. Concentrations of radioactivity in pup livers were similar to or slightly higher than plasma concentrations in pups. Excretion of radioactivity into milk was observed at both dose levels but never exceeded 0.4%of the dose of benzyl alcohol, with peaks occurring in suckling pups at 4 h post maternal dosing with the amount excreted being higher at the dose of 0.6 ml/kg (~5 mg BA).

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Metabolic disposition of ^{14}C-Sucrose Acetate Isobutyrate (SAIB) following oral and subcutaneous administration to male rats (study 7116-109 - GLP) - The pharmacokinetics (PK) of SAIB and tissue distribution, excretion (urine, feces, and expired air), and metabolism (ADME) of radioactivity of ^{14}C-SAIB were evaluated after a single subcutaneous (SC) or oral dose to male Sprague Dawley rats in a GLP study. SAIB is the depot component of SABER-Bupivacaine, the proposed drug product. SC dose site histopathology was also evaluated. Determinations for PK and radioactivity were for 48 hours (oral) and 1008 hours (6 weeks – SC) post dose. The non-radiolabeled test article was SAIB (b) (4) The proposed solvent for NDA 204803 (SABER-Bupivacaine) is Benzyl Alcohol, but this difference is not anticipated to effect SAIB radiolabel ADME to any significant degree.

Group Designations and Dose Levels

Group	Number of Males	Target SAIB Dose Level (mg/kg)	Dose Route	Target Dose Volume (mL/kg)	Target Radioactivity (µCi/kg)	Group Description
1	3	280	Oral	0.4	100	Excretion/Mass Balance
2	15	280	Oral	0.4	100	PK/Profiling
3	5	280	SC	0.4	500	Excretion/Mass Balance
4	18	280	SC	0.4	500	PK/Profiling
5	6	280	SC	0.4	100	WBA

PK Pharmacokinetic analyses.
SAIB Sucrose Acetate Isobutyrate.
SC Subcutaneous.
WBA Whole-body autoradiography.

Radioactivity in blood and plasma reached maximum level at 4 hours post-dose. While the SC Cmax was 4-fold lower that the oral Cmax, the SC systemic exposure was 2-fold higher than the oral systemic exposure due to a much longer SC dose plasma half-life. This indicates that the SC exposure results in a prolonged systemic exposure (see table).

Summary of pharmacokinetic parameters after administration of ^{14}C-SAIB

Group	Dose Route	Matrix	Dose Level (mg/kg)	C_{max} (µg equiv/mL)	T_{max} (hours)	AUC_{0-t} (µg equiv*hr/g)	$AUC_{0-\infty}$ (µg equiv*hr/g)	k_e (1/Hour)	$t_{1/2}$ (Hours)
2	Oral	Blood	309	9.42	4	214	385	0.0162	42.7
2	Oral	Plasma	309	13.8	4	306	403	0.0303	22.9
4	SC	Blood	299	2.70	336	2360	5290	0.0006	1130
4	SC	Plasma	299	3.29	1	714	839	0.0016	425
1	Oral	Urine	313	NA	NA	NA	NA	0.0253	27.5
1	Oral	Feces	313	NA	NA	NA	NA	0.0424	17.2
1	Oral	Expired Air	313	NA	NA	NA	NA	0.0175	41.1
3	SC	Urine	311	NA	NA	NA	NA	0.0014	500
3	SC	Feces	311	NA	NA	NA	NA	0.0016	441
3	SC	Expired Air	311	NA	NA	NA	NA	0.0013	544

NA Not applicable.
SC Subcutaneous.

Radioactivity was largely eliminated by 24 hours after oral dosing with the elimination half-life ranging from 17-41 hours (66.2% feces – unabsorbed drug, 13.6% expired air, 8.7% urine). After SC dosing, the elimination half-life was prolonged over the 6 week study period (500 hours urine, 441 hours feces, 544 hours expired air). Elimination was 21.6% in urine, 13.7% in expired air, and 3.7% in feces with the remainder of the radioactivity at the dose site indication SAIB largely remains at the dose site past 6 weeks after dosing.

Histological evaluation of the SC dosing site at 1 week after dosing identified subacute inflammation, characterized by slight to moderate lymphohistiocytic infiltrates and reactive fibrosis with or without aggregates of fibrin. At 2 weeks, subacute to chronic inflammation with slight lymphohistiocytic infiltrates, reactive fibroplasia, and fibrosis were observed. At 4 and 6 weeks, the SC dosing sites were surrounded by chronic inflammation, characterized by fibrosis and minimal lymphohistiocytic inflammation. Reversibility was not evident while inflammation persisted with severity similar at 4 and 6 weeks after dosing.

Microscopic Incidence and Mean Severity ( ) of Inflammatory Responses[a]

Sex	Male			
Time of sacrifice (weeks)	1	2	4	6
Number Examined	3	3	3	3
Inflammation, subacute	3 (2.3)	0	0	0
Inflammation, subacute-chronic	0	2 (2.0)	0	0
Inflammation, chronic	0	1 (1.0)	2 (1.0)	3 (1.0)

a Incidence and mean severity ( ) based on number of animals with finding.

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Two, Six and Ten-Week Subcutaneous Distribution Study of ^{14}C-SAIB in Male Sprague-Dawley Rats (SRI study No. B167-05 - non-GLP) - The study objective was to determine the effect of formulation components on ^{14}C-SAIB (sucrose acetate isobutyrate) absorption and/or elimination from a single subcutaneous (SC) injection

or intrascapular, back implantation of 0.1 mL as measured at 0, 2, 6 and 10 weeks post-dose in male Sprague-Dawley rats.

Two pilot studies of 30 minutes duration with 3 male rats/group were conducted prior to the start of the definitive study. They were designed to evaluate and develop the formulation procedures, dose administration and dose site recovery techniques of different, selected proposed vehicle formulations. As a result of the pilot studies, the following approaches were used for the definitive study: collection of dose site area of 2 x 2 in; tattooing the area boundaries for easy recovery determination; limiting handling of the dose site; suturing the dose site skin to underlying muscle just prior to collection. These studies allowed the development of techniques that resulted in essentially complete recovery of radioactivity from the dose site using a 0.1 mL dose volume and dose site collection within 30 minutes of dose administration. One test group in each pilot contained a group relevant to the proposed drug product containing benzyl alcohol (^{14}C-SAIB/BA - 70:30, SC). These groups are the focus of this review as they are most relevant to the proposed drug product.

The definitive study consisted of 14 dose formulation groups with 3 rats/group/time point. Solvents varied and included (b) (4) ethanol, Tween 80, polyethylene glycol, and others. Control tissues were collected at approximately 30 minutes post dose administration with other collection times of 2, 6, or 10 weeks. On the day of dose administration (Week 0), more than 70% of the administered dose was recovered at the dose site in all groups except Group 10 , which had the lowest initial recovery, 56.3 ± 2.0. Mean recovery at Week 0 for the other study groups (Groups 1-9 and 11-14) ranged from 73.0 ± 44.3% (Group 5) to 103.0 ± 1.5% (Group 12). Most notable were the results for the Benzyl Alcohol (BA) solvent group 4 (^{14}C-SAIB/BA - 70:30 %, SC) and 100% SAIB groups 5 (^{14}C-SAIB, SC Implant). Only these groups will be reported.

Retention of radioactivity at the dose site varied among the formulation groups. No group remained above 60% for the duration of the study to Week 6 or Week 10 see table). Groups 5, 6 (100% SAIB), 12, and 14 retained the greatest percentage of the dose at Week 6, approximately 50%. Groups 1 and 14 retained approximately 50% of the administered dose of radioactivity at Week 10. The loss of radioactivity was greatest at Week 2 for Group 9, from 82.8 ± 2.8 (Week 0) to 5.2 ± 4.4 (Week 2). Groups 3, 4 (SAIB/BA), 7, 8, 10, 11 and 13 all decreased to approximately 20-50% by Week 6. Recovery of radioactivity for Groups 2 and 9 was less than approximately 20% by Week 2 or 6. In conclusion, marked differences were observed in the rate of loss of radioactivity from the dose site of the various formulation groups but a significant amount of the SAIB remained at the injection site 6 weeks after dosing.

RECOVERY OF RADIOACTIVITY AT THE DOSE SITE

Dose Group	Test Formulation Route & Dilution Ratios	Radioactivity (% of Administered Dose)			
		Week 0 (Mean ± SD)	Week 2 (Mean ± SD)	Week 6 (Mean ± SD)	Week 10 (Mean ± SD)
1	^{14}C-SAIB (b) (4) SC (70:30)	91.4 ± 10.6	59.8 ± 16.7	44.5 ± 9.7	51.7 ± 4.8
2	^{14}C-SAIB (b) (4) SC Implant (70:30)	74.4 ± 27.0	40.1 ± 27.2	11.0 ± 17.1	NS
3	^{14}C-SAIB/EtOH SC (70:30)	92.9 ± 10.5	63.1 ± 2.7	32.6 ± 27.8	NS
4 (PG-5)	^{14}C-SAIB/BA SC (70:30)	97.5 ± 1.4	56.8 ± 13.3	38.9 ± 13.7	NS
5	^{14}C-SAIB/BB SC (70:30)	73.0 ± 44.3	42.3 ± 21.0	51.2 ± 4.8	NS
6	^{14}C-SAIB SC Implant (100%)	89.1 ± 14.7	63.9 ± 6.4	58.4 ± 2.5	36.3 ± 31.9
7 (PG-1)	^{14}C-SAIB/Tween 80 SC (70:30)	89.5 ± 3.9	40.4 ± 4.6	32.1 ± 5.2	NS
8	^{14}C-SAIB/Tween 80/PEG 300 SC (33:33:33)	77.5 ± 19.5	37.4 ± 8.5	28.0 ± 20.3	NS
9 (PG-3)	^{14}C-SAIB/Sorbitol F68/H20 SC Implant (25:12.5:37.5:25)	82.8 ± 2.8	5.2 ± 4.4	1.6 ± 0.3	NS
10	^{14}C-SAIB/Solutol HS-15 (+/- H20) SC Implant (70:30)	56.3 ± 2.0	21.0 ± 14.7	23.8 ± 4.2	NS
11 (PG-2)	^{14}C-SAIB/Mannitol/ F68/Sorbitan Oleate SC Implant (25:50:18.75:6.25)	80.3 ± 12.6	18.3 ± 9.6	22.2 ± 2.8	NS
12	^{14}C-SAIB (b) (4) 750-90:10 PEG-PLGA SC (53.8:37.1:9.1)	103.0 ± 1.5	74.8 ± 7.6	46.4 ± 6.3	33.5 ± 27.3
13 (PG-4)	^{14}C-SAIB (b) (4) 65:35 PLGA SC (55:40:5)	92.2 ± 6.2	66.4 ± 16.1	31.7 ± 15.1	39.1 ± 23.3
14	^{14}C-SAIB (b) (4) (b) (4) SC (52.1:40.9:7)	92.1± 1.4	46.3 ± 22.8	46.6 ± 15.4	51.0 ± 5.1

PG = Pilot study group number with corresponding formulation.
NS = Not sampled as per protocol

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Determination of ^{14}C-SAIB in the Skin and Subcutaneous Tissues of Male Sprague-Dawley Rats Following Application to a Surgical Wound or Subcutaneous Injection (study 8255730, 12-11-803-R-SC-AD - GLP) - The purpose of this study was to assess the disappearance of radioactivity after a single subcutaneous injection or surgical wound application of ^{14}C-Sucrose Acetate Isobutyrate (^{14}C-SAIB, lot 3614087) administered as ^{14}C-SAIB/Benzyl Alcohol (BA) (SAIB/BA; 75/25 (w/w)) to rats. The non-radiolabeled test article was SAIB/BA (lot 7211003F). The average radiopurity values of pre-dose and post-dose samples of the dose formulations were 95.8% and 96.1%, respectively, for Group 1 and 98.2% and 96.7%, respectively, for Groups 2 and 3.

Group	Phase	Number of Male Animals	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (µL/animal)	Samples Collected
1	1	3	Subcutaneous	440	100	Skin section and underlying muscle
2	2	25	Application to a Surgical Wound	440	100	Skin section and underlying muscle
3	2	25	Subcutaneous	440	100	Skin section and underlying muscle

Notes: The dose was approximately 1.56 µCi/animal based on a prepared test article formulation (1:100 dilution of ^{14}C-SAIB into SAIB/BA) of 0.0156 mCi/mL.
Phase 2 was dosed approximately 2 weeks after Phase 1 to allow for evaluation of the sample solubilization method.

A fixed volume (100 µL) of radiolabeled dose formulation was administered to each animal. The application of dose to a surgical wound was performed via syringe and needle into a surgical incision (approximately 0.5 inches in length) made down to the fascia in the interscapular region. The disposition of radioactivity from dose sites was determined after an interscapular subcutaneous injection (Groups 1 and 3) or by application to an interscapular incision (Group 2) using liquid scintillation counting (LSC). After dosing, skin and subcutaneous thoracic tissues from Group 1 animals were collected immediately for analysis of radioactivity. In Groups 2 and 3, skin and subcutaneous thoracic tissues were collected at 0, 7, 14, 42, and 70 days post-dose for determination of radioactivity concentration. Subcutaneous dosing of ^{14}C-SAIB to Group 1 animals was conducted to determine the best dosing technique.

The decreases in radioactivity at the dose sites for both Groups 2 and 3 were biphasic over the course of the study, with a relatively rapid decrease in radioactivity through 14 days post-dose followed by a relatively slower decrease through 70 days (10 weeks) post-dose (see tables and figures). The mean percentage of dose remaining in the skin and subcutaneous tissues were 54 and 60% in Groups 2 and 3, respectively, at 70 days post-dose. No substantive differences in disposition of radioactivity were evident between Groups 2 and 3. Overall, ^{14}C-SAIB-dependent radioactivity was slowly and incompletely removed from the dose sites after a subcutaneous dose or after application to a surgical incision by 70 days (10 weeks) post-dose, and disposition of ^{14}C-SAIB-dependent radioactivity was independent of the route of dosing.

Percent of radioactive dose in skin and subcutaneous tissues immediately after a single subcutaneous dose of ^{14}C-SAIB to male rats (Group 1, Phase 1, 440 mg/kg)

Percent of Radioactive Dose				
Animal Number				
B29552	B29553	B29554	Mean	SD
75.6	80.5	92.4	82.9	8.64

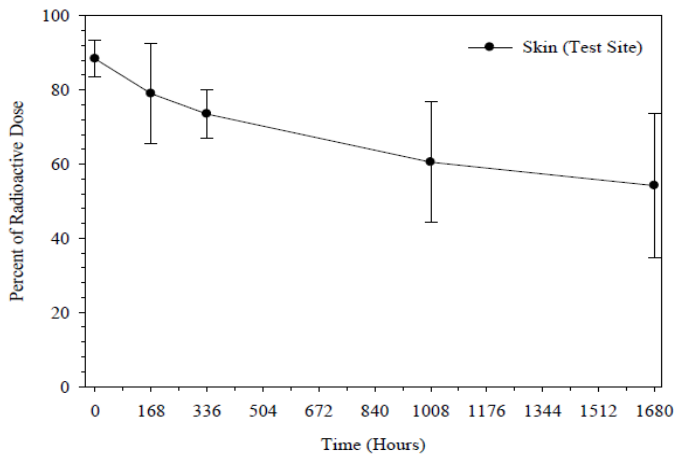
SD Standard deviation.

Percent of radioactive dose in skin and subcutaneous tissues at specified times after a surgical wound administration of ^{14}C-SAIB to male rats (Group 2, Phase 2, 440 mg/kg)

	Percent of Radioactive Dose						
	Animal Number						
Time Point	B29819	B29820	B29821	B29822	B29823	Mean	SD
0 h	89.6	82.0	93.3	92.6	84.4	88.4	4.99
	Animal Number						
	B29824	B29825	B29826	B29827	B29828	Mean	SD
168 h	82.1	84.6	86.7	55.1	86.5	79.0	13.5
	Animal Number						
	B29829	B29830	B29831	B29832	B29833	Mean	SD
336 h	74.0	64.0	81.8	71.4	76.1	73.5	6.53
	Animal Number						
	B29834	B29835	B29836	B29837	B29838	Mean	SD
1008 h	38.6[a]	65.3	51.9	81.8	64.9	60.5	16.2
	Animal Number						
	B29839	B29840	B29841	B29842	B29843	Mean	SD
1680 h	69.1	52.0	61.6	67.0	21.4	54.2	19.5

h Hours.
SD Standard deviation.
a Metal staples were used along with the glue to insure the incision remained closed.

Mean percent of radioactive dose in skin and subcutaneous tissues at specified times after a surgical wound administration of ^{14}C-SAIB to male rats (Group 2, Phase 2, 440 mg/kg)

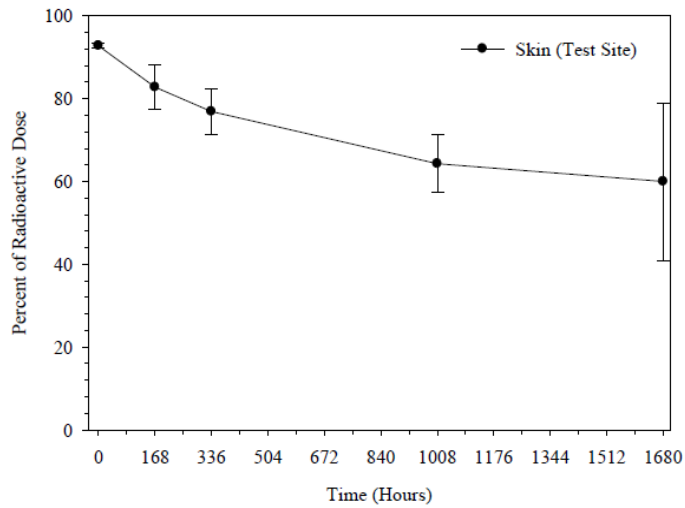


Percent of radioactive dose in skin and subcutaneous tissues at specified times after a single subcutaneous administration of ^{14}C-SAIB to male rats (Group 3, Phase 2, 440 mg/kg)

	Percent of Radioactive Dose						
	Animal Number						
Time Point	B29844	B29845	B29846	B29847	B29848	Mean	SD
0 h	93.2	93.3	93.5	92.4	91.8	92.8	0.712
	Animal Number						
	B29849	B29850	B29851	B29852	B29853	Mean	SD
168 h	73.5	83.7	85.8	87.1	84.1	82.8	5.40
	Animal Number						
	B29854	B29855	B29856	B29857	B29858	Mean	SD
336 h	80.2	68.5	77.8	75.4	82.7	76.9	5.44
	Animal Number						
	B29859	B29860	B29861	B29862	B29863	Mean	SD
1008 h	57.7	75.4	64.2	65.1	59.1	64.3	6.97
	Animal Number						
	B29864	B29865	B29866	B29867	B29868	Mean	SD
1680 h	78.3	52.6	58.9	77.4	32.7	60.0	19.0

h Hours.
SD Standard deviation.

Mean percent of radioactive dose in skin and subcutaneous tissues at specified times after a single subcutaneous administration of ^{14}C-SAIB to male rats (Group 3, Phase 2, 440 mg/kg)



In summary, ^{14}C-SAIB-dependent radioactivity was slowly and incompletely removed from the dose sites after a subcutaneous dose or after application to a surgical incision by 10 weeks post-dose. The apparent rates of decrease of radioactivity from the dose sites were biphasic and similar between the dose groups. Disposition of ^{14}C-SAIB-dependent radioactivity was independent of the route of dosing.

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Status of depot material and solvent 12 months after injection (studies 04-1885 & 04-1200) – As reported as appendices in study (b) (4)-434007 regarding Sucrose Acetate Isobutyrate (SAIB) and solvent (b) (4) after as single SC dose with SABER-Bupivacaine, the physical condition of SAIB was found to be essentially unchanged and still present 12 months after injection. The material was described as viscous material with essentially no (b) (4) present ((b) (4)%) at the injection site. The solvent for the proposed drug is benzyl alcohol but no substantial difference in migrating activity is anticipated compared to the (b) (4).

5.2 Toxicokinetics

(Most data included in individual toxicity study reviews)

Study title: Pharmacokinetic comparison study in rats of SABER-Bupivacaine formulations administered subcutaneously vs. intra-wound administration (non-GLP)

Study no.:	11519.01.08
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 21, 2005
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Bupivacaine/SAIB/BA (12/66/22, % w/w), lot 046-05, purity not reported - aka SABER-Bupivacaine Control – Sensorcaine-MPF 0.5%, lot LB2271, purity not reported

Key Study Findings

- Male SD rats received a single injection with Sensorcaine (4.2 mg/kg of bupivacaine) by subcutaneous (SC) administration, SABER-Bupivacaine (60 mg/kg of bupivacaine) SC, or SABER-Bupivacaine (60 mg/kg of bupivacaine) SC over an incision. Plasma levels of bupivacaine were measured at 4 and 7 days.
- The results of this study indicated that peak plasma concentrations of bupivacaine and AUC values were similar for rats given the SAIB/BA-12% formulations (aka SABER-Bupivacaine) SC or by installation into a SC incision site. These data indicated that the extent of absorption of bupivacaine was similar for rats given the SAIB/BA-12% formulation by SC injection or by application into a SC incision site.
- For rats given the SABE-bupivacaine formulations, mean peak plasma concentrations of bupivacaine were highest for rats dosed with the SAIB/BA-12% formulation and were 725 ng/mL at 1 hour after SC administration of the SAIB/BA-12% formulation and 714 ng/mL at 2 hours after instillation of the SAIB/BA-12% formulation into an SC incision site. AUC_{INF} values were similar

for animals given the SAIB/BA-12% formulation (SC or SC incision) at 17611 and 18317 ng·hr/mL, respectively.

- In addition, the plasma time course of bupivacaine supports the intended delayed release of bupivacaine using the depot product containing SAIB. Also, nonclinical SC studies are appropriate for describing systemic exposure to bupivacaine compared to human wound dosing.

Methods

Doses:

Group Number	Number of Animals	Formulation Name	Route	Dose Volume (mL/kg)	Dose mg/kg
1	6	0.5% Sensorcaine-MPF	injection	0.83	4.2
2	6	SAIB/BA-12%	injection	0.50	60
3	6	SAIB/BA-12%	incision	0.50	60

Note that the amount of bupivacaine in Sensorcaine group is 14-fold lower than in the SAIB/BA groups

Frequency of dosing: Single
Route of administration: Subcutaneous (SC) for groups 1 & 2 and dripped on the incision for group 3
Dose volume: 0.5 mL/kg for SC and incision injection
Formulation/Vehicle: Benzyl alcohol for test article
Species/Strain: Charles River Sprague Dawley
Number/Sex/Group: 6 males/group
Age: 11 weeks at initiation
Weight: 341-375 g at initiation
Satellite groups: None
Unique study design: Purpose of this study was to compare pharmacokinetics of SABER-Bupivacaine at the same dose by SC dosing compared to SC dosing over an incision (proposed clinical use)
Deviation from study protocol: Nothing significant.

Observations and Results

Mortality

All rats were observed twice daily throughout the quarantine and study periods for signs of moribundity and mortality.

No signs were observed.

Clinical Signs

Rats were removed from their cages on Days 0-7 and observed for clinical signs of toxicity, with particular attention paid to the injection or incision site.

Swelling at the site of injection was observed on one or more days between Days 1 and 5 for 4/6 rats dosed SC with the SAIB/BA-12% formulation (Group 2). No other adverse clinical signs were observed for any other rats on the study.

Body Weights

Measured on days 0 (prior to dosing), 4 & 7.

No treatment-related effects on body weight were apparent. The group mean body weights of animals in each dose group increased between Days 0 and 4 and between Days 4 and 7.

Feed Consumption, Ophthalmoscopy, ECG, Hematology, Clinical Chemistry, Urinalysis, Gross Pathology, Organ Weights, and Histopathology

Not evaluated.

Special Evaluation

None.

Toxicokinetics

Blood was sampled from the retro-orbital plexus from 3 animals/group on days 4 & 7 before sacrifice. Sampling times were as follows with times being combined for the final overall calculations:

Subgroup	Collection Times
A	0.5, 2, 8, 48, and 96 hours
B	1, 4, 24, 72, and 168 hours

Mean peak plasma concentrations of bupivacaine were 725 ng/mL at 1 hour after dosing for rats given the SAIB/BA-12% formulation SC (Group 2) and 714 ng/mL at 2 hours after dosing for rats given the SAIB/BA-12% SC into an incision site (Group 3) indicating a more rapid absorption after the SC dose alone (see table).

Time	Plasma Concentration (ng/mL) Mean ± SD		
	Group 1	Group 2	Group 3
0.5 hour	266 ± 123	458 ± 75.5	396 ± 85.3
1 hour	444 ± 16.6	725 ± 139	692 ± 217
2 hours	213 ± 50.8	441 ± 36.3	714 ± 243
4 hours	39.6 ± 11.8	546 ± 218	666 ± 97.2
8 hours	4.63 ± 2.67	376 ± 35.2	502 ± 79.0
24 hours	BQL	237 ± 53.7	329 ± 151
48 hours	BQL	102 ± 16.7	97.3 ± 69.8
72 hours	BQL	54.5 ± 14.1	8.52 ± 4.74
96 hours	BQL	38.8 ± 7.80	7.64 ± 5.93
168 hours	BQL	5.62 ± 3.02	0.38 ± 0.46

- BQL (below quantitation limit of 0-.5 ng/mL

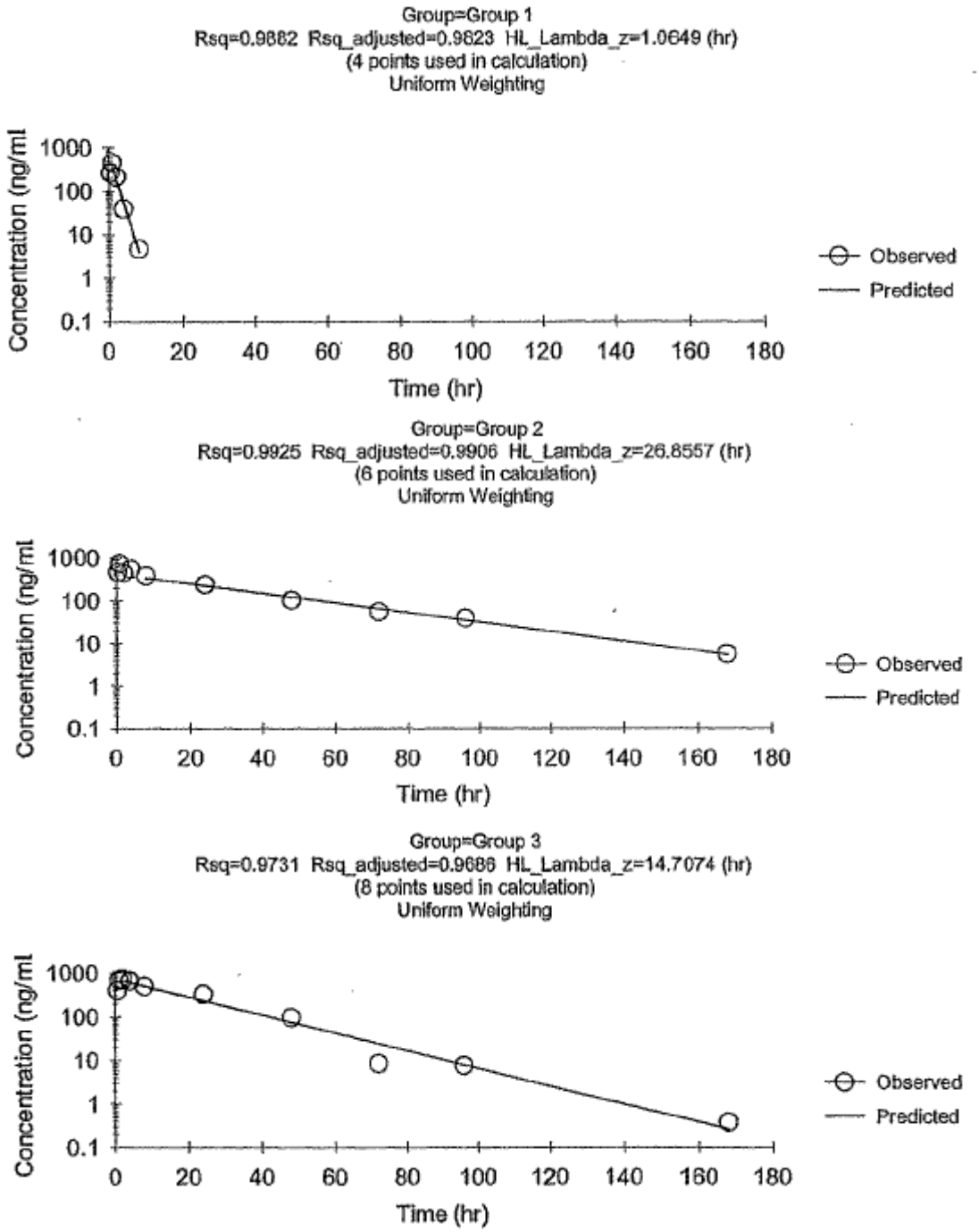
The area under the plasma drug concentration versus time curve was similar for animals given the SAIB/BA-12% formulation (SC or SC incision). (see table)

Pharmacokinetic Parameters Calculated from Plasma Concentrations of Bupivacaine

Group	Dose (mg/kg)	Tmax[a] (hr)	Cmax[b] (ng/mL)	HL_Lambda_z[c] (hr)	AUClast[d] (hr·ng/mL)	AUCINF_obs[e] (hr·ng/mL)
1	4.2	1	444	1.1	914	921
2	60	1	725	26.9	17393	17611
3	60	2	714	14.7	18306	18314

[a]Time of maximum plasma concentration of bupivacaine
[b]Maximum plasma concentration of bupivacaine
[c]Terminal half-life of bupivacaine in plasma
[d]Area under the plasma drug concentration versus time curve calculated from 0 to the last time point the mean plasma concentration of bupivacaine was above the limit of reliable quantitation
[e]Area under the plasma drug concentration time curve calculated from 0 to infinity

Comparison of time curves of bupivacaine in plasma indicated the prolonged release of bupivacaine in SABER-Bupivacaine (groups 2 & 3) compared to the bupivacaine in Sensorcaine (group 1), the purpose of the proposed drug.



Dosing Solution Analysis

No. Test articles used as provided by sponsor/supplier.

In summary - The results of this study indicated that peak plasma concentrations of bupivacaine and AUC values were similar for rats given the SAIB/BA-12% formulations SC or by installation into a SC incision site. These data indicated that

the extent of absorption of bupivacaine was similar for rats given the SAIB/BA-12% formulation by SC injection or by application into a SC incision site.

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6 General Toxicology

6.1 Single-Dose Toxicity

Acute oral toxicity of POSIDUR™ (SABER-Bupivacaine) in albino rats (study 04-07-803-R-PO-ATX; (b) (4) -4340400 - GLP) – Posidur (lot 079-05C) was administered once orally via gavage to a single group of 5 fasted male and 5 fasted female albino rats at a dosage level of 500 mg/kg. A concurrent placebo group received Posidur placebo (SABER placebo, lot 036-06A) as a single dose. The dose volume was 0.5 mL/kg for both groups. Mortality, clinical observations, and body weight changes were evaluated over a 14-day observation period. All animals were subjected to a gross necropsy. Acceptable analytical and stability data was provided by the sponsor.

There were no deaths or notable body weight changes (animals gained weight), clinical findings, or gross necropsy findings. There were no notable differences between the placebo and test article-treated groups. Based on the results of this study, Posidur™ (SABER-Bupivacaine) administered orally (gavage) to Crl:CD(SD) rats as a single dose at 500 mg/kg resulted in no mortality and was well tolerated.

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Study title: **Six-week toxicity study of SABER-Bupivacaine Injectable Formulation in Sprague Dawley rats**

Study no.	11519.01.04
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 22, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Test article – lot 015-04, 12% bupivacaine (97.2% purity post-dose), 66% SAIB, 22% BA Placebo – lot GLP 803-17, 75% SAIB, 25% BA

Key Study Findings

- Groups of 15 male and females SD rats received single subcutaneous injections of vehicle (SABER, 2 mL/kg), saline (2 mL/kg), 24 mg/kg SABER-Bupivacaine (0.2 mL/kg), 72 mg/kg SABER-Bupivacaine (0.6 mL/kg), or 240 mg/kg SABER-

Bupivacaine (2 mL/kg). Ten (10) animals per group were sacrificed on day 15 and the other remaining animals were sacrificed on day 43 (6 weeks).

- One high dose female was found dead on the day of dosing from unknown cause. This death, which occurred after the 8 hour blood sampling was considered a spurious/chance occurrence by the reviewer as this animal's clinical symptoms (none), bupivacaine blood levels, and histopathology were no different than the other 29 animals (females and males) in the high dose group. In addition, no mortality was observed in rats dosed at 250 mg/kg using the original (b) (4)-based SABER-Bupivacaine (study A624.1.1). All other animals survived until their scheduled sacrifice.
- Only clinical observations associated with treatment included discoloration, swelling, sore/ulcer, discharge, scab, and discolored scabrous areas at the injection site.
- No treatment related effects on body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, and macroscopic and microscopic pathology (other than injection sites).
- All injection site effects were in vehicle control (SABER) and SABER-Bupivacaine groups with no effects in saline control group.
- Subcutaneous injections of either the vehicle or of any of the three concentrations of bupivacaine (in the same vehicle) produced localized inflammatory reactions characterized primarily by panniculitis with subcutaneous cyst formation and reactive fibrosis. However, all of the injection site reactions were qualitatively and quantitatively similar between the sites injected with the vehicle and those injected with similar volumes of the vehicle and bupivacaine. Moderate to marked cutaneous necrosis was observed on day 15 in 1 mid dose male, 3 high dose males, and 4 vehicle (SABER) females but none was observed on day 43.
- There was evidence of reduction of the various inflammatory and reactive processes between the Day 15 and Day 43 necropsy time points, although marked subcutaneous cysts persisted in most males and females of the vehicle control (SABER) and high dose groups. These dose groups were administered the same, largest dose volume (2 mL/kg). The SABER-Bupivacaine test article is the same composition as the proposed drug product. The low SABER-Bupivacaine dose of 0.2 mL/kg (~0.1 mL) was not a NOAEL for injection site effects.
- Toxicokinetic (TK) data collected identified exposure levels that caused no systemic toxicity, but local toxicity similar to the vehicle (SABER). Peak blood levels were within 1-2 hours with levels maintaining similar, higher exposure to bupivacaine for 8-24 hours. Regarding the lack of systemic toxicity, TK values at the high dose were a gender-combined Cmax of 1432 ng/mL and $AUC_{0\text{-}INF}$ of 74423 ng·h/mL.

Methods

Doses:

Dose Group	Article Administered	Dose Level (mg/kg)	Dose Volume (mL/kg)	No. of Rats	
				Male	Female
1	Vehicle Control[a]	0	2	15	15
2	Saline Control	0	2	15	15
3	Bupivacaine	24	0.2	15	15
4	Bupivacaine	72	0.6	15	15
5	Bupivacaine	240	2	15	15

[a] 75% sucrose acetate isobutyrate (SAIB): 25% benzyl alcohol

Frequency of dosing: Single dose at a clipped site near the middle of the back
Route of administration: Subcutaneous (SC)
Dose volume: See above
Formulation/Vehicle: SAIB/BA (vehicle control), Saline (negative control)
Species/Strain: Sprague Dawley [Crl:CD®(SD)IGSBR]
Number/Sex/Group: 15
Age: 9-12 weeks at study initiation
Weight: 296.9-407.2 g (males) and 218.0-269.2 g (females) at study initiation
Satellite groups: Test animals used for toxicokinetic analysis
Unique study design: 15 day sacrifice(10/sex/group) then 6 week recovery group (5/sex/group)
Deviation from study protocol: Nothing significant

Observations and Results

Mortality

All surviving rats were observed twice daily throughout the quarantine and study periods for signs of moribundity and mortality.

One female from the high dose group was found dead on day 1 from unknown causes. This animal exhibited no adverse clinical symptoms through at least 8 hours after treatment, the time of the second blood sampling for plasma drug levels. All other animals survived until scheduled sacrifice.

Another note is that dosing rats with 250 mg/kg of the original (b) (4) formulation of SABER-Bupivacaine resulted in no mortality (study A624.1.1).

Clinical Signs

All surviving rats were removed from their cages on Days 1 (after dosing), 8, 15, 22, 29, 36, and 43 and examined closely for clinical signs of toxicity. The injection site was also specifically observed on those days.

Clinical observations associated with treatment included discoloration, swelling, sore/ulcer, discharge, scab, and discolored scabrous areas at the injection site. Clinical observations associated with the injection site were observed in all SABER™-Bupivacaine-treated and vehicle control males and females. There were no abnormal treatment-related clinical observations in the male or female saline control group. Discoloration of the injection site was observed predominantly on Day 1 in 3/15 males in Group 1**,** 2/15 males in Group 3, 2/15 males in Group 4, 6/15 males in Group 5, 5/15 females in Group 1, and 2/15 females in Group 5. Swelling at the injection site was observed in males in Groups 1, 3, 4, and 5 and females in Groups 1 and 5. Incidence of all events generally reduced after treatment and by 6 weeks after treatment.

Body Weights

Animals were weighed at randomization (days -3 or -4 before dosing), prior to dosing (day 1), and on days 8, 15, 22, 29, 36, and 43.

No test article-related changes in mean body weights in the male or female rats were observed throughout the study.

Feed Consumption

Feed consumption was measured weekly and reported as g/animal/day.
No test article-related changes in food consumption were observed during the study.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on both eyes during Week -1, Week 2, and Week 6.

No test article-related changes in the ophthalmic examinations were observed during the study although there were adverse effects due to the blood collection procedures.

ECG – none conducted

Blood sampling for hematology, clinical chemistry, and toxicokinetics

Rats from each group were subdivided into three subgroups of 5 rats/sex for each blood sample collection. For clinical pathology (hematology and clinical chemistry), blood samples were collected on Day 15 (10 rats/sex/group; Subgroups A & C) and on Day 43 (surviving rats/sex/group; Subgroup B). For toxicokinetics, blood was taken

from a subgroup of rats for toxicokinetic analysis at 0.5, 1, 2, 4, 8, 24, 48,and 72 hours and at 7, 15, 22, 29, 36, and 43 days after dosing.

Subgroup	Collection Times
A	0.5, 4, and 48 hours; Day 15
B	1, 8, and 72 hours; Days 22, 29, 36, and 43
C	2 and 24 hours; Day 7

Hematology

The following indices were evaluated with units of measurement:

Hematology

WBC	Total leukocyte count	$10^3/mm^3$
RBC	Erythrocyte count	$10^6/mm^3$
HGB	Hemoglobin	g/dL
HCT	Hematocrit	%
MCV	Mean corpuscular volume	fL
MCH	Mean corpuscular hemoglobin	pg
MCHC	Mean corpuscular hemoglobin concentration	g/dL
Retic	Reticulocyte count	$10^5/mm^3$
PLT	Platelet count	$10^3/mm^3$
	Differential leukocyte count	$10^3/mm^3$
nRBC	Nucleated red blood cell count	N/100 WBC

Based on comparison of group mean results for Groups 3, 4, and 5 with Group 1 (SABER control) and Group 2 (vehicle control) there were no hematologic changes at Day 15 or 43 for males or females attributable to SABER™- Bupivacaine administration.

Clinical Chemistry

The following indices were evaluated with units of measurement:

Clinical Chemistry

NA	Sodium	mEq/L
K	Potassium	mEq/L
CL	Chloride	mEq/L
TP	Total protein	g/dL
Alb	Albumin	g/dL
BUN	Blood urea nitrogen	mg/dL
Crea	Creatinine	mg/dL
AST	Aspartate aminotransferase	U/L
ALT	Alanine aminotransferase	U/L
ALP	Alkaline phosphatase	U/L
Glob	Globulin	g/dL
A/G Ratio	Albumin/globulin ratio	
Gluc	Glucose	mg/dL

Based on comparison of group mean results for Groups 3, 4, and 5 with Group 1 (SABER control) and Group 2 (vehicle control) there were no clinical chemistry changes at Day 15 or 43 for males or females attributable to SABER™- Bupivacaine administration.

Urinalysis – none conducted

Gross Pathology

All rats, whether found dead, euthansized *in extremis*, on scheduled days 15 (subgroups A & C) or 43 (subgroup B) received a complete postmortem examination. The postmortem examination included, but was not limited to, the examination of the external surfaces and orifices. The cranial, thoracic, abdominal, and pelvic cavities were opened, the organs/tissues within each cavity were inspected, and the organs/tissues collected. Tissues/organs (see list in histopathology section) were fixed appropriately.

Macroscopic findings at the injection sites included crust, mass, and nodules with subcutaneous tissue exhibiting gelatinous, thick, mottled mass and nodule in the vehicle control (SABER) and SABER-Bupivacaine treated groups at day 15. Masses and nodules persisted at day 43. No such effects were observed in the saline control group. No other macroscopic changes were treatment related. Any individual observation occurred in no more than 9 of 30 high dose animals (skin crust) and 7 of 30 high dose animals (subcutaneous nodule). Gross observations of the eyes and Harderian glands were not treatment-specific and considered related to the retro-orbital technique for blood collection and therefore not listed here. No different findings were observed in the female of the high dose group that was found dead compared to other high dose animals.

Organ Weights – none weighed

Histopathology

Adequate Battery – yes

The following tissues were collected and evaluated:

Adrenals [2]
All gross lesions
Aorta
Bone with bone marrow (femur and sternum)
Bone marrow smear (contralateral femur)
Brain (fore-, mid-, and hind-)
Esophagus
Eyes [2]
Harderian gland
Heart
Kidneys [2]
Large intestine, cecum
Large intestine, colon
Large intestine, rectum
Liver
Lungs (with mainstem bronchi)
Lymph node (mesenteric)
Lymph node (submandibular)
Ovaries [2]
Pancreas
Pituitary gland
Prostate
Salivary gland (submaxillary) [2]
Sciatic nerve
Seminal vesicle [2]
Skeletal muscle (thigh)
Skin (injection site; ventral abdomen, including mammary gland)
Small intestine, duodenum
Small intestine, ileum
Small intestine, jejunum
Spinal cord (cervical, thoracic, lumbar)
Spleen
Stomach
Testes/epididymis [2]
Thymus
Thyroid/parathyroid glands [2]
Tongue
Trachea
Urinary bladder
Uterus

All tissues from all rats in Groups 1 and 2 (controls) and 5 (high dose group) from the Day 15 necropsy were evaluated. In addition, target tissues and gross lesions (including the injection site) were evaluated from Groups 3 and 4 (low and mid dose groups) from the Day 15 necropsy and from all Groups from the Day 43 necropsy.

Peer Review – no for organs/tissues but yes for injection site (biocompatibility evaluation)

Histological Findings – The high dose female found dead on day 1 exhibited no unique histological findings compared to other high dose animals.

The microscopic observations closely follow the gross observations in that the lesions of the eyes, retro-orbital tissues, and Harderian glands were all considered to be the result of the retro-orbital phlebotomy procedures. Other than the injection sites, no other microscopic observations were considered treatment related, which included the saline control group. There was no evidence, under the conditions of this study, that the vehicle (SABER) or the SABER-Bupivacaine resulted in any systemic toxicity.

Subcutaneous injections of either the vehicle or of any of the three concentrations of bupivacaine (in the same vehicle) produced localized inflammatory reactions characterized primarily by panniculitis with subcutaneous cyst formation and reactive fibrosis. However, all of the injection site reactions were qualitatively and quantitatively similar between the sites injected with the vehicle and those injected with similar volumes of the vehicle and bupivacaine. Moderate to marked cutaneous necrosis was observed on day 15 in 1 mid dose male, 3 high dose males, and 4 vehicle (SABER) females but none was observed on day 43. There was evidence of reduction of the various inflammatory and reactive processes between the Day 15 and Day 43 necropsy time points, although marked subcutaneous cysts persisted in most males and females of the vehicle control (SABER) and high dose groups. These dose groups were administered the same, largest dose volume (2 mL/kg).

Tables follow to illustrate the injections site effects differing for SABER only and SABER-Bupivacaine treatment compared to the saline control. No other observed effects differed from those observed in saline controls.

SUMMARY OF MICROSCOPIC DIAGNOSES
MALE RATS FROM DAY 15 NECROPSY

		GROUP: 1	2	3	4	5
Number of animals included		10	10	10	10	10
INJECTION SITE						
Number of Tissues Examined		10	10	10	10	10
Microscopically Normal		0	9	6	1	1
No. With Microscopic Diagnoses		10	1	4	9	9
SUBCUTANEOUS CYST(S)		10	1b	1b	7	9
	mild	-	1	1	5	-
	moderate	3	-	-	2	3
	marked	7	-	-	-	6
ACANTHOSIS		7	0b	3	6	8
	minimal	-	-	-	1	-
	mild	1	-	2	2	1
	moderate	4	-	1	2	4
	marked	2	-	-	1	3
DERMATITIS		2	0	3	1	2
	minimal	-	-	1	-	-
	mild	2	-	2	-	-
	moderate	-	-	-	1	2
ULCERATIVE DERMATITIS		7	0b	0b	2a	6
	minimal	-	-	-	-	1
	mild	1	-	-	1	1
	moderate	2	-	-	1	2
	marked	4	-	-	-	2
CUTANEOUS NECROSIS		4	0a	0a	0a	0a
	mild	2	-	-	-	-
	moderate	2	-	-	-	-
SURFACE INFLAMMATORY CRUST		7	0b	3	4	7
	minimal	-	-	-	1	-
	mild	2	-	1	1	2
	moderate	1	-	1	1	1
	marked	4	-	1	1	4
PANNICULITIS		10	0b	2b	8	9
	minimal	-	-	1	2	-
	mild	-	-	-	4	1
	moderate	10	-	1	2	7
	marked	-	-	-	-	1
DERMAL FIBROSIS		1	0	0	0	2
	mild	1	-	-	-	2
FIBROSIS, SUBCUTIS		8	0b	0b	0b	5
	mild	8	-	-	-	5

Group Legend: 1 is Vehicle Control, 2 is Saline Control, 3 is 24 mg/kg, 4 is 72 mg/kg, 5 is 240 mg/kg
Statistics performed using Fisher's exact (1-tail)
a = Significantly different from GROUP 1 at P<=0.05
b = Significantly different from GROUP 1 at P<=0.01

SUMMARY OF MICROSCOPIC DIAGNOSES
MALE RATS FROM DAY 43 NECROPSY

		GROUP:	1	2	3	4	5
Number of animals included			5	5	5	5	5
INJECTION SITE							
Number of Tissues Examined			5	5	5	5	5
Microscopically Normal			0	5	2	0	0
No. With Microscopic Diagnoses			5	0	3	5	5
SUBCUTANEOUS CYST(S)			4	0a	3	5	5
	mild		-	-	2	-	1
	moderate		4	-	1	5	-
	marked		-	-	-	-	4
ACANTHOSIS			0	0	1	1	0
	mild		-	-	-	1	-
	moderate		-	-	1	-	-
DERMATITIS			0	0	0	1	0
	mild		-	-	-	1	-
PANNICULITIS			4	0a	3	5	5
	minimal		-	-	-	3	2
	mild		4	-	3	2	3
DERMAL FIBROSIS			0	0	1	1	2
	minimal		-	-	-	-	1
	mild		-	-	-	-	1
	moderate		-	-	1	1	-
FIBROSIS, SUBCUTIS			5	0b	3	5	5
	mild		4	-	3	4	5
	moderate		1	-	-	1	-

SUMMARY OF MICROSCOPIC DIAGNOSES
FEMALE RATS FROM DAY 15 NECROPSY

		GROUP: 1	2	3	4	5
Number of animals included		10	10	10	10	10
INJECTION SITE						
Number of Tissues Examined		10	10	10	10	10
Microscopically Normal		0	9	6	1	1
No. With Microscopic Diagnoses		10	1	4	9	9
SUBCUTANEOUS CYST(S)		10	1b	1b	7	9
	mild	-	1	1	5	-
	moderate	3	-	-	2	3
	marked	7	-	-	-	6
ACANTHOSIS		7	0b	3	6	8
	minimal	-	-	-	1	-
	mild	1	-	2	2	1
	moderate	4	-	1	2	4
	marked	2	-	-	1	3
DERMATITIS		2	0	3	1	2
	minimal	-	-	1	-	-
	mild	2	-	2	-	-
	moderate	-	-	-	1	2
ULCERATIVE DERMATITIS		7	0b	0b	2a	6
	minimal	-	-	-	-	1
	mild	1	-	-	1	1
	moderate	2	-	-	1	2
	marked	4	-	-	-	2
CUTANEOUS NECROSIS		4	0a	0a	0a	0a
	mild	2	-	-	-	-
	moderate	2	-	-	-	-
SURFACE INFLAMMATORY CRUST		7	0b	3	4	7
	minimal	-	-	-	1	-
	mild	2	-	1	1	2
	moderate	1	-	1	1	1
	marked	4	-	1	1	4
PANNICULITIS		10	0b	2b	8	9
	minimal	-	-	1	2	-
	mild	-	-	-	4	1
	moderate	10	-	1	2	7
	marked	-	-	-	-	1
DERMAL FIBROSIS		1	0	0	0	2
	mild	1	-	-	-	2
FIBROSIS, SUBCUTIS		8	0b	0b	0b	5
	mild	8	-	-	-	5

Group Legend: 1 is Vehicle Control, 2 is Saline Control, 3 is 24 mg/kg, 4 is 72 mg/kg, 5 is 240 mg/kg
Statistics performed using Fisher's exact (1-tail)
a = Significantly different from GROUP 1 at P<=0.05
b = Significantly different from GROUP 1 at P<=0.01

SUMMARY OF MICROSCOPIC DIAGNOSES
MALE RATS FROM DAY 43 NECROPSY

		GROUP:	1	2	3	4	5
Number of animals included			5	5	5	5	5
INJECTION SITE							
Number of Tissues Examined			5	5	5	5	5
Microscopically Normal			0	5	2	0	0
No. With Microscopic Diagnoses			5	0	3	5	5
SUBCUTANEOUS CYST(S)			4	0a	3	5	5
	mild		-	-	2	-	1
	moderate		4	-	1	5	-
	marked		-	-	-	-	4
ACANTHOSIS			0	0	1	1	0
	mild		-	-	-	1	-
	moderate		-	-	1	-	-
DERMATITIS			0	0	0	1	0
	mild		-	-	-	1	-
PANNICULITIS			4	0a	3	5	5
	minimal		-	-	-	3	2
	mild		4	-	3	2	3
DERMAL FIBROSIS			0	0	1	1	2
	minimal		-	-	-	-	1
	mild		-	-	-	-	1
	moderate		-	-	1	1	-
FIBROSIS, SUBCUTIS			5	0b	3	5	5
	mild		4	-	3	4	5
	moderate		1	-	-	1	-

Special Evaluation – Biocompatibility Evaluation

The vehicle control (SABER) and SABER-Bupivacaine injection showed similar, anticipated findings after such subcutaneous injections with foreign matter. Minimal to mild granulation tissue, foreign body reaction, and fibrous capsule formation were observed at 15 and 43 days. Normal tissue was observed in the saline control.

Toxicokinetics

Blood was taken from a subgroup of rats for toxicokinetic analysis at 0.5, 1, 2, 4, 8, 24, 48, and 72 hours after dosing and at 7, 15, 22, 29, 36, and 43 days after dosing.

Plasma values of bupivacaine over 43 days after dosing are listed in the following tables for males and females. Test groups are 1 (vehicle control - SABER), 2 (saline

control), 3 (24 mg/kg bupivacaine in SABER), 4 (72 mg/kg bupivacaine in SABER), and 5 (240 mg/kg bupivacaine in SABER). BQL is below the quantitation limit of 0.5 ng/mL. Peak blood levels were within 1-2 hours with levels maintaining similar, high exposure to bupivacaine for 8-24 hours. Plasma levels in the high dose female that was found dead on day 1 were no different than other high dose group animals.

Summary of Plasma Concentrations of Bupivacaine (ng/mL): Males

Time	Plasma Concentration (ng/mL) Mean ± SD				
	Group 1	Group 2	Group 3	Group 4	Group 5
0.5 hour	BQL	BQL	314 ± 120	522 ± 151	933 ± 270
1 hour	BQL	BQL	342 ± 127	752 ± 385	1031 ± 340
2 hours	BQL	BQL	377 ± 222	514 ± 155	1330 ± 300
4 hours	--	--	316 ± 117	538 ± 164	857 ± 209
8 hours	--	--	275 ± 111	422 ± 97.0	776 ± 118
24 hours	--	--	76.0 ± 8.64	224 ± 48.0	655 ± 320
48 hours	--	--	24.0 ± 6.10	100 ± 41.1	391 ± 66.9
72 hours	--	--	15.8 ± 8.28	106 ± 30.0	320 ± 64.2
Day 7	--	--	1.92 ± 1.94	23.2 ± 4.89	114 ± 36.6
Day 15	--	--	BQL	1.72 ± 2.79	22.3 ± 4.85
Day 22	--	--	BQL	BQL	2.55 ± 3.05
Day 29	--	--	BQL	BQL	0.536 ± 0.753
Day 36	--	--	BQL	BQL	0.127 ± 0.284
Day 43	--	--	BQL	BQL	BQL

Summary of Plasma Concentrations of Bupivacaine (ng/mL): Females

Time	Plasma Concentration (ng/mL) Mean ± SD				
	Group 1	Group 2	Group 3	Group 4	Group 5
0.5 hour	BQL	BQL	315 ± 120	625 ± 208	1132 ± 230
1 hour	BQL	BQL	378 ± 99.1	713 ± 276	1534 ± 257
2 hours	2.18 ± 2.34	0.484 ± 0.663	233 ± 76.8	408 ± 23.6	800 ± 325
4 hours	--	--	227 ± 129	387 ± 134	613 ± 165
8 hours	--	--	255 ± 29.8	457 ± 140	1103 ± 202
24 hours	--	--	76.9 ± 22.2	250 ± 42.9	644 ± 190
48 hours	--	--	30.5 ± 10.9	130 ± 29.1	551 ± 114
72 hours	--	--	7.00 ± 5.31	57.1 ± 29.7	331 ± 92.4[a]
Day 7	--	--	0.376 ± 0.519	9.92 ± 3.41	138 ± 44.9
Day 15	--	--	BQL	0.284 ± 0.635	8.85 ± 3.59
Day 22	--	--	BQL	BQL	1.36 ± 2.20[a]
Day 29	--	--	BQL	BQL	0.270 ± 0.540[a]
Day 36	--	--	BQL	BQL	BQL[a]
Day 43	--	--	BQL	BQL	BQL[a]

Toxicokinetic values for blood sampled through 72 hours after dosing are reported in the following table. There were no major gender differences but bupivacaine did appear to be present longer in males. Plasma levels increased with dose in a less than dose-response manner but plasma levels for the different doses were measured

over differing time periods. Calculation would have been of greater real value if they were based on proposed time of drug effectiveness (i.e., 24-72 hours).

Mean Toxicokinetic Values in Male and Female Rats after Single Subcutaneous Doses with SABER Bupivacaine (study11519.01.04)							
Dose (mg/kg)	gender	Tmax (hour)	Cmax (ng/mL)	Terminal $t_{1/2}$ (hour)	T_{last}[a] (hour)	AUC_{last}[b] (ng·hr/mL)	AUC_{inf}[c] (ng·hr/mL)
24	female	1	378	15.6	144	6646	6658
	male	2	377	23.8	144	7602	7670
72	female	1	713	25.8	144	18405	18774
	male	1	752	46.1	336	22635	22756
240	female	1	1534	53.0	504	77706	77814
	male	1	1330	67.2	672	70966	71033

a – last time point mean plasma level was above lower reliable quantitation limit
b – AUC from 0 time until T_{last}
c – AUC from time 0 to infinity

Dosing Solution Analysis

Dose concentration, homogeneity, and stability analysis were performed by the Sponsor and indicated that test materials were acceptable.

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Study title: A six-week toxicity study of Bupivacaine in SAIB in New Zealand White Rabbits

Study no.: A784.6.1
Study report location: eCTD in DARRTS
Conducting laboratory and location: (b) (4)

Date of study initiation: April 16, 2004
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SABER Bupivacaine (12 wt% Bupivacaine in 66:22 SAIB:BA) Lot GLP-803-19, 98.4% purity
SABER Bupivacaine Placebo (75:25 SAIB:BA) Lot GLP-803-17, purity NA

Key Study Findings

- Male and female New Zealand White rabbits received single subcutaneous doses (split to each side of the back) of SABER-Bupivacaine or SABER placebo at bupivacaine doses of 0 mg/kg (0.25 mL/kg placebo), 0 mg/kg (1.0 mL/kg placebo), 30 mg/kg (0.25 mL/kg), or 120 mg/kg (1.0 mL/kg). Animals were sacrificed on day 15/18 and 44 post-dose.

- Two male rabbits in the high dose group were sacrificed as moribund 3-4 hours after dosing on Day 1. The sacrificed animals had apparently aspirated stomach contents. Bupivacaine treatment-related mortality cannot be ruled out.
- No treatment-related or toxicologically relevant effects were observed for the biological indices evaluated (i.e., clinical signs, body weights, food consumption, ophthalmoscopy, hematology, and clinical chemistry).
- Injection site effects were most common in animals sacrificed 15 days after dosing (interim sacrifice). The most common findings in the injection sites of animals from all groups were chronic inflammation and the presence of clear empty spaces (space, vacant, presumed to contain the test article). In all groups, the inflammation was usually of trace or mild severity and consisted of variable degrees of early fibrosis with infiltration of histiocytes and small mononuclear cells.
- Observations were generally less common and severe after the 44 day terminal sacrifice, suggesting healing, recovery, and/or reversibility of local effects.
- In a separate biocompatibility evaluation of injection sties, the test and control articles elicited a normal inflammatory and wound healing sequence of events with a normal foreign body reaction consistent with a 6-week implant time and both control and treated groups were considered to be biocompatible**.**
- This study demonstrated a prolonged release of Bupivacaine up to 2 days for the 30 mg/kg dose level and up to 4 days for the 120 mg/kg dose level with no efficacy implied.
- Toxicokinetic (TK) data collected identified exposure levels that caused no systemic toxicity (NOAEL), but local toxicity similar to the vehicle (SABER placebo). Peak blood levels were within 1-2 hours with levels maintaining similar, higher exposure to bupivacaine for 8-24 hours. Regarding the lack of systemic toxicity, TK values at the high dose were a gender-combined Cmax of 3,033 ng/mL and $AUC_{0-\infty}$ of 48,645 ng•h/mL.

Methods

Doses:

Group	Test Material	Dose Level (mg/kg)	Dose Volume (mL/kg)
1	Vehicle Control	0	0.25
2	Vehicle Control	0	1.0
3	Bupivacaine	30	0.25
4	Bupivacaine	120	1.0

- vehicle control group is SABER-Bupivacaine placebo

Frequency of dosing: Single dose (1/2 to each side of upper back)
Route of administration: Subcutaneous to intrascapular region
Dose volume: See table
Formulation/Vehicle: Benzyl alcohol
Species/Strain: New Zealand White rabbits
Number/Sex/Group: 6

Age:	3-4 months at dosing
Weight:	2.9-3.3 kg at dosing
Satellite groups:	none
Unique study design:	Single dose with day 15 and 44 (6 week) sacrifices
Deviation from study protocol:	Nothing significant

Observations and Results

Mortality

All rabbits in this study were observed twice daily during the quarantine and study periods for signs of mortality and moribundity.

Two high dose males were sacrificed in moribund condition after day 1 dosing due to aspiration (confirmed by autopsy). Based on these deaths after a SC dose, the deaths are considered treatment-related by reviewer. All other animals survived to scheduled sacrifice.

Clinical Signs

Detailed clinical observations were recorded during Week -1, on Day 1 (prior to and approximately 3-5 hours after dosing) and on Days 3, 8, 15, 22, 29, 36, and 44, or more often as clinical signs warranted.

The clinical observations during the study consisted of hypoactivity, prostration, nasal discharge, scabs, and discolored scabrous areas. These observations were noted primarily in the high dose treated group (Group 4) and to a lesser degree in the high dose control group (Group 2) which received the same dose volume. There were no abnormal clinical findings in any of the low dose animals in either the control or treated groups (Groups 1 and 3).

Summary of Clinical Observations: Males

		Day numbers relative to Start Date					
		-3	1 predose	1 3-6 hrs postdose	2	3	8
Group Sex	Clinical Sign						
1M	Unremarkable	6	6	6	NR	6	6
2M	Unremarkable	6	6	6	NR	6	6
	Scab	.	.	.	.	.	.
	Discolored scabrous area	.	.	.	.	.	.
3M	Unremarkable	6	6	6	NR	6	6
4M	Unremarkable	6	6	2	2	4	4
	Scab	.	.	.	.	.	.
	Discharge	.	.	2	.	.	.
	Hypoactive	.	.	3	.	.	.
	Prostrate	.	.	1	.	.	.

Day numbers relative to Start Date

Group Sex	Clinical Sign	11	15	18	22	29	36	44
1M	Unremarkable	NR	6	NR	3	3	3	3
2M	Unremarkable	NR	5	NR	3	2	3	3
	Scab	.	1	.	.	.	.	.
	Discolored scabrous area	.	.	.	.	1	.	.
3M	Unremarkable	NR	6	NR	3	3	3	3
4M	Unremarkable	NR	2	1	1	2	2	2
	Scab	.	2	.	1	.	.	.
	Discharge	.	.	.	.	.	.	.
	Hypoactive	.	.	.	.	.	.	.
	Prostrate	.	.	.	.	.	.	.

Summary of Clinical Observations: Females

Day numbers relative to Start Date

Group Sex	Clinical Sign	-4	1 predose	1 3-4 hrs postdose	2	3	8
1F	Unremarkable	6	6	6	NR	6	6
2F	Unremarkable	6	6	6	NR	6	6
	Discolored scabrous area	.	.	.	.	.	.
3F	Unremarkable	6	6	6	NR	6	6
4F	Unremarkable	6	6	6	NR	3	4
	Discolored scabrous area	.	.	.	.	2	2
	Discoloration	.	.	.	.	1	.

Day numbers relative to Start Date

Group Sex	Clinical Sign	11	15	18	22	29	36	44
1F	Unremarkable	NR	6	NR	3	3	3	3
2F	Unremarkable	NR	6	NR	2	2	2	2
	Discolored scabrous area	.	.	.	1	1	1	1
3F	Unremarkable	NR	6	NR	3	3	3	3
4F	Unremarkable	.	3	NR	3	3	3	3
	Discolored scabrous area	1	3	.	.	.	.	.
	Discoloration	.	.	.	.	.	.	.

Body Weights

Individual body weights were recorded for each surviving rabbit during baseline (randomization), on Day 1 prior to dosing, and on Days 8, 15, 22, 29, 36, and 44.

There were treatment-related effects on body weight or any differences among any of the groups.

Feed Consumption

Individual quantitative food consumption was recorded daily for each study animal throughout the study. Average weekly food consumption (g/anima/day) was calculated for each rabbit on study.

There were treatment-related effects on food consumption or any differences among any of the groups.

Ophthalmoscopy

Prior to dosing and on Day 38 (females) or Day 39 (males), both eyes of each surviving rabbit were examined by indirect ophthalmoscopy, slit lamp examination, and direct focal illumination.

There were no abnormal findings noted in any animal during the course of this study.

ECG

No ECG evaluation conducted.

Hematology

Blood samples were collected from fasted animals during weeks -1 from all rabbits and on days 15 & 44 from all rabbits scheduled for a day 44 sacrifice.

The following indices were measured:

WBC	Total leukocyte count	$10^3/mm^3$
RBC	Erythrocyte count	$10^6/mm^3$
HGB	Hemoglobin	g/dL
HCT	Hematocrit	%
MCV	Mean corpuscular volume	fL
MCH	Mean corpuscular hemoglobin	pg
MCHC	Mean corpuscular hemoglobin concentration	g/dL
PLT	Platelet count	$10^3/mm^3$
RETIC	Reticulocyte count	$10^5/mm^3$
	Differential leukocyte counts	$10^3/mm^3$
	RBC morphology	

No treatment-related or toxicologically relevant changes in hematology parameters were observed.

Clinical Chemistry

Blood samples were collected from fasted animals during weeks -1 from all rabbits and on days 15 & 44 from all rabbits scheduled for a day 44 sacrifice.

The following indices were measured:

BUN	Blood urea nitrogen	mg/dL
Crea	Creatinine	mg/dL
BUN/Crea	BUN/creatinine ratio	
Gluc	Glucose	mg/dL
TP	Total protein	g/dL
Alb	Albumin	g/dL
Glob	Globulin	g/dL
A/G	Albumin/globulin ratio	
ALT	Serum alanine aminotransferase	U/L
AST	Serum aspartate aminotransferase	U/L
ALP	Alkaline phosphatase	U/L
Na	Sodium	mEq/L
K	Potassium	mEq/L
Cl	Chloride	mEq/L
Ca	Calcium	mg/dL
Phos	Phosphorus	mg/dL
TBIL	Bilirubin (total)	mg/dL
Chol	Cholesterol	mg/dL

No treatment-related or toxicologically relevant changes in clinical chemistry parameters were observed.

Urinalysis

No urine was sampled/evaluated.

Gross Pathology

Animals sacrificed on day 15 or 44 and those sacrificed as moribund received a complete postmortem exam. The complete postmortem necropsy examination included a thorough inspection of all external surfaces, organs, and orifices. The cranial, thoracic, abdominal, and pelvic cavities were opened, and the tissues/organs within each cavity were inspected.

Skin surrounding the injection sites was cut, flipped back to reveal the underlying fascia/musculature, and inspected for any SAIB depots. If the SAIB depot could not be found directly under the injection site, both the injection site, the tissue surrounding the depot, and the SIB depot were taken.

All of the organs and tissues listed below were appropriately collected and fixed.

All gross lesions including tumors & masses
Adrenal [2]
Aorta
Bone marrow (femur)
Brain (fore, mid, and hind)
Epididymis [2]
Esophagus
Gallbladder
Eye [2]
Harderian gland
Heart
Injection site
Kidney [2]
Large intestine, cecum
Large intestine, colon
Large intestine, rectum
Liver
Lung
Lymph node, bronchial
Lymph node, mesenteric
Lymph node, mandibular
Nerve, right sciatic
Ovary [2]
Pancreas
Pituitary gland
Prostate gland (including seminal vesicles [2])
Salivary gland, mandibular, right
Salivary gland, parotid, right
Salivary gland, sublingual, right
Skeletal muscle (thigh)
Skin (ventral abdomen, including mammary gland)
Small intestine, duodenum
Small intestine, jejunum
Small intestine, ileum
Spinal cord, cervical
Spinal cord, thoracolumbar
Spleen
Stomach, cardiac
Stomach, fundic
Stomach, pyloric
Testis [2]
Thymus
Thyroid gland [2]/ Parathyroid gland [2]
Trachea
Urinary bladder
Uterus (with cervix)
Vagina

Observations in the injection sites of male and/or female rabbits included discoloration with thick subcutis with crust. There appeared to be no definitive patterns of lesion incidence in the injection sites of male or female rabbits to indicate a treatment effect at 15 days and 44 days after treatment. The high dose males sacrificed as moribund on day 1 exhibited a higher incidence of local effects plus lung discoloration, as would be expected.

Organ Weights

No organ weights were taken.

Histopathology

All fixed tissues from the day 15 sacrifice were processed and evaluated. High dose groups 2 (SABER placebo) and 4 (SABER-Bupivacaine) were processed first and then the remaining as appropriate. Day 44 tissues evaluated were target organs based on day 15 results, gross lesions, and injection sites.
Adequate Battery - yes

Peer Review – no

Histological Findings - Injection site effects were most common in animals sacrificed 15 days after dosing (interim sacrifice). The most common findings in the injection sites of animals from all groups were chronic inflammation and the presence of clear

empty spaces (space, vacant, presumed to contain the test article). In all groups, the inflammation was usually of trace or mild severity and consisted of variable degrees of early fibrosis with infiltration of histiocytes and small mononuclear cells. The vacant spaces consisted of clear, round to oval cavitations surrounded by varying degrees of fibrosis and a layer of simple squamous epithelium. These spaces occurred mainly in the subcutaneous tissue immediately beneath the dermis or, less frequently, within the dermis. The inflammation usually occurred in the areas surrounding the vacant spaces, but not always. Exudate was observed in the subcutis just beneath the dermis of a few animals. The exudate in the spaces contained eosinophilic, proteinaceous material with mixed inflammatory cells and were lined by simple squamous epithelium and early fibrous tissue. Hemorrhage, usually of minimal or mild severity, was sporadically observed in the dermis or subcutis of all groups. One high dose bupivacaine male exhibited an ulcer. The high dose group appeared to exhibit a higher incidence of chronic inflammation. In the table, each column is an individual animal with the incidence following (e.g., 1/3).

Microscopic Observations: Males (Interim Sacrifice)

Dose Level (mg/kg)	0 (vehicle)		0 (vehicle)	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	1 1 1 M M M 3 3 3 7 7 7 2 3 3 6 2 6		2 2 2 M M M 3 3 3 7 7 7 2 2 2 2 4 7	
Day of Death/Sacrifice	1 1 1 5 5 5	I	1 1 1 5 5 5	I
Tissue -lesion				
Lung -inflammation, subacute -inflammation, suppurative -edema -foreign body	 1 0 0 0 0 0 0 0 0 0 0 0	 1/3 0/3 0/3 0/3	 0 1 1 0 0 0 0 0 0 0 0 0	 2/3 0/3 0/3 0/3
Lymph node -hyperplasia, lymphoid, mesenteric -cellular infiltrate, heterophil, bronchial	 0 0 0 0 M 0	 0/3 0/3	 0 2 0 0 0 0	 1/3 0/3
Liver -congestion	 0 0 0	 0/3	 0 0 0	 0/3
Pancreas -accessory spleen	 X 0 0	 0/3	 0 0 0	 0/3
Testis -degeneration, germinal epithelium	 0 2 2	 2/3	 0 2 1	 2/3
Epididymis -degeneration, spermatids	 0 3 2	 2/3	 0 3 0	 1/3
Thyroid gland -persistent thyroglossol duct -degeneration, focal, follicle	 0 0 X 0 0 0	 0/3 0/3	 0 X X 0 0 0	 0/3 0/3
Adrenal gland -accessory structure, cortex	 X 0 0	 0/3	 0 X 0	 0/3

Dose Level (mg/kg)	0 (vehicle)		0 (vehicle)	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	1 1 1 M M M 3 3 3 7 7 7 2 3 3 6 2 6		2 2 2 M M M 3 3 3 7 7 7 2 2 2 2 4 7	
Day of Death/Sacrifice	1 1 1 5 5 5	I	1 1 1 5 5 5	I
Tissue -lesion				
Skeletal Muscle -inflammation, chronic -hemorrhage	 0 0 0 0 0 0	 0/3 0/3	 2 0 0 0 0 0	 1/3 0/3
Injection site, right -inflammation, chronic -inflammation, granulomatous -ulcer -hemorrhage -acanthosis -hyperkeratosis -space, vacant -space, exudate containing	 3 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 3 1 3 3 0 0	 2/3 0/3 0/3 1/3 0/3 0/3 3/3 1/3	 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 3 2 3 0 3 0	 3/3 0/3 0/3 0/3 0/3 0/3 3/3 1/3
Injection site, left -inflammation, chronic -hemorrhage -hyperkeratosis -space, vacant -space, exudate containing	 3 0 0 0 0 0 0 0 0 2 2 1 3 0 0	 1/3 0/3 0/3 3/3 1/3	 1 2 2 0 1 0 0 0 0 1 3 3 0 2 3	 3/3 1/3 0/3 3/3 2/3

Dose Level (mg/kg)	30		120	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	3 3 3 M M M 3 3 3 7 7 7 2 2 2 3 5 8		4 4 4 4 M M M M 3 3 3 3 7 7 7 7 3 3 3 4 1 4 8 2	
Day of Death/Sacrifice	1 1 1 5 5 5	I	0 0 1 1 1 1 5 8	I
Tissue -lesion				
Lung -inflammation, subacute -inflammation, suppurative -edema -foreign body	 1 0 0 0 0 0 0 0 3 0 0 0	 1/3 0/3 1/3 0/3	 1 0 0 1 0 3 0 0 0 0 0 0 0 X 0 0	 2/4 1/4 0/4 0/4
Lymph node -hyperplasia, lymphoid, mesenteric -cellular infiltrate, heterophil, bronchial	 0 0 2 M 0 0	 1/3 0/3	 3 3 0 0 0 3 0 0	 2/4 1/4
Liver -congestion	 0 0 0	 0/3	 0 4 0 0	 0/4
Pancreas -accessory spleen	 0 0 X	 0/3	 0 0 0 0	 0/4
Testis -degeneration, germinal epithelium	 0 2 2	 2/3	 2 3 2 1	 4/4
Epididymis -degeneration, spermatids	 0 2 3	 2/3	 3 4 2 2	 4/4
Thyroid gland -persistent thyroglossol duct -degeneration, focal, follicle	 0 X 0 0 0 0	 0/3 0/3	 X 0 0 0 0 0 0 2	 0/4 0/4
Adrenal gland -accessory structure, cortex	 0 0 0	 0/3	 0 0 0 0	 0/4

Dose Level (mg/kg)	30		120	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	3 3 3 M M M 3 3 3 7 7 7 2 2 2 3 5 8		4 4 4 4 M M M M 3 3 3 3 7 7 7 7 3 3 3 4 1 4 8 2	
Day of Death/Sacrifice	1 1 1 5 5 5	I	0 0 1 1 1 1 5 8	I
Tissue -lesion				
Skeletal Muscle -inflammation, chronic -hemorrhage	 2 0 3 1 0 0	 2/3 1/3	 0 3 2 3 0 0 0 0	 3/4 0/4
Injection site, right -inflammation, chronic -inflammation, granulomatous -ulcer -hemorrhage -acanthosis -hyperkeratosis -space, vacant -space, exudate containing	 0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 2 1 0 0 0	 1/3 0/3 0/3 1/3 0/3 0/3 2/3 0/3	 2 0 1 1 0 0 0 0 0 0 0 0 3 4 1 0 0 0 2 0 0 0 2 0 0 0 3 2 0 0 0 0	 3/4 0/4 0/4 3/4 1/4 1/4 2/4 0/4
Injection site, left -inflammation, chronic -hemorrhage -hyperkeratosis -space, vacant -space, exudate containing	 0 2 1 1 0 0 0 0 0 2 2 2 0 0 0	 2/3 0/3 0/3 3/3 0/3	 1 1 1 1 0 1 0 0 0 0 2 0 0 0 0 3 0 0 0 0	 4/4 1/4 1/4 1/4 0/4

0 = Lesion not observed
1 = Lesion of minimal severity
2 = Lesion of mild severity
3 = Lesion of moderate severity
4 = Lesion of marked severity

I - Incidence; number of animals with lesion/number of animals with tissue examined
X - Lesion severity not graded
M - Missing
NA - Not applicable
* - Tissue not examined

Microscopic Observations: Females (Interim Sacrifice)

Dose Level (mg/kg)	0 (vehicle)		0 (vehicle)	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	1 1 1 F F F 3 3 3 7 7 7 5 5 6 7 9 0		2 2 2 F F F 3 3 3 7 7 7 5 6 6 0 2 5	
Day of Death/Sacrifice	1 1 1 5 5 5	I	1 1 1 5 5 5	I
Tissue -lesion				
Lung -inflammation, subacute	1 0 0	1/3	0 0 0	0/3
Lymph node -hyperplasia, lymphoid, mesenteric	0 0 0	0/3	0 2 3	2/3
Spleen -hematopoietic cell, proliferation	0 0 0	0/3	0 0 0	0/3
Liver -inflammation, chronic	2 0 0	1/3	0 0 0	0/3
Pancreas -accessory spleen	0 0 0	0/3	X 0 0	0/3
Kidney -inflammation, chronic	2 0 0	1/3	0 0 0	0/3
Thyroid gland -persistent thyroglossal duct -degeneration, focal, follicle	 X 0 0 0 2 0	 0/3 1/3	 0 0 0 0 0 0	 0/3 0/3
Adrenal gland -accesssory structure, cortex	0 0 0	0/3	0 0 0	0/3
Mammary gland -hyperplasia	0 0 0	0/3	0 0 0	0/3

Dose Level (mg/kg)	0 (vehicle)		0 (vehicle)	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	1 1 1 F F F 3 3 3 7 7 7 5 5 6 7 9 0		2 2 2 F F F 3 3 3 7 7 7 5 6 6 0 2 5	
Day of Death/Sacrifice	1 1 1 5 5 5	I	1 1 1 5 5 5	I
Tissue -lesion				
Skeletal muscle -inflammation, chronic -hemorrhage	 0 0 0 0 0 0	 0/3 0/3	 0 2 0 0 2 0	 1/3 1/3
Injection site, right -inflammation, chronic -inflammation, granulomatous -ulcer -hemorrhage -acanthosis -hyperkeratosis -space, vacant -space, exudate containing	 1 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 1 1 0 0 0 0	 1/3 0/3 0/3 1/3 0/3 0/3 2/3 0/3	 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 2 0 0 0 0 0	 1/3 0/3 0/3 1/3 0/3 0/3 1/3 0/3
Injection site, left -inflammation, chronic -hemorrhage -hyperkeratosis -space, vacant -space, exudate containing	 1 1 2 0 1 1 0 0 0 2 1 3 0 0 0	 3/3 2/3 0/3 3/3 0/3	 3 0 0 2 0 0 0 0 0 3 0 0 2 0 0	 1/3 1/3 0/3 1/3 1/3

Dose Level (mg/kg)	30		120	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	3 3 3 F F F 3 3 3 7 7 7 5 5 5 1 2 3		4 4 4 F F F 3 3 3 7 7 7 5 5 5 5 6 8	
Day of Death/Sacrifice	1 1 1 5 5 5	I	1 1 1 5 5 5	I
Tissue -lesion				
Lung -inflammation, subacute	1 1 0	2/3	0 0 1	1/3
Lymph node -hyperplasia, lymphoid, mesenteric	2 2 0	2/3	3 3 2	3/3
Spleen -hematopoietic cell, proliferation	2 0 0	1/3	0 3 3	2/3
Liver -inflammation, chronic	0 0 0	0/3	0 0 0	0/3
Pancreas -accessory spleen	0 0 X	0/3	X 0 0	0/3
Kidney -inflammation, chronic	0 0 0	0/3	0 0 0	0/3
Thyroid gland -persistent thyroglossal duct -degeneration, focal, follicle	 0 0 X 0 0 0	 0/3 0/3	 0 0 0 0 3 0	 0/3 1/3
Adrenal gland -accesssory structure, cortex	0 0 0	0/3	X 0 0	0/3
Mammary gland -hyperplasia	0 2 2	2/3	0 2 0	1/3

Dose Level (mg/kg)	30		120	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	3 3 3 F F F 3 3 3 7 7 7 5 5 5 1 2 3		4 4 4 F F F 3 3 3 7 7 7 5 5 5 5 6 8	
Day of Sacrifice	1 1 1 5 5 5	I	1 1 1 5 5 5	I
Tissue -lesion				
Skeletal muscle				
-inflammation, chronic	0 0 0	0/3	0 0 0	0/3
-hemorrhage	0 0 0	0/3	0 0 0	0/3
Injection site, right				
-inflammation, chronic	0 0 0	0/3	3 1 4	3/3
-inflammation, granulomatous	0 0 0	0/3	3 0 0	1/3
-ulcer	0 0 0	0/3	0 0 4	1/3
-hemorrhage	0 0 0	0/3	0 0 2	1/3
-acanthosis	0 0 0	0/3	0 0 3	1/3
-hyperkeratosis	0 0 0	0/3	0 0 0	0/3
-space, vacant	2 0 0	1/3	3 0 3	2/3
-space, exudate containing	0 0 0	0/3	0 0 0	0/3
Injection site, left				
-inflammation, chronic	3 0 1	2/3	3 0 2	2/3
-hemorrhage	0 0 0	0/3	2 0 0	1/3
-hyperkeratosis	0 0 0	0/3	0 0 0	0/3
-space, vacant	0 1 0	1/3	3 0 3	2/3
-space, exudate containing	3 0 1	2/3	2 0 0	1/3

At 44 days after treatment (terminal sacrifice), the most commonly observed injection site effects were chronic inflammation and vacant spaces with no spaces containing exudate. Ulcer, erosion, epidermal hyperplasia/thickening, and hemorrhage were observed, but strictly related to the active ingredient but more to the SABER (SAIB/BA). A separate evaluation (Biocompatibility Evaluation in next heading) described effects as anticipated foreign body reaction.

Although the cause of death for the high dose males sacrificed as moribund at necropsy as "drug toxicity", no changes directly attributable to drug treatment were evident on microscopic evaluation. Lung lesions of suppurative inflammation and foreign material were suggestive of aspirated stomach contents. These changes were considered secondary to the moribund state of the animal rather than treatment-related lesions. Heterophil infiltration in the bronchial lymph node was reported as secondary to the lung lesions, and the liver congestion observed was reported as an agonal change. This reviewer considers the deaths, possibly related to bupivacaine.

Microscopic Observations: Males (Terminal Sacrifice)

Dose Level (mg/kg)	0 (vehicle)		0 (vehicle)	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	1 1 1 M M M 3 3 3 7 7 7 3 4 4 9 7 8		2 2 2 M M M 3 3 3 7 7 7 3 3 4 3 7 0	
Day of Death/Sacrifice	4 4 4 4 4 4	I	4 4 4 4 4 4	I
Tissue -lesion				
Injection site, right -inflammation, chronic -ulcer -erosion -hemorrhage -acanthosis -space, vacant	 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2	 1/3 0/3 0/3 0/3 0/3 2/3	 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 1	 0/3 0/3 0/3 0/3 0/3 2/3
Injection site, left -inflammation, chronic -hemorrhage -acanthosis -space, vacant	 0 0 0 0 0 0 0 0 0 2 0 3	 0/3 0/3 0/3 2/3	 1 1 0 0 0 0 0 0 0 3 0 2	 2/3 0/3 0/3 2/3
Lung -Inflammation, chronic -congestion	 * * * * * *	 NA NA	 1 * * 0 * *	 1/1 0/1

Dose Level (mg/kg)	30		120	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	3 3 3 M M M 3 3 3 7 7 7 3 4 4 5 3 5		4 4 M M 3 3 7 7 4 4 4 6	
Day of Death/Sacrifice	4 4 4 4 4 4	I	4 4 4 4	I
Tissue -lesion				
Injection site, right -inflammation, chronic -ulcer -erosion -hemorrhage -acanthosis -space, vacant	 2 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 2	 1/3 0/3 0/3 0/3 1/3 1/3	 0 0 0 0 0 0 0 0 0 0 0 3	 0/2 0/2 0/2 0/2 0/2 1/2
Injection site, left -inflammation, chronic -hemorrhage -acanthosis -space, vacant	 2 0 0 0 0 0 1 0 0 0 2 2	 1/3 0/3 1/3 2/3	 0 0 0 0 0 0 1 0	 0/2 0/2 0/2 1/2
Lung -Inflammation, chronic -congestion	 * * 0 * * 2	 0/1 1/1	 * * * *	 NA NA

0 = Lesion not observed
1 = Lesion of minimal severity
2 = Lesion of mild severity
3 = Lesion of moderate severity
4 = Lesion of marked severity

I - Incidence; number of animals with lesion/number of animals with tissue examined
X - Lesion severity not graded
M - Missing
NA - Not applicable
* - Tissue not examined

Microscopic Observations: Females (Terminal Sacrifice)

Dose Level (mg/kg)	0 (vehicle)		0 (vehicle)	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	1 1 1 F F F 3 3 3 7 7 7 6 6 7 1 7 3		2 2 2 F F F 3 3 3 7 7 7 6 7 7 9 4 5	
Day of Death/Sacrifice	4 4 4 4 4 4	I	4 4 4 4 4 4	I
Tissue -lesion				
Injection site, right -inflammation, chronic -ulcer -erosion -hemorrhage -acanthosis -space, vacant	 2 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 0 2	 1/3 0/3 0/3 1/3 1/3 1/3	 3 0 0 0 4 0 0 0 0 0 0 0 1 3 0 0 0 1	 1/3 1/3 0/3 0/3 2/3 1/3
Injection site, left -inflammation, chronic -hemorrhage -acanthosis -space, vacant	 1 0 0 0 0 0 0 0 0 1 2 4	 1/3 0/3 0/3 3/3	 3 0 0 0 0 0 1 0 0 0 0 0	 1/3 0/3 1/3 0/3
Lung -inflammation, chronic -congestion -edema	 * * * * * * * * *	 NA NA NA	 1 * 0 0 * 2 3 * 0	 1/2 1/2 1/2
Spleen -accessory spleen	 * * *	 NA	 * X *	 1/1

Dose Level (mg/kg)	30		120	
Dose Volume (mL/kg)	0.25		1.0	
Animal Number	3 3 3 F F F 3 3 3 7 7 7 6 6 7 4 8 0		4 4 4 F F F 3 3 3 7 7 7 6 7 7 6 1 2	
Day of Death/Sacrifice	4 4 4 4 4 4	I	4 4 4 4 4 4	I
Tissue -lesion				
Injection site, right -inflammation, chronic -ulcer -erosion -hemorrhage -acanthosis -space, vacant	 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0	 0/3 0/3 1/3 0/3 0/3 0/3	 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	 0/3 0/3 0/3 0/3 0/3 0/3
Injection site, left -inflammation, chronic -hemorrhage -acanthosis -space, vacant	 0 0 0 0 0 0 0 0 0 0 2 0	 0/3 0/3 0/3 1/3	 0 0 0 0 0 1 0 0 0 3 0 3	 0/3 1/3 0/3 2/3
Lung -inflammation, chronic -congestion -edema	 * 0 * * 2 * * 0 *	 0/1 1/1 0/1	 1 * * 1 * * 0 * *	 1/1 1/1 0/1
Spleen -accessory spleen	 * * *	 NA	 * 0 *	 1/1

Special Evaluation (A Biocompatibility Evaluation)

In a separate evaluation, histology slides from male and female rabbit implant sites were evaluated. The test and control articles elicited a normal inflammatory and wound healing sequence of events with a normal foreign body reaction consistent with a 6-week implant time and both were considered to be biocompatible. No significant differences within or between the control and treatment groups were identified.

Toxicokinetics

Blood samples were collected prior to dosing (0 hour), 0.5, 1, 2, 4, 8, 24 (day 2), 48 (day 3) hours after dosing and on days 4, 7, 15, 22, 29, 36, & 44. The rabbits scheduled to be sacrificed on Day 44 (the second 3 rabbits/sex/treated group) were designated for toxicokinetic analysis.

Plasma levels increased with increasing dose but appeared quite variable. What is noted is that increased blood levels are observed at ½ hour after dosing lasting for 24-48 hours at clearly elevated levels.

Mean Plasma Concentrations of Bupivacaine (ng/mL): Males

Time after the end of injection	Group 3	Group 4
0 hour	BQL	BQL
0.5 hours	390 ± 69.7	1201 ± 766
1 hour	530 ± 92.8	913 ± 250
2 hours	662 ± 133	2789 ± 3491
4 hours	560 ± 82.3	1774 ± 629
8 hours	424 ± 109	2314 ± 1253
24 hours	209.3 ± 31.0	1238 ± 368
48 hours	42.6 ± 12.1	554 ± 313
Day 4	17.3 ± 8.00	106 ± 86.1
Day 7	BQL	9.26 ± 7.17
Day 15	BQL	0.380 ± 0.658
Day 22	BQL	BQL[a]
Day 29	BQL	BQL[a]
Day 36	BQL	BQL[a]
Day 44	BQL	BQL[a]

Mean Plasma Concentrations of Bupivacaine (ng/mL): Females

Time after the end of injection	Group 3	Group 4
0 hour	BQL	BQL
0.5 hours	859 ± 321	1333 ± 1024
1 hour	1827 ± 806	855 ± 79
2 hours	1027 ± 105	1719 ± 1384
4 hours	718 ± 374	1650 ± 287
8 hours	1019 ± 715	1512 ± 506
24 hours	1143 ± 791	575 ± 365
48 hours	73.1 ± 49.5	655 ± 110
Day 4	23.0 ± 27.2	158 ± 52.2
Day 7	1.71 ± 1.92	20.3 ± 12.6
Day 15	BQL	0.86 ± 1.49
Day 22	BQL	BQL
Day 29	BQL	BQL
Day 36	BQL	BQL
Day 44	BQL	BQL

n = 3, except as noted
[a] n = 2
BQL = All samples below quantifiable limit (<1 ng/mL)

Nominal Dose: Group 1 - 0 mg/kg (0.25 mL/kg) Group 2 - 0 mg/kg (1.0 mL/kg)
Group 3 - 30 mg/kg (0.25 mL/kg) Group 4 - 120 mg/kg (1.0 mL/kg)

Pharmacokinetic values generally increase with increasing dose, but are again quite variable, with increases not consistently dose-responsive. AUC values are until the last time bupivacaine is measureable in the plasma (BQL of <1 ng/mL). After a single SC dose in rabbits (study A784.6.1), the highest bupivacaine exposure level was 3,033 ng/mL (Cmax) and 48,465 ng*h/mL (AUC_{last}). The AUC was adjusted from ng*days/mL considering that the last time point that drug was found was day 15.

A Six-Week Toxicity Study of Bupivacaine in SAIB in New Zealand White Rabbits

Pharmacokinetic Parameters

Males

Animal Number	Tmax[a] (hours)	Cmax[b] (ng/mL)	Tlast[c] (days)	AUC·last[d] (ng·days/mL)
3M3735	2.00	815	4	576
3M3743	2.00	595	4	570
3M3745	4.00	655	4	559
Mean	2.67	688	4	568
SD	1.16	114	0	9
4M3742	2.00	6820	7	4557
4M3744	0.50	1994	7	3471
4M3746	8.00	1640	7	2636
Mean	3.50	3485	7	3555
SD	3.97	2894	0	963

Females

Animal Number	Tmax[a] (hours)	Cmax[b] (ng/mL)	Tlast[c] (days)	AUC·last[d] (ng·days/mL)
3F3764	1.00	2200	7	1922
3F3768	24.0	1930	7	2035
3F3770	1.00	2380	4	1354
Mean	8.67	2170	6	1770
SD	13.3	226	2	365
4F3766	2.00	3300	7	3294
4F3771	0.50	2460	15	2811
4F3772	8.00	1986	7	2689
Mean	3.50	2582	10	2931
SD	3.97	665	5	320

[a]Time of maximum plasma concentration
[b]Maximum plasma concentration
[c]Last time point test article was measureable in plasma
[d]Area under the plasma drug concentration versus time curve from 0 to the last time point the test article was measureable in plasma.
Nominal Dose - Group 3 – 30 mg/kg Group 4 – 120 mg/kg

Dosing Solution Analysis
Test article received from sponsor two days before dosing and were determined to be stable.

=========

Drug product is original composition containing solvent [redacted] instead of current solvent of Benzyl Alcohol (BA)

Study title: A two-week toxicity study of bupivacaine in Sprague Dawley rats

Note:

1) original review conducted by Timothy J. McGovern, Ph.D. and placed in current review format with noted changes to original review
2) drug product is original composition containing solvent [redacted] instead of current solvent of Benzyl Alcohol (BA)

Study no.:	A624.1.1
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	[redacted]
Date of study initiation:	June 2002
GLP compliance:	GLP compliance statement was originally unsigned (now signed)
QA statement:	Not originally (now present)
Drug, lot #, and % purity:	bupivacaine, Lot X02521 (5% bupivacaine in 70:30 SAIB: [redacted]), NA, 97.7%

Key Study Findings

- Single SC injections of up to 250 mg/kg bupivacaine produced no findings that are definitively related to bupivacaine through days 4 and 15 of observation.
- Gross and microscopic findings related to inflammation and necrosis at the injection site were observed in all groups including vehicle control group. The findings tended to increase in severity and incidence as injection volume increased and were not completely resolved within 15 days. Vacant spaces at the injection site, indicative of deposited vehicle with or without bupivacaine, were still present at day 15.
- The NOAEL for bupivacaine can be considered 250 mg/kg; a NOAEL for the vehicle was not identified.

Methods	
Doses:	0, 25, 75, 250 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Subcutaneous – slow push injection
Dose volume:	5, 0.5, 1.5 or 5 ml/kg
Formulation/Vehicle:	5% bupivacaine (50 mg free base/ml) depot formulation prepared in 70% sucrose acetate isobutyrate (SAIB): 30% (b) (4) . Vehicle control formulation consisted of 70% SAIB:30% (b) (4).
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	8
Age:	7-8 weeks (males), 8 to 9 weeks (females)
Weight:	194.4-329.7 g
Satellite groups:	9/sex/group used for TK
Unique study design:	On day 1, each animal received a slow push, single SC injection of vehicle or 5% bupivacaine formulation. The dose was administered into the upper back (subscapular region). Doses were based upon body weights taken on day 1. Injection sites were shaved and marked to facilitate evaluation and collection at necropsy. Three/sex/group from the main study groups were sacrificed on day 4 and 5/sex/group were sacrificed on Day 15.
Deviation from study protocol:	None reported.

Observations and Results

Mortality

Twice daily on days 1, 4, 8 and 15.

All animals survived to day 15.

Clinical Signs

Twice daily on days 1, 4, 8 and 15.

Transient hypoactivity was observed at the highest dose on day 1 from 1hour after dosing. The effect was not reported on other study dates. Sores on the skin over the injection site were observed from day 4 onward with resolution in some animals on day 15.

Clinical signs	3 hrs post-dose		Day 4		Day 8		Day 15	
	M	F	M	F	M	F	M	F
Sores (inject site)								
0 mg/kg	0/8	0/8	1/8	2/8	2/5	3/5	1/5	2/5
25 mg/kg	0/17	0/17	6/17	2/17	5/14	2/14	2/14	1/14
75 mg/kg	0/17	0/17	5/17	3/17	6/14	5/14	3/14	2/14
250 mg/kg	0/17	0/17	4/17	2/17	10/14	8/14	5/14	3/14
Hypoactive								
0 mg/kg	0/8	0/8	0/8	0/8	0/5	0/5	0/5	0/5
25 mg/kg	0/17	0/17	0/17	0/17	0/14	0/14	0/14	0/14
75 mg/kg	0/17	0/17	0/17	0/17	0/14	0/14	0/14	0/14
250 mg/kg	17/17	11/17	0/17	0/17	0/14	0/14	0/14	0/14

Body Weights

Week -1, day 1 prior to dosing, day 4, day 8, and day 15.

No significant drug-related effects were noted.

Feed Consumption

Weekly.

No significant drug-related effects were noted.

Ophthalmoscopy

Not assessed.

ECG

Not assessed.

Hematology

Days 4 (3/sex/group) and 15 (5/sex/group) after dosing.

No significant drug-related effects were noted. However, rats receiving the highest dosing volumes (control and HD) were generally observed to have the lowest reticulocyte percents and counts when compared to in-house historical data. Low reticulocyte counts were observed on day 4 in 3/3, 0/3, 1/3, and 2/2 male rats in the control, LD, MD and HD groups and 3/3, 1/3, 2/3, and 3/3 female rats in the control, LD, MD and HD. No significant changes were noted on day 15.

Clinical Chemistry

Days 4 (3/sex/group) and 15 (5/sex/group) after dosing.

Mild increases in mean total protein and globulin values were observed in high-dose males on day 4. These changes may be consistent with the observed scores and were resolved by day 15.

Hematology and clinical chemistry findings on Day 4

Hematology	Males			Females		
Dose (mg/kg)	25	75	250	25	75	250
Reticulocyte % % Δ vs control	↑113	↑60	↓6	↑62	↑41	↑21
Reticulocyte count % Δ vs control	↑104	↑58	↓5	↑67	↑44	↑18
Clinical Chemistry						
Total protein % Δ vs control	↑2	↑2	↑8	↑4	↑8	↑6
Globulin % Δ vs control	↑5	↑18	↑22	no Δ	↑3	↑5

Urinalysis

Day 12 (5/sex/group).

No significant drug-related effects were noted.

Gross Pathology

Days 4 (3/sex/group) and 15 (5/sex/group) after dosing.

No systemic gross changes were observed. Injection site changes were observed in all groups including controls. Gross changes were generally attributed to scratching or rubbing against cage and were indicative of a generally mild, vehicle-volume related subchronic inflammatory response.

Macroscopic findings related to the injection site.

	Males				Females			
Day 4 Dose	0	25	75	250	0	25	75	250
Volume	5	0.5	1.5	5	5	0.5	1.5	5
n	3	3	3	3	3	3	3	3
Crust	1	1	2	0	0	1	1	1
Discoloration, subcutis	0	0	0	1	0	0	0	1
Focus, sc	0	0	0	1	0	0	0	0
Gelatinous, sc	1	0	0	0	0	0	0	0
Thick, sc	0	0	0	1	0	0	0	0
Day 15 n	5	5	5	5	5	5	5	5
Crust	1	0	0	3	2	0	0	1
nodule	0	0	1	0	0	0	0	0

Organ Weights

Days 4 (3/sex/group) and 15 (5/sex/group) after dosing.

No significant drug-related effects were noted.

Histopathology

Days 4 (3/sex/group) and 15 (5/sex/group) after dosing; performed on all fixed tissues of control and high dose animals for the toxicity study; injection sites and lesions processed and examined in the low- and mid-dose groups. See histopathology inventory table at end of rabbit study A624.1.2 that follows.

Adequate Battery - yes

Peer Review – yes (added by current reviewer)

Histological Findings

Microscopic changes related to vehicle/drug treatment were limited to the injection site and were consistent with subchronic inflammation. Findings at day 4 included exudate, ulceration, inflammation, edema, and necrosis/degeneration of the paniliculus muscle and other underlying muscle. The inflammation appears to be volume related as control and high-dose groups generally demonstrated more severe results. The findings appeared to resolve at least partially by day 15. There were vacant spaces in the injection site subcutis of animals of all groups. These spaces were consistent with deposition of an exogenous material (either vehicle alone or with bupivacaine) that did not survive histological processing. These findings were still present at day 15 indicating that injected material was still present at the injection site 15 days after administration.

There were no systemic drug-related histologic findings (see tables).

Microscopic findings related to the injection site: Day 4.

	Males				Females			
Day 4 Dose	0	25	75	250	0	25	75	250
Volume	5	0.5	1.5	5	5	0.5	1.5	5
n	3	3	3	3	3	3	3	3
Exudate, escharotic								
Trace	0	0	0	0	0	0	0	1
Mild	0	0	1	0	0	1	0	1
Moderate	0	0	0	1	0	0	1	1
Severe	1	1	1	0	0	0	0	0
Ulcer								
Trace	0	0	0	0	0	0	0	1
Mild	0	0	0	1	0	0	1	0
Moderate	1	0	0	0	0	1	0	0
Severe	0	1	0	0	0	0	0	0
Inflammation, sub-chronic, subcutaneous	0	1	0	0	1	2	1	0
Trace	3	1	3	2	2	1	2	3
Mild	0	1	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Calcification, mild								
Hemorrhage, SC	1	0	2	1	0	0	1	0
Trace	0	0	1	1	0	0	2	1
Mild	0	0	0	0	0	0	0	1
Moderate								
Edema, SC	0	1	2	0	2	1	1	1
Trace	2	1	0	2	0	0	1	0
Mild	1	0	0	1	0	0	0	0
Moderate								
Debris, eosinophilic, SC, mild/moderate	0	0	0	1	0	0	0	1
Necrosis/degeneration, panniculus muscle	0	0	0	1	0	0	0	0
Trace	2	0	1	0	1	0	0	0
Mild	1	0	0	1	0	0	0	1
Moderate								
Space, vacant, SC	0	2	1	0	0	2	1	0
Mild	3	1	0	0	0	0	2	0
Moderate	0	0	0	3	2	0	0	2
Severe								
Inflammation, underlying muscle	0	0	0	1	0	0	0	0
Trace	0	0	0	1	0	1	0	0
Mild	0	0	0	1	0	0	0	1
Moderate								
Necrosis/degeneration, underlying muscle moderate	0	0	0	1	0	0	0	1

Microscopic findings related to the injection site: Day 15.

	Males				Females			
Day 15 Dose	0	25	75	250	0	25	75	250
Volume	5	0.5	1.5	5	5	0.5	1.5	5
n	5	5	5	5	5	5	5	5
Exudate, escharotic								
Trace	0	0	0	1	1	0	0	0
Mild	0	0	0	1	1	0	0	0
Moderate	1	0	0	0	0	0	0	0
Severe	1	0	0	0	0	0	0	0
Ulcer								
Mild	0	0	0	1	1	0	0	0
Moderate	1	0	0	0	0	0	0	0
Inflammation, sub-chronic, subcutaneous	1	1	1	0	1	1	1	0
Trace	2	2	4	1	1	1	3	3
Mild	2	0	0	3	2	1	1	1
Moderate severe	0	0	0	1	1	0	0	1
Calcification	0	0	0	1	0	0	0	0
Trace	0	0	0	4	0	0	0	1
Mild moderate	0	0	1	0	0	0	0	0
Hemorrhage, SC	1	0	1	1	0	1	1	2
Trace Mild	1	0	0	0	0	0	0	0
Debris, eosinophil, SC	1	0	0	4	1	0	0	1
trace/mild moderate	0	0	0	0	0	0	0	1
Necrosis/degeneration, panniculus muscle	0	0	0	0	1	0	0	0
Trace	1	0	0	1	0	0	0	0
Mild Moderate	0	0	0	0	2	0	0	0
Space, vacant, SC	0	1	1	0	0	1	1	0
Trace	0	0	1	0	0	1	2	0
Mild	1	0	0	1	1	0	1	3
Moderate Severe	1	0	2	4	3	0	0	1
Inflammation, underlying muscle Moderate	0	0	0	0	0	0	0	1
Necrosis/degeneration, underlying muscle moderate	0	0	0	0	0	0	0	1

Special Evaluation

None.

Toxicokinetics

Blood collected at 0, 0.5, 1, 2, 4, 8, 24, and 48 hours on days 4, 7 and 15 after dosing. Plasma levels increased in a dose-proportional manner. Plasma bupivacaine levels were observed for 7 days in rats given 25 mg/kg, 7 to 15 days in rats given 75 mg/kg and 15 days in rats given 250 mg/kg. Tmax ranged from 0.5-8 hrs with the time decreasing with increasing dose. Elimination half-life was 23-34 hours at the low dose and 38-62 hours at the two highest doses.

	Males			Females		
Dose (mg/kg)	25	75	250	25	75	250
Cmax (ng/ml)	233	393	778	261	448	1082
AUC 0-24 hr (ng.day/ml)	147	277	498	182	320	634
AUC 0-ltp* (ng.day/ml)	311	737	2870	294	818	2812
Tmax (hr)	4	1	0.5	8	2	1
T half-life (hr)	33.6	45.6	62.4	23.3	43.2	38.4

*AUC from 0 to last time point bupivacaine was detectable in plasma. LTP was 7 days for the LD and MD groups and 15 days for the HD group.

Dosing Solution Analysis (not addressed by original reviewer)

Dose concentration analysis and homogeneity analysis was performed by the sponsor.

Results appear acceptable to current reviewer.

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Study title: A two-week toxicity study of bupivacaine in rabbits

Note:

1) original review conducted by Timothy J. McGovern, Ph.D. and placed in current review format with noted changes to original review
2) drug product is original composition containing solvent (b) (4) (b) (4) instead of current solvent of Benzyl Alcohol (BA)

Study no.:	A624.1.2
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 2002
GLP compliance:	GLP compliance statement was originally unsigned (but now signed)
QA statement:	Not originally present (now present)
Drug, lot #, and % purity:	bupivacaine, Lot X02521 (5% bupivacaine in 70:30 SAIB (b) (4)), NA, 97.7%

Key Study Findings

- A single dose injection of SABER-bupivacaine resulted in the death of one high-dose (125 mg/kg) female within two hours of dosing; no obvious cause of death was determined.
- No systemic findings that could be definitively considered related to bupivacaine administration were observed.
- Gross and microscopic findings related to inflammation at the injection site were observed in all groups including vehicle group at day 4 after administration and the findings were not resolved but worsened in some cases after 15 days. Necrosis of underlying muscle tissue was observed at Day 15.
- The NOAEL for bupivacaine can be considered 37.5 mg/kg, but a NOAEL for the vehicle was not identified. The Sponsor concludes that the NOAEL dose is 37.5 mg/kg.

Methods

Doses:	0, 12.5, 37.5, 125 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Subcutaneous
Dose volume:	2.5, 0.25, 0.75 and 2.5 ml/kg
Formulation/Vehicle:	5% bupivacaine (50 mg free base/ml) depot formulation prepared in 70% sucrose acetate isobutyrate (SAIB): 30% (b) (4)

	([(b) (4)]). Vehicle control formulation consisted of 70% SAIB:30% [(b) (4)].
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	5
Age:	Not provided
Weight:	3-3.3 kg
Satellite groups:	None.
Unique study design:	On day 1, each animal received a slow push, single SC injection of vehicle or 5% bupivacaine formulation. The dose volume in each group was split such that approximately one-half of the dose was administered into each side of the animal's upper back (subscapular region). Doses were based upon body weights taken on day 1. Injection sites were shaved and marked to facilitate evaluation and collection at necropsy.
Deviation from study protocol:	None reported.

Observations and Results

Mortality

Twice daily on days 1, 4, 11 and 15.

One high dose female died on study day 1, approximately 3 hours after injection. Ataxia, dyspnea and hypoactivity occurred prior to death. A ball of what appeared to be compacted, partially digested food was found in the esophagus. The cause of death was not determined. An additional HD female was added to the study on day 1.

Clinical Signs

Twice daily on days 1, 4, 11 and 15.

No test-article-related clinical signs were reported with the exception of those reported above for the HD female that died. However, an injection site sore and injection site swelling were reported in 1 and 2 vehicle control males, respectively from days 2 onward. Similarly, one HD female also exhibited an injection site sore and swelling from day 2 onward.

Body Weights

Week -1, day 1 prior to dosing, day 4, day 11, and day 15.

No significant drug-related effects were noted.

Feed Consumption

Recorded daily; average weekly consumption calculated.

A transient decrease in mean food consumption (43%) occurred in the 2 high dose males during the study interval day 1 through day 4. No significant findings were noted in males at later time points or in females.

Ophthalmoscopy

Not assessed.

ECG

Not assessed.

Hematology

During week -1, days 4 (2/sex/group) and 15 (3/sex/group) after dosing.

A significant increase in neutrophil count was observed in HD males on day 4 but was resolved by day 15. Slightly lower RBC counts at all doses, and HGB and HCT levels, were noted in high dose females on day 15 only. Lymphocyte counts were also significantly reduced, though not dose-dependently in females.

Hematology	Males			Females		
Day 4	12.5	37.5	125	12.5	37.5	125
Neutrophils % ch vs control	↑4	↑33	↑79	↓4	↓10	↑14
Day 15						
RBC % ch vs control	↓2	↓3	↓2	↓7	↓11	↓12
Hemoglobin % ch vs control	↓3	↓2	↓1	↓1	↓8	↓10
Hematocrit % ch vs control	↑1	0	↑2	↓1	↓6	↓7
Lymphocyte % ch vs control	↓8	↓20	↓17	↓34	↓23	↓34

Clinical Chemistry

During week -1, days 4 (2/sex/group) and 15 (3/sex/group) after dosing.

No significant drug-related effects were noted.

Urinalysis

Not assessed.

Gross Pathology

Days 4 (2/sex/group) and 15 (3/sex/group) after dosing.

No definitive treatment-related systemic gross changes were observed. Injection site changes were observed in all groups including controls and were present at both Day 4 and Day 15. Gross changes were generally indicative of a subchronic inflammatory response (see table below).

Macroscopic findings following administration of SABER-bupivacaine in rabbits.

		Males				Females			
Day 4	Dose	0	12.5	37.5	125	0	12.5	37.5	125
	Volume	2.5	0.25	0.75	2.5	2.5	0.25	0.75	2.5
	n	2	2	2	2	2	2	2	3
Injection site, SC									
Brown/red/green		0	0	0	0	1	0	1	2
Skin									
Dorsal thoracic crust		1	0	1	0	0	0	0	0
Uterus									
Bilateral horn, thick									
Oviduct						0	0	0	1
Right cyst clear									
Left cyst clear						0	0	0	1
Ovary						0	0	0	1
Bilateral, enlarged									
Vagina						0	0	0	1
Red mucosa									
Gall bladder						0	0	0	1
Lumen, fluid, opaque, yellow		0	0	0	0	0	0	0	1
Day 15	n	3	3	3	3	3	3	3	3
Injection site, SC									
Brown/red/green		0	0	0	2	0	0	1	1
Dark		3	0	0	1	1	0	0	1
Thick		1	0	0	1	2	0	1	0
Mottled		0	0	1	0	0	0	0	0
Mass		1	0	0	0	0	0	0	1
Crust		0	0	0	0	0	0	0	1
Skin									
Neck, alopecia		0	0	0	0	0	1	0	0
Neck, crust		0	0	0	0	0	0	1	1
Shoulder, mass		0	0	0	0	0	0	0	1
Oviduct									
Left cyst clear						0	0	0	1

Organ Weights

Days 4 (2/sex/group) and 15 (3/sex/group) after dosing.

No significant drug-related effects were noted.

Histopathology

Days 4 (2/sex/group) and 15 (3/sex/group) after dosing; performed on all fixed tissues of animals from all dose groups; see histopathology inventory table at end of the study review.

Adequate Battery - yes

Peer Review – No.

Histological Findings

Microscopic changes related to drug treatment were limited to the injection site and were consistent with subchronic inflammation. The frequency and severity of the findings were related to dose volume and the response to the vehicle alone was indistinguishable from that of the high dose group. The inflammatory cells consisted of PMNLs, lymphocytes, and macrophages. There was no evidence of exogenous material in the tissues examined which was expected since the vehicle is soluble in the solvents used for processing specimens for slide preparation (according to the study report). There were, however, at one or both injection sites of some animals, spaces within the subcuticular connective tissue or within pools of proteinaceous material considered to be a combination of edema and degenerating red blood cells from local hemorrhage. The spaces were sometimes quiescent and sometimes rimmed with a narrow, 1-4 cell deep, band of mixed inflammatory cells. The vacant spaces were presumed to have held either the vehicle alone or vehicle with bupivacaine.

Inflammation was largely confined to the subcutis. Escharotic exudate and ulceration in high dose animals are likely the result of scratching or rubbing against cage. The underlying dermis was free of inflammation and there was no evidence of an inflammatory response in the subcutis sufficient to erupt to the epidermis and cause even a minor ulceration. In contrast to the rat, findings at day 15 appeared to worsen in severity rather than partially resolve. Additionally, findings of necrosis were present at Day 15 but not at Day 4.

Microscopic findings in rabbits on Day 4.

	Males				Females			
Day 4 Dose	0	12.5	37.5	125	0	12.5	37.5	125
Volume	2.5	0.25	0.75	2.5	2.5	0.25	0.75	2.5
n	2	2	2	2	2	2	2	3
Right injection site								
Exudate, escharotic								
Mild	0	0	0	1	0	0	0	0
Ulcer								
Moderate	0	0	0	0	0	0	0	1
Hemorrhage, SC								
Trace	1	1	0	0	0	0	0	0
Mild	0	0	0	0	2	1	0	1
Moderate Inflammation, sub-chronic	1	0	0	0	0	0	1	1
Trace	0	0	1	1	1	2	1	0
Mild	2	1	1	1	1	0	1	1
Moderate	0	0	0	0	1	1	1	1
Calcification, mild	0	0	0	0	0	0	1	1
Edema, mild	0	1	0	0	2	0	2	1
Space, vacant, SC								
Trace	1	0	0	2	0	0	0	0
Mild	0	0	0	0	2	0	0	0
Moderate	1	0	0	0	0	1	0	0
Severe	0	0	0	0	0	0	1	0
Left injection site								
Exudate, escharotic								
Trace	0	0	0	1	0	0	0	0
Ulcer								
Mild	0	0	0	0	0	0	0	1
Hemorrhage, SC								
Trace	0	0	0	0	0	1	0	0
Mild	1	1	0	1	0	1	0	0
Moderate	1	0	0	0	1	0	1	2
Inflam., sub-chronic								
Trace	0	0	0	1	0	0	0	0
Mild	2	2	2	1	2	2	2	1
Moderate	0	0	0	0	0	0	0	1
Calcification								
Mild	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	0	1
Edema								
Mild	0	0	0	1	1	0	2	0
Moderate	1	0	0	0	0	0	0	1
Space, vacant, SC								
Mild	0	0	1	1	0	0	0	2
Moderate	2	1	0	0	0	0	2	0

Microscopic findings in rabbits on Day 15.

	Males				Females			
Day 15 Dose	0	12.5	37.5	125	0	12.5	37.5	125
Volume	2.5	0.25	0.75	2.5	2.5	0.25	0.75	2.5
n	3	3	3	3	3	3	3	3
Right injection site								
Exudate, escharotic								
Severe	0	0	0	0	0	0	0	1
Hyperkeratosis								
Trace	0	0	0	0	1	0	0	0
Ulcer								
Severe	0	0	0	0	0	0	0	1
Dermatitis								
Severe	0	0	0	0	0	0	0	1
Hemorrhage, SC								
Trace	0	1	0	1	0	0	1	0
Mild	1	0	0	1	0	0	0	0
Moderate	0	0	0	0	1	0	0	0
Inflammation, sub-chronic								
Trace	0	0	0	1	1	0	1	0
Mild	0	1	1	1	0	1	2	0
Moderate	2	0	0	0	0	0	0	1
Severe	1	0	0	1	2	0	0	1
Giant cells								
Trace	1	0	0	0	0	0	0	0
Mild	1	0	0	0	1	0	0	0
Moderate	1	0	0	1	1	0	0	0
Edema								
Trace	0	1	0	0	1	0	0	0
Mild	2	1	1	1	1	0	0	1
Moderate	1	0	0	1	0	1	0	1
Space, vacant, SC								
Trace	0	0	0	2	0	0	0	0
Mild	0	0	1	1	1	1	1	2
Moderate	1	0	0	0	1	0	2	0
Severe	2	0	0	0	1	0	0	0
Debris, eosinophilic								
Trace	0	0	0	0	0	0	1	0
Mild	0	0	0	1	0	1	0	0
Moderate	3	0	0	0	0	0	0	1
Severe	0	0	0	0	1	0	0	1
Necrosis/degeneration, panniculus muscle								
Trace	0	0	0	0	0	0	2	0
Mild	0	0	0	0	0	0	0	1
Moderate	2	0	0	1	1	0	0	1
Left injection site								
Ulcer								
Severe	1	0	0	0	0	0	0	0
Hemorrhage, SC								
Trace	0	0	0	1	1	1	0	0
Mild	2	0	0	0	0	0	1	0
Moderate	1	0	0	0	0	0	0	0

Inflammation, sub-chronic								
Trace	1	1	1	0	1	0	1	0
Mild	0	0	1	1	1	2	1	1
Moderate	1	0	0	2	1	0	1	1
Severe	1	0	0	0	0	0	0	0
Giant cells								
Trace	1	0	0	1	0	1	2	0
Mild	0	0	0	1	1	0	0	1
Moderate	0	0	0	0	1	0	0	0
Edema								
Trace	0	1	0	1	1	1	1	0
Mild	0	1	1	1	1	1	1	2
Space, vacant, SC								
Trace	0	0	1	0	1	1	2	0
Mild	1	0	0	0	0	0	0	0
Moderate	1	0	0	2	1	0	0	0
Severe	1	0	0	0	1	1	0	1
Debris, eosinophilic								
Mild	1	0	0	1	0	0	0	0
Severe	1	0	0	1	0	0	0	1
Necrosis/degeneration, panniculus muscle								
Trace	0	0	0	1	0	1	0	0
Moderate	0	0	0	1	0	0	0	1
Severe	1	0	0	1	0	0	0	0
Calcification								
Trace	0	0	0	0	1	0	0	0
Mild	0	0	0	0	0	0	1	0

Special Evaluation

None.

Toxicokinetics

Blood collected from 3 animals/sex/group at 0, 0.5, 1, 2, 4, 8, 24, and 48 hours after dosing and on days 4, 7 and 15 after dosing.

Plasma levels increased in a sub-proportional manner from the low- to mid-dose and generally proportional at the highest dose. Plasma bupivacaine levels were observed for 4-7 days in rabbits given 12.5 and 27.5 mg/kg, and 15 days in rabbits given 125 mg/kg. No significant gender differences were observed. Tmax occurred between 1 and 4 hours and elimination half-life was 13-23 hours at the lowest dose, increasing to ~45 hours at the highest dose.

	Males			Females		
Dose (mg/kg)	12.5	37.5	125	12.5	37.5	125
Cmax (ng/ml)	603	999	2199	482	709	1608
AUC 0-24 (ng.day/ml)	232	421	1165	185	446	784
AUC 0-ltp* (ng.day/ml)	333	727	3258	298	752	2811
Tmax (hr)	2.3	2.3	1.8	3	4.2	2.2
T half-life (hr)	13.6	19.1	42.4	23.5	19.3	44.2

*AUC from 0 to last time point bupivacaine was detectable in plasma. LTP was 7 days for the LD and MD groups and 15 days for the HD group.

Dosing Solution Analysis (not addressed by original reviewer)

Dose concentration analysis and homogeneity analysis was performed by the sponsor. Results appear acceptable to current reviewer.

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Histopathology Inventory for studies A624.1.1 & A624.1.2

Study	A624.1.1 Single SC injection	A624.1.2 Single SC injection
Species	Rat	Rabbit
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)		X
Brain	X*	X*
Cecum	X	X
Cervix	X	
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions	X	X
Harderian gland	X	X
Heart	X*	X*
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland		
Larynx		
Liver	X*	X*
Lungs	X*	X*
Lymph nodes, bronchial	X	X
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland		X
Nasal cavity		
Optic nerves		
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve		
Pharynx		
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles		X
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum		
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X	X
Tongue		
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		
Standard List		

X, histopathology performed
*, organ weight obtained

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Study title: A repeated histopathological injection site evaluation up to 12 months following a single subcutaneous administration of SABER-Bupivacaine in the rabbit

- [redacted] vehicle

Study no.:	[redacted]-434007
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	[redacted]
Date of study initiation:	February 26, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Test article – lot A02005, 96.8% - 5% bupivacaine, 28.5% [redacted] and 66.5% Sucrose Acetate Isobutyrate (SAIB) Placebo – lot A02007, NA - 30% [redacted] and 70% SAIB

Key Study Findings

- The purpose of reviewing this study was to evaluate the duration of local effects of SAIB after a single SC dose of SABER-Bupivacaine with the observation period continuing up to one year after a single injection.
- Single doses of SABER-Bupivacaine did not result in test article-related injection site reactions up to 52 weeks post-injection. Macroscopic and microscopic effects indicative of inflammation at the injection sites were attributed to the injection procedure and/or the placebo and were typical of a normal reaction to a foreign body and subsequent wound healing (i.e., local effects SABER-Bupivacaine comparable to those of SABER placebo).
- SAIB was found to be essentially unchanged and still present 12 months after injection described as viscous material with essentially no [redacted] present [redacted]%).

Methods

Doses:	0 (placebo) & 37.5 mg/kg (selected based on earlier studies)
Frequency of dosing:	Single dose over 2 injection sites
Route of administration:	Subcutaneous (back – intrascapular)
Dose volume:	0.75 mL/kg
Formulation/Vehicle:	[redacted]
Species/Strain:	New Zealand White Rabbits
Number/Sex/Group:	21 males/group

Age:	6.5 months
Weight:	2.9-3.6 kg
Satellite groups:	3 males/group were sacrificed during study on weeks 2, 4, 6, 12, 26, 39, & 52 preceded by sampling of blood for toxicokinetic analysis
Unique study design:	Injections sites were evaluated for presence of test article. Study mainly only evaluating physical signs, body weights, injection site effects and plasma levels
Deviation from study protocol:	Nothing significant

Observations and Results

Mortality

All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity.

All animals survived to the scheduled necropsies with no apparent increased moribundity for either group.

Clinical Signs

Clinical examinations were performed twice on the day of dosing, at the time of dose administration and approximately 2 hours following dose administration. During the recovery period, the animals were observed once daily.

There were no test article-related clinical signs as all observations were similar between test article and placebo groups and what was observed was not notable as it was common for laboratory rabbits.

Body Weights

Individual body weights were recorded at least weekly, beginning 1 week prior to test article administration (study week -1).

Body weights were unaffected by test article administration as there were no remarkable including statistically significant differences between the placebo and test article groups. Animals gained weight during the course of the study. Noting that the group size decreased by 3 at every interval, with only 3 animals in the final measurement, body weight increases over the course of the study were 29% for the treated group and 38% for the placebo group.

Feed Consumption, Ophthalmoscopy, ECG, Hematology, Clinical Chemistry, and Urinalysis not observed/analyzed

Gross Pathology and Tissue Processing

Three (3) males/group were sacrificed on weeks 2, 4, 6, 12, 26, 39, & 52. Both injection sites, including the subcutis and any potentially attached muscle were collected. Starting at the study week 39 examination, if test article was suspected to be present, the site containing the test article was divided, and one half was placed in 10% neutral-buffered formalin. The other half was used for analysis of injected test article.

There were no test article-related macroscopic findings at the scheduled necropsies. All macroscopic changes noted were attributed to the injection procedure and/or the placebo and were unrelated to test article administration. With that stated, as both groups were injected, not unanticipated gross observations occurred post injection in the placebo and treated groups, respectively. Observations included:

2-weeks - scabbing in 1/3 and 3/3, dark red areas in 2/3 and 0/3
4-weeks - scabbing in 1/3 and 2/3, a raised area containing the test article at the injection site in 0/3 and 1/3
6-weeks - cysts containing the test article in 0/3 and 2/3.
12-weeks - none
26-weeks - raised areas in 2/3 and 2/3, a white area in 0/3 and 1/3
39-weeks - raised areas in 3/3 and 2/3, viscous contents in 2/3 and 1/3
52-weeks - white areas in 1/3 and 2/3, viscous contents in 2/3 and 1/3

Organ Weights

Not evaluated.

Histopathology

Adequate Battery – No, only injection sites examined

Peer Review – Yes with concurrence.

Histological Findings – While there were incidences of adverse histopathology, there were no test article-related microscopic differences at the injection sites of the test article-treated groups when compared to the placebo group. Therefore, microscopic changes are likely attributable to the injection procedure and/or the placebo. The progression of microscopic findings over this 12-month study was described by the pathologist and peer reviewer as typical of a normal reaction to a foreign substance and subsequent wound healing which is not contested by this reviewer. Three animals per group were evaluated each time period.

At 52 weeks, other than fibrosis, severe in 1 of 3 placebo animals, observed histology was as described by the pathologists:

(WEEK 52 RECOVERY NECROPSY)
PROJECT NO.: (b) (4)134007A SINGLE SQ ADMINISTRATION OF SABER BUPIVACAINE IN THE RABBIT
SPONSOR:DURECT CORP. SUMMARY OF MICROSCOPIC FINDINGS

----- MALE -----

GROUP:	1	2
NUMBER OF ANIMALS IN DOSE GROUP	21	21
NUMBER OF ANIMALS EXAMINED	3	3
INJ. SITE- CAUD.		
TOTAL NUMBER EXAMINED	3	3
EXAMINED, UNREMARKABLE	1	0
-SPACES	2	3
PRESENT	2	3
-INFLAMMATION, CHRONIC	1	0
MINIMAL	1	NONE
-FIBROSIS	2	2
MINIMAL	1	1
MILD	1	1
-HYPERKERATOSIS	0	1
MINIMAL	NONE	1
INJ. SITE- ROST.		
TOTAL NUMBER EXAMINED	3	3
EXAMINED, UNREMARKABLE	1	0
-DEGENERATION, MUSCLE	1	0
MILD	1	NONE
-REGENERATION, MUSCLE	1	0
MILD	1	NONE
-SPACES	2	3
PRESENT	2	3
-INFLAMMATION, GRANULOMATOUS	1	1
MINIMAL	1	1
INJ. SITE- ROST. - CONTINUED		
-INFLAMMATION, SUBACUTE	1	0
MINIMAL	1	NONE
-FIBROSIS	2	2
MINIMAL	1	NONE
MILD	NONE	2
SEVERE	1	NONE
-HYPERKERATOSIS	0	1
MILD	NONE	1
-MINERALIZATION	0	1
MINIMAL	NONE	1

1-0 MG/KG PLACEBO 2-37.5 MG/KG BUPI

Epidermal ulceration was present at 2 weeks (1 of 3 placebo and 3 of 3 article-treated animals) and 4 weeks (2 of 3 article-treated animals) post-injection evaluations. Full-thickness epidermal necrosis was present in three of these animals (1/3 males in the placebo group and 2/3 males in the 37.5 mg/kg group). Epidermal hyperplasia was a common finding in the intact epidermis adjacent to the ulcer. All animals with ulcerative and necrotic lesions had spaces in the epidermis, dermis and/or subcutis, suggesting that the presence of ulcers may have been related to local irritation and self-trauma.

(b) (4) the test article solvent in this study that is not present in the proposed drug product, has been reported to cause such reactions so the toxicological relevance of this finding in this particular study is in question relative to the proposed drug product using benzyl alcohol versus (b) (4).

Variably sized, discrete ovoid spaces, interpreted as the site of placebo or test article deposition, were noted in skin sections of all animals at all post-injection evaluations. Spaces were present in the subcutis only (13 animals), both subcutis and dermis (26 animals), dermis only (one animal), or epidermis, dermis and subcutis (two animals). Spaces were surrounded by thin bands of fibrous connective tissue and variable numbers of inflammatory cells. As the months progressed, the fibrous connective tissue surrounding the spaces tended to thicken.

Inflammation was generally oriented around the spaces. Incidence and severity of inflammation did not increase with test article administration. Early inflammation was predominantly granulomatous, of mild to moderate severity, and characterized by a mixture of mononuclear cells and multinucleated giant cells. Granulomatous inflammation was present in animals throughout the study but decreased in severity and incidence as the months progressed. Chronic active inflammation was present in 13 animals through the 12-week post-injection evaluation and was characterized by an infiltrate containing mononuclear cells, heterophils and lymphocytes. Chronic inflammation, consisting of mononuclear cells, lymphocytes and often associated with fibrosis, was present in seven animals starting at the 4-week post-injection evaluation and was noted in only one animal each at the 39- and 52-week post-injection valuations. Acute inflammation was observed in animals with epidermal ulceration and was characterized by a predominance of heterophils near the ulcer.

Granulation tissue, characterized by loosely arranged fibrous connective tissue, with plump fibroblasts and neovascularization, was noted in all animals at the 2- and 4-week post-injection evaluations and in 1/3 males in the 37.5 mg/kg group at the 6-week post-injection evaluation. The presence of granular tissue was considered a normal feature of wound healing.

Dermal fibrosis was present in several animals at all post-injection evaluations from 4 to 52 weeks following dose administration and was characterized by abundant mature collagen, often with a marked reduction in adnexal structures. In all occurrences of dermal fibrosis, the animal had spaces in the dermis and/or subcutis; therefore, the presence of dermal fibrosis was associated with injections in the dermis.

Minimal to moderate degeneration and regeneration of the panniculus muscle adjacent to spaces was present in some animals through the 6-week post-injection and in one animal at the 52-week post-injection evaluation.

All other microscopic changes were consistent with normal background lesions in clinically normal rabbits of the strain and age used in this study, and were considered to be spontaneous and/or incidental in nature and unrelated to test article administration (i.e., bupivacaine component of SABER-Bupivacaine).

Special Evaluation

No.

Toxicokinetics

Prior to euthanasia, blood was collected from each animal scheduled for necropsy. Blood frozen and not analyzed.

Dosing Solution Analysis

A single sample received from supplier and tested with purity reported at 96.8% for test article. Concentration, homogeneity, and stability information was provided by the sponsor.

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Intra-Articular Dosing

Two-week pilot toxicity study of SABER-Bupivacaine injectable formulation in New Zealand White rabbits (study 02-06-803-B-IJ-TXR, non-GLP) - This pilot study for the six-week study evaluated the potential toxicity and plasma bupivacaine levels after a single injection of SABER-Bupivacaine into the stifle joint (intra-articular) followed by 2 weeks of observation then necropsy. Three (3) male New Zealand White rabbits received the following:

Dose Group	Article Administered	Dose Level (mg)	Dose Volume (mL)	No. of Male Rabbits
1	Saline Control	0	0.5	3
2	Vehicle Control	0	0.5	3
3	SABER-Bupivacaine	66	0.5	3

SABER-Bupivacaine was lot GLP-803-19Feb04-01 and the placebo (SABER placebo) was lot GLP-803-17Feb04-01. Benzyl alcohol was the excipient. Dosing solution and homogeneity analyses were not conducted. A 66 mg dose is 22 mg/kg (0.17 mL/kg) for the 3 kg rabbits. A full set of biological indices were evaluated, the same as in the six-week study, except that only the stifle joint was evaluated histologically.

No effects on mortality, clinical signs, body weight, food consumption, clinical pathology (hematology and clinical chemistry), or stifle joint circumference were observed. Histological evaluation of the stifle joint identified similar responses to SABER-Bupivacaine and placebo (SABER placebo). Subacute inflammation and synovial hyperplasia of the stifle joint were observed with an increase in incidence and severity for the SABER-Bupivacaine group (see table). No treatment-related effects were observed in the saline control animals.

Dose Level (mg)	0 (Saline)		0 (75% SAIB:25% Benzyl Alcohol)		66 (Bupivacaine)	
Animal Number	1 1 1 M M M 6 6 6 6 6 6 7 7 8 7 9 6		2 2 2 M M M 6 6 6 6 6 6 8 8 8 0 4 7		3 3 3 M M M 6 6 6 6 6 6 8 8 8 1 2 5	
Day of Euthanasia	1 1 1 4 4 4	I	1 1 1 4 4 4	I	1 1 1 4 4 4	I
Tissue -lesion						
Stifle Joint -inflammation, subacute -hyperplasia, synovium	0 0 0 0 0 0	0/3 0/3	1 3 1 0 0 1	3/3 1/3	2 3 1 1 2 1	3/3 3/3

Maximum plasma levels were observed 4 hours after treatment with toxicokinetic values for the 3 males in the SABER-Bupivacaine as follows:

Rabbit	Tmax[a] (hr)	Cmax[b] (ng/mL)	HL_Lambda_z[c] (hr)	AUC_{last}[d] (hr·ng/mL)	AUC_{INF_obs}[e] (hr·ng/mL)
3M6681	4	638	9.9	7513	7796
3M6682	4	307	11.2	5136	5261
3M6685	4	681	6.0	7208	7245

[a]Time of maximum plasma concentration of bupivacaine
[b]Maximum plasma concentration of bupivacaine
[c]Half-life of the terminal elimination phase
[d]Area under the plasma drug concentration versus time curve calculated from 0 to the last time point bupivacaine was quantifiable in plasma
[e]Area under the plasma drug concentration versus time curve calculated from 0 to infinity

In summary, SABER-Bupivacaine (66 mg, 3 mg/kg) and SABER placebo caused subacute inflammation and synovial hyperplasia of the stifle joint after single intra-articular injection of 0.5 mL followed by a two-week recovery period. Incidence and severity were observed in the SABER-Bupivacaine group suggesting a bupivacaine component of the observed histopathology. Bupivacaine was absorbed into the blood stream. No injection site effects were observed in the saline controls indicating that

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Study title: Six-Week Intra-Articular Toxicity Study of SABER-Bupivacaine Injectable Formulation in New Zealand White Rabbits

Study no.:	02-07-803-B-IJ-TX
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 30, 2007
GLP compliance:	Yes.
QA statement:	Yes.
Drug, lot #, and % purity:	SABER-Bupivacaine, lot 079-05B, 98.2% 12% Bupivacaine, 66% Sucrose Acetate Isobutyrate (SAIB), 22% Benzyl Alcohol (BA) SABER Bupivacaine Placebo, lot 076-05-A, 75% Sucrose Acetate Isobutyrate (SAIB)/25% Benzyl Alcohol (BA) 0.9% saline, lot 6071169, Certificate of Analysis

Key Study Findings

- The objective of this study was to investigate the potential toxicity and plasma bupivacaine levels of SABER-Bupivacaine injectable formulation following a single dose into a stifle joint (intra-articular) of New Zealand White rabbits.
- The study consisted of six rabbits/sex/group assigned to treatment with saline (negative control), SABER placebo (vehicle control) and 13.2, 33, or 66 mg bupivacaine in SABER-Bupivacaine.
- No remarkable treatment-related effects were observed for mortality/moribundity, clinical signs, body weights, food consumption, ophthalmic examinations, hematology, clinical chemistry, coagulation, macroscopic pathology, ands absolute and relative organ weights. Recoverable clinical signs (e.g. scabs, inflammation, sore/ulcer, injection site discoloration) were reported in both males and females.
- Synovial hyperplasia, fatty degeneration, inflammation, fibrosis, and osseous metaplasia were the microscopic lesions observed in the stifle joints of rabbits treated with SABER placebo or SABER-Bupivacaine. Both treatments (drug and placebo) resulted in comparable joint effects. The incidence and the severity of the lesions were greatest in the high dose groups at Days 14 and 42 of euthanasia. **No necrosis was observed in the joints of any treatment group.** Rabbits injected with saline alone did not show any microscopic lesions in their stifle joints. In addition, no microscopic lesions were present in the right stifle joint (non-injected) of any rabbits.
- Overall, the data indicated that the extent of absorption and plasma kinetics of bupivacaine were similar among rabbits and gender given stifle joint injections of 13.2, 33, or 66 mg of bupivacaine. The Tmax (hours) for peak plasma concentrations of bupivacaine (Cmax) observed were variable with a value for most rabbits at 2-4 hours after dosing. Mean Cmax values and AUCs were dose responsive but not dosed proportional. Most half-lives ranged for 7-9 hours.
- Plasma bupivacaine levels declined gradually over the 72-hour period, and they were not detectable at 168 hours post treatment. Mean Cmax values were 140, 404, or 503 ng/mL for female rabbits, and 218, 375, or 543 ng/mL for male rabbits administered 13.2, 33, or 66 mg of bupivacaine in SABER-Bupivacaine, respectively. Mean AUC values were 1538, 5336, or 8800 hr·ng/mL for female rabbits, and 2122, 4716, or 8132 hr·ng/mL for male rabbits given 13.2, 33, or 66 mg of bupivacaine, respectively.

Methods

Doses:

Dose Group	Article Administered	Dose Level of Bupivacaine (mg)	Dose Volume (mL)	No. of Male Rabbits	No. of Female Rabbits
1	Saline Control	0	0.50	6	6
2	Vehicle Control	0	0.50	6	6
3	SABER-Bupivacaine	13.2	0.10	6	6
4	SABER-Bupivacaine	33	0.25	6	6
5	SABER-Bupivacaine	66	0.50	6	6

Frequency of dosing: Single dose.

Route of administration: Intra-articular (left stifle joint)
- right stifle joint not injected

Dose volume:	See table.
Formulation/Vehicle:	SABER placebo (75% SAIB/25% BA)
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	3/sex/group for 14 and 42 day sacrifices
Age:	22-23 weeks on day 1
Weight:	2.8-3.5 kg (males) and 2.6-3.5 kg (females) on day 1
Satellite groups:	None.
Unique study design:	None.
Deviation from study protocol:	Nothing significant.

Observations and Results

Mortality

Each animal was observed twice daily throughout the quarantine and study periods for signs of mortality and moribundity.

No treatment-related moribundity or mortality was reported during the 14- or 42-day period.

Clinical Signs

Each rabbit was removed from its cage daily and examined closely for clinical signs of toxicity, with particular attention paid to the dose site, gait, and the use of the hind limbs.

Clinical observations reported in both males and females for all groups included but were not limited to scab and sore/ulcer in the left hind limb/hind foot, mottled redness in the left hindlimb, discoloration at the injection site, and left hind limb inflammation, which was most noticeable in the high dose females. While the time course to recovery of clinical signs for the different groups was somewhat varied, recovery was complete for all groups by day 42.

Body Weights

Each animal was weighed during Week -1 (for randomization), prior to dosing on Day 1, weekly thereafter, and prior to sacrifice on days 14 and 42.

No treatment-related differences in body weight were observed in any of the groups. Group means for both sexes increased form day 1 to 42 except for placebo males where the body weight remained about the same.

Feed Consumption

Food consumption was measured and recorded once weekly throughout the study.

No notable treatment-related differences in food consumption were observed in any of the treated groups. Mean food consumption for the high dose group males and females was less than other groups in the first week, but subsequently recovered.

Ophthalmoscopy

Both eyes were examined during week -1 and 2-3 days prior to scheduled sacrifice.

No abnormal findings during the ophthalmic examinations were recorded at baseline, prior to interim, or prior to terminal euthanasia for any of the groups.

ECG – no evaluation conducted.

Hematology

Blood samples were collected from each rabbit after overnight fasting during week -1 and on days 14 and 42. The following parameters were evaluated:

WBC	Total leukocyte count
RBC	Erythrocyte count
HGB	Hemoglobin
HCT	Hematocrit
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
Retic	Reticulocyte count
PLT	Platelet count
	Differential leukocyte count
nRBC	Nucleated red blood cell count
	RBC Morphology

No remarkable treatment-related differences were observed in hematology values.

Clinical Chemistry

Blood samples were collected from each rabbit after overnight fasting during week -1 and on days 14 and 42. The following parameters were evaluated:

NA	Sodium
K	Potassium
CL	Chloride
TP	Total protein
Alb	Albumin
BUN	Blood urea nitrogen
Crea	Creatinine
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
Glob	Globulin
ALP	Alkaline phosphatase
A/G Ratio	Albumin/globulin ratio
Gluc	Glucose
Tbil	Total Bilirubin
Chol	Cholesterol
Ca	Calcium
Phos	Phosphorus

No treatment-related differences were observed in clinical chemistry values.

Coagulation

Blood samples were collected from each rabbit after overnight fasting during week -1 and on days 14 and 42. The following parameters were evaluated:

Coagulation

FBGN	Fibrinogen
PT	Prothrombin time
APTT	Activated partial thromboplastin time

No treatment-related differences were observed for coagulation values.

Urinalysis – no evaluation conducted.

Gross Pathology

Rabbits that were sacrificed for a scheduled necropsy on days 14 and 42. The post-mortem examination included, but was not limited to, the examination of the external surfaces and orifices. The cranial, thoracic, abdominal, and pelvic cavities were opened and the organs/tissues within each cavity were inspected. The animal's identification was retained with its tissues collected during necropsy. Samples of all tissue/organs, except eyes, were saved in 10% neutral buffered formalin for histopathological evaluation. Eyes were saved in Davidson's fixative. Bone marrow smears were prepared, stained, and held for possible future examination. The following tissues listed below were collected from each rabbit:

Adrenals [2]
All gross lesions
Aorta
Bone with bone marrow (femur and sternum)
Bone marrow smear (contralateral femur)
Brain (fore-, mid-, and hind-)
Kidneys [2]
Large intestine, cecum
Large intestine, colon
Large intestine, rectum
Liver
Lungs (with mainstem bronchi)
Lymph node (mesenteric)
Lymph node (submandibular)
Ovaries [2]
Pancreas
Pituitary gland
Prostate
Salivary gland (submaxillary) [2]
Sciatic nerve
Seminal vesicle [1]
Skeletal muscle (thigh)
Esophagus
Eyes [2]
Gallbladder
Harderian gland
Heart
Dosing sites [stifle joints; 2, including treated leg and non-treated leg]
Skin (ventral abdomen, including mammary gland)
Small intestine, duodenum
Small intestine, ileum
Small intestine, jejunum
Spinal cord (cervical, thoracic, lumbar)
Spleen
Stomach
Testes/epididymis [2]
Thymus
Thyroid/parathyroid glands [2]
Tongue
Trachea
Urinary bladder
Uterus

No remarkable gross differences were observed between any of the groups on days 14 or 42.

Organ Weights

The following organs were weighed after sacrifice:

Adrenal [2]	Liver	Spleen
Brain	Lungs	Testes [2]
Heart	Ovary [2]	Thyroid/parathyroid [2]
Kidney [2]		

No notable treatment-related differences in the organ weights/relative organ to body weight ratios for both sexes were observed in the saline, vehicle, or the three SABER-Bupivacaine groups.

Histopathology

All available tissues from all rabbits in Groups 1 (saline control), 2 (SABER placebo – vehicle control), and 5 (SABER-Bupivacaine – high dose) were processed into slides. In addition, any target tissues for the low and mid dose SABER-Bupivacaine groups and all stifle joints were processed. All stifle joint slides from all animals were also submitted for peer review.

Adequate Battery - yes

Peer Review – yes (stifle joints only)

1) (b) (4) – histology of stifle joint for comparison to original histology review

Histological Findings – No notable histology was observed in the organs of any of the evaluated treatment groups (saline, vehicle, and high dose) other than for the stifle joint (all groups evaluated). No histological effects were observed in the left stifle joint of rabbits injected with saline or in the right stifle joint (non-injected) of any rabbits from all groups.

No microscopic lesions of the stifle joint were observed in approximately half of the vehicle (SABER placebo) or test article (SABER-Bupivacaine) rabbits.

Day 14 – Minimal synovial hyperplasia (3/6), minimal inflammation (3/6) (subacute -2/6, chronic -1/6), minimal fibrosis (1/6), and minimal fatty degeneration (2/6) were present in the synovium of the left stifle joint of the vehicle control rabbits (SABER placebo). In the low dose group, minimal synovial hyperplasia (1/6), minimal chronic inflammation (1/6) and minimal fatty degeneration (1/6) were observed in one rabbit. No lesions were observed in the stifle joint of five of the rabbits. In the mid dose group, minimal synovial hyperplasia (3/6), minimal (1/6) to mild chronic inflammation (2/6), minimal fibrosis (3/6)

and minimal (1/6) to mild (2/6) fatty degeneration were present in the male rabbits. No lesions were observed in the joints of the three female rabbits. In the high dose group, minimal (1/6), mild (1/6), and moderate (1/6) synovial hyperplasia, minimal (2/6) to mild (1/6) chronic inflammation, minimal (2/6) to moderate (1/6) fibrosis, and minimal (1/6) to mild (2/6) fatty degeneration were present in the left synovial joint of three rabbits. No lesions were observed in the joints of three rabbits.

Microscopic Observations: Day 14 Euthanasia

Dose Level (mg)	0 (Saline Control)		0 (Vehicle Control)		13.2 (SABER-Bupivacaine)	
Animal Number	1 1 1 1 1 1 M M M F F F 6 6 6 6 6 6 7 8 8 8 8 8 9 1 1 6 6 6 9 6 7 5 8 9		2 2 2 2 2 2 M M M F F F 6 6 6 6 6 6 7 7 8 8 8 8 9 9 0 8 8 8 5 7 4 1 3 5		3 3 3 3 3 3 M M M F F F 6 6 6 6 6 6 7 8 8 8 8 8 9 0 0 7 7 7 4 2 9 0 5 7	
Day of Euthanasia	1 1 1 1 1 1 4 4 4 4 4 4	I	1 1 1 1 1 1 4 4 4 4 4 4	I	1 1 1 1 1 1 4 4 4 4 4 4	I
Tissue -lesion						
Stifle joint, left synovium -hyperplasia	0 0 0 0 0 0	0/6	0 1 1 0 0 1	3/6	0 0 0 1 0 0	1/6
-inflammation, subacute	0 0 0 0 0 0	0/6	0 1 1 0 0 0	2/6	0 0 0 0 0 0	0/6
-inflammation, chronic	0 0 0 0 0 0	0/6	0 0 0 0 0 1	1/6	0 0 0 1 0 0	1/6
-fibrosis	0 0 0 0 0 0	0/6	0 0 0 0 0 1	1/6	0 0 0 0 0 0	0/6
-fatty degeneration	0 0 0 0 0 0	0/6	0 1 1 0 0 0	2/6	0 0 0 1 0 0	1/6

Dose Level (mg)	33 (SABER-Bupivacaine)		66 (SABER-Bupivacaine)	
Animal Number	4 4 4 4 4 4 M M M F F F 6 6 6 6 7 7 8 8 9 8 0 0 0 1 6 8 0 3 7 0 3 6 0 3		5 5 5 5 5 5 M M M F F F 6 6 6 6 6 6 8 8 8 8 8 8 0 0 0 6 7 8 3 5 6 7 4 7	
Day of Euthanasia	1 1 1 1 1 1 4 4 4 4 4 4	I	1 1 1 1 1 1 4 4 4 4 4 4	I
Tissue -lesion				
Stifle joint, left synovium -hyperplasia	1 1 1 0 0 0	3/6	3 0 0 0 1 2	3/6
-inflammation, subacute	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6
-inflammation, chronic	1 2 2 0 0 0	3/6	2 0 0 0 1 1	3/6
-fibrosis	1 1 1 0 0 0	3/6	2 0 0 0 1 1	3/6
-fatty degeneration	2 2 1 0 0 0	3/6	2 0 0 0 1 2	3/6

Day 42 – Minimal synovial hyperplasia (1/6), minimal subacute inflammation (1/6), mild fibrosis (1/6), minimal (1/6) to mild (116) fatty degeneration were observed in the left stifle joints of the vehicle control rabbits. No lesions were observed in the stifle joints of three of the vehicle control rabbits. In the low dose group, minimal synovial hyperplasia (2/6), mild subacute inflammation (2/6), minimal (116) to mild (2/6) fatty degeneration, and mild osseous metaplasia (1/6) were present in the left stifle joints of three female rabbits. No lesions were observed in the stifle joints of the three male rabbits. Minimal synovial hyperplasia (1/6), mild fibrosis (1/6), minimal subacute inflammation (1/6), and mild (2/6) to moderate (1/6) fatty degeneration were observed in the left stifle joints of the mid dose group of rabbits. No lesions were observed in the joints of three of the rabbits. Minimal (3/6) to mild (1/6) synovial hyperplasia, minimal chronic inflammation (1/6), mild fibrosis (1/6), minimal (2/6) to mild (3/6) fatty degeneration, and mild osseous

metaplasia (3/6) were observed in the left stifle joints of the high dose group. No left stifle joints were completely devoid of lesions.

Microscopic Observations: Day 42 Euthanasia

Dose Level (mg)	0 (Saline Control)		0 (Vehicle Control)		13.2 (SABER-Bupivacaine)	
Animal Number	1 1 1 1 1 1 M M M F F F 6 6 6 7 7 7 9 9 9 0 0 0 4 5 5 3 3 3 8 6 8 0 2 4		2 2 2 2 2 2 M M M F F F 6 6 6 7 7 7 9 9 9 0 0 0 5 6 9 0 2 3 2 5 3 1 7 1		3 3 3 3 3 3 M M M F F F 6 6 6 6 7 7 9 9 9 8 0 0 4 6 7 7 0 2 3 6 5 8 8 0	
Day of Euthanasia	4 4 4 4 4 4 2 2 2 2 2 2	I	4 4 4 4 4 4 2 2 2 2 2 2	I	4 4 4 4 4 4 2 2 2 2 2 2	I
Tissue -lesion						
Stilfe joint, left synovium						
-hyperplasia	0 0 0 0 0 0	0/6	0 0 0 0 0 1	1/6	0 0 0 0 1 1	2/6
-inflammation, subacute	0 0 0 0 0 0	0/6	0 0 0 1 0 0	1/6	0 0 0 2 2 0	2/6
-inflammation, chronic	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6
-fibrosis	0 0 0 0 0 0	0/6	0 2 0 0 0 0	1/6	0 0 0 0 0 0	0/6
-degeneration, fatty	0 0 0 0 0 0	0/6	0 2 0 1 0 0	2/6	0 0 0 2 2 1	3/6
-metaplasia, osseous	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6	0 0 0 2 0 0	1/6

Dose Level (mg)	33 (SABER-Bupivacaine)		66 (SABER-Bupivacaine)	
Animal Number	4 4 4 4 4 4 M M M F F F 6 6 6 7 7 7 9 9 9 0 0 0 7 7 8 4 4 4 0 4 3 3 5 7		5 5 5 5 5 5 M M M F F F 6 6 6 7 7 7 8 8 8 0 0 0 1 1 1 1 3 4 1 3 4 9 5 6	
Day of Euthanasia	4 4 4 4 4 4 2 2 2 2 2 2	I	4 4 4 4 4 4 2 2 1 2 2 2	I
Tissue -lesion				
Stilfe joint, left synovium				
-hyperplasia	0 0 1 0 0 0	1/6	1 0 2 1 0 1	4/6
-inflammation, subacute	0 0 0 1 0 0	1/6	0 0 0 0 0 0	0/6
-inflammation, chronic	0 0 0 0 0 0	0/6	0 0 0 0 1 0	1/6
-fibrosis	0 0 2 0 0 0	1/6	0 0 2 0 0 0	1/6
-degeneration, fatty	0 0 2 2 3 0	3/6	2 1 2 0 2 1	5/6
-metaplasia, osseous	0 0 0 0 0 0	0/6	0 0 2 2 2 0	3/6

In summary, microscopic lesions that were observed in the stifle joints included the following: synovial hyperplasia, fatty degeneration, inflammation, fibrosis, and osseous metaplasia. The intra-articular injection of SABER-Bupivacaine in conjunction with the vehicle, SABER placebo, resulted in joint changes that were comparable to those sustained by injection of vehicle alone except in the high dose group. For the high dose SABER-Bupivacaine group, the incidence and severity of the lesions were greater on both days 14 and 42 of evaluation.

Peer review of stifle joint - The results of the peer review, along with original diagnoses confirmed the reported histopathological changes in the stifle joints of rabbits. Main intra-articular changes were hyperplasia, inflammation, fatty degeneration, and fibrosis of the synovium. Although minor differences in terminology and severity grading may have occurred, the original histological observations were confirmed by the peer review of the stifle joints.

Special Evaluation – Histological characterization of stifle joint effects as anticipated foreign body reaction and not potentially progressive effect.
- Dr. Anderson

Based on Dr. Anderson's report, only a foreign body reaction at the tissue/material interface (up to minimal severity in placebo and SABER-Bupivacaine groups except for minimal up to moderate in severity in high dose SABER-Bupivacaine group) and synovitis (up to minimal in severity) were observed. Both of these observations were focal with the foreign body reaction being located in the synovium and subsynovium and the focal synovitis being present in the synovial lining of the joint space. No other pathological findings were identified. No acute and/or chronic inflammation was identified. No degenerative osteoarthritis or pathological changes in the articular cartilage were identified. In the groups where foreign body reaction and synovitis were identified, these findings were considered to be those consistent with an injected biocompatible material. No effects were observed in saline controls.

Toxicokinetics

Blood samples (approximately 1.0 mL) were collected from the central ear artery of each rabbit prior to dosing and at approximately 0.5, 1, 2, 4, 8, 24, 48, 72, and 168 hours (7 days) after dosing.

Overall, the data indicated that the extent of absorption and plasma kinetics of bupivacaine were similar among rabbits and gender given stifle joint injections of 13.2, 33, or 66 mg of bupivacaine (see tables for males and females). The Tmax (hours) for peak plasma concentrations of bupivacaine (Cmax) observed were variable with a value for most rabbits at 2-4 hours after dosing. Mean Cmax values and AUCs were dose responsive but not dosed proportional. Most half-lives ranged for 7-9 hours. Plasma bupivacaine levels declined gradually over the 72-hour period, and they were not detectable at 168 hours post treatment.

Summary of Pharmacokinetic Parameters Calculated from Plasma Concentrations of Bupivacaine: Males

Group Sex		Tmax[a] (hr)	Cmax[b] (ng/mL)	HL_Lambda_z[c] (hr)	AUC_{last}[d] (hr·ng/mL)	AUC_{INF_obs}[e] (hr·ng/mL)
3M	Mean	1.3	218	7.4	2122	2169
	SD	0.6	31.2	1.0	135	131
4M	Mean	2.5	375	7.2	4716	4757
	SD	1.2	83.0	1.2	1014	1019
5M	Mean	3.4	543	9.4	8132	8210
	SD	2.6	144	1.5	1612	1613

Summary of Pharmacokinetic Parameters Calculated from Plasma Concentrations of Bupivacaine: Females

Group Sex		Tmax[a] (hr)	Cmax[b] (ng/mL)	HL_Lambda_z[c] (hr)	AUC_{last}[d] (hr·ng/mL)	AUC_{INF_obs}[e] (hr·ng/mL)
3F	Mean	2.1	140	7.9	1538	1579
	SD	1.5	34.2	1.7	146	145
4F	Mean	3.2	404	7.6	5336	5380
	SD	1.3	103	1.8	955	948
5F	Mean	2.7	503	13.3	8800	9862
	SD	1.0	141	10.7	2480	3864

[a] Time of maximum plasma concentration of bupivacaine
[b] Maximum plasma concentration of bupivacaine
[c] Half-life of the terminal elimination phase
[d] Area under the plasma drug concentration versus time curve calculated from 0 to the last time point bupivacaine was quantifiable in plasma
[e] Area under the plasma drug concentration versus time curve calculated from 0 to infinity
NA = Not applicable
Nominal Dose: Group 3 - 13.2 mg (SABER-Bupivacaine) Group 4 - 33 mg (SABER-Bupivacaine) Group 5 - 66 mg (SABER-Bupivacaine)

Dosing Solution Analysis – Both pre-dose and post-dose active formulation samples were analyzed for appearance, potency, and degradation products. The results show that the SABER-Bupivacaine remained clear, light yellow brown in color, and percent label strength assays show that the product was chemically stable during the animal dosing period. Total degradation of pre-dose samples was 0.53% and that of post-dose samples was 0.65%. Both pre-dose and post-dose SABER placebo samples were analyzed for appearance. The placebo formulation remained as clear, colorless solution, and absence of bupivacaine was confirmed.

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Study title: Six-Week Intra-Articular Toxicity Study of SABER-Bupivacaine Injectable Formulation in Beagle Dogs

Study no.:	03-07-803-D-IJ-TX
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 16, 2007
GLP compliance:	Yes.
QA statement:	Yes.
Drug, lot #, and % purity:	SABER-Bupivacaine, lot 079-05B, 98.2% 12% Bupivacaine, 66% Sucrose Acetate Isobutyrate (SAIB), 22% Benzyl Alcohol (BA) SABER Bupivacaine Placebo, lot 076-05-A, 75% Sucrose Acetate Isobutyrate (SAIB)/25% Benzyl Alcohol (BA), NA

0.9% saline, lot 6071169, Certificate of Analysis

Key Study Findings

- The objective of this study was to investigate the potential toxicity and plasma bupivacaine levels of SABER-Bupivacaine injectable formulation following a single dose into a stifle joint (intra-articular) of Beagle dogs.
- The study consisted of six dogs/sex/group assigned to treatment with saline (negative control), SABER placebo (vehicle control) and 19.8, 66, or 198 mg bupivacaine in SABER-Bupivacaine (test article).
- No remarkable treatment-related effects were observed for mortality/moribundity, clinical signs, body weights, ophthalmic examinations, electroencephalograms, hematology, clinical chemistry, coagulation, macroscopic pathology, and absolute and relative organ weights. Food consumption was decreased up to twelve days after dosing compared to saline controls with no corresponding decrease in body weights. Recoverable clinical signs (e.g. edema, swelling, limping in left hindlimb, and loose stool) were reported in both males and females.
- Hyperplasia, fatty degeneration, inflammation, fibrosis, and a fibrinous exudate of the synovium, **necrosis** and fibrosis of the joint cartilage, and fibrosis of the subchondral bone were the microscopic lesions observed in the stifle joints of dogs treated with SABER placebo or SABER-Bupivacaine. Both treatments resulted in comparable joint effects. Except for the fibrinous exudate on days 14 and 42, synovial lesions in the low and mid dose test article groups were similar in incidence, but less severe than those in the vehicle and high dose groups. In addition, no microscopic lesions were present in the right stifle joint (non-injected) of any dogs except for the high dose group which was explained as due to shift in weight bearing resulting from dosed joint compensation.
- At day 14, joint cartilage necrosis was present in one vehicle control animal (moderate) and one high dose animal (marked), but not in the other two test article groups. **Joint cartilage necrosis of marked severity was observed in all vehicle and high dose test article dogs at 42 days after dosing.**
- Peer review confirmed original histopathology observations and second peer review indicated only foreign body reaction and no progressive, degenerative processes.
- Overall, the data indicated that the extent of absorption and plasma kinetics of bupivacaine were similar among dogs and gender given stifle joint injections of 19.8, 66, or 198 mg of bupivacaine in SABER-Bupivacaine. Overall exposure (AUC) was dose responsive but Cmax did not noticeably increase from the mid to high dose.
- The Tmax (hours) for peak plasma concentrations of bupivacaine (Cmax) observed were 0.5, 0.5, and 2 hours after dosing, respectively. Mean AUCs were dose responsive but not dose proportional. Most half-lives were ~3, 14, & 19 hours, respectively.
- Plasma bupivacaine levels generally declined over a 24-hour period for the low dose and for a 72-hour period for the mid and high doses with bupivacaine not detectable at 168 hours post treatment. Mean Cmax values were 876, 1386, or 1879 ng/mL for female dogs, and 808, 1524, or 1099 ng/mL for male dogs administered 19.8, 66, or

198 mg of bupivacaine in SABER-Bupivacaine, respectively. Mean AUC values were 2961, 9146, or 36227 hr·ng/mL for female dogs, and 2750, 8831, or 23261 hr·ng/mL for male dogs given 19.8, 66, or 198 mg of bupivacaine, respectively.

Methods

Doses:

Dose Group	Article Administered	Dose Level of Bupivacaine (mg)	Dose Volume (mL)	Number of Dogs	
				Males	Females
1	Saline Control	0	1.5	5	5
2	Vehicle Control	0	1.5	5	5
3	SABER-Bupivacaine	19.8	0.15	5	5
4	SABER-Bupivacaine	66.0	0.5	5	5
5	SABER-Bupivacaine	198.0	1.5	5	5

Frequency of dosing: Single dose
Route of administration: Intra-articular (left stifle joint); right joint not injected
Dose volume: See above
Formulation/Vehicle: SABER placebo (75% SAIB/25% BA)
Species/Strain: Beagle dogs
Number/Sex/Group: 5
Age: 10-11 months
Weight: 9.2-11.3 kg (males) and 6.6-9.1 (females) on day 1
Satellite groups: None.
Unique study design: None.
Deviation from study protocol: Nothing significant.

Observations and Results

Mortality

Each animal was observed at least twice daily throughout the quarantine and study periods for signs of mortality and moribundity.

No moribundity or mortality occurred during the 14- or 42-day observation period.

Clinical Signs

Each dog was removed from its cage daily and examined closely for clinical signs of toxicity, with particular attention paid to the dosing site, gait, and the use of the hind limbs.

No notable treat-related clinical observations occurred. In both males and females that included all groups, edema, swelling, and limping in left hindlimb were observed. Most notable effects were observed early (first 9 days).

Summary of Clinical Observations: Males

Group Sex	Clinical Sign, Site	Day numbers relative to Start Date 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1M	Unremarkable	4	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	2	2
	Swelling, Left hindlimb	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Emesis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.
2M	Unremarkable	.	.	.	.	.	.	.	.	4	5	5	5	5	5	2	2	2	2	2	2	2
	Diarrhea	.	.	.	.	.	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.
	Loose Stool	.	.	.	.	1	3	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	Edema, Left hindlimb	.	.	1	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Swelling, Left hindlimb	5	5	5	5	5	5	5	5	.	.	.	.	.	.	.	.	.	.	.	.	.
	Limping, Left hindlimb	4	5	4	4	4	4	3	3	1	.	.	.	.	.	.	.	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
3M	Unremarkable	5	4	5	5	5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	2	2
	Swelling, Left hindlimb	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Limping, Left hindlimb	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
4M	Unremarkable	4	.	.	2	3	3	3	2	5	5	5	5	5	5	2	2	2	2	2	2	2
	Loose Stool	.	.	.	.	.	1	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.
	Edema, Left hindlimb	.	.	.	2	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Swelling, Left hindlimb	1	5	5	3	2	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	Limping, Left hindlimb	1	4	3	2	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
5M	Unremarkable	4	3	1	3	3	3	2	2	3	3	3	3	3	5	2	2	2	2	2	2	2
	Edema, Left hindlimb	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Swelling, Left hindlimb	1	2	4	2	2	1	3	3	1	1	1	1	1	.	.	.	.	.	.	.	.
	Hypoactive	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Limping, Left hindlimb	1	2	4	2	2	2	3	3	2	2	2	2	2	.	.	.	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.

Summary of Clinical Observations: Females

Group Sex	Clinical Sign, Site	Day numbers relative to Start Date 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1F	Unremarkable	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	2	2
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
2F	Unremarkable	.	.	.	.	.	.	.	3	4	4	4	4	5	5	2	2	2	2	2	2	2
	Loose Stool	.	.	.	.	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Edema, Left hindlimb	.	.	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Swelling, Left hindlimb[a]	5	5	5	5	5	5	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	Hypoactive	.	.	.	.	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Limping, Left hindlimb	5	5	5	5	5	4	4	2	1	1	1	1	.	.	.	.	.	.	.	.	.
	Excessive salivation	.	.	2	1	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
3F	Unremarkable	4	2	4	5	4	4	4	5	5	5	5	5	5	5	2	1	2	2	2	2	2
	Loose Stool	.	.	.	.	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Swelling, Left hindlimb	1	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Limping, Left hindlimb[b]	1	3	1	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
4F	Unremarkable	.	.	.	2	1	3	3	5	5	5	5	5	5	5	2	2	2	2	2	2	2
	Loose Stool	.	.	.	.	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Edema, Left hindlimb	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Swelling, Left hindlimb	5	5	5	3	3	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Emesis	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Limping, Left hindlimb	3	4	2	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
5F	Unremarkable	.	1	2	2	3	2	2	4	4	4	4	4	5	5	2	2	2	2	2	2	2
	Loose Stool	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Swelling, Left hindlimb	5	4	3	3	2	3	3	1	1	1	1	1	.	.	.	.	.	.	.	.	.
	Limping, Left hindlimb[c]	2	1	1	2	2	3	3	1	1	1	1	1	.	.	.	.	.	.	.	.	.
	Excessive salivation	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.

[a] Swelling was also observed in the left hindfoot of an animal in Group 2F.
[b] Limping was also observed in the right forelimb of an animal in Group 3F.
[c] Limping was also observed in the left forefoot and forelimb of an animal in Group 5F.

Nominal Dose: Group 1 - 0 mg (Saline control) Group 2 - 0 mg (Vehicle control) Group 3 - 19.8 mg (SABER-Bupivacaine)
Group 4 - 66.0 mg (SABER-Bupivacaine) Group 5 - 198.0 mg (SABER-Bupivacaine)

Body Weights

Each animal was weighed during week -1 (for randomization), prior to dosing on day 1, and on days 8, 14, 22, 29, 36, and 42.

No treatment-related differences in the body weights of males and females were seen in any of the treated groups.

Feed Consumption

Beginning on day 1, quantitative food consumption was measured and recorded daily throughout the study.

Food consumption of males and females was reduced with no reduction in body weights for the SABER placebo (vehicle) and SABER-Bupivacaine groups up to the first twelve days after dosing.

Summary of Food Consumption Results (g/animal/day): Males

Day numbers relative to Start Date

Group	From:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sex	To:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1M	Mean	239.4	222.2	206.2	194.2	154.6	201.2	258.0	197.8	249.2	164.8	221.8	225.0	205.2	268.5	185.0
	S.D.	100.5	51.7	42.3	66.6	40.4	60.0	34.9	28.8	33.1	58.5	53.4	76.5	58.5	23.3	17.0
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2
2M	Mean	276.0	101.2	7.0	29.6	23.8	70.2	106.2	129.6	231.6	220.8	235.2	234.6	241.8	303.5	340.5
	S.D.	134.5	111.2	14.0	43.4	23.8	59.5	31.1	22.8	56.2	43.0	30.1	80.4	62.1	119.5	87.0
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2
3M	Mean	338.0	248.2	118.4	212.4##	166.2##	207.6#	183.0#	192.4	246.0	247.8	255.4	285.0	269.4	356.5	390.5**
	S.D.	99.6	110.6	121.3	113.1	82.0	91.9	65.6	85.5	89.5	85.6	83.3	84.2	97.0	14.8	43.1
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2
4M	Mean	354.4	120.0	69.4	69.2	46.8*	93.0	131.6**	145.4	267.4	232.0	297.0	303.6	287.6	339.5	341.5**
	S.D.	71.8	94.3	59.2	76.8	43.1	67.6	37.8	48.9	51.3	40.4	29.5	41.0	44.4	30.4	24.7
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2
5M	Mean	340.6	258.8	94.2	34.4*	33.0*	85.8*	138.8**	139.4	177.0	236.0	225.2	273.0	250.2	303.0	344.0**
	S.D.	58.9	141.8	128.9	63.7	71.0	47.2	41.6	79.3	79.7	98.1	33.0	77.0	45.7	66.5	2.8
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2

Summary of Food Consumption Results (g/animal/day): Females

Day numbers relative to Start Date

Group	From:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sex	To:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1F	Mean	188.6	188.6	195.6	158.8	205.2	169.8	209.4	250.4	223.2	260.2	205.2	227.8	298.0	226.0	191.0
	S.D.	29.3	26.2	55.1	42.7	63.5	34.1	21.3	70.8	51.3	31.6	45.1	41.5	55.8	48.1	4.2
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2
2F	Mean	197.2	75.8	22.0	11.4	74.0	65.4	90.8	173.8	211.0	204.6	214.0	201.0	285.6	233.5	194.0
	S.D.	21.1	64.6	37.4	15.8	80.7	57.3	34.7	40.6	42.2	36.1	59.1	82.5	55.0	0.7	12.7
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2
3F	Mean	219.6	158.6	16.8**	27.6**	42.0**	65.0*	90.8*	247.4	212.4	227.8	228.0	210.8	242.2	178.5	245.0
	S.D.	106.1	112.0	34.3	46.0	57.0	58.0	60.8	101.2	39.1	43.5	32.4	57.1	62.1	2.1	65.1
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2
4F	Mean	250.2	131.8	61.2**	50.4**	122.8	125.6	155.4	259.0	199.0	222.4	235.4	202.8	263.4	195.0	165.5
	S.D.	62.3	87.1	55.9	53.0	62.6	24.6	69.2	74.1	67.9	61.8	87.0	52.5	82.1	79.2	64.3
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2
5F	Mean	181.4	135.2	36.2**	26.0**	72.4*	80.8	89.8*	165.4	157.0	176.8	180.4	192.6	260.0	205.0	233.5
	S.D.	61.4	81.7	45.7	57.0	78.5	82.0	83.9	104.8	72.6	80.6	70.0	67.8	96.0	56.6	41.7
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2

Statistical Analysis (Dunnett's):
* = $p \leq 0.05$; ** = $p \leq 0.01$ (Groups 3, 4, and 5 compared to Group 1)
# = $p \leq 0.05$; ## = $p \leq 0.01$ (Groups 3, 4, and 5 compared to Group 2)

Nominal Dose: Group 1 - 0 mg (Saline control) Group 2 - 0 mg (Vehicle control) Group 3 - 19.8 mg (SABER-Bupivacaine)
Group 4 - 66.0 mg (SABER-Bupivacaine) Group 5 - 198.0 mg (SABER-Bupivacaine)

Ophthalmoscopy

Both eyes of each dog were evaluated by direct and indirect ophthalmoscopy and slit lamp examination during week -1 and prior to scheduled sacrifice.

No abnormal ophthalmic findings were observed in any of the groups.

ECG

A 10-lead ECG recording was obtained for each dog during quarantine (week -1) and on day 1 (approximately 4 hours after dosing).

Electrocardiograms for all groups of males and females were within the normal limits.

Hematology

Dogs were fasted overnight and blood samples were collected for hematology determinations during week -1 and on days 14 and 42. The following parameters were evaluated:

WBC	Total leukocyte count
RBC	Erythrocyte count
HGB	Hemoglobin
HCT	Hematocrit
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
Retic	Reticulocyte count
PLT	Platelet count
	Differential leukocyte count
nRBC	Nucleated red blood cell count
	RBC morphology

No notable treatment-related differences in hematology values were observed in this study. Any values when compared to the saline or vehicle control groups were not considered test article related due to the lack of a dose or time relationship, the small magnitude of the differences, and/or comparability of individual data.

Clinical Chemistry

Dogs were fasted overnight and blood samples were collected for clinical chemistry determinations during week -1 and on days 14 and 42. The following parameters were evaluated:

NA	Sodium
K	Potassium
CL	Chloride
TP	Total protein
Alb	Albumin
BUN	Blood urea nitrogen
Crea	Creatinine
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
Glob	Globulin
ALP	Alkaline phosphatase
A/G Ratio	Albumin/globulin ratio
Gluc	Glucose
Tbil	Total bilirubin
Chol	Cholesterol
Ca	Calcium
Phos	Phosphorus

No notable treatment-related differences in clinical chemistry values were observed in this study. Any values when compared to the saline or vehicle control groups were not considered test article related due to the lack of a dose or time relationship, the small magnitude of the differences, and/or comparability of individual data.

Coagulation

Dogs were fasted overnight and blood samples were collected for coagulation determinations during week -1 and on days 14 and 42. The following parameters were evaluated:

PT	Prothrombin time
APTT	Activated partial thromboplastin time
FBGN	Fibrinogen

No notable treatment-related differences in coagulation values were observed in this study. Any values when compared to the saline or vehicle control groups were not considered test article related due to the lack of a dose or time relationship, the small magnitude of the differences, and/or comparability of individual data.

Urinalysis – no evaluation conducted.

Gross Pathology

On day 14, 3 dogs/sex/group were sacrificed. The surviving 2 dogs/sex/group were sacrificed on day 42 in the same manner. The postmortem examination included, but was not limited to, the examination of the external surfaces and orifices. The cranial, thoracic, abdominal, and pelvic cavities were opened and the organs/tissues within each cavity were inspected. Bone marrow smears were prepared, stained, and held for possible future examination. Samples of all tissues/organs, except eyes, were saved in 10% neutral buffered formalin for histopathological evaluation. Eyes were saved in Davidson's fixative.

The following tissues were collected:

Adrenals [2]
All gross lesions
Aorta
Bone with bone marrow (femur and sternum)
Bone marrow smear (contralateral femur)
Brain (fore-, mid-, and hind-)
Esophagus
Eyes [2]
Gallbladder
Heart
Dosing sites [stifle joints; 2, including treated leg and non-treated leg]
Kidneys [2]
Large intestine, cecum
Large intestine, colon
Large intestine, rectum
Liver
Lungs (with mainstem bronchi)
Lymph node, mesenteric
Lymph node, submandibular
Ovaries [2]
Pancreas
Pituitary gland
Prostate
Salivary gland (submaxillary) [2]
Sciatic nerve
Skeletal muscle (thigh)
Skin (ventral abdomen, including mammary gland)
Small intestine, duodenum
Small intestine, ileum
Small intestine, jejunum
Spinal cord (cervical, thoracic, lumbar)
Spleen
Stomach
Testes/epididymides [2]
Thymus
Thyroid/parathyroid glands [2]
Tongue
Trachea
Urinary bladder
Uterus

No notable treatment-related differences in macroscopic observations were observed in this study.

Organ Weights

The organs listed below were weighed for each respective dog sacrificed on day 14 or day 42.

Adrenals [2]
Brain
Heart
Kidneys [2]
Liver
Lungs
Ovaries [2]
Spleen
Testes/epididymides [2]
Thyroid/parathyroid glands [2]

No treatment-related differences in the organ weights or organ-to-body weight ratios of males and females were seen in any of the three SABER-Bupivacaine dose groups when compared to animals in the saline and SABER placebo (vehicle) control groups. Any values when compared to the saline or vehicle control groups were not considered test article related due to the lack of a dose or time relationship, the small magnitude of the differences, and/or comparability of individual data.

Histopathology

Adequate Battery - yes

All available tissues from all dogs in all groups were processed into slides and evaluated. All stifle joint slides from all animals were also submitted for peer review.

Peer Review – yes (stifle joints only)

2) (b) (4) – histology of stifle joint for comparison to original histology review

Histological Findings – No notable histology was observed in the organs of any of the evaluated treatment groups other than for the stifle joint (all groups evaluated). No notable histological effects were observed in the left stifle joint of dogs injected with saline or in the right stifle joint (non-injected) of any dogs from all groups.

The following microscopic lesions were observed in the stifle joints of dogs treated with SABER placebo (vehicle) or SABER-Bupivacaine (test article): hyperplasia, fatty degeneration, inflammation, fibrosis, and a fibrinous exudate of the synovium, necrosis and fibrosis of the joint cartilage, and fibrosis of the subchondral bone. It was reported that on days 14 and 42, the intra-articular injection of the high dose of the test article in conjunction with the vehicle resulted in joint changes that were comparable to those sustained by injection of the vehicle alone. Except for the fibrinous exudate on days 14 and 42, synovial lesions in the low and mid dose test article groups were similar in incidence, but less severe than those in the vehicle and high dose groups. Joint cartilage and subchondral bone lesions were present in the vehicle and high dose test article groups, but not in the low and mid dose groups. On Day 42, osteophytes were present in the high dose group alone. No microscopic lesions were noted in the stifle joints of dogs injected with saline.

Day 14 - No microscopic lesions were observed in the left stifle joint of the saline control animals or the right stifle joint of any of the animals, except for one animal in the mid dose group. No differences in the incidence of hyperplasia, inflammation, fibrosis, and fatty degeneration of the synovium were observed in SABER placebo (vehicle) and SABER-Bupivacaine (test article) dogs from day 14. All six animals in each of the vehicle and test article groups had four or more of the previously described lesions, varying from minimal to moderate. However, the incidence and severity of a fibrinous exudate was greater in the vehicle control (6/6) and the high dose group (6/6) than in

the low (2/6) and mid (4/6) dose groups. A higher incidence of moderate fibrinous exudate was present in the vehicle (6/6) and high (5/6) dose groups, compared to the low (0/6) and mid (2/6) dose groups. The severity of synovial fibrosis (moderate) was greater in the vehicle (4/6) and high (6/6) dose groups, compared to the low (0/6) and mid (3/6) dose groups. At day 14, joint cartilage necrosis and subchondral bone fibrosis were present in one vehicle control animal (moderate) and one high dose animal (marked), but not in the other two test article groups. (see tables)

Microscopic Observations: Day 14 Euthanasia

Dose: mg	0 (Saline control)		0 (Vehicle control)		19.8 (SABER-Bupivacaine)	
Animal Number	1 1 1 1 1 1 M M M F F F 7 7 7 7 7 7 4 4 4 4 4 4 5 6 6 8 9 9 8 0 7 7 0 1		2 2 2 2 2 2 M M M F F F 7 7 7 7 7 7 4 4 4 4 4 5 6 6 6 8 9 0 1 4 8 5 6 3		3 3 3 3 3 3 M M M F F F 7 7 7 7 7 7 4 4 4 4 4 5 6 6 6 8 9 0 2 3 5 8 4 6	
Day of Euthanasia	1 1 1 1 1 1 4 4 4 4 4 4	I	1 1 1 1 1 1 4 4 4 4 4 4	I	1 1 1 1 1 1 4 4 4 4 4 4	I
Tissue -lesion						
Stifle joint, left						
-hyperplasia, synovium	0 0 0 0 0 0	0/6	1 1 1 2 2 1	6/6	1 1 1 2 1 1	6/6
-inflammation, chronic, synovium	0 0 0 0 0 0	0/6	2 2 2 2 2 2	6/6	1 1 1 2 1 2	6/6
-inflammation, chronic active, synovium	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6
-fibrosis, synovium	0 0 0 0 0 0	0/6	3 3 3 1 3 2	6/6	1 1 1 2 2 1	6/6
-degeneration, fatty, synovium	0 0 0 0 0 0	0/6	3 2 2 2 2 2	6/6	1 2 2 2 2 2	6/6
-exudate, fibrinous, synovium	0 0 0 0 0 0	0/6	3 3 3 3 3 3	6/6	2 0 0 0 0 1	2/6
-necrosis, joint cartilage	0 0 0 0 0 0	0/6	0 0 0 0 0 3	1/6	0 0 0 0 0 0	0/6
-fibrosis, subchondral bone	0 0 0 0 0 0	0/6	0 0 0 0 0 3	1/6	0 0 0 0 0 0	0/6
Stifle joint, right synovium						
-inflammation, chronic	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6
-fibrosis	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6
-exudate, fibrinous	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6	0 0 0 0 0 0	0/6

Dose: mg	66.0 (SABER-Bupivacaine)		198.0 (SABER-Bupivacaine)	
Animal Number	4 4 4 4 4 4 M M M F F F 7 7 7 7 7 7 4 4 4 4 4 4 5 6 6 9 9 9 9 6 9 2 5 7		5 5 5 5 5 5 M M M F F F 7 7 7 7 7 7 4 4 4 4 4 5 7 7 7 9 9 0 1 6 8 3 9 0	
Day of Euthanasia	1 1 1 1 1 1 4 4 4 4 4 4	I	1 1 1 1 1 1 4 4 4 4 4 4	I
Tissue -lesion				
Stifle joint, left				
-hyperplasia, synovium	1 1 1 2 2 1	6/6	1 1 1 2 1 1	6/6
-inflammation, chronic, synovium	2 2 3 2 3 3	6/6	2 2 0 3 3 3	5/6
-inflammation, chronic active, synovium	0 0 0 0 0 0	0/6	0 0 2 0 0 0	1/6
-fibrosis, synovium	3 2 3 1 2 3	6/6	3 3 3 3 3 3	6/6
-degeneration, fatty, synovium	2 2 1 1 2 2	6/6	3 2 1 2 1 3	6/6
-exudate, fibrinous, synovium	0 2 3 2 0 3	4/6	3 3 2 3 3 3	6/6
-necrosis, joint cartilage	0 0 0 0 0 0	0/6	0 0 0 0 0 4	1/6
-fibrosis, subchondral bone	0 0 0 0 0 0	0/6	0 0 0 0 0 3	1/6
Stifle joint, right synovium				
-inflammation, chronic	0 1 0 0 0 0	1/6	0 0 0 0 0 0	0/6
-fibrosis	0 1 0 0 0 0	1/6	0 0 0 0 0 0	0/6
-exudate, fibrinous	0 2 0 0 0 0	1/6	0 0 0 0 0 0	0/6

0 = Lesion not observed
1 = Lesion of minimal severity
2 = Lesion of mild severity
3 = Lesion of moderate severity
4 = Lesion of marked severity
X = Lesion not graded

N = Not applicable to animal
M = Tissue missing
* = Nonprotocol-specified tissue
I = Incidence: Number of animals with lesion/number of animals examined

Day 42 – No microscopic lesions were observed in the stifle joints of the saline control group or untreated right stifle joint, except for two animals in the high dose group. This observation was described as weight bearing compensation for the affected other, treated joint. Not all vehicle and high dose test article animals were affected so this reviewer considers these observations on minimal to mild severity as spurious. Some small differences in the incidence of hyperplasia (4/4, 3/4, 3/4, 4/4), inflammation (4/4 for all groups), fibrosis (3/4, 4/4, 4/4, 4/4), and fatty degeneration (4/4, 3/4, 4/4, 4/4) were observed in the synovium of the left stifle joint of the vehicle and test article animals. Small differences in the severity of hyperplasia, inflammation, and fibrosis were observed in the left synovium of the vehicle and test article animals, but fatty degeneration was slightly more severe in the mid and high dose groups (2/4 with moderate fatty degeneration). **Joint cartilage necrosis (marked)**, joint cartilage fibrosis (minimal to marked), and subchondral bone fibrosis (minimal to marked) were observed in the vehicle (4/4, 2/4, and 4/4, respectively) and high (4/4, 3/4, and 4/4, respectively) dose groups, but not in the low and mid dose groups. At day 42, a fibrinous exudate was present in the vehicle (1/4) and high (2/4) dose groups and osteophytes (2/4) were present in the high dose group, but not in any of the other groups. Osteophytes, representing fragments of necrotic cartilage, were present loose in the joints of two high dose animals at day 42 (not on table). In chronic joint disease, osteophytes occasionally become intra-articular loose bodies or "joint mice". Joint mice are fragments of cartilage and/or bone that originate from synovial membranes or articular plates that are lying free in the joint space. (see tables)

Microscopic Observations: Day 42 Euthanasia

Dose: mg	0 (Saline control)		0 (Vehicle control)		19.8 (SABER-Bupivacaine)	
Animal Number	1 1 1 1 M M F F 7 7 7 7 4 4 4 5 8 8 9 0 0 4 8 8		2 2 2 2 M M F F 7 7 7 7 4 4 5 5 7 7 0 0 2 3 5 9		3 3 3 3 M M F F 7 7 7 7 4 4 5 5 7 7 0 1 0 5 7 2	
Day of Euthanasia	4 4 4 4 2 2 2 2	I	4 4 4 4 2 2 2 2	I	4 4 4 4 2 2 2 2	I
Tissue -lesion						
Stifle joint, left						
-hyperplasia, synovium	0 0 0 0	0/4	1 1 2 2	4/4	1 1 1 0	3/4
-inflammation, chronic, synovium	0 0 0 0	0/4	1 1 2 2	4/4	3 2 2 1	4/4
-fibrosis, synovium	0 0 0 0	0/4	1 0 1 1	3/4	2 1 1 2	4/4
-degeneration, fatty, synovium	0 0 0 0	0/4	1 1 2 2	4/4	0 2 2 2	3/4
-exudate, fibrinous, synovium	0 0 0 0	0/4	0 0 1 0	1/4	0 0 0 0	0/4
-necrosis, joint cartilage	0 0 0 0	0/4	4 4 4 4	4/4	0 0 0 0	0/4
-fibrosis, joint cartilage	0 0 0 0	0/4	0 3 2 0	2/4	0 0 0 0	0/4
-fibrosis, subchondral bone	0 0 0 0	0/4	3 4 3 3	4/4	0 0 0 0	0/4
Stifle joint, right synovium						
-hyperplasia	0 0 0 0	0/4	0 0 0 0	0/4	0 0 0 0	0/4
-inflammation, chronic	0 0 0 0	0/4	0 0 0 0	0/4	0 0 0 0	0/4
-fibrosis	0 0 0 0	0/4	0 0 0 0	0/4	0 0 0 0	0/4
-degeneration, fatty	0 0 0 0	0/4	0 0 0 0	0/4	0 0 0 0	0/4

Dose: mg	66.0 (SABER-Bupivacaine)		198.0 (SABER-Bupivacaine)	
Animal Number	4 4 4 4 M M F F 7 7 7 7 4 4 5 5 7 8 1 1 4 1 0 1		5 5 5 5 M M F F 7 7 7 7 4 4 5 5 8 8 0 0 2 3 2 4	
Day of Euthanasia	4 4 4 4 2 2 2 2	I	4 4 4 4 2 2 2 2	I
Tissue -lesion				
Stifle joint, left				
-hyperplasia, synovium	1 1 0 1	3/4	1 1 1 2	4/4
-inflammation, chronic, synovium	3 2 2 1	4/4	1 1 2 3	4/4
-fibrosis, synovium	2 2 2 1	4/4	1 2 2 3	4/4
-degeneration, fatty, synovium	3 3 1 2	4/4	1 3 1 3	4/4
-exudate, fibrinous, synovium	0 0 0 0	0/4	0 1 2 0	2/4
-necrosis, joint cartilage	0 0 0 0	0/4	4 4 4 4	4/4
-fibrosis, joint cartilage	0 0 0 0	0/4	0 1 1 4	3/4
-fibrosis, subchondral bone	0 0 0 0	0/4	1 3 3 3	4/4
Stifle joint, right synovium				
-hyperplasia	0 0 0 0	0/4	2 0 2 0	2/4
-inflammation, chronic	0 0 0 0	0/4	2 0 2 0	2/4
-fibrosis	0 0 0 0	0/4	1 0 0 0	1/4
-degeneration, fatty	0 0 0 0	0/4	1 0 0 0	1/4

0 = Lesion not observed
1 = Lesion of minimal severity
2 = Lesion of mild severity
3 = Lesion of moderate severity
4 = Lesion of marked severity
X = Lesion not graded

N = Not applicable to animal
M = Tissue missing
* = Nonprotocol-specified tissue
I = Incidence: Number of animals with lesion/number of animals examined

Peer review (b) (4) – confirmed similar evaluation to primary pathologist.

Special Evaluation - Histological characterization of stifle joint effects as anticipated foreign body reaction and not potentially progressive effect.
- Dr. Anderson

Evaluation showed only foreign body reaction with no indication of degenerative osteoarthritis or other pathological changes.

Toxicokinetics

Blood samples were collected from each dog prior to dosing and at approximately 0.5, 1, 2, 4, 8, 24, 48, 72, and 168 hours (7 days) after dosing.

Overall, the data suggested that the extent absorption of bupivacaine was similar among female and male dogs given an intra-articular (stifle joint) dose of 19.8, 66.0, or 198.0 mg of bupivacaine in SABER-Bupivacaine. Overall exposure (AUC) was dose responsive but Cmax did not noticeably increase from the mid to high dose.

Mean peak plasma concentrations (Cmax) of bupivacaine observed at 0.5 hours (Tmax) after dosing in the low dose group (group 3 - 16.8 mg bupivacaine) were 876 ng/mL and 808 ng/mL for female and male dogs, respectively. Mean AUC values were 2961 hr·ng/mL and 2750 hr·ng/mL for female and male dogs, respectively. Mean Cmax values for the mid dose group (group 4 – 66 mg bupivacaine) observed at 0.5 to 1 hour after dosing were 1386 ng/mL and 1524 ng/mL for female and male dogs, respectively. Mean AUC values were 3.1- and 3.2-fold higher than those observed in the low dose group for female and male dogs (8831 and 9146 hr·ng/mL), respectively. Mean Cmax values in the high dose group (group 5 – 198 mg bupivacaine) were only slightly higher (females, 1879 ng/mL) or were lower (males, 1099 ng/mL) than those observed for the mid dose group with Tmax at 2 hours. Mean AUC values for female and male dogs were approximately 8- to 12-fold higher than those observed for the 10-fold lower dose group (23261 and 36227 hr·ng/mL, respectively). Estimated mean half-lives of bupivacaine in plasma were 3.1-3.4 hours for the low dose groups, 13.8-15 hours for the mid dose groups, and 18.7-18.9 hours for the high dose groups of males and females. These differences in the apparent half-life of bupivacaine among animals in the different dose groups were reported to be reflective of differences in the phase of elimination characterized, and not to dose-related changes in the elimination/metabolism of bupivacaine. (see tables – half-lives not listed)

Summary of Pharmacokinetic Parameters Calculated from Plasma Concentrations of Bupivacaine: Males

Group Sex		Tmax[a] (hr)	Cmax[b] (ng/mL)	HL_Lambda_z[c] (hr)	AUC_{last}[d] (hr·ng/mL)	AUC_{INF_obs}[e] (hr·ng/mL)
3M	Mean	0.5	808	3.1	2750	2800
	SD	0.0	182	0.9	773	729
4M	Mean	0.7	1524	13.8	8831	8964
	SD	0.3	391	12.6	1593	1526
5M	Mean	1.9	1099	18.9	23261	23686
	SD	1.3	379	6.4	7042	7275

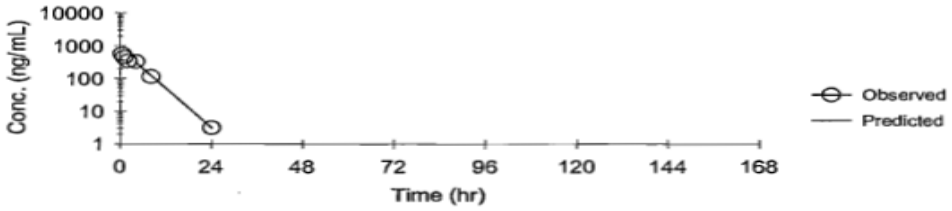
Summary of Pharmacokinetic Parameters Calculated from Plasma Concentrations of Bupivacaine: Females

Group Sex		Tmax[a] (hr)	Cmax[b] (ng/mL)	HL_Lambda_z[c] (hr)	AUC_{last}[d] (hr·ng/mL)	AUC_{INF_obs}[e] (hr·ng/mL)
3F	Mean	0.5	876	3.4	2961	2986
	SD	0.0	333	0.4	1033	1053
4F	Mean	0.7	1386	15.0	9146	9188
	SD	0.3	334	6.7	2353	2354
5F	Mean	2	1879	18.7	36227	36556
	SD	1.2	1122	3.4	12592	12433

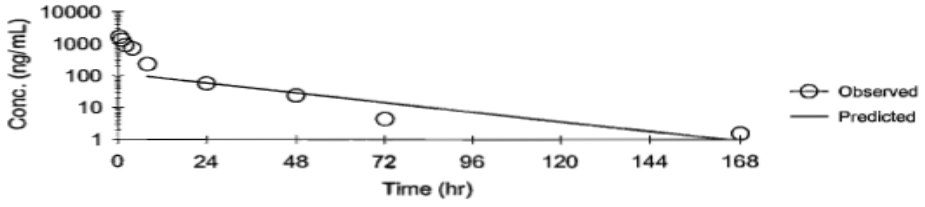
[a] Time of maximum plasma concentration of bupivacaine
[b] Maximum plasma concentration of bupivacaine
[c] Half-life of the terminal elimination phase
[d] Area under the plasma drug concentration versus time curve calculated from 0 to the last time point bupivacaine was quantifiable in plasma
[e] Area under the plasma drug concentration versus time curve calculated from 0 to infinity
Nominal Dose: Group 3 – 19.8 mg (SABER-Bupivacaine) Group 4 – 66.0 mg (SABER-Bupivacaine) Group 5 – 198.0 mg (SABER-Bupivacaine)

Plasma bupivacaine levels generally declined over a 24-hour period for the low dose and for a 72-hour period for the mid and high doses with bupivacaine not detectable at 168 hours post treatment as evidence by the figures of select individual animals.

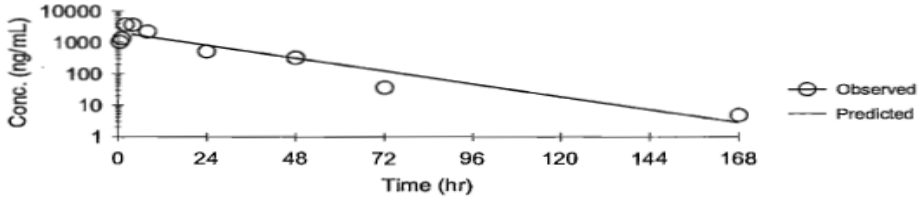
Low dose male



Mid dose female



High dose female



Dosing Solution Analysis - Both pre-dose and post-dose active formulation samples were analyzed for appearance, potency, and degradation products. The results show that the SABER-Bupivacaine remained clear, light yellow brown in color, and percent label strength assays show that the product was chemically stable during the animal dosing period. Total degradation of pre-dose samples was 0.53% and that of post-dose samples was 0.65%. Both pre-dose and post-dose SABER placebo samples were analyzed for appearance. The placebo formulation remained as clear, colorless solution, and absence of bupivacaine was confirmed.

Peri-sciatic nerve administration

A single-dose toxicity study with peri-sciatic nerve administration of bupivacaine solutions, pastes, or solids in the Sprague Dawley rat followed by a 7-day recovery period (study 022-010 – non-GLP) - Five rats per groups served in each of 14 dose groups with each animal receiving either a bupivacaine solution, solid, or paste formulation near the area of the sciatic nerve in the left thigh according to the following table with attention to groups 1 to 5 as being most relevant to the proposed SABER-Bupivacaine drug product:

Study Design

Group	Treatment	Bupivacaine Dose (mg)	Dose Amount	Number of Animals
1	0.9% Sodium Chloride for injection	0	250 µl	5
2	Sensorcaine®-MPF (0.5%)	1.25	250 µl	5
3	SAIB/BA – Placebo	0	200 µl	5
4	SAIB/BA – 12%	24	200 µl	5
5	SAIB/BA – 12%	48	400 µl	5
6	Vehicle control (solid form) SAIB/Span 80/F68/Mannitol (25 / 6.25 / 18.75 / 50%)	0	250 mg	5
7	SAIB/Span 80/F68/Mannitol/Bupivacaine (20 / 5 / 15 / 40 / 20%)	25	125 mg	5
8	SAIB/Span 80/F68/Mannitol/Bupivacaine (20 / 5 / 15 / 40 / 20%)	50	250 mg	5
9	SAIB/Span 80/F68/PVP-C15 (solid form) (31.25 / 6.25 / 18.75 / 43.75%)	0	250 mg	5
10	SAIB/Span 80/F68/PVP-C15/Bupivacaine (25 / 5 / 15 / 35 / 20%)	50	250 mg	5
11	Vehicle control (thick paste) SAIB/H_2O/Solutol HS-15 (25 / 25 / 25%)	0	250 µl	5
12	SAIB/H_2O/Solutol HS-15/Bupivacaine (20 / 20 / 40 / 20%)	50	250 µl	5
13	Vehicle control (thick paste) SAIB/H_2O/F68/Sorbitol (25 / 25 / 37.5 / 12.5%)	0	250 µl	5
14	SAIB/H_2O/F68/Sorbitol/Bupivacaine (20 / 20 / 30 / 10 / 20%)	50	250 µl	5

Body weights were recorded on the day of dosing and at necropsy. Food consumption values were recorded 5 days prior to dosing and for the week following test article administration. Clinical signs and a neurological examination were recorded daily. The neurological evaluation

Body weights were recorded on the day of dosing and at necropsy. Food consumption values were recorded 5 days prior to dosing and for the week following test article administration. Clinical signs and a neurological examination were recorded daily. The neurological evaluation included evaluation of toe pinch, proprioception, and flexor withdrawal reflex as well as an observation of gait.

All the animals were sacrificed 7 days (Day 8) after dose administration and the muscles and sciatic nerves from both hind legs were collected and placed into 10% neutral buffered formalin. The treated legs from animals in Groups 1-3, 5, 6, 8, 13, and 14 were analyzed histopathologically by PAI. One control leg from each group was also evaluated.

The administration of bupivacaine by both peri-sciatic injection and placement of a solid rod or paste produced a nerve block 3 hours post administration. The neurological signs appeared to resolve in most animals by Day 2. Some animals receiving 50 mg bupivacaine paste continued to have a proprioceptive deficit through Day 4. The gait of animals that received 24 mg or 48 mg by peri-sciatic injection appeared to be the most severely affected with 3 of 5 24 mg animals and 1 of 5 48 mg animals displaying an abnormal gait through Day 8 (proposed drug product).

Local administration of SABER-Bupivacaine as a single injection at a dose of 48 mg followed by a seven day recovery period resulted in an increased frequency of neuronal inflammation and axonal degeneration compared to untreated (right leg), saline treated, or Sensorcaine® treated groups. This effect appeared to result from both the presence of the vehicle as well as the bupivacaine since the frequency and/or severity of axonal changes showed incremental increases from control (right leg), to saline, to Sensorcaine®, to bupivacaine-treated groups. In addition, the dosage form appeared to play a role with the greatest neuronal inflammation and axonal degeneration occurring in the solution administration group. Inflammation within the intermuscular fasciae and perineural connective tissues and myofiber degeneration and regeneration also showed incremental increases in frequency and severity between the untreated control (right leg), vehicle, and test article groups. In contrast, these extra-neural changes were less frequent and severe compared to the paste/solid forms.

Histopathological evaluation revealed the highest frequency of neuronal inflammation and axonal degeneration and the lowest frequency of extraneural changes in the bupivacaine treated peri-sciatic injection groups.

	Group 1, 0 mg						Group 2, 1.25 mg						Group 3, 0 mg						Group 5, 48 mg					
Tissue/Diagnosis	001	002	003	004	005	INC	006	007	008	009	010	INC	011	012	013	014	015	INC	021	022	023	024	025	INC
SCIATIC NERVE	N		N		N	3	N	N		N	N	4	N				N	2		N				1
Axonal Degeneration		1>		1)		2						0			2>	2>		2			1>	3>	4]	3
Inflammation, Subacute, Lymphohistiocytic, Perineurial Connective Tissue						0			1)			1		1)		2)		2			2>	3]	4]	3
Inflammation, Subacute, Mixed, Perineurial Connective Tissue						0						0			3]			1	1>					1
Inflammation, Subacute, Lymphohistiocytic, Endoneurium						0						0			1]	2>		2			1>	2>	2]	3
Inflammation, Subacute, Lymphohistiocytic, Epineurium-Perineurium						0						0			1]	2>		2			1>	2>	2]	3
Hemorrhage, Perineurial Connective Tissue						0			1>			1						0						0
MUSCLE	N	N	N	N	N	5		N		N	N	3	N	N	N	N	N	5		N	N			2
Increased Endomyseal and Perimyseal Space						0			1)			1						0				2]	1]	2
Degeneration, Myofiber						0						0						0				1>	1>	2
Regeneration, Myofiber						0	1>		2]			2						0				3]	2]	2
Inflammation, Subacute, Lymphohistiocytic, Endo-Perimycium						0			1>			1						0				3]	1]	2
Inflammation, Subacute, Lymphohistiocytic, Intermuscular Fascia						0						0						0	1>			3]	1]	3
Hemorrhage, Intermuscular Fascia						0						0						0				1>		1

The animals receiving either the paste or solid dosage forms had swelling of the affected limb on Day 2 but not thereafter. The incidence of swelling appeared greater in the animals receiving bupivacaine compared to the control animals. The animals receiving the peri-sciatic injection did not experience swelling.

Mean body weights for all animals increased during the study. The animals showing the least amount of weight gain (24.2 g and 24.8 g) were animals in Groups 4 and 5 dosed with 24 mg or 48 mg 12% SAIB/BA by peri-sciatic injection compared with a 35.4 gram gain for the SAIB/BA Placebo. Food consumption values were also slightly lower in these peri-sciatic injection animals. Animals in Groups 4 and 5 (24 mg or 48 mg 12% SAIB/BA) had the lowest mean food consumption at 182.0 and 189.6 grams/week, respectively compared to 195.9 grams/week for the SAIB/BA Placebo group.
There were no gross lesions observed at necropsy. The test material was not evident at the time of dissection.

The peri-sciatic injection of bupivacaine resulted in the greatest neuronal inflammation and axonal degeneration. All treated groups showed an increase in neuronal inflammation and axonal degeneration with increased severity in the test article treated animals compared to the control animals receiving the same form of test article. The local administration of the bupivacaine paste showed the least amount of neuronal inflammation and axonal degeneration and the neuronal response of the animals receiving the solid form of bupivacaine was intermediate between the peri-sciatic injection and the paste dosage forms.

Conversely, extraneural inflammation and myofiber degeneration/regeneration appeared to have the greatest effect in the bupivacaine paste groups (control and treated). The bupivacaine solid groups had an intermediate effect and the peri-sciatic injection groups had the least frequent and severe changes of all the dosage forms. However, changes in the surrounding muscle appeared to be reversible given the large percentage of regenerating myofibers in the affected areas.

Administration of bupivacaine solutions, solids, and pastes produced an expected nerve block as evidenced by the neurological examination at least 3 hours after dosing. The block appeared to have resolved by Day 2. Swelling was evident in the peri-sciatic placement animals in both the control and treated animals on Day 2. The peri-sciatic injection animals did not have swelling of the treated leg. There were minor decreases in body weight gain and food consumption in the 12% SAIB/BA peri-sciatic injection animals. Histopathological evaluation revealed the highest frequency of neuronal inflammation and axonal degeneration and the lowest frequency of extraneural changes in the bupivacaine treated peri-sciatic injection groups. By contrast, the peri-sciatic placement animals receiving the paste showed the greatest extraneural inflammation and myofiber degeneration/regeneration and the least amount of neuronal inflammation and axonal degeneration.

The changes in the surrounding muscle however, appear to be reversible. The peri-sciatic placement of the solid form of bupivacaine resulted in neuronal inflammation, axonal degeneration, extraneural inflammation and myofiber degeneration/regeneration that were intermediate between the peri-sciatic injection and the paste forms of bupivacaine. Therefore, it is estimated that the peri-sciatic placement of bupivacaine paste produces a nerve block with the least amount of damage to the nerve.

In summary, relative to the proposed drug product, injected liquids SABER-Bupivacaine and SABER placebo produced increased local effects to the sciatic nerve compared to the saline, Bupivacaine HCl, and negative control (untreated nerve) groups. This has been described in other studies as an anticipated foreign body reaction due to the SAIB, but in this case, the bupivacaine combined with SAIB and BA (SABER-Bupivacaine) appears to contribute to a greater local reaction.

6.2 Repeat-Dose Toxicity

Study title: 4-Week Toxicity and Toxicokinetic Study of SABER™-Bupivacaine after Subcutaneous Administration in the Rat

- “aged”, yellow drug product

Study no.:	BR1265 (b) (4)
Study report location:	eCTD (DARRTS SDN 64, submitted June 9, 201), volume 1 (page 68) through volume 2
Conducting laboratory and location:	(b) (4)

(b) (4)

Date of study initiation:	November 26, 2008
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	SABER-Bupivacaine - batch GLP-803-19Feb04-01 - pre-aged drug 12% Bupivacaine, 66% Sucrose Acetate Isobutyrate (SAIB), and 22% Benzyl alcohol (BA), the release specification of the proposed drug product

The test material is “aged” (stored for 54 months) and exceeds the yellow color specifications of released drug product with increased levels of degradants. This situation is intentional in order to qualify the aged, more yellow drug product.

Each vial contains 8 mL liquid containing 120 mg bupivacaine/g liquid, which is equivalent to 132 mg bupivacaine/mL liquid (label strength).

According to the testing results the solution contains:

Bupivacaine - (b) (4)% of label strength

(b) (4)

SAIB (sucrose acetate isobutyrate) – (b) (4)% by weight ((b) (4)% of label strength)

Benzyl alcohol (BA) – apparently not analyzed

(b) (4)

2) <u>SABER placebo</u>
- batch GLP-803-17Feb04-01
- release composition 75% SAIB and 25% BA

Each vial contains 8 mL liquid. According to the documentation, the liquid contains no bupivacaine.

According to the testing results the solution contains:

Bupivacaine - absent

SAIB (sucrose acetate isobutyrate) – (b) (4)% by weight ((b) (4)% of label strength

Benzyl alcohol (BA) – apparently not analyzed

Key Study Findings

- Groups of 10 male and female SD rats received weekly subcutaneous injections of 0 (2.0 mL/kg), 102 (0.75 mL/kg), or 240 (2.0 mL/kg) mg/kg SABER-Bupivacaine for 4 weeks (days 1, 8, 15, & 22) followed by sacrifice on days 29-30 (1 week after last dose) and day 85 (8-week after terminal sacrifice recovery group, n = 8 for placebo and high dose group). The test material was an “aged” material (54 months) that exceeded acceptable yellow color specifications and had increased levels of impurities. At release, the test material met recipe specifications of the proposed drug product (12% bupivacaine, 66% Sucrose Acetate Isobutyrate, & 22% Benzyl Alcohol). At testing, the aged test material contained (b) (4)% bupivacaine label strength and (b) (4)% of label strength for SAIB.
- No bupivacaine-related deaths or clinical symptoms were observed.
- The only notable treatment-related effect was at the site of injection where all dose groups were affected.
- Weekly injections of the placebo or the SABER-Bupivacaine solutions resulted in the macroscopic findings of small wounds, cysts or nodules at the injection sites. These findings were diagnosed as chronic granulomatous inflammations.
- After an 8-week recovery period, the incidence and severity of these findings were reduced to a maximum of moderate severity compared to the animals autopsied in week 5 which exhibited up to a severe severity. Injections site effects were reversing but were still present 8 weeks after dosing. The sponsor described this as a normal wound healing process.

- The systemic NOAEL is 240 mg/kg and there was no local toxicity/injection site NOAEL. The low dose of 102 mg/kg could be a LOAEL with reversal of local toxicity, but local toxicity was not fully reversed at 8 weeks after the last dose.
- Based on reported blood levels with no toxicokinetic value calculations, blood levels are no greater than 441 & 695 ng/mL in males and 491 & 768 ng/mL in females for the low and high dose groups, respectively,. The high dose mean value was 731 ng/mL

Methods

Doses: 0, 102, & 240 mg/kg

Frequency of dosing: Weekly for 4 weeks (days 1, 8, 15, & 22)

Group	Label	Dose of SABER-Bupivacaine® (GLP-803-19Feb04-01) mg/kg	Administration Volume ml/kg	Animals (n) M/F
1		0 (vehicle)	2.0	10/10
	0-0	0 (vehicle)	2.0	8/8
2		102	0.75	10/10
3		240	2.0	10/10
	240-0	240	2.0	8/8
4	TK	0 (vehicle)	2.0	5/5
5	TK	102	0.75	10/10
6	TK	240	2.0	10/10

- 0-0 animals from group 1 & 240-0 animals from group 3 were recovery animals

Route of administration: Subcutaneous

Injections were rotated each day:
Day 1 – over the right hind leg
Day 8 – over the right foreleg
Day 15 – over the lift hind leg
Day 22 – over the left foreleg

Dose volume: 0 & 240 mg/kg (2.0 mL/kg), 102 mg/kg (0.75 mL/kg)

Formulation/Vehicle: Placebo SABER-Bupivacaine
- drug supplied for use as is by sponsor DURECT Corporation (aged 54 months)

Species/Strain: Sprague Dawley (Crl:CD(SD)IGSBR)

Number/Sex/Group: 10/sex/group for main study animals (0, 102, &

	240 mg/kg groups sacrificed on day 28 – 1) and 8/sex/group for recovery animals (0 and 240 mg/kg sacrificed on day 85 – 8 weeks after last dose)
Age:	6 weeks old at start of study
Weight:	Males on day 1: 171 – 241 g Females on day 1: 130 – 190 g
Satellite groups:	Toxicokinetic (TK) groups at 5/sex for placebo and 10/sex for 1-02 and 240 mg/kg dose groups
Unique study design:	Note that there is BA and (b) (4) in the placebo drug
Deviation from study protocol:	The test item SABER-Bupivacaine GLP-803-19Feb04-01 contained (b) (4) mg bupivacaine/g liquid, which is equivalent to 132 mg bupivacaine/mL liquid (label strength). As the animals were dosed according to volume (0.75 mL/kg or 2 mL/kg) and not weight, the animals in groups 2 and 5 actually received doses of 99 mg/kg (instead of (b) (4) mg/kg) and the animals in groups 3 and 6 received doses of 264 mg/kg (instead of (b) (4) mg/kg).

Methods, Observations, and Results

All animals were checked immediately, 0.5, 3, 6 and 24 hours after administration on treatment days, or 3 times a day on treatment-free days or once daily on weekends.

Mortality

One male (day 22) and the 1 female (day 1) from that low dose TK group died after convulsions just after dosing on the listed days. As these two animals were toxicokinetic animals for bupivacaine, tissues were fixed but not evaluated after gross necropsy. While symptoms were consistent with opioid effects, these mortalities were not considered of toxicological relevance and could have been a result of handling during dosing as no dose response was evident, controls were affected, and no other animals were affected.

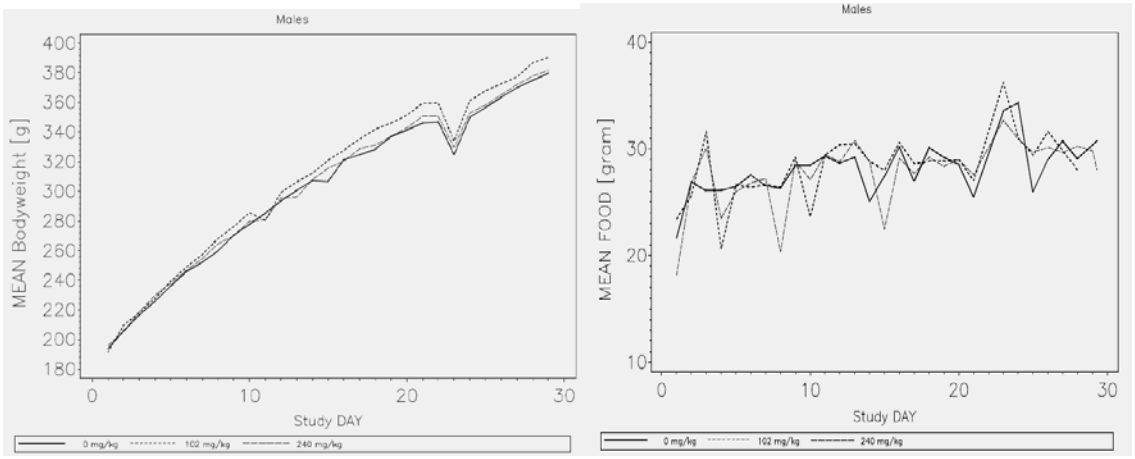
Clinical Signs

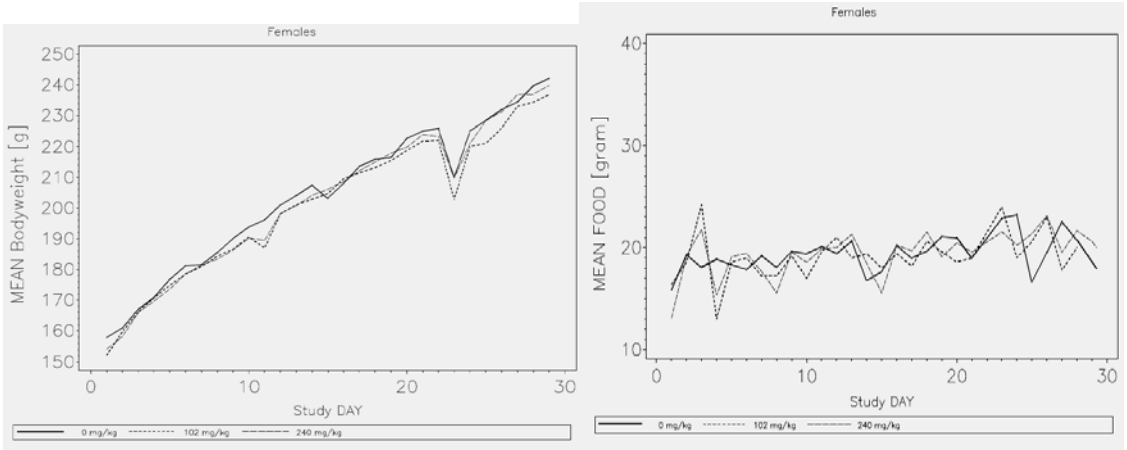
The toxicological relevance of observed clinical is unknown because there was a lack of any dose-response and controls were affected for the clinical signs of marked transient ataxia, convulsions, lying in an abdominal position, and lying in a lateral position observed after dosing in 2 control females and 2 low dose toxicokinetic (TK) rats (1 female and 1 male).

Body Weights and Food Consumption

Body weights were measured on days 1–29 daily for all animals and then weekly on days 29–85 (recovery groups) and days 29–42 (toxicokinetic groups). Food consumption was measured on days 1–29 daily for all main study animals and then weekly on days 29–85 (recovery groups). No food consumption was measured for toxicokinetic groups.

Mean food consumption was reduced at times during the study in a dose-responsive manner, mostly in the high dose animals, without an associated effect on body weights (see tables), putting in question the toxicological relevance of this observation. Some animals were without water for days 22 & 23 possibly resulting in a body weight downward spike at that time.





Water Consumption

Water consumption was measured on days 1–29 daily for all main study animals and then weekly on days 29–85 (recovery groups). No water consumption was measured for toxicokinetic groups.

No treatment related effects were observed for water consumption.

Ophthalmoscopy – not evaluated

ECG – not evaluated

Hematology

Parameters evaluated only in groups 1, 2, & 3 on days 6 or 7, 23, & 85 included hematocrit, erythrocytes, leukocytes, hemoglobin, thrombocytes, MCHC, MCH, MCV, reticulocytes, differential blood count, thromboplastin time and partial thromboplastin time.

Slight changes were observed with no dose response pattern. In the recovery period, no significant increases or decreases in these parameters were observed. Changes observed in the hematology parameters were considered to be of no toxicological relevance as effects were slight, not dose responsive, and reversible.

Clinical Chemistry

Parameters evaluated only in groups 1, 2, & 3 on days 6 or 7, 23, & 85 (no TK animals) included glucose, total protein, albumin, urea, creatinine, bilirubin, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (AP), glutamate dehydrogenase (GLDH), sodium (Na+), potassium (K+), chloride (Cl-), and calcium (Ca++).

Blood glucose increases of ~10-20% compared to control were observed in males and females of all dose groups with the increase at 15% after recovery. Creatinine was increased 42% compared to controls in both dose groups of males with no difference at recovery. These changes were not considered toxicologically relevant by the reviewer as they were isolated and had no correlation with other toxicological parameters (e.g., histology).

Urinalysis – not evaluated

Gross Pathology

Necropsies were conducted Groups 1, 2, & 3 on days 29/30, 7 or 8 days after the last administration. The animals in the recovery groups were necropsied on day 85/86, 8 weeks after the last treatment day. Surviving TK animals were sacrificed on day 42 after

the final blood TK sampling without necropsy. Adipose tissue around the kidneys and adrenals was graded for adiposity.

Gross observations were almost entirely related to the treatment site and there appeared to be no difference between placebo and treated animals (0 mg/kg is placebo).

Removal Reason: Terminal kill	MALES			FEMALES		
	0 mg/kg	102 mg/kg	240 mg/kg	0 mg/kg	102 mg/kg	240 mg/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(9)	(9)	(10)	(10)
SKIN;						
Submitted	(0)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	3	5	1	2	5
Adhesion; left; Foreleg	0	0	0	1	0	0
Alopecia	1	0	0	0	0	0
Alopecia; dorsal	0	0	0	0	1	1
Alopecia; right	1	0	0	0	0	0
Cyst; Foreleg; single	1	0	0	0	0	0
Cyst; firm; Hind leg; left; single	2	0	0	0	0	0
Cyst; left; Foreleg	0	0	0	1	0	0
Cyst; left; Foreleg; single	1	0	0	0	0	0
Cyst; left; Foreleg; few	1	0	0	2	0	0
Cyst; left; Hind leg; single	2	0	0	3	0	0
Cyst; left; Hind leg; few	2	0	0	1	0	0
Cyst; right; Foreleg; single	1	0	0	3	0	0
Cyst; right; Foreleg; few	2	0	0	1	0	0
Cyst; right; Foreleg; multiple	1	0	0	0	0	0
Cyst; right; Hind leg; single	1	0	0	3	0	0
Cyst; right; Hind leg; few	1	0	0	1	0	0
Cyst; right; Hind leg; multiple	1	0	0	0	0	0
Focus (Spot); black; Hind leg; right; single	0	0	0	1	0	0
SKIN; (continued)						
Focus (Spot); brown; Foreleg; left	0	0	2	0	0	1
Focus (Spot); brown; Hind leg; right	0	0	0	0	0	1
Hardened (Firm); roughened; Foreleg; right; single	0	0	0	1	0	0
Hardened (Firm); left; Foreleg; single	1	0	0	0	0	0
Hematoma; brown; Foreleg; right	1	0	0	0	0	0
Hematoma; brown; Hind leg; left; single	0	0	0	1	0	0
Hematoma; left; Foreleg	0	0	0	1	0	0
Hematoma; left; Foreleg; few	0	0	0	1	0	0
Hematoma; right; Foreleg; single	1	0	0	0	0	0
Necrosis	0	0	0	1	0	0
Nodule; blue; Foreleg; right	0	0	0	1	0	0
Nodule; brown; Foreleg; left	0	0	1	0	0	0
Nodule; brown; Foreleg; right	0	0	1	0	0	0
Nodule; brown; Hind leg; left	0	0	1	0	0	0
Nodule; brown; Hind leg; left; single	0	0	0	0	0	1
Nodule; dark; Foreleg; hemorrhagic; left; single	0	0	0	1	0	0
Nodule; firm; single	1	0	0	0	0	0
Nodule; firm; Foreleg; glassy; right; single	0	0	0	1	0	0
Nodule; firm; Foreleg; left; single	1	3	0	2	0	0
Nodule; firm; Foreleg; left; few	1	0	0	0	0	0
Nodule; firm; Foreleg; right; single	0	3	0	0	0	0
Nodule; firm; Hind leg; glassy; left; single	0	0	0	1	0	0
Nodule; firm; Hind leg; left; single	0	2	0	1	1	0
Nodule; left; Foreleg	0	0	1	0	0	0
Nodule; left; Foreleg; few	1	0	0	0	0	0
Injury/Wound/Scratch; left; Foreleg	0	0	1	0	4	2
Injury/Wound/Scratch; left; Foreleg; single	0	1	0	0	2	0
Injury/Wound/Scratch; left; Foreleg; few	0	0	0	0	1	0
Injury/Wound/Scratch; left; Hind leg	0	1	1	0	0	1
Injury/Wound/Scratch; left; Hind leg; single	0	1	0	0	0	0
Injury/Wound/Scratch; right; Foreleg	0	0	0	0	0	2
Thickened; brown; Foreleg; right; single	1	0	0	0	0	0
Thickened; dark; Hind leg; left; single	1	0	0	0	0	0

For recovery animals, there was a significant decrease in gross observations, suggesting recovery ongoing eight weeks after the last dose.

Removal Reasons: All of those SELECTED	MALES 0 mg/kg	MALES 240 mg/kg	FEMALES 0 mg/kg	FEMALES 240 mg/kg
Number of Animals on Study :	8	8	8	8
Number of Animals Completed:	(8)	(7)	(7)	(8)
SKIN;				
Submitted	(0)	(0)	(0)	(0)
No Visible Lesions	6	3	6	5
Alopecia; Head; single	0	1	0	0
Alopecia; Tail	0	1	0	0
Alopecia; dorsal	0	1	0	1
Cyst; dorsal; single	1	0	0	0
Cyst; dorsal; few	0	1	0	0
Cyst; left; Foreleg; single	0	0	1	0
Cyst; left; Hind leg; single	0	0	1	0
Cyst; right; Foreleg; few	0	1	0	0
Nodule; brown; Foreleg; left; single	0	1	0	0
Nodule; brown; Hind leg; fluid-filled; glassy; yellow; left; right; single	0	0	0	1
Nodule; granular; Foreleg; left; single	0	0	0	1
Injury/Wound/Scratch; dorsal	0	1	0	0
Injury/Wound/Scratch; left; Foreleg	0	1	0	0
Injury/Wound/Scratch; right; Hind leg	1	0	0	0

Organ Weights

The following organ weights were measured: adrenals, brain, heart (without blood), kidneys, liver (without blood), lungs, ovaries, spleen, stomach, testes, thymus, and uterus. The pituitary, seminal vesicles, prostate, and thyroids were measured after fixation.

Decreases of absolute and relative heart, liver, lung, and kidney weights were observed in both dose groups compared to placebo that ranged from <10 up to 20% during treatment and less after recovery. These changes were not always dose-responsive. In the clinical observations and in the histopathology, no functional or morphological correlations were observed for the organ weight changes. Therefore the changes in organ weights were considered to be of no toxicological relevance.

Histopathology

The following organs of groups 1 & 3 were fully evaluated (see table). For other groups, including recovery animals, only the injection sites were evaluated.

Organ / Tissue	Histopathology	
	Groups 1 + 3 (terminal kill) All animals	Groups 1 + 3 (recovery kill) Group 2 Animal number
ADMINISTRATION SITE	X	X
ADRENAL GLAND	X	
AORTA	X	
BONE	X	
BONE MARROW	X	
BRAIN	X	
CERVIX	X	
COAGULATING GLAND	X	
EPIDIDYMIS	X	
ESOPHAGUS	X	
EYE	X	
HARDERIAN GLAND	X	
HEART	X	
INTESTINE	X	
JOINT	X	
KIDNEY	X	
LACRIMAL GLAND	X	
LIVER	X	9
LUNG	X	
LYMPH NODE (mesenteric)	X	
MAMMARY GLAND	X	
NERVE; SCIATIC	X	
OVARY	X	
OVIDUCT	X	
PANCREAS	X	
PARATHYROID GLAND	X	
PITUITARY GLAND	X	
PROSTATE GLAND	X	
SALIVARY GLAND (parotid, mandibular + sublingual)	X	

Organ / Tissue	Histopathology	
	Groups 1 + 3 (terminal kill) All animals	Groups 1 + 3 (recovery kill) Group 2 Animal number
SEMINAL VESICLE	X	
SKELETAL MUSCLE	X	
SKIN	X	3, 12
SPINAL CORD	X	
SPLEEN	X	
STOMACH	X	
TESTIS	X	
THYMUS	X	
THYROID GLAND	X	
TONGUE	X	
TRACHEA	X	
URINARY BLADDER	X	
UTERUS	X	
VAGINA	X	

X = all animals examined

Adequate Battery – yes Peer Review – yes (internal)

Histological Findings

There was no evidence of systemic toxicity with the drug or with the vehicle. The only notable histological findings that are considered to be treatment related were observed at the injection site. To this end, evaluation of only the injection sites of recovery animals was at least partly justified, but that's speculation. Subcutaneous tissue exhibited granulomatous inflammation (foreign body granuloma). This minimal to severe granulation tissue (inflammatory changes consistent with wound healing) was observed a week after the last dose (5 weeks) with no clear difference between placebo, low dose, and high dose groups (see main study table).

Main study (week 5) results were as follows (0 mg/kg is placebo):

Observations: Neo-Plastic and Non Neo-Plastic	MALES			FEMALES		
Removal Reasons: All of those SELECTED	0 mg/kg	102 mg/kg	240 mg/kg	0 mg/kg	102 mg/kg	240 mg/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
ADMINISTRATION SITE;						
Examined	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	1	0	0	1	0	0
Granulation Tissue; subcutis; Foreleg; pseudocyst; left; focal	(6)	(8)	(8)	(8)	(9)	(10)
minimal	0	0	3	1	2	0
mild	0	3	3	3	2	4
moderate	3	5	2	2	3	5
severe	3	0	0	2	2	1
Granulation Tissue; subcutis; Foreleg; pseudocyst; right; focal	(6)	(8)	(10)	(8)	(8)	(9)
minimal	2	7	2	0	0	0
mild	1	1	3	2	6	5
moderate	3	0	4	5	1	3
severe	0	0	1	1	1	1
Granulation Tissue; subcutis; Hind leg; pseudocyst; left; focal	(7)	(10)	(9)	(6)	(9)	(7)
minimal	4	5	3	0	1	1
mild	0	4	3	4	4	4
moderate	3	1	3	1	3	1
severe	0	0	0	1	1	1
Granulation Tissue; subcutis; Hind leg; pseudocyst; right; focal	(1)	(6)	(7)	(8)	(6)	(9)
minimal	1	5	1	0	1	1
mild	0	1	4	2	5	3
moderate	0	0	2	6	0	4
severe	0	0	0	0	0	1

The recovery groups showed a reduction in the incidence and severity of inflammatory cell infiltrates and granulation tissue as only 1 of 24 (4%) recovery animals exhibited severe effects compared to 8 of 60 (13%) main study animals (see table). The sponsor described this as the normal wound healing process. Additionally, no signs of cellular death (i.e. necrotic changes) were observed histologically. However, there is concern that 7 of 32 (22%) animals exhibit moderate effects 8 weeks after the last injection.

Recovery (week 13) results were as follows:

Observations: Neo-Plastic and Non Neo-Plastic	MALES		FEMALES	
Removal Reasons: All of those SELECTED	0 mg/kg	240 mg/kg	0 mg/kg	240 mg/kg
Number of Animals on Study :	8	8	8	8
Number of Animals Completed:	(8)	(8)	(8)	(8)
ADMINISTRATION SITE;				
Examined	(8)	(8)	(8)	(8)
Within Normal Limits	0	1	0	0
Granulation Tissue; subcutis; Foreleg; pseudocyst; left; focal	(4)	(6)	(5)	(8)
minimal	0	1	0	1
mild	2	5	2	5
moderate	2	0	3	2
Granulation Tissue; subcutis; Foreleg; pseudocyst; right; focal	(5)	(5)	(6)	(7)
minimal	0	3	1	2
mild	3	0	2	5
moderate	2	2	3	0
Granulation Tissue; subcutis; Hind leg; pseudocyst; left; focal	(6)	(6)	(4)	(8)
minimal	2	4	1	2
mild	3	2	2	5
moderate	1	0	0	1
severe	0	0	1	0
Granulation Tissue; subcutis; Hind leg; pseudocyst; right	(1)	(0)	(0)	(0)
mild	1	0	0	0
Granulation Tissue; subcutis; Hind leg; pseudocyst; right; focal	(7)	(7)	(6)	(7)
minimal	0	3	3	3
mild	3	4	2	4
moderate	4	0	1	0

In summary, injection site effects were reduced in incidence and severity form week 5 to week 13, with no apparent difference between the placebo, low dose, and high dose groups, suggesting the vehicle and the incision may be responsible for the prolonged inflammation.

Special Evaluation - none

Toxicokinetics

Blood levels of bupivacaine in TK groups were measured at 0, 0.5, & 4 hours and 3, 7, 14, 21, 35, 42 days post-dose as follows:

Test day	Time and number of animals
1	0 h (predose): blood from all rats in groups 4, 5 and 6
1	0.5 h after 1st dose: blood from 1 M/1 F in group 4 and 2 M/2 F each from groups 5 and 6
1	4 h after 1st dose: blood from 1 M/1 F in group 4 and 2 M/2 F each from groups 5 and 6
2	24 h (1 day) after 1st dose: blood from 1 M/1 F in group 4 and 2 M/2 F each from groups 5 and 6
3	48 h (2 days) after 1st dose: blood from 1 M/1 F in group 4 and 2 M/2 F each from groups 5 and 6
7	144 h (6 days) after 1st dose (1 day before 2nd dose): blood from 1 M/1 F in group 4 and 2 M/2 F each from groups 5 and 6
14	144 h (6 days) after 2nd dose (1 day before 3rd dose): blood from all rats in groups 4, 5 and 6
21	144 h (6 days) after 3rd dose (1 day before 4th dose): blood from all rats in groups 4, 5 and 6
28	144 h (6 days) after 4th dose: blood from all rats in groups 4, 5 and 6
35	312 h (13 days) after 4th dose: blood from all rats in groups 4, 5 and 6
42	480 h (20 days) after 4th dose: blood from all rats in groups 4, 5 and 6

Plasma concentrations of bupivacaine were highest following the first subcutaneous dose of SABER-Bupivacaine to male and female rats at both dose levels. Levels were the highest up to 48 hours following the subcutaneous dose (see table). After 6 days (144 hours after the first injection), 7 of 8 treated rats in groups 5 and 6 still had measurable plasma levels of bupivacaine. At 144 hours after the 2nd, 3rd and 4th injections, the mean values of the animals in groups 5 and 6 were comparable to the values measured at 144 hours after the first injection, demonstrating the continued

exposure to the test item over time. The plasma concentrations then fell considerably at 312 and 480 hours after the last administration, and for most rats in group 5, the plasma concentrations were below the detection limit at these later times.

Plasma concentrations (µg/L) of bupivacaine in rats

Mean values for day 1 at 0 h (predose) and for days 14 through 42. For other times, values are from 1 animal (group 4) or a mean of 2 animals (groups 5 and 6).

Day	Hours after injection	Males / Group number			Females / Group number		
		4	5	6	4	5	6
1	0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
1	0.5	< 1.0	437	695	7.1	435	768
1	4	< 1.0	441	568	< 1.0	491 [3)]	481
2	24	< 1.0	321	435	< 1.0	157	505
3	48	< 1.0	84.9	231	< 1.0	79.6	278
7	144	< 1.0	6.8 [1)]	18.4	< 1.0	2.8	59.4
14	144	< 1.0	11.5	46.2	< 1.0	12.1	34.3
21	144	< 1.0	9.6	73.6	< 1.0	6.4	62.8
28	144	< 1.0	14.2	92.4	< 1.0	13.8	64.2
35	312	< 1.0	2.9 [2)]	10.6	< 1.0	1.3 [4)]	3.8
42	480	< 1.0	< 1.0	2.2	< 1.0	< 1.0	1.3 [2)]

1) 1 rat; the other rat had a value of < 1.0
2) mean of 2 rats (8 rats had a value of < 1.0)
3) 1 rat
4) 1 rat (8 rats had a value of < 1.0)

NOTE: units of µg/L = ng/mL

Cmax appears to be no greater than 441 & 695 ng/mL in males and 491 & 768 ng/mL in females for the low and high dose groups, respectively. Other than blood levels, no other TK values were reported/calculated. An approximately proportional increase in 2 out of 4 animals was seen shortly after the first dose, when the dose was increased from 102 to 240 mg/kg. At later time points and after three additional administrations, plasma concentrations of bupivacaine increased more than proportionally with the dose. The last dose of SABER-Bupivacaine was on day 21. There were no major gender differences in plasma concentrations of bupivacaine.

Dosing Solution Analysis

For BA and (b) (4), dosing was as follows:

Placebo: 2.0 mL/kg at 1.3 mg/mL BA (2.6 mg/kg total) and 0.9 mg/mL (b) (4) (1.8 mg/kg total)
Low dose: 0.75 mL/kg at 21.0 mg/mL BA (15.8 mg/kg total) and 9.3 mg/mL (b) (4) (7.0 mg/kg total)
High dose: 2.0 mL/kg at 21.0 mg/mL (42 mg/kg total) and 9.3 mg/mL (b) (4) (18.6 mg/kg total)

7 Genetic Toxicology

Note: For the pharmacological active ingredient, bupivacaine, a 505(b)(2) reference is made to the approved Marcaine (bupivacaine hydrochloride - NDA 16-964) label which has no genotoxicity data. The listed genetic toxicology studies reviewed in this section include *in vitro* Ames and chromosomal aberration assays for Bupivacaine free base and the degradants (b) (4). An *in vivo* micronucleus assay was conducted for SABER-Bupivacaine and SABER placebo.

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

Study title: *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay With A Confirmatory Assay
- with Bupivacaine Free Base

Study no.:	7116-116
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 5, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Bupivacaine Base, batch 971526, lot F0083, purity 99.0%

Key Study Findings

- Bupivacaine base was tested for mutagenicity at 33.3, 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of S9 mix in tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and in *Escherichia coli* WP2*uvr*A (initial and confirmatory assays).
- The results of the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay indicate that under the conditions of this study, the test article, Bupivacaine free base, did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9) in a valid assay.

Methods

Strains:	tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 and the *Escherichia coli* tryptophan auxotroph WP2*uvr*A
Concentrations in definitive study:	33.3, 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of S9 mix

(initial and confirmatory assays).

Basis of concentration selection: Doses tested in the mutagenicity assay were selected based on the results of the dose range-finding assay conducted on the test article using tester strains TA100 and WP2*uvr*A in both the presence and absence of S9 mix with one plate per dose. Ten doses of test article, Bupivacaine free base, from 6.67 to 5000 µg per plate. Since little or no cytotoxicity was observed in the dose range-finding study, the highest dose level of test article used in the mutagenicity assays was the same dose as that tested in the range-finding study.

Negative control: Dimethylsulfoxide (DMSO)

Positive control:

Positive Controls			
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*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Formulation/Vehicle: DMSO

Incubation & sampling time: 52 ± 4 hours at 37 ± 2 ^{o}C
(plates not immediately evaluated were stored at 0 to 10 ^{o}C)

Study Validity

Study was considered valid as tester strain integrity (*rfa* Wall Mutation was present for *Salmonella typhimurium*, pKM101 plasmid presence for TA98 & TA100, characteristic number of spontaneous revertants), tester strain culture density, positive control values in absence of S9 (mutagen identification) and presence of S9 (S9 mix integrity), and a minimum of three non-toxic doses for evaluation occurred.

Results

Dose Range-finding Assay – No cytotoxicity was observed with tester strain WP2*uvr*A in the presence or absence of S9 mix as evidenced by no dose-related decrease in the number of revertants per plate and normal bacterial background lawns. Slight decreases in revertant counts were observed with tester strain TA100 in the presence and absence of S9 mix (see table).

Dose Range-finding Study

Test Article ID: Bupivacaine free base

Assay No.: 28280-0-409OECD | Trial No.: A1

Date Plated: 19-Apr-06 | Vehicle: DMSO

Date Counted: 24-Apr-06 | Plating Aliquot: 50 µL

		Revertants per Plate			
	Dose/Plate	TA100	Background Lawn[a]	WP2*uvr*A	Background Lawn[a]
Microsomes: Rat Liver					
Vehicle Control		89	N	21	N
Test Article	6.67 µg	98	N	8	N
	10.0 µg	80	N	9	N
	33.3 µg	84	N	15	N
	66.7 µg	81	N	10	N
	100 µg	79	N	14	N
	333 µg	81	N	7	N
	667 µg	71	N	10	N
	1000 µg	72	N	15	N
	3330 µg	78	N	8	N
	5000 µg	61	N	11	N
Microsomes: None					
Vehicle Control		82	N	10	N
Test Article	6.67 µg	70	N	17	N
	10.0 µg	95	N	9	N
	33.3 µg	72	N	18	N
	66.7 µg	69	N	12	N
	100 µg	78	N	8	N
	333 µg	78	N	12	N
	667 µg	60	N	9	N
	1000 µg	64	N	7	N
	3330 µg	60	N	11	N
	5000 µg	51	N	6	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

Initial Mutagenicity Assay - The results of the dose range-finding study were used to select the doses tested in the mutagenicity assay. The doses tested with all tester strains were 33.3, 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of S9 mix. No positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix (see table).

Mutagenicity Assay Results – Summary

Test Article ID: Bupivacaine free base

Assay No.: 28280-0-409OECD | Trial No.: B1

Date Plated: 02-May-06 | Vehicle: DMSO

Date Counted: 05-May-06 | Plating Aliquot: 50 µL

	Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
		TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		23	8	115	3	10	3	6	1	15	3	N
Test Article	33.3 µg	21	5	98	9	7	4	9	2	13	3	N
	100 µg	18	2	95	7	9	4	5	3	12	4	N
	333 µg	23	2	105	9	7	1	7	1	8	6	N
	1000 µg	21	3	114	11	11	2	3	1	7	5	N
	3330 µg	20	5	92	22	7	3	5	4	8	2	N
	5000 µg	19	2	84	4	8	2	3	3	5	1	N
Positive Control[b]		236	35	506	49	88	9	78	10	214	14	N
Microsomes: None												
Vehicle Control		12	5	76	14	6	4	5	1	14	6	N
Test Article	33.3 µg	19	7	86	9	11	3	3	1	10	2	N
	100 µg	10	2	72	9	10	4	4	2	13	4	N
	333 µg	12	4	69	13	7	2	6	3	10	1	N
	1000 µg	13	5	70	13	9	1	2	2	11	5	N
	3330 µg	13	4	59	8	9	5	3	4	8	2	N
	5000 µg	9	2	69	14	9	3	2	1	7	2	N
Positive Control[c]		305	28	657	77	537	35	291	53	205	64	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b]
TA98	benzo[a]pyrene	2.5 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate

[c]
TA98	2-nitrofluorene	1.0 µg/plate
TA100	sodium azide	2.0 µg/plate
TA1535	sodium azide	2.0 µg/plate
TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Confirmatory Mutagenicity Assay - The results of the dose range-finding study were used to select the doses tested in the mutagenicity assay. The doses tested with all tester strains were 33.3, 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of S9 mix. No positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix (see table). Yes, same text as for Initial Mutagenicity Assay.

Mutagenicity Assay Results – Summary

Test Article ID: Bupivacaine free base

Assay No.: 28280-0-409OECD | Trial No.: C1

Date Plated: 16-May-06 | Vehicle: DMSO

Date Counted: 02-Jun-06 | Plating Aliquot: 50 µL

	Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
		TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		15	3	92	6	12	6	5	3	16	3	N
Test Article	33.3 µg	20	4	88	13	10	1	6	1	15	2	N
	100 µg	13	5	79	4	16	4	5	3	16	2	N
	333 µg	12	2	87	5	10	1	4	1	15	3	N
	1000 µg	18	5	92	11	8	2	6	3	18	1	N
	3330 µg	13	4	79	14	14	6	3	2	14	5	N
	5000 µg	17	5	73	16	11	5	4	4	8	1	N
Positive Control[b]		116	14	483	52	57	9	58	11	206	39	N
Microsomes: None												
Vehicle Control		12	5	81	6	12	5	7	5	10	1	N
Test Article	33.3 µg	7	2	91	17	15	3	5	3	16	9	N
	100 µg	8	2	75	7	8	2	6	2	14	6	N
	333 µg	10	5	81	3	9	7	4	2	14	4	N
	1000 µg	9	3	64	13	15	4	4	3	14	2	N
	3330 µg	9	2	64	4	16	5	5	2	9	0	N
	5000 µg	8	2	63	10	15	9	6	1	9	3	N
Positive Control[c]		230	74	794	181	504	64	205	37	221	8	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b]

TA98	benzo[a]pyrene	2.5 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate

[c]

TA98	2-nitrofluorene	1.0 µg/plate
TA100	sodium azide	2.0 µg/plate
TA1535	sodium azide	2.0 µg/plate
TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

CONCLUSION - The results of the *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay indicate that under the conditions of this study, the test article, Bupivacaine free base, did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9) in a valid assay.

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Study title: Bacterial Reverse Mutation Assay with a Confirmatory Assay - with (b) (4)

Study no.: (05-10-803-Y-VO-AM; 8223757)
Study report location: eCTD in DARRTS

Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 8, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Dimethylsulfoxide (DMSO), lot 07796KK, 99.97% pure

Key Study Findings

- (b) (4) was tested for mutagenicity at 1.60, 5.00, 16.0, 50.0, 160, 500, 1600, & 5000 µg/plate (initial assay) and 50.0, 160, 500, 1600, & 5000 µg/plate (confirmatory assay) in the presence and absence of S9 mix in tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and in E*scherichia coli* WP2*uvr*A.
- The results of the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay indicate that under the conditions of this study, the test article (b) (4), did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9) in a valid assay.

Methods

Strains:	tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 and the *Escherichia coli* tryptophan auxotroph WP2*uvr*A
Concentrations in definitive study:	1.60, 5.00, 16.0, 50.0, 160, 500, 1600, & 5000 µg/plate (initial assay) and 50.0, 160, 500, 1600, & 5000 µg/plate (confirmatory assay) in the presence and absence of S9 mix
Basis of concentration selection:	Log doses with testing at maximum limit dose of 5000 µg/plate for initial assay then 50.0, 160, 500, 1600, and 5000 µg/plate (confirmatory assay) based on initial assay results
Negative control:	Dimethylsulfoxide (DMSO)

Positive control:

Positive Control Articles

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)	CAS No.	Lot No.
TA98	-	2-nitrofluorene	1.0	607-57-8	01508BE
TA100, TA1535	-	sodium azide	2.0	26628-22-8	017K0136
TA1537	-	ICR-191	2.0	17070-45-0	116K1026
WP2*uvr*A	-	4-nitroquinoline-N-oxide	1.0	56-57-5	117K1485
TA98	+	benzo[a]pyrene	2.5	50-32-8	087K0733
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5	613-13-8	12317CE
WP2*uvr*A	+	2-aminoanthracene	25.0	613-13-8	12317CE

Formulation/Vehicle: DMSO

Incubation & sampling time: 52 ± 4 hours at 37 ± 2 ^{o}C
(plates not immediately evaluated were stored at 0 to 10 ^{o}C)

Study Validity

Study was considered valid as tester strain integrity (*rfa* Wall Mutation was present for *Salmonella typhimurium*, pKM101 plasmid presence for TA98 & TA100, characteristic number of spontaneous revertants), tester strain culture density, positive control values in absence of S9 (mutagen identification) and presence of S9 (S9 mix integrity), and a minimum of three non-toxic doses for evaluation occurred.

Results

Dose Range-finding Assay – None conducted.

Initial Mutagenicity Assay - Inhibited growth (characterized by a reduced background lawn and/or a decrease in revertant frequency) was observed in all five tester strains at the highest one or two doses evaluated +/- S9, with the exception of tester strain TA98 with S9, in which inhibited growth was observed at the top three doses. Revertant frequencies for all doses of [redacted] (b) (4), in all tester strains with and without S9, approximated or were less than those observed in the concurrent vehicle control cultures (see tables 1 and 2).

Table 1
Initial Mutagenicity Assay Results with S9

Study No.: 8223757
Trial No.: 8223757-B1
Plating Method: Plate incorporation assay

Date Plated: 2/11/2010
Date Counted: 2/15/2010 to 2/16/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*16.5*	*2.1*	*1.1*	18 R, 15 R
		1600	*17.5*	*2.1*	*1.2*	16 R, 19 R
		500	*23.5*	*9.2*	*1.6*	30 M R, 17 M R
		160	*20.0*	*5.7*	*1.3*	24 M N, 16 M N
		50.0	*17.5*	*2.1*	*1.2*	19 N, 16 N
		16.0	*16.5*	*2.1*	*1.1*	18 N, 15 N
		5.00	*14.0*	*0.0*	*0.9*	14 N, 14 N
		1.60	*22.0*	*4.2*	*1.5*	25 M N, 19 N
	Dimethyl Sulfoxide		*15.0*	*2.8*		13 M N, 17 N
TA100	(b) (4)	5000	*76.0*	*19.8*	*0.9*	90 R, 62 M R
		1600	*80.0*	*8.5*	*1.0*	86 R, 74 R
		500	*77.0*	*2.8*	*1.0*	75 N, 79 N
		160	*93.0*	*18.4*	*1.2*	106 N M, 80 N M
		50.0	*84.0*	*4.2*	*1.0*	87 N, 81 N
		16.0	*70.0*	*4.2*	*0.9*	67 M N, 73 N
		5.00	*82.0*	*8.5*	*1.0*	76 N, 88 N
		1.60	*94.5*	*12.0*	*1.2*	86 N M, 103 N M
	Dimethyl Sulfoxide		*80.5*	*3.5*		83 M N, 78 N
TA1535	(b) (4)	5000	*8.5*	*3.5*	*0.7*	6 N, 11 N
		1600	*7.0*	*2.8*	*0.6*	9 N, 5 N
		500	*11.0*	*2.8*	*0.9*	13 N, 9 M N
		160	*9.5*	*0.7*	*0.8*	9 M N, 10 M N
		50.0	*8.5*	*0.7*	*0.7*	8 M N, 9 N
		16.0	*10.5*	*0.7*	*0.8*	11 N, 10 N
		5.00	*14.5*	*0.7*	*1.2*	15 N, 14 N
		1.60	*10.0*	*2.8*	*0.8*	12 M N, 8 N
	Dimethyl Sulfoxide		*12.5*	*6.4*		17 M N, 8 M N

Key to Plate Postfix Codes

R	Reduced background bacterial lawn
M	Plate counted manually
N	Normal background bacterial lawn

Table 1 (cont.)
Initial Mutagenicity Assay Results with S9

Study No.: 8223757
Trial No.: 8223757-B1 — Date Plated: 2/11/2010
Plating Method: Plate incorporation assay — Date Counted: 2/15/2010 to 2/16/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA1537	(b) (4)	5000	*7.0*	*2.8*	*1.8*	5 R M, 9 R M
		1600	*6.0*	*1.4*	*1.5*	5 M R, 7 M R
		500	*4.5*	*0.7*	*1.1*	5 M N, 4 M N
		160	*4.5*	*2.1*	*1.1*	3 M N, 6 M N
		50.0	*3.0*	*0.0*	*0.8*	3 N, 3 N
		16.0	*6.0*	*1.4*	*1.5*	5 M N, 7 M N
		5.00	*6.5*	*0.7*	*1.6*	6 N, 7 N
		1.60	*6.5*	*2.1*	*1.6*	5 N, 8 N
	Dimethyl Sulfoxide		*4.0*	*1.4*		3 M N, 5 M N
WP2*uvr*A	(b) (4)	5000	*14.0*	*1.4*	*0.8*	13 M N, 15 N
		1600	*22.5*	*0.7*	*1.3*	23 N, 22 N
		500	*17.5*	*0.7*	*1.0*	17 N, 18 N
		160	*17.5*	*0.7*	*1.0*	17 N, 18 N
		50.0	*14.5*	*2.1*	*0.8*	16 N, 13 N
		16.0	*10.5*	*0.7*	*0.6*	11 N, 10 N
		5.00	*16.5*	*2.1*	*0.9*	15 N, 18 N
		1.60	*13.0*	*4.2*	*0.7*	16 N, 10 N
	Dimethyl Sulfoxide		*17.5*	*6.4*		22 N, 13 M N
TA98	**BP**	2.5	*340.5*	*89.8*	*22.7*	277 N, 404 N
TA100	**2AA**	2.5	*1043.5*	*188.8*	*13.0*	910 N, 1177 N
TA1535	**2AA**	2.5	*129.0*	*11.3*	*10.3*	137 N, 121 N
TA1537	**2AA**	2.5	*97.5*	*17.7*	*24.4*	110 N, 85 M N
WP2*uvr*A	**2AA**	25.0	*444.0*	*103.2*	*25.4*	371 N, 517 N

Key to Positive Controls		Key to Plate Postfix Codes	
BP	Benzo{a}pyrene	R	Reduced background bacterial lawn
2AA	2-aminoanthracene	M	Plate counted manually
		N	Normal background bacterial lawn

Table 2
Initial Mutagenicity Assay Results without S9

Study No.: 8223757
Trial No.: 8223757-B1
Plating Method: Plate incorporation assay
Date Plated: 2/11/2010
Date Counted: 2/15/2010 to 2/16/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*2.0*	*0.0*	*0.2*	2 M R, 2 M R
		1600	*3.0*	*2.8*	*0.3*	5 M R, 1 M R
		500	*8.0*	*1.4*	*0.7*	7 N, 9 N
		160	*16.0*	*8.5*	*1.4*	10 N, 22 M N
		50.0	*11.0*	*1.4*	*1.0*	10 N, 12 M N
		16.0	*12.0*	*1.4*	*1.0*	11 N, 13 N
		5.00	*12.5*	*4.9*	*1.1*	16 M N, 9 N
		1.60	*13.5*	*0.7*	*1.2*	14 N, 13 N
	Dimethyl Sulfoxide		*11.5*	*3.5*		14 N, 9 M N
TA100	(b) (4)	5000	*0.5*	*0.7*	*0.0*	1 M R, 0 M R
		1600	*58.0*	*5.7*	*0.8*	54 R, 62 R
		500	*62.5*	*6.4*	*0.9*	58 M N, 67 N
		160	*76.0*	*18.4*	*1.1*	89 N, 63 N
		50.0	*66.0*	*18.4*	*0.9*	53 M N, 79 N
		16.0	*65.5*	*0.7*	*0.9*	66 N, 65 N
		5.00	*74.0*	*2.8*	*1.0*	76 N, 72 N
		1.60	*78.0*	*2.8*	*1.1*	80 N, 76 N
	Dimethyl Sulfoxide		*72.0*	*2.8*		74 N, 70 N
TA1535	(b) (4)	5000	*0.0*	*0.0*	*0.0*	0 M R, 0 M R
		1600	*6.0*	*5.7*	*0.6*	10 M R, 2 M R
		500	*11.0*	*0.0*	*1.2*	11 N, 11 N
		160	*14.5*	*0.7*	*1.5*	15 N, 14 N
		50.0	*13.0*	*2.8*	*1.4*	15 M N, 11 M N
		16.0	*14.0*	*5.7*	*1.5*	18 N, 10 N
		5.00	*10.0*	*0.0*	*1.1*	10 N, 10 N
		1.60	*11.0*	*2.8*	*1.2*	9 N, 13 M N
	Dimethyl Sulfoxide		*9.5*	*0.7*		10 N, 9 M N

Key to Plate Postfix Codes

M	Plate counted manually
R	Reduced background bacterial lawn
N	Normal background bacterial lawn

Table 2 (cont.)
Initial Mutagenicity Assay Results without S9

Study No.: 8223757
Trial No.: 8223757-B1 Date Plated: 2/11/2010
Plating Method: Plate incorporation assay Date Counted: 2/15/2010 to 2/16/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA1537	(b) (4)	5000	*0.0*	*0.0*	*0.0*	0 R M, 0 R M
		1600	*3.0*	*0.0*	*0.5*	3 M R, 3 M R
		500	*5.0*	*0.0*	*0.8*	5 N, 5 N
		160	*2.0*	*0.0*	*0.3*	2 N, 2 N
		50.0	*5.5*	*0.7*	*0.8*	6 N, 5 M N
		16.0	*2.0*	*1.4*	*0.3*	3 N, 1 N
		5.00	*4.0*	*1.4*	*0.6*	3 N, 5 N
		1.60	*6.5*	*0.7*	*1.0*	6 N, 7 N
	Dimethyl Sulfoxide		*6.5*	*0.7*		6 N, 7 N
WP2*uvrA*	(b) (4)	5000	*6.5*	*4.9*	*0.7*	3 M R, 10 M R
		1600	*13.5*	*3.5*	*1.4*	16 N, 11 N
		500	*11.5*	*3.5*	*1.2*	9 M N, 14 N
		160	*24.0*	*0.0*	*2.5*	24 N, 24 N
		50.0	*15.0*	*2.8*	*1.6*	17 N, 13 N
		16.0	*7.5*	*0.7*	*0.8*	7 N, 8 N
		5.00	*13.5*	*3.5*	*1.4*	11 N, 16 N
		1.60	*18.0*	*0.0*	*1.9*	18 N, 18 N
	Dimethyl Sulfoxide		*9.5*	*0.7*		9 N, 10 N
TA98	**2NF**	1.0	*301.5*	*27.6*	*26.2*	321 N, 282 N
TA100	**SA**	2.0	*975.0*	*58.0*	*13.5*	934 N, 1016 N
TA1535	**SA**	2.0	*843.0*	*4.2*	*88.7*	840 N, 846 N
TA1537	**ICR**	2.0	*144.0*	*12.7*	*22.2*	153 N, 135 N
WP2*uvrA*	**4NQO**	1.0	*252.5*	*47.4*	*26.6*	286 N, 219 N

Key to Positive Controls		Key to Plate Postfix Codes	
2NF	2-nitrofluorene	M	Plate counted manually
SA	sodium azide	R	Reduced background bacterial lawn
ICR	ICR-191	N	Normal background bacterial lawn
4NQO	4-nitroquinoline-N-oxide		

Confirmatory Mutagenicity Assay – As in the initial assay, inhibited growth was observed in all five tester strains at the highest 1 or 2 doses evaluated +/- S9. Of note is that tester strain TA1537 exhibited no growth at doses ≥500 µg/plate with S9, proposed to be technical error. Revertant frequencies for all doses of (b) (4) in all tester strains +/- S9, were again approximate or less than control values (see tables 3 and 4). Based upon the assumed technical error in the trial with TA1537, (b) (4) was re-evaluated in tester strain TA1537 with S9. This time, only slightly reduced revertant number and reduced background lawn was observed at the highest dose tested. Regardless, revertant frequencies for all doses of (b) (4) again approximated control values (see table 5).

Table 3
Confirmatory Mutagenicity Assay Results with S9

Study No.: 8223757
Trial No.: 8223757-C1
Plating Method: Plate incorporation assay
Date Plated: 2/17/2010
Date Counted: 2/19/2010 to 2/22/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*15.7*	*6.5*	*0.7*	16 R, 9 R, 22 R
		1600	*23.0*	*5.2*	*1.1*	26 R, 26 R, 17 R
		500	*20.7*	*4.2*	*1.0*	16 N, 22 N, 24 N
		160	*28.7*	*1.5*	*1.3*	29 N, 30 N, 27 N
		50.0	*20.3*	*5.1*	*1.0*	16 N, 26 N, 19 N
		16.0	*20.7*	*1.5*	*1.0*	22 N, 21 N, 19 N
	Dimethyl Sulfoxide		*21.3*	*4.6*		24 N, 16 N, 24 N
TA100	(b) (4)	5000	*84.0*	*8.7*	*0.8*	94 R, 79 R, 79 R
		1600	*97.3*	*4.9*	*0.9*	95 N, 103 N, 94 N
		500	*107.7*	*12.2*	*1.0*	97 N, 121 N, 105 N
		160	*101.3*	*2.5*	*0.9*	101 N, 104 N, 99 N
		50.0	*90.7*	*10.1*	*0.8*	92 N, 80 N, 100 N
		16.0	*93.7*	*11.5*	*0.8*	94 N, 105 N, 82 N
	Dimethyl Sulfoxide		*110.7*	*0.6*		110 N, 111 N, 111 N
TA1535	(b) (4)	5000	*11.7*	*1.2*	*1.0*	13 R, 11 R, 11 R
		1600	*11.3*	*1.5*	*1.0*	11 M R, 10 R, 13 R
		500	*14.3*	*4.2*	*1.2*	13 N, 11 N, 19 N
		160	*9.7*	*4.5*	*0.8*	14 N, 5 N, 10 N
		50.0	*13.3*	*6.4*	*1.1*	17 N, 17 N, 6 N
		16.0	*10.7*	*4.2*	*0.9*	12 N, 14 N, 6 M N
	Dimethyl Sulfoxide		*11.7*	*6.0*		11 N, 18 N, 6 N

Key to Plate Postfix Codes

R	Reduced background bacterial lawn
N	Normal background bacterial lawn
M	Plate counted manually
A	Absence of background bacterial lawn

Table 3 (cont.)
Confirmatory Mutagenicity Assay Results with S9

Study No.: 8223757
Trial No.: 8223757-C1
Plating Method: Plate incorporation assay
Date Plated: 2/17/2010
Date Counted: 2/19/2010 to 2/22/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA1537	(b) (4)	5000	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		1600	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		500	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		160	*8.7*	*1.5*	*0.9*	7 N, 9 N, 10 N
		50.0	*12.0*	*2.0*	*1.2*	10 N, 12 N, 14 N
		16.0	*13.0*	*2.0*	*1.3*	13 N, 11 N, 15 N
	Dimethyl Sulfoxide		*10.0*	*2.6*		7 N, 11 N, 12 N
WP2*uvr*A	(b) (4)	5000	*11.7*	*3.5*	*0.8*	15 R, 8 R, 12 R
		1600	*13.0*	*2.0*	*0.8*	11 R, 13 R, 15 R
		500	*16.7*	*2.1*	*1.1*	16 N, 19 N, 15 N
		160	*18.0*	*8.5*	*1.2*	10 N, 17 N, 27 N
		50.0	*18.3*	*3.2*	*1.2*	16 N, 17 N, 22 N
		16.0	*14.3*	*1.2*	*0.9*	15 N, 13 N, 15 N
	Dimethyl Sulfoxide		*15.3*	*2.1*		13 N, 16 N, 17 N
TA98	**BP**	2.5	*322.3*	*48.1*	*15.1*	376 N, 308 N, 283 N
TA100	**2AA**	2.5	*1427.7*	*73.2*	*12.9*	1344 N, 1480 N, 1459 N
TA1535	**2AA**	2.5	*197.0*	*11.5*	*16.9*	206 N, 201 N, 184 N
TA1537	**2AA**	2.5	*152.3*	*29.7*	*15.2*	118 N, 170 N, 169 N
WP2*uvr*A	**2AA**	25.0	*379.7*	*28.7*	*24.8*	357 N, 412 N, 370 N

Key to Positive Controls	
BP	Benzo{a}pyrene
2AA	2-aminoanthracene

Key to Plate Postfix Codes	
R	Reduced background bacterial lawn
N	Normal background bacterial lawn
M	Plate counted manually
A	Absence of background bacterial lawn

Table 4
Confirmatory Mutagenicity Assay Results without S9

Study No.: 8223757
Trial No.: 8223757-C1
Plating Method: Plate incorporation assay
Date Plated: 2/17/2010
Date Counted: 2/19/2010 to 2/22/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*2.0*	*1.0*	*0.1*	2 M R, 3 M R, 1 M R
		1600	*6.3*	*1.5*	*0.4*	6 M R, 8 M R, 5 M R
		500	*18.3*	*9.1*	*1.0*	10 N, 28 N, 17 N
		160	*20.0*	*7.5*	*1.1*	12 N, 21 N, 27 N
		50.0	*17.3*	*3.1*	*1.0*	20 N, 14 N, 18 N
		16.0	*12.3*	*4.7*	*0.7*	16 N, 14 N, 7 M N
	Dimethyl Sulfoxide		*17.7*	*6.5*		24 N, 11 N, 18 N
TA100	(b) (4)	5000	*11.3*	*7.6*	*0.1*	13 M R, 18 M R, 3 M R
		1600	*76.7*	*3.2*	*0.9*	78 R, 79 R, 73 R
		500	*77.0*	*6.2*	*0.9*	79 N, 82 N, 70 N
		160	*88.0*	*3.6*	*1.0*	85 N, 87 N, 92 N
		50.0	*72.7*	*9.8*	*0.8*	67 N, 84 N, 67 N
		16.0	*86.3*	*15.3*	*1.0*	77 N, 78 N, 104 N
	Dimethyl Sulfoxide		*87.0*	*16.4*		101 N, 91 N, 69 N
TA1535	(b) (4)	5000	*3.3*	*3.1*	*0.2*	0 M R, 4 M R, 6 M R
		1600	*10.7*	*2.1*	*0.6*	13 R, 10 R, 9 M R
		500	*13.0*	*4.6*	*0.7*	8 M N, 17 N, 14 N
		160	*13.0*	*3.6*	*0.7*	17 N, 12 N, 10 N
		50.0	*8.3*	*4.5*	*0.4*	4 M N, 8 M N, 13 M N
		16.0	*19.0*	*1.7*	*1.0*	17 N, 20 N, 20 N
	Dimethyl Sulfoxide		*18.7*	*3.8*		17 N, 23 N, 16 N
TA1537	(b) (4)	5000	*3.0*	*1.7*	*0.3*	1 M R, 4 M R, 4 M R
		1600	*4.0*	*0.0*	*0.4*	4 M R, 4 M R, 4 M R
		500	*8.7*	*0.6*	*0.9*	9 M N, 8 M N, 9 N
		160	*9.0*	*3.0*	*0.9*	12 N, 9 N, 6 N
		50.0	*7.3*	*4.5*	*0.8*	12 N, 7 N, 3 N
		16.0	*5.3*	*0.6*	*0.6*	5 N, 6 M N, 5 M N
	Dimethyl Sulfoxide		*9.7*	*4.5*		5 N, 14 N, 10 N

Key to Plate Postfix Codes

M	Plate counted manually
R	Reduced background bacterial lawn
N	Normal background bacterial lawn

Table 4 (cont.)
Confirmatory Mutagenicity Assay Results without S9

Study No.: 8223757
Trial No.: 8223757-C1
Plating Method: Plate incorporation assay
Date Plated: 2/17/2010
Date Counted: 2/19/2010 to 2/22/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2*uvrA*	(b) (4)	5000	*10.0*	*1.7*	*0.7*	8 M R, 11 M R, 11 R
		1600	*9.7*	*4.0*	*0.7*	9 R, 6 M R, 14 R
		500	*12.7*	*3.2*	*0.9*	15 N, 9 N, 14 N
		160	*19.0*	*4.4*	*1.3*	17 N, 16 N, 24 N
		50.0	*12.7*	*3.1*	*0.9*	12 N, 16 N, 10 N
		16.0	*15.3*	*1.5*	*1.1*	17 N, 14 N, 15 N
	Dimethyl Sulfoxide		*14.3*	*2.9*		16 N, 11 N, 16 N
TA98	**2NF**	1.0	*195.7*	*47.6*	*11.1*	150 N, 192 N, 245 N
TA100	**SA**	2.0	*1093.0*	*135.7*	*12.6*	969 N, 1238 N, 1072 N
TA1535	**SA**	2.0	*607.3*	*518.3*	*32.5*	817 N, 988 N, 17 N
TA1537	**ICR**	2.0	*167.3*	*19.7*	*17.3*	145 N, 182 N, 175 N
WP2*uvrA*	**4NQO**	1.0	*143.0*	*7.0*	*10.0*	140 N, 151 N, 138 N

Key to Positive Controls		Key to Plate Postfix Codes	
2NF	2-nitrofluorene	M	Plate counted manually
SA	sodium azide	R	Reduced background bacterial lawn
ICR	ICR-191	N	Normal background bacterial lawn
4NQO	4-nitroquinoline-N-oxide		

Table 5
Re-test with S9

Study No.: 8223757
Trial No.: 8223757-D1
Plating Method: Plate incorporation assay
Date Plated: 2/25/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA1537	(b) (4)	5000	*5.3*	*2.3*	*0.9*	8 M R, 4 M R, 4 M R
		1600	*5.3*	*4.2*	*0.9*	10 M N, 4 M N, 2 M N
		500	*4.3*	*1.5*	*0.7*	4 M N, 3 M N, 6 M N
		160	*6.0*	*1.0*	*1.0*	6 M N, 7 M N, 5 M N
		50.0	*9.0*	*4.0*	*1.5*	9 M N, 13 M N, 5 M N
		16.0	*6.3*	*4.5*	*1.1*	11 M N, 2 M N, 6 M N
	Dimethyl Sulfoxide		*6.0*	*1.7*		5 M N, 5 M N, 8 M N
TA1537	**2AA**	2.5	*81.3*	*18.9*	*13.6*	103 M N, 73 M N, 68 M N

2AA	2-aminoanthracene	M	Plate counted manually
		R	Reduced background bacterial lawn
		N	Normal background bacterial lawn

CONCLUSION – (b) (4) was negative in the Bacterial Reverse Mutation Assay with a Confirmatory Assay under the conditions, and according to the criteria, of the test protocol in a valid assay.

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Study title: Bacterial Reverse Mutation Assay with a Confirmatory Assay
- with (b) (4)

Study no.:	03-10-803-X-VO-HCA; 8223262
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 8, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4), lot 6540-9, 99.98% pure (white powder) Dimethylsulfoxide (DMSO), lot 07796KK, 99.97% pure

Key Study Findings

- (b) (4) was tested for mutagenicity at 1.60, 5.00, 16.0, 50.0, 160, 500, 1600, & 5000 µg/plate (initial assay) and 50.0, 160, 500, 1600, & 5000 µg/plate (confirmatory assay) in the presence and absence of S9 mix in tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and in *Escherichia coli* WP2*uvr*A.
- The results of the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay indicate that under the conditions of this study, the test article (b) (4), did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9) in a valid assay.

Methods

Strains:	tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 and the *Escherichia coli* tryptophan auxotroph WP2*uvr*A
Concentrations in definitive study:	1.60, 5.00, 16.0, 50.0, 160, 500, 1600, & 5000 µg/plate (initial assay) and 50.0, 160, 500, 1600, & 5000 µg/plate (confirmatory assay) in the presence and absence of S9 mix
Basis of concentration selection:	Log doses with testing at maximum limit dose of 5000 µg/plate for initial assay then 50.0, 160, 500, 1600, and 5000 µg/plate (confirmatory assay) based on initial assay results
Negative control:	Dimethylsulfoxide (DMSO)

Positive control:

Positive Control Articles

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)	CAS No.	Lot No.
TA98	-	2-nitrofluorene	1.0	607-57-8	01508BE
TA100, TA1535	-	sodium azide	2.0	26628-22-8	017K0136
TA1537	-	ICR-191	2.0	17070-45-0	116K1026
WP2*uvr*A	-	4-nitroquinoline-N-oxide	1.0	56-57-5	117K1485
TA98	+	benzo[a]pyrene	2.5	50-32-8	087K0733
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5	613-13-8	12317CE
WP2*uvr*A	+	2-aminoanthracene	25.0	613-13-8	12317CE

Formulation/Vehicle: DMSO

Incubation & sampling time: 52 ± 4 hours at 37 ± 2 ^{o}C
(plates not immediately evaluated were stored at 0 to 10 ^{o}C)

Study Validity

Study was considered valid as tester strain integrity (*rfa* Wall Mutation was present for *Salmonella typhimurium*, pKM101 plasmid presence for TA98 & TA100, characteristic number of spontaneous revertants), tester strain culture density, positive control values in absence of S9 (mutagen identification) and presence of S9 (S9 mix integrity), and a minimum of three non-toxic doses for evaluation occurred.

Results

Dose Range-finding Assay – None conducted.

Initial Mutagenicity Assay - Normal growth was observed in all five tester strains, and the test article was found to be freely soluble in the aqueous top agar, at all doses evaluated with and without S9. Revertant frequencies for all doses of (b) (4) (b) (4) in all tester strains with and without S9, approximated or were less than those observed in the concurrent vehicle control cultures. (see table 1 for +S9 and table 2 for -S9 numerical results).

Table 1
Initial Mutagenicity Assay Results with S9

Study No.: 8223262
Trial No.: 8223262-B1
Plating Method: Plate incorporation assay
Date Plated: 2/11/2010
Date Counted: 2/15/2010 to 2/16/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*15.0*	*4.2*	*1.0*	12 N, 18 N
		1600	*16.0*	*4.2*	*1.0*	19 N, 13 M N
		500	*16.5*	*2.1*	*1.1*	15 M N, 18 N
		160	*15.5*	*3.5*	*1.0*	18 N, 13 N
		50.0	*15.5*	*4.9*	*1.0*	19 N, 12 M N
		16.0	*17.0*	*2.8*	*1.1*	19 N, 15 N
		5.00	*21.0*	*0.0*	*1.4*	21 N, 21 M N
		1.60	*22.0*	*1.4*	*1.4*	21 M N, 23 M N
	Dimethyl Sulfoxide		*15.5*	*3.5*		18 N, 13 N
TA100	(b) (4)	5000	*93.5*	*10.6*	*0.9*	101 N, 86 N
		1600	*82.0*	*7.1*	*0.8*	87 N, 77 N
		500	*98.0*	*9.9*	*1.0*	91 N, 105 N
		160	*90.5*	*12.0*	*0.9*	82 N, 99 N
		50.0	*100.5*	*2.1*	*1.0*	102 N, 99 M N
		16.0	*94.0*	*9.9*	*0.9*	87 N, 101 N
		5.00	*96.5*	*10.6*	*0.9*	104 N, 89 N
		1.60	*90.0*	*21.2*	*0.9*	75 M N, 105 N
	Dimethyl Sulfoxide		*102.5*	*7.8*		97 N, 108 N
TA1535	(b) (4)	5000	*12.5*	*2.1*	*1.1*	14 N, 11 N
		1600	*10.0*	*4.2*	*0.9*	7 N, 13 N
		500	*8.5*	*12.0*	*0.7*	0 M Ra, 17 N
		160	*6.0*	*1.4*	*0.5*	5 M N, 7 N
		50.0	*16.0*	*1.4*	*1.4*	15 N, 17 N
		16.0	*14.5*	*3.5*	*1.3*	17 N, 12 N
		5.00	*16.0*	*1.4*	*1.4*	17 N, 15 N
		1.60	*14.0*	*2.8*	*1.2*	12 N, 16 M N
	Dimethyl Sulfoxide		*11.5*	*2.1*		10 N, 13 N
TA1537	(b) (4)	5000	*5.0*	*1.4*	*0.8*	6 N, 4 N
		1600	*5.0*	*1.4*	*0.8*	4 M N, 6 N
		500	*3.5*	*0.7*	*0.6*	4 N, 3 N
		160	*7.5*	*0.7*	*1.3*	8 N, 7 N
		50.0	*2.5*	*2.1*	*0.4*	1 N, 4 M N
		16.0	*4.0*	*0.0*	*0.7*	4 M N, 4 N
		5.00	*8.0*	*1.4*	*1.3*	7 N, 9 N
		1.60	*5.5*	*2.1*	*0.9*	7 N, 4 M N
	Dimethyl Sulfoxide		*6.0*	*2.8*		4 N, 8 N

Key to Plate Postfix Codes

M	Plate counted manually
N	Normal background bacterial lawn
R	Reduced background bacterial lawn

a = The lone plate with reduced background is considered to be a spurious result, likely due to technical error.

Table 1 (cont.)
Initial Mutagenicity Assay Results with S9

Study No.: 8223262
Trial No.: 8223262-B1
Plating Method: Plate incorporation assay

Date Plated: 2/11/2010
Date Counted: 2/15/2010 to 2/16/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2*uvr*A	(b) (4)	5000	*11.5*	*2.1*	*1.0*	10 N, 13 N
		1600	*12.0*	*2.8*	*1.0*	10 M N, 14 N
		500	*13.5*	*2.1*	*1.1*	15 N, 12 M N
		160	*15.5*	*3.5*	*1.3*	18 N, 13 N
		50.0	*13.5*	*4.9*	*1.1*	10 M N, 17 N
		16.0	*13.0*	*0.0*	*1.1*	13 M N, 13 N
		5.00	*14.0*	*4.2*	*1.2*	11 M N, 17 N
		1.60	*15.0*	*8.5*	*1.3*	9 N, 21 N
	Dimethyl Sulfoxide		*12.0*	*4.2*		9 N, 15 N
TA98	**BP**	2.5	*342.0*	*31.1*	*22.1*	320 N, 364 N
TA100	**2AA**	2.5	*616.0*	*722.7*	*6.0*	1127 N, 105 M N[b]
TA1535	**2AA**	2.5	*260.5*	*24.7*	*22.7*	243 M N, 278 M N
TA1537	**2AA**	2.5	*111.5*	*23.3*	*18.6*	128 N, 95 N
WP2*uvr*A	**2AA**	25.0	*380.5*	*47.4*	*31.7*	347 N, 414 N

Key to Positive Controls		Key to Plate Postfix Codes	
BP	Benzo{a}pyrene	N	Normal background bacterial lawn
2AA	2-aminoanthracene	M	Plate counted manually
		R	Reduced background bacterial lawn

b = One replicate positive control value (TA100, 2AA) is considered to be a spurious result, likely due to technical error

Table 2
Initial Mutagenicity Assay Results without S9

Study No.: 8223262
Trial No.: 8223262-B1
Plating Method: Plate incorporation assay

Date Plated: 2/11/2010
Date Counted: 2/15/2010 to 2/16/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*14.5*	*6.4*	*1.2*	19 N, 10 N
		1600	*10.5*	*0.7*	*0.9*	10 N, 11 N
		500	*13.0*	*8.5*	*1.1*	7 N, 19 N
		160	*16.0*	*2.8*	*1.3*	18 N, 14 N
		50.0	*13.5*	*4.9*	*1.1*	10 N, 17 N
		16.0	*9.0*	*1.4*	*0.8*	8 M N, 10 N
		5.00	*14.5*	*4.9*	*1.2*	18 N, 11 N
		1.60	*12.5*	*0.7*	*1.0*	12 N, 13 N
	Dimethyl Sulfoxide		*12.0*	*4.2*		15 M N, 9 N
TA100	(b) (4)	5000	*77.5*	*2.1*	*1.1*	79 M N, 76 M N
		1600	*63.0*	*5.7*	*0.9*	67 M N, 59 M N
		500	*67.5*	*9.2*	*1.0*	74 M N, 61 M N
		160	*71.5*	*0.7*	*1.1*	72 M N, 71 N
		50.0	*87.5*	*6.4*	*1.3*	92 M N, 83 M N
		16.0	*78.5*	*4.9*	*1.2*	82 M N, 75 N
		5.00	*75.0*	*19.8*	*1.1*	61 M N, 89 M N
		1.60	*67.5*	*2.1*	*1.0*	66 M N, 69 M N
	Dimethyl Sulfoxide		*68.0*	*1.4*		69 N, 67 M N
TA1535	(b) (4)	5000	*13.5*	*0.7*	*1.5*	13 N, 14 N
		1600	*12.5*	*3.5*	*1.4*	10 N, 15 N
		500	*13.0*	*8.5*	*1.4*	7 N, 19 N
		160	*17.5*	*0.7*	*1.9*	18 N, 17 N
		50.0	*10.5*	*2.1*	*1.2*	12 N, 9 M N
		16.0	*11.5*	*2.1*	*1.3*	13 N, 10 M N
		5.00	*12.5*	*0.7*	*1.4*	12 N, 13 N
		1.60	*9.5*	*0.7*	*1.1*	10 N, 9 N
	Dimethyl Sulfoxide		*9.0*	*2.8*		11 N, 7 N
TA1537	(b) (4)	5000	*4.0*	*0.0*	*1.1*	4 M N, 4 N
		1600	*5.0*	*2.8*	*1.4*	7 N, 3 M N
		500	*5.5*	*2.1*	*1.6*	4 M N, 7 N
		160	*3.5*	*0.7*	*1.0*	3 N, 4 M N
		50.0	*5.0*	*1.4*	*1.4*	4 M N, 6 N
		16.0	*4.5*	*0.7*	*1.3*	4 M N, 5 M N
		5.00	*4.0*	*1.4*	*1.1*	5 M N, 3 M N
		1.60	*3.0*	*0.0*	*0.9*	3 M N, 3 N
	Dimethyl Sulfoxide		*3.5*	*3.5*		6 M N, 1 M N

Key to Plate Postfix Codes

M	Plate counted manually
N	Normal background bacterial lawn

Table 2 (cont.)
Initial Mutagenicity Assay Results without S9

Study No.: 8223262
Trial No.: 8223262-B1
Plating Method: Plate incorporation assay
Date Plated: 2/11/2010
Date Counted: 2/15/2010 to 2/16/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2*uvrA*	(b) (4)	5000	*11.5*	*2.1*	*1.0*	10 N, 13 N
		1600	*9.5*	*0.7*	*0.8*	10 N, 9 N
		500	*13.5*	*3.5*	*1.1*	11 M N, 16 N
		160	*18.5*	*4.9*	*1.5*	15 N, 22 N
		50.0	*20.0*	*4.2*	*1.7*	17 N, 23 M N
		16.0	*10.5*	*0.7*	*0.9*	11 N, 10 N
		5.00	*16.0*	*1.4*	*1.3*	17 N, 15 N
		1.60	*10.0*	*1.4*	*0.8*	9 N, 11 N
	Dimethyl Sulfoxide		*12.0*	*1.4*		13 N, 11 N
TA98	**2NF**	1.0	*215.5*	*29.0*	*18.0*	236 N, 195 N
TA100	**SA**	2.0	*916.5*	*149.2*	*13.5*	1022 N, 811 N
TA1535	**SA**	2.0	*813.0*	*7.1*	*90.3*	808 N, 818 N
TA1537	**ICR**	2.0	*194.0*	*18.4*	*55.4*	181 N, 207 N
WP2*uvrA*	**4NQO**	1.0	*176.5*	*37.5*	*14.7*	203 N, 150 N

Key to Positive Controls		Key to Plate Postfix Codes	
2NF	2-nitrofluorene	M	Plate counted manually
SA	sodium azide	N	Normal background bacterial lawn
ICR	ICR-191		
4NQO	4-nitroquinoline-N-oxide		

Confirmatory Mutagenicity Assay - Normal growth again was observed in all five tester strains, and the test article again was found to be freely soluble, at all doses evaluated with and without S9. Revertant frequencies for all doses of (b) (4), in all tester strains with and without S9, again approximated or were less than control values. All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met. (see table 3 +S9 and table 4 -S9 for numerical results).

Table 3
Confirmatory Mutagenicity Assay Results with S9

Study No.: 8223262
Trial No.: 8223262-C1
Plating Method: Plate incorporation assay
Date Plated: 2/17/2010
Date Counted: 2/25/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*20.0*	*6.0*	*1.1*	26 N, 20 N, 14 N
		1600	*27.3*	*11.0*	*1.5*	36 N, 15 N, 31 N
		500	*26.0*	*4.4*	*1.5*	24 N, 31 M N, 23 N
		160	*21.7*	*4.0*	*1.2*	21 N, 18 N, 26 N
		50.0	*19.7*	*3.1*	*1.1*	23 N, 19 N, 17 N
	Dimethyl Sulfoxide		*17.7*	*0.6*		17 N, 18 N, 18 N
TA100	(b) (4)	5000	*134.7*	*11.1*	*1.2*	123 N, 136 N, 145 N
		1600	*120.3*	*7.2*	*1.1*	125 N, 124 N, 112 N
		500	*127.0*	*8.7*	*1.1*	131 N, 133 N, 117 M N
		160	*120.7*	*13.5*	*1.1*	134 N, 107 N, 121 N
		50.0	*120.0*	*16.1*	*1.1*	115 N, 138 N, 107 N
	Dimethyl Sulfoxide		*111.0*	*2.0*		109 M N, 111 N, 113 N
TA1535	(b) (4)	5000	*9.3*	*3.1*	*0.9*	6 N, 10 N, 12 N
		1600	*13.7*	*3.1*	*1.4*	17 M N, 11 N, 13 N
		500	*11.0*	*1.7*	*1.1*	10 N, 13 N, 10 N
		160	*9.7*	*4.6*	*1.0*	15 N, 7 N, 7 N
		50.0	*12.0*	*4.4*	*1.2*	7 N, 14 N, 15 N
	Dimethyl Sulfoxide		*10.0*	*5.2*		7 M N, 16 M N, 7 N
TA1537	(b) (4)	5000	*6.3*	*1.5*	*0.7*	8 M N, 5 M N, 6 N
		1600	*8.0*	*2.0*	*0.9*	6 M N, 8 N, 10 N
		500	*8.7*	*2.1*	*1.0*	11 N, 8 M N, 7 N
		160	*11.0*	*4.4*	*1.2*	14 N, 13 N, 6 N
		50.0	*9.7*	*2.9*	*1.1*	8 N, 13 N, 8 N
	Dimethyl Sulfoxide		*9.0*	*1.7*		10 M N, 10 N, 7 M N
WP2*uvrA*	(b) (4)	5000	*15.0*	*2.6*	*1.0*	12 M N, 16 M N, 17 M N
		1600	*16.7*	*1.5*	*1.1*	17 N, 18 M N, 15 N
		500	*17.3*	*3.5*	*1.1*	14 N, 17 N, 21 M N
		160	*13.0*	*1.0*	*0.8*	13 N, 14 N, 12 M N
		50.0	*14.0*	*1.7*	*0.9*	12 N, 15 N, 15 N
	Dimethyl Sulfoxide		*15.3*	*3.8*		18 M N, 11 M N, 17 M N
TA98	**BP**	2.5	*425.3*	*15.2*	*24.1*	409 N, 428 N, 439 N
TA100	**2AA**	2.5	*2061.0*	*114.2*	*18.6*	2167 N, 2076 N, 1940 N
TA1535	**2AA**	2.5	*217.0*	*9.2*	*21.7*	225 N, 207 N, 219 N
TA1537	**2AA**	2.5	*166.0*	*19.3*	*18.4*	149 N, 187 N, 162 N
WP2*uvrA*	**2AA**	25.0	*389.7*	*28.9*	*25.4*	412 N, 400 N, 357 N

Key to Positive Controls		Key to Plate Postfix Codes	
BP	Benzo{a}pyrene	N	Normal background bacterial lawn
2AA	2-aminoanthracene	M	Plate counted manually

Table 4
Confirmatory Mutagenicity Assay Results without S9

Study No.: 8223262
Trial No.: 8223262-C1 Date Plated: 2/17/2010
Plating Method: Plate incorporation assay Date Counted: 2/25/2010

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*19.3*	*2.9*	*1.5*	21 N, 21 N, 16 M N
		1600	*11.3*	*1.5*	*0.9*	13 N, 10 N, 11 N
		500	*14.0*	*3.6*	*1.1*	10 N, 15 N, 17 N
		160	*16.3*	*2.1*	*1.3*	18 N, 14 N, 17 N
		50.0	*13.3*	*2.9*	*1.0*	15 N, 10 M N, 15 N
	Dimethyl Sulfoxide		*13.0*	*4.4*		18 N, 10 N, 11 N
TA100	(b) (4)	5000	*96.3*	*3.8*	*1.2*	92 N, 98 N, 99 N
		1600	*86.0*	*3.0*	*1.1*	89 N, 83 N, 86 N
		500	*88.0*	*16.8*	*1.1*	107 N, 75 M N, 82 N
		160	*88.3*	*10.5*	*1.1*	99 N, 88 M N, 78 M N
		50.0	*88.7*	*14.2*	*1.1*	86 M N, 104 N, 76 M N
	Dimethyl Sulfoxide		*81.0*	*1.7*		82 M N, 82 N, 79 N
TA1535	(b) (4)	5000	*15.7*	*5.0*	*1.5*	11 N, 15 M N, 21 N
		1600	*13.7*	*5.5*	*1.3*	10 N, 20 M N, 11 N
		500	*13.0*	*5.6*	*1.3*	19 M N, 12 M N, 8 N
		160	*12.7*	*2.1*	*1.2*	12 N, 15 N, 11 N
		50.0	*12.3*	*1.5*	*1.2*	14 N, 11 N, 12 N
	Dimethyl Sulfoxide		*10.3*	*4.7*		5 M N, 12 M N, 14 M N
TA1537	(b) (4)	5000	*6.7*	*2.5*	*1.2*	4 M N, 7 N, 9 M N
		1600	*7.3*	*3.1*	*1.3*	4 N, 10 N, 8 N
		500	*4.0*	*2.6*	*0.7*	3 M N, 7 N, 2 N
		160	*7.0*	*3.6*	*1.2*	10 N, 8 N, 3 M N
		50.0	*7.7*	*3.1*	*1.4*	5 N, 11 N, 7 N
	Dimethyl Sulfoxide		*5.7*	*1.2*		5 N, 7 N, 5 N
WP2*uvrA*	(b) (4)	5000	*14.7*	*1.5*	*1.1*	13 M N, 16 M N, 15 M N
		1600	*13.7*	*2.5*	*1.0*	11 N, 14 N, 16 M N
		500	*12.7*	*2.5*	*0.9*	13 M N, 15 N, 10 N
		160	*17.0*	*2.6*	*1.2*	18 N, 19 N, 14 N
		50.0	*11.7*	*1.5*	*0.9*	12 N, 10 N, 13 N
	Dimethyl Sulfoxide		*13.7*	*2.5*		14 M N, 16 M N, 11 M N
TA98	**2NF**	1.0	*241.0*	*29.5*	*18.5*	217 N, 274 N, 232 N
TA100	**SA**	2.0	*1145.3*	*57.7*	*14.1*	1127 N, 1099 N, 1210 N
TA1535	**SA**	2.0	*757.7*	*22.1*	*73.3*	737 N, 755 N, 781 N
TA1537	**ICR**	2.0	*180.0*	*11.1*	*31.8*	190 N, 182 N, 168 N
WP2*uvrA*	**4NQO**	1.0	*490.0*	*21.8*	*35.9*	505 N, 500 N, 465 N

Key to Positive Controls		Key to Plate Postfix Codes	
2NF	2-nitrofluorene	N	Normal background bacterial lawn
SA	sodium azide	M	Plate counted manually
ICR	ICR-191		
4NQO	4-nitroquinoline-N-oxide		

CONCLUSION - (b) (4) was negative in the Bacterial Reverse Mutation Assay with a Confirmatory Assay under the conditions, and according to the criteria, of the test protocol in a valid assay.

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7.2 *In Vitro* Assays in Mammalian Cells

Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

- with Bupivacaine Free Base

Study no.:	7116-117
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 3, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Bupivacaine free base – lot F0083, 98% Dimethylsulfoxide (DMSO) - Lots A0204801001 and A0225973,

Key Study Findings

- Bupivacaine free base was tested for the potential to cause chromosomal aberrations in cultured human in peripheral blood lymphocytes in an *in vitro* assay. In the definitive study, doses were 29.8, 59.5, 119, 178, 237, 356, 475, 633, 844, 1130, and 1500 µg/mL without metabolic activation (3 hour exposure) and 119, 237, 475, 633, 844, 1130, and 1500 µg/mL with metabolic activation (22 hour exposure).
- Bupivacaine free base did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes with or without metabolic activation in a valid assay.

Methods

Cell line:	Human peripheral blood lymphocyctes
Concentrations in definitive study:	29.8, 59.5, 119, 178, 237, 356, 475, 633, 844, 1130, and 1500 µg/mL without metabolic activation (-S9) and 119, 237, 475, 633, 844, 1130, and 1500 µg/mL with metabolic activation (+S9).
Basis of concentration selection:	Highest dose of 1500 µg/mL based on solubility limitations and 48% reduction in mitotic index at 1050 µg/mL (-S9) and 45% reduction in mitotic index at 1500 µg/mL (+S9) from the initial assay. See tables 1, 3, 5, & 7 in results section.
Negative control:	In the assays conducted without metabolic activation, negative controls were cultures, which contained only cells and culture medium. Vehicle controls were cultures containing DMSO at 10.0 µL/mL. In the assays conducted with metabolic activation, the negative and vehicle controls were the same, but with the S9 activation mix included.
Positive control:	The positive control agents used in the

assays were mitomycin C (MMC) for the assays without metabolic activation and cyclophosphamide (CP) in the assays with metabolic activation. In the chromosomal aberrations assays, concentrations of MMC (0.750, 1.00, and 1.50 µg/mL, 3-hour treatment; 0.200, 0.300, and 0.400 µg/mL, ~22-hour treatment) and CP (20.0, 25.0, and 40.0 µg/mL) were used to induce chromosomal aberrations.

Formulation/Vehicle: DMSO for bupivacaine and sterile, deionized water for the positive controls.

Incubation & sampling time: Initial assay - 2 days after culture initiation, cells were incubated at 37 ± 2°C with the test article, vehicle, or positive controls for 3 hours (±S9). Cultures were then washed and incubated for the rest of the culture period with 0.1 µg/mL Colcemid added for the last 2 ± 0.5 hours of incubation before harvesting.

Summary of Chromosomal Aberrations Assay Treatment Schedule in Hours

Activation Conditions	Test Article Added	Treatment Completed	Colcemid® Added	Harvest Started
-S9	0	3	~20	~22
+S9	0	3	~20	~22

Confirmatory assay – Cultures were handled as in the initial assay for cultures +S9. For -S9 cultures, cells were incubated at 37 ± 2°C with the test article, vehicle, or positive controls for ~22 hours then washed, with 0.1 µg/mL Colcemid added for the last 2 ± 0.5 hours of incubation before harvesting.

Summary of Confirmatory Chromosomal Aberrations Assay Treatment Schedule in Hours

Activation Conditions	Test Article Added	Treatment Completed	Colcemid® Added	Harvest Started
-S9	0	~22	~20	~22
+S9	0	3	~20	~22

Study Validity Valid – assay acceptable for evaluation of test results according to observed acceptable assay guidelines regarding controls, high concentrations, and number of concentrations.

Results

Initial chromosomal aberration assay - In the assay without metabolic activation, a precipitate was observed after dosing at ≥735 µg/mL, and at wash at ≥1050 µg/mL. Hemolysis was observed at wash and at harvest at 1500 µg/mL. Mitotic index data are provided in Table 1. Chromosomal aberrations were analyzed from the cultures treated with 360, 515, 735, and 1050 µg/mL (Table 2). The high dose selected for analysis, 1050 µg/mL, had a precipitate at the end of the treatment period and a 48% reduction in mitotic index as compared with the vehicle control cultures. No significant increase in chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed.

In the assay with metabolic activation, a precipitate was observed after dosing at ≥735 µg/mL, and at wash at ≥1050 µg/mL. Mitotic index data are provided in Table 3. Chromosomal aberrations were analyzed from the cultures treated with 515, 735, 1050, and 1500 µg/mL (Table 4). The high dose selected for analysis, 1500 µg/mL, had a precipitate at the end of the treatment period and a 45% reduction in mitotic index as compared with the vehicle control cultures. No significant increase in chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed.

Table 1: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 28280-0-449OECD Trial No.: B1 Date: 04/12/06 Lab No.: CY041306
Test Article: Bupivacaine free base

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Negative Control	RPMI 1640		9.9	10.9	10.4	--
Vehicle Control	DMSO	10.0 µL/mL	11.1	10.5	10.8	0
Test Article		360 µg/mL	11.2	9.4	10.3	5
		515 µg/mL	8.4	9.4	8.9	18
		735 µg/mL[a]	5.8	8.4	7.1	34
		1050 µg/mL[b]	5.9	5.2	5.6	48
		1500 µg/mL[b, c]	1.2	1.2	1.2	89

[a] Precipitate observed at dose.
[b] Precipitate observed at dose and wash.
[c] Hemolysis observed at wash and harvest.
RPMI 1640 = culture medium DMSO = dimethylsulfoxide

Table 2: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 28280-0-449OECD Trial No.: B1 Date: 04/12/06 Lab No.: CY041306 Test Article: Bupivacaine free base

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judgement (+/-)[d]
										gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	
Controls																	
Negative:	RPMI 1640		A	100		100	0	0							0	0	
			B	100		100	0	0		1	1				1	2	
			Total	200		200				1	1				1	2	
			Average	%	--		0.0	0.0		0.5	0.5				0.5	1.0	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0							0	0	
			Total	200		200				1					0	1	
			Average	%	0		0.0	0.0		0.5					0.0	0.5	
Positive:	MMC	1.00 µg/mL	A	50		100	0	0		3	13	4			16	18	
			B	50		100	0	0		6	20	8		3	26	29	
			Total	100		200				9	33	12		3	42	47	
			Average	%	--		0.0	0.0	-	9.0	33.0	12.0		3.0	42.0	47.0	+
Test Article		360 µg/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0		1					0	1	
			Total	200		200				2					0	2	
			Average	%	5		0.0	0.0	-	1.0					0.0	1.0	-
		515 µg/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0							0	0	
			Total	200		200				1					0	1	
			Average	%	18		0.0	0.0	-	0.5					0.0	0.5	-
		735 µg/mL	A	100		100	0	0		2	1		1		2	4	
			B	100		100	0	0		2					0	2	
			Total	200		200				4	1		1		2	6	
			Average	%	34		0.0	0.0	-	2.0	0.5		0.5		1.0	3.0	-
		1050 µg/mL	A	100		100	0	0		1	1				1	1	
			B	100		100	0	0		1	2				2	3	
			Total	200		200				2	3				3	4	
			Average	%	48		0.0	0.0	-	1.0	1.5				1.5	2.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = dimethylsulfoxide MMC = Mitomycin C

Table 3: Assessment of Toxicity for Chromosomal Aberrations Assay - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 28280-0-449OECD Trial No.: B1 Date: 04/12/06 Lab No.: CY041306
Test Article: Bupivacaine free base

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Negative Control	RPMI 1640		12.8	11.9	12.4	--
Vehicle Control	DMSO	10.0 µL/mL	9.5	10.1	9.8	0
Test Article		515 µg/mL	11.1	12.0	11.6	0
		735 µg/mL[a]	9.2	8.4	8.8	10
		1050 µg/mL[b]	9.4	6.9	8.2	16
		1500 µg/mL[b]	4.5	6.2	5.4	45

[a] Precipitate observed at dose.

[b] Precipitate observed at dose and wash.

RPMI 1640 = culture medium DMSO = dimethylsulfoxide

Table 4: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 28280-0-449OECD Trial No.: B1 Date: 04/12/06 Lab No.: CY041306 Test Article: Bupivacaine free base

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judgement (+/-)[d]
										gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	
Controls																	
Negative:	RPMI 1640		A	100		100	0	0		2					0	2	
			B	100		100	0	0		1					0	1	
			Total	200		200				3					0	3	
			Average	%	--		0.0	0.0		1.5					0.0	1.5	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		2					0	2	
			B	100		100	0	0		1					0	1	
			Total	200		200				3					0	3	
			Average	%	0		0.0	0.0		1.5					0.0	1.5	
Positive:	CP	25.0 µg/mL	A	50		100	0	0		4	18	5			20	23	
			B	50		100	0	0		5	19	5			21	23	
			Total	100		200				9	37	10			41	46	
			Average	%	--		0.0	0.0	-	9.0	37.0	10.0			41.0	46.0	+
Test Article		515 µg/mL	A	100		100	0	0			1				1	1	
			B	100		100	0	0		2	1				1	3	
			Total	200		200				2	2				2	4	
			Average	%	0		0.0	0.0	-	1.0	1.0				1.0	2.0	-
		735 µg/mL	A	100		100	0	0							0	0	
			B	100		100	0	0		3					0	3	
			Total	200		200				3					0	3	
			Average	%	10		0.0	0.0	-	1.5					0.0	1.5	-
		1050 µg/mL	A	100		100	0	0		2	1				1	3	
			B	100		100	0	0		2	1				1	3	
			Total	200		200				4	2				2	6	
			Average	%	16		0.0	0.0	-	2.0	1.0				1.0	3.0	-
		1500 µg/mL	A	100		100	0	0		2	2				2	4	
			B	100		100	0	0		1	2				2	2	
			Total	200		200				3	4				4	6	
			Average	%	45		0.0	0.0	-	1.5	2.0				2.0	3.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = dimethylsulfoxide CP = Cyclophosphamide

Confirmatory chromosomal assay - In the assay without metabolic activation, a precipitate was observed after dosing at ≥844 µg/mL. Hemolysis was observed at harvest at ≥1130 µg/mL. Mitotic index data are provided in Table 5. Chromosomal aberrations were analyzed from the cultures treated with 119, 178, 237, and 356 µg/mL (Table 6). The high dose selected for analysis, 356 µg/mL, had a 55% reduction in mitotic index as compared with the vehicle control cultures. No significant increase in chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed.

In the assay with metabolic activation, a precipitate was observed after dosing at ≥844 µg/mL and at wash at ≥1130 µg/mL. Slight hemolysis was observed at wash at 1500 µg/mL. Mitotic index data are provided in Table 7. Chromosomal aberrations were analyzed from the cultures treated with 633, 844, 1130, and 1500 µg/mL (Table 8). The high dose selected for analysis, 1500 µg/mL, had a precipitate at the end of the treatment period and 51% reduction in mitotic index as compared with the vehicle control cultures. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

Table 5: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest

Assay No.: 28280-0-449OECD Trial No.: C1 Date: 05/18/06 Lab No.: CY052306
Test Article: Bupivacaine free base

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Negative Control	RPMI 1640		7.4	6.3	6.9	--
Vehicle Control	DMSO	10.0 µL/mL	5.8	5.2	5.5	0
Test Article		59.5 µg/mL	5.2	5.6	5.4	2
		119 µg/mL	4.3	4.8	4.6	16
		178 µg/mL	4.1	4.4	4.3	22
		237 µg/mL	2.8	3.3	3.1	44
		356 µg/mL	2.5	2.4	2.5	55
		475 µg/mL	2.6	2.4	2.5	55
		633 µg/mL	1.8	2.1	2.0	64
		844 µg/mL[a]	0.2	0.3	0.3	95
		1130 µg/mL[a, b]	0.0	0.0	0.0	100
		1500 µg/mL[a, b]	0.0	0.0	0.0	100

[a] Precipitate observed at dose.
[b] Hemolysis observed at harvest.
RPMI 1640 = culture medium DMSO = dimethylsulfoxide

Table 6: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest

Assay No.: 28280-0-449OECD Trial No.: C1 Date: 05/18/06 Lab No.: CY CY052306 Test Article: Bupivacaine free base

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
										gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	
Controls																	
Negative:	RPMI 1640		A	100		100	0	0							0	0	
			B	100		100	0	0		1					0	1	
			Total	200		200				1					0	1	
			Average	%	--		0.0	0.0		0.5					0.0	0.5	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		1	1				1	2	
			B	100		100	0	0		3					0	3	
			Total	200		200				4	1				1	5	
			Average	%	0		0.0	0.0		2.0	0.5				0.5	2.5	
Positive:	MMC	0.300 µg/mL	A	50		100	0	0		5	11	9			17	21	
			B	75		100	0	0		2	13	8			20	20	
			Total	125		200				7	24	17			37	41	
			Average	%	--		0.0	0.0	-	5.6	19.2	13.6			29.6	32.8	+
Test Article		119 µg/mL	A	100		100	0	0							0	0	
			B	100		100	0	0		1	1				1	2	
			Total	200		200				1	1				1	2	
			Average	%	16		0.0	0.0	-	0.5	0.5				0.5	1.0	-
		178 µg/mL	A	100		100	0	0			1				1	1	
			B	100		100	0	0			1				1	1	
			Total	200		200					2				2	2	
			Average	%	22		0.0	0.0	-		1.0				1.0	1.0	-
		237 µg/mL	A	100		100	0	0			2				2	2	
			B	100		100	0	0							0	0	
			Total	200		200					2				2	2	
			Average	%	44		0.0	0.0	-		1.0				1.0	1.0	-
		356 µg/mL	A	100		100	0	0							0	0	
			B	100		100	0	0			1				1	1	
			Total	200		200					1				1	1	
			Average	%	55		0.0	0.0	-		0.5				0.5	0.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = dimethylsulfoxide MMC = Mitomycin C

Table 7: Assessment of Toxicity for Chromosomal Aberrations Assay - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 28280-0-449OECD Trial No.: C1 Date: 05/18/06 Lab No.: CY052306
Test Article: Bupivacaine free base

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Negative Control	RPMI 1640		8.8	9.3	9.1	--
Vehicle Control	DMSO	10.0 µL/mL	7.8	8.4	8.1	0
Test Article		633 µg/mL	6.9	8.1	7.5	7
		844 µg/mL[a]	6.1	7.8	7.0	14
		1130 µg/mL[b]	4.4	5.6	5.0	38
		1500 µg/mL[b, c]	3.8	4.1	4.0	51

[a] Precipitate observed at dose.
[b] Precipitate observed at dose and wash.
[c] Slight hemolysis observed at wash.
RPMI 1640 = culture medium DMSO = dimethylsulfoxide

Table 8: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 28280-0-449OECD Trial No.: C1 Date: 05/18/06 Lab No.: CY052306 Test Article: Bupivacaine free base

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judgement (+/-)[d]
Controls																	
Negative:	RPMI 1640		A	100		100	0	0		2					0	2	
			B	100		100	0	0		1	1				1	2	
			Total	200		200				3	1				1	4	
			Average	%	--		0.0	0.0		1.5	0.5				0.5	2.0	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	2	0		1					0	1	
			B	100		100	0	0		2					0	2	
			Total	200		200				3					0	3	
			Average	%	0		1.0	0.0		1.5					0.0	1.5	
Positive:	CP	25.0 µg/mL	A	50		100	0	0		3	15	3			16	19	
			B	75		100	0	0		6	24	6			28	34	
			Total	125		200				9	39	9			44	53	
			Average	%	--		0.0	0.0	-	7.2	31.2	7.2			35.2	42.4	+
Test Article		633 µg/mL	A	100		100	0	0		1	1				1	2	
			B	100		100	2	0		1					0	1	
			Total	200		200				2	1				1	3	
			Average	%	7		1.0	0.0	-	1.0	0.5				0.5	1.5	-
		844 µg/mL	A	100		100	2	0							0	0	
			B	100		100	1	0		1					0	1	
			Total	200		200				1					0	1	
			Average	%	14		1.5	0.0	-	0.5					0.0	0.5	-
		1130 µg/mL	A	100		100	0	0		1					0	1	
			B	100		100	1	0			1				1	1	
			Total	200		200				1	1				1	2	
			Average	%	38		0.5	0.0	-	0.5	0.5				0.5	1.0	-
		1500 µg/mL	A	100		100	0	0			1				1	1	
			B	100		100	1	0		1	2				2	3	
			Total	200		200				1	3				3	4	
			Average	%	51		0.5	0.0	-	0.5	1.5				1.5	2.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = dimethylsulfoxide CP = Cyclophosphamide

Conclusion

Bupivacaine free base did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes with or without an exogenous metabolic activation system in a valid assay.

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Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

- with (b) (4)

Study no.:	06-10-803-X-VO-HCA; 8223278
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 17, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Dimethylsulfoxide (DMSO) – Lot 35596LK, 99.92%

Key Study Findings

- (b) (4) was tested for the potential to cause chromosomal aberrations in cultured human in peripheral blood lymphocytes in an *in vitro* assay. Doses were up to 1000 µg/mL based on solubility of the test article. Cultures were treated for 3 hours (±S9) and 22 hours (-S9).
- (b) (4) did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes with or without metabolic activation in a valid assay.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	Up to 1000 µg/mL with and without metabolic activation (3 hour dosing) and without metabolic activation (22 hour dosing).
Basis of concentration selection:	Solubility
Negative control:	In the assays conducted without metabolic activation, negative controls were cultures, which contained only cells and culture medium. Vehicle controls were cultures containing DMSO at 10.0 µL/mL. In the assays conducted with metabolic activation, the negative and vehicle controls were the same, but with the S9 activation mix included.
Positive control:	The positive control agents used in the assays were mitomycin C (MMC) for the assays without metabolic activation and

cyclophosphamide (CP) in the assays with metabolic activation. In the chromosomal aberrations assays, concentrations of MMC (0.750, 1.00, and 1.50 µg/mL, 3-hour treatment; 0.200, 0.300, and 0.400 µg/mL, ~22-hour treatment) and CP (20.0, 25.0, and 40.0 µg/mL) were used to induce chromosomal aberrations.

Formulation/Vehicle: DMSO for test article and sterile, deionized water for the positive controls.

Incubation & sampling time: In this single assay study (no initial and confirmatory assays), 2 days after culture initiation, cells were incubated at 37 ± 2°C with the test article, vehicle, or positive controls for 3 hours (±S9) or 22 hours (-S9). Cultures were then washed and incubated for the rest of the culture period with 0.1 µg/mL Colcemid added for the last 2 ± 0.5 hours of incubation before harvesting.

Summary of Treatment Schedule in Hours (approximate)

S9Activation Mix	Test Article Added	Exposure Completed	Colcemid® Added	Harvest Started
Without	0	3	20	22
Without	0	22	20	22
With	0	3	20	22

Study Validity Valid – assay acceptable for evaluation of test results according to observed acceptable assay guidelines regarding controls, high concentrations, and number of concentrations.

Results

In the assay without metabolic activation with a 3-hour treatment, the mitotic index was reduced by 54% at the dose of 512 µg/mL (Table 1). Therefore, chromosomal aberrations were analyzed from the cultures treated at the doses of 262, 410, and 512 µg/mL (Table 2). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in the cultures analyzed**.**

Table 1: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8223758 Trial No.: B1 Date: 03/04/10
Test Article: (b) (4)

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0 µL/mL	12.9	13.2	13.1	0
Test Article		20.0 µg/mL	--[a]	--[a]	--	--
		32.0 µg/mL	--[a]	--[a]	--	--
		46.0 µg/mL	--[a]	--[a]	--	--
		66.0 µg/mL	--[a]	--[a]	--	--
		94.0 µg/mL	--[a]	--[a]	--	--
		134 µg/mL	--[a]	--[a]	--	--
		168 µg/mL	--[a]	--[a]	--	--
		210 µg/mL	--[a]	--[a]	--	--
		262 µg/mL[b]	11.6	12.3	12.0	8
		328 µg/mL[b]	9.4	10.1	9.8	25
		410 µg/mL[b]	7.8	8.3	8.1	38
		512 µg/mL[b]	6.3	5.7	6.0	54
		640 µg/mL[b,c]	5.8	5.1	5.5	58
		800 µg/mL[b,d]	5.3	4.5	4.9	63
		1000 µg/mL[b,d]	4.1	4.7	4.4	66

[a] Not analyzed since non-toxic dose levels were achieved (≤15% mitotic index reduction).
[b] Precipitate observed at dose.
[c] Hemolysis observed at wash.
[d] Hemolysis observed at wash and harvest.
DMSO = dimethylsulfoxide

Table 2: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8223758 Trial No.: B1 Date: 03/04/10 Test Article: (b) (4)

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: simple gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judge-ment (+/-)[d]
Controls																	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0		2					0	2	
			Total	200		200				3					0	3	
			Average	%	0		0.0	0.0		1.5					0.0	1.5	
Positive:	MMC	1.00 µg/mL	A	75		100	0	0		5	11	15			22	25	
			B	50		100	1	0		4	13	12			23	25	
			Total	125		200				9	24	27			45	50	
			Average	%	--		0.5	0.0	-	7.2	19.2	21.6			36.0	40.0	+
Test Article		262 µg/mL	A	100		100	1	0			1				1	1	
			B	100		100	0	0							0	0	
			Total	200		200					1				1	1	
			Average	%	8		0.5	0.0	-		0.5				0.5	0.5	-
		410 µg/mL	A	100		100	0	0							0	0	
			B	100		100	1	0			1				1	1	
			Total	200		200					1				1	1	
			Average	%	38		0.5	0.0	-		0.5				0.5	0.5	-
		512 µg/mL	A	100		100	0	0		5					0	5	
			B	100		100	0	0		2					0	2	
			Total	200		200				7					0	7	
			Average	%	54		0.0	0.0	-	3.5					0.0	3.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

In the assay without metabolic activation with a ~22-hour treatment, the mitotic index was reduced 57% at 410 µg/mL compared with the vehicle control cultures (Table 3). On this basis, chromosomal aberrations were analyzed from the cultures treated with

168, 328, and 410 µg/mL (Table 4). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

Table 3: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest

Study No.: 8223758 Trial No.: B1 Date: 03/04/10
Test Article: (b) (4)

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0 µL/mL	8.6	7.9	8.3	0
Test Article		3.00 µg/mL	--[a]	--[a]	--	--
		6.00 µg/mL	--[a]	--[a]	--	--
		12.0 µg/mL	--[a]	--[a]	--	--
		20.0 µg/mL	--[a]	--[a]	--	--
		32.0 µg/mL	--[a]	--[a]	--	--
		57.0 µg/mL	--[a]	--[a]	--	--
		94.0 µg/mL	--[a]	--[a]	--	--
		168 µg/mL	8.9	8.1	8.5	0
		262 µg/mL[c]	6.3	7.8	7.1	14
		328 µg/mL[c]	4.7	5.1	4.9	41
		410 µg/mL[c]	3.9	3.2	3.6	57
		512 µg/mL[c]	3.1	2.7	2.9	65
		640 µg/mL[c]	2.1	2.7	2.4	71
		800 µg/mL[c]	1.2	1.9	1.6	81
		1000 µg/mL[c]	--[b]	--[b]	--	--

[a] Not analyzed since non-toxic dose levels were achieved (≤15% mitotic index reduction).
[b] Only dead cells present on slide.
[c] Precipitate observed at dose.
DMSO = dimethylsulfoxide

Table 4: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest

Study No.: 8223758 Trial No.: B1 Date: 03/04/10 Test Article: (b) (4)

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: simple gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judge-ment (+/-)[d]
Controls																	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		3	1				1	4	
			B	100		100	0	0		2	2				2	4	
			Total	200		200				5	3				3	8	
			Average	%	0		0.0	0.0		2.5	1.5				1.5	4.0	
Positive:	MMC	0.300 µg/mL	A	75		100	0	0		5	13	7			18	23	
			B	75		100	0	0		5	11	5			15	20	
			Total	150		200				10	24	12			33	43	
			Average	%	--		0.0	0.0	-	6.7	16.0	8.0			22.0	28.7	+
Test Article		168 µg/mL	A	100		100	0	0		4	1				1	5	
			B	100		100	0	0		2	1				1	3	
			Total	200		200				6	2				2	8	
			Average	%	0		0.0	0.0	-	3.0	1.0				1.0	4.0	-
		328 µg/mL	A	100		100	0	0		4					0	4	
			B	100		100	0	0		5	2				2	7	
			Total	200		200				9	2				2	11	
			Average	%	41		0.0	0.0	-	4.5	1.0				1.0	5.5	-
		410 µg/mL	A	100		100	0	0		9	2				2	10	
			B	100		100	0	0		8	1				1	8	
			Total	200		200				17	3				3	18	
			Average	%	57		0.0	0.0	-	8.5	1.5				1.5	9.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

In the assay with metabolic activation with a 3-hour treatment, the mitotic index was reduced by 55% at the highest dose of 1000 µg/mL (Table 5). Therefore, chromosomal aberrations were analyzed from the cultures treated with 410, 640, and 1000 µg/mL (Table 6). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed**.**

Table 5: Assessment of Toxicity for Chromosomal Aberrations Assay - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8223758 Trial No.: B1 Date: 03/04/10
Test Article: (b) (4)

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0 µL/mL	12.6	11.2	11.9	0
Test Article		20.0 µg/mL	--[a]	--[a]	--	--
		32.0 µg/mL	--[a]	--[a]	--	--
		46.0 µg/mL	--[a]	--[a]	--	--
		66.0 µg/mL	--[a]	--[a]	--	--
		94.0 µg/mL	--[a]	--[a]	--	--
		134 µg/mL	--[a]	--[a]	--	--
		168 µg/mL	--[a]	--[a]	--	--
		210 µg/mL	--[a]	--[a]	--	--
		262 µg/mL	--[a]	--[a]	--	--
		328 µg/mL	--[a]	--[a]	--	--
		410 µg/mL	10.5	12.1	11.3	5
		512 µg/mL	8.5	9.4	9.0	24
		640 µg/mL[b]	6.8	5.8	6.3	47
		800 µg/mL[b]	6.2	7.3	6.8	43
		1000 µg/mL[b]	5.9	4.7	5.3	55

[a] Not analyzed since non-toxic dose levels were achieved (≤15% mitotic index reduction).
[b] Precipitate observed at dose.
DMSO = dimethylsulfoxide

Table 6: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8223758 Trial No.: B1 Date: 03/04/10 Test Article: (b) (4)

					# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
											simple					Totals[c]		
											gaps	breaks	chte	chre	mab	-g	+g	
Controls																		
Vehicle:	DMSO	10.0	µL/mL	A	100		100	0	0		3	1				1	4	
				B	100		100	0	0		2					0	2	
				Total	200		200				5	1				1	6	
				Average	%	0		0.0	0.0		2.5	0.5				0.5	3.0	
Positive:	CP	25.0	µg/mL	A	50		100	1	0		10	14	3		2	19	24	
				B	50		100	0	0		3	16	2	1		17	19	
				Total	100		200				13	30	5	1	2	36	43	
				Average	%	--		0.5	0.0	-	13.0	30.0	5.0	1.0	2.0	36.0	43.0	+
Test Article		410	µg/mL	A	100		100	0	0		1					0	1	
				B	100		100	0	0		1			1		1	2	
				Total	200		200				2			1		1	3	
				Average	%	5		0.0	0.0	-	1.0			0.5		0.5	1.5	-
		640	µg/mL	A	100		100	0	0		2	2				2	4	
				B	100		100	0	0		4	1				1	5	
				Total	200		200				6	3				3	9	
				Average	%	47		0.0	0.0	-	3.0	1.5				1.5	4.5	-
		1000	µg/mL	A	100		100	0	0							0	0	
				B	100		100	0	0							0	0	
				Total	200		200									0	0	
				Average	%	55		0.0	0.0	-						0.0	0.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide CP = Cyclophosphamide

Conclusion

(b) (4) did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes with or without an exogenous metabolic activation system in a valid assay.

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Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

- with (b) (4)

Study no.: 04-10-803-X-VO-HCA; 8223263
Study report location: eCTD in DARRTS
Conducting laboratory and location: (b) (4)
Date of study initiation: February 17, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4)
Dimethylsulfoxide (DMSO) – Lot 35596LK, 99.92%

Key Study Findings

- [redacted] was tested for the potential to cause chromosomal aberrations in cultured human in peripheral blood lymphocytes in an *in vitro* assay. Doses were up to the limit dose of 10 mM (3100 µg/mL). Cultures were treated for 3 hours (±S9) and 22 hours (-S9).
- [redacted] did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes with or without metabolic activation in a valid assay.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	Up to 3100 µg/mL with and without metabolic activation.
Basis of concentration selection:	Highest dose of 3100 µg/mL was ~10 mM, the limit dose per protocol guidelines.
Negative control:	In the assays conducted without metabolic activation, negative controls were cultures, which contained only cells and culture medium. Vehicle controls were cultures containing DMSO at 10.0 µL/mL. In the assays conducted with metabolic activation, the negative and vehicle controls were the same, but with the S9 activation mix included.
Positive control:	The positive control agents used in the assays were mitomycin C (MMC) for the assays without metabolic activation and cyclophosphamide (CP) in the assays with metabolic activation. In the chromosomal aberrations assays, concentrations of MMC (0.750, 1.00, and 1.50 µg/mL, 3-hour treatment; 0.200, 0.300, and 0.400 µg/mL, ~22-hour treatment) and CP (20.0, 25.0, and 40.0 µg/mL) were used to induce chromosomal aberrations.
Formulation/Vehicle:	DMSO for test article and sterile, deionized water for the positive controls.
Incubation & sampling time:	In this single assay study (no initial and confirmatory assays), 2 days after culture initiation, cells were incubated at 37 ± 2°C with the test article, vehicle, or positive controls for 3 hours (±S9) or 22 hours (-S9). Cultures were then washed and incubated for the rest of the culture period with 0.1 µg/mL Colcemid added for the last 2 ± 0.5 hours of incubation before harvesting.

Summary of Treatment Schedule in Hours (approximate)

S9Activation Mix	Test Article Added	Exposure Completed	Colcemid® Added	Harvest Started
Without	0	3	20	22
Without	0	22	20	22
With	0	3	20	22

Study Validity Valid – assay acceptable for evaluation of test results according to observed acceptable assay guidelines regarding controls, high concentrations, and number of concentrations.

Results

In the assay without metabolic activation with a 3-hour treatment, the mitotic index was only reduced by 8% at the highest dose of 3100 µg/mL (Table 1). Therefore, chromosomal aberrations were analyzed from the cultures treated at the three highest doses of 1520, 2170, and 3100 µg/mL (Table 2). The high dose selected for analysis, 3100 µg/mL is approximately 10 mM, the high dose recommended for this assay by the OECD Testing Guidelines. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed**.**

Table 1: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8223263 Trial No.: B1 Date: 03/03/10
Test Article: (b) (4)

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0 µL/mL	12.6	11.3	12.0	0
Test Article		21.0 µg/mL	--[a]	--[a]	--	--
		32.0 µg/mL	--[a]	--[a]	--	--
		43.0 µg/mL	--[a]	--[a]	--	--
		63.0 µg/mL	--[a]	--[a]	--	--
		88.0 µg/mL	--[a]	--[a]	--	--
		125 µg/mL	--[a]	--[a]	--	--
		180 µg/mL	--[a]	--[a]	--	--
		255 µg/mL	--[a]	--[a]	--	--
		365 µg/mL	--[a]	--[a]	--	--
		521 µg/mL	--[a]	--[a]	--	--
		745 µg/mL	--[a]	--[a]	--	--
		1070 µg/mL	--[a]	--[a]	--	--
		1520 µg/mL	12.7	11.2	12.0	0
		2170 µg/mL	10.9	11.5	11.2	7
		3100 µg/mL	10.5	11.4	11.0	8

[a]Not analyzed since non-toxic dose levels were achieved (≤15% mitotic index reduction).
DMSO = dimethylsulfoxide

Table 2: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8223263 Trial No.: B1 Date: 03/03/10 Test Article: (b) (4)

					# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
												simple				Totals[c]		
											gaps	breaks	chte	chre	mab	-g	+g	
Controls																		
Vehicle:	DMSO	10.0	µL/mL	A	100		100	0	0							0	0	
				B	100		100	0	0		4	1				1	5	
				Total	**200**		**200**				4	1				1	5	
				Average	%	**0**		**0.0**	**0.0**		**2.0**	**0.5**				**0.5**	**2.5**	
Positive:	MMC	1.00	µg/mL	A	50		100	0	0		9	19	10			25	29	
				B	50		100	0	0		10	19	11			25	30	
				Total	**100**		**200**				19	38	21			50	59	
				Average	%	--		**0.0**	**0.0**	-	**19.0**	**38.0**	**21.0**			**50.0**	**59.0**	+
Test Article		1520	µg/mL	A	100		100	0	0		1					0	1	
				B	100		100	0	0		2					0	2	
				Total	**200**		**200**				3					0	3	
				Average	%	**0**		**0.0**	**0.0**	-	**1.5**					**0.0**	**1.5**	-
		2170	µg/mL	A	100		100	0	0							0	0	
				B	100		100	0	0			1				1	1	
				Total	**200**		**200**					1				1	1	
				Average	%	**7**		**0.0**	**0.0**	-		**0.5**				**0.5**	**0.5**	-
		3100	µg/mL	A	100		100	0	0		1	2				2	3	
				B	100		100	0	0		1					0	1	
				Total	**200**		**200**				2	2				2	4	
				Average	%	**8**		**0.0**	**0.0**	-	**1.0**	**1.0**				**1.0**	**2.0**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a]% Mitotic index reduction as compared to the vehicle control.
[b]Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c]-g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d]Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

In the assay without metabolic activation with a ~22-hour treatment, the mitotic index was reduced 53% at 745 µg/mL compared with the vehicle control cultures (Table 3). On this basis, chromosomal aberrations were analyzed from the cultures treated with

365, 521, and 745 µg/mL (Table 4). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

Table 3: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest

Study No.: 8223263 Trial No.: B1 Date: 03/03/10
Test Article: (b) (4)

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0 µL/mL	7.3	8.2	7.8	0
Test Article		2.00 µg/mL	--[a]	--[a]	--	--
		4.00 µg/mL	--[a]	--[a]	--	--
		8.00 µg/mL	--[a]	--[a]	--	--
		16.0 µg/mL	--[a]	--[a]	--	--
		32.0 µg/mL	--[a]	--[a]	--	--
		63.0 µg/mL	--[a]	--[a]	--	--
		125 µg/mL	--[a]	--[a]	--	--
		180 µg/mL	--[a]	--[a]	--	--
		255 µg/mL	8.9	8.1	8.5	0
		365 µg/mL	6.8	7.3	7.1	9
		521 µg/mL	4.5	5.1	4.8	38
		745 µg/mL	3.4	3.9	3.7	53
		1070 µg/mL	2.3	2.9	2.6	67
		1520 µg/mL	2.1	1.8	2.0	74
		2170 µg/mL	1.5	1.1	1.3	83
		3100 µg/mL	--[b]	--[b]	--	--

[a] Not analyzed since non-toxic dose levels were achieved (≤15% mitotic index reduction).
[b] Only dead cells present on slide.
DMSO = dimethylsulfoxide

Table 4: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest

Study No.: 8223263 Trial No.: B1 Date: 03/03/10 Test Article: (b) (4)

					# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
											simple					Totals[c]		
											gaps	breaks	chte	chre	mab	-g	+g	
Controls																		
Vehicle:	DMSO	10.0	µL/mL	A	100		100	1	0		3	3				3	6	
				B	100		100	1	0		1	1				1	2	
				Total	200		200				4	4				4	8	
				Average	%	0		1.0	0.0		2.0	2.0				2.0	4.0	
Positive:	MMC	0.300	µg/mL	A	50		100	0	0		8	7	11			15	20	
				B	50		100	0	0		8	15	13			22	25	
				Total	100		200				16	22	24			37	45	
				Average	%	--		0.0	0.0	-	16.0	22.0	24.0			37.0	45.0	+
Test Article		365	µg/mL	A	100		100	0	0		4	1				1	5	
				B	100		100	1	0		4	1				1	5	
				Total	200		200				8	2				2	10	
				Average	%	9		0.5	0.0	-	4.0	1.0				1.0	5.0	-
		521	µg/mL	A	100		100	2	0		5	6				6	10	
				B	100		100	0	0		9	2				2	10	
				Total	200		200				14	8				8	20	
				Average	%	38		1.0	0.0	-	7.0	4.0				4.0	10.0	-
		745	µg/mL	A	100		100	0	0		3	2				2	5	
				B	100		100	0	0		6	5				5	10	
				Total	200		200				9	7				7	15	
				Average	%	53		0.0	0.0	-	4.5	3.5				3.5	7.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

In the assay with metabolic activation with a 3-hour treatment, the mitotic index was only reduced by 7% at the highest dose of 3100 µg/mL (Table 5). Therefore, chromosomal aberrations were analyzed from the cultures treated with 1520, 2170, and 3100 µg/mL (Table 6). The high dose selected for analysis, 3100 µg/mL is approximately 10 mM, the high dose recommended for this assay by the OECD Testing Guidelines, which also exceeds the current ICHS2(R1) dose limit for the assay (1 mM or 0.5 mg/mL, whichever is lower).

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No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed**.**

Table 5: Assessment of Toxicity for Chromosomal Aberrations Assay - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8223263 Trial No.: B1 Date: 03/03/10
Test Article: (b) (4)

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0 µL/mL	11.2	10.6	10.9	0
Test Article		21.0 µg/mL	--[a]	--[a]	--	--
		32.0 µg/mL	--[a]	--[a]	--	--
		43.0 µg/mL	--[a]	--[a]	--	--
		63.0 µg/mL	--[a]	--[a]	--	--
		88.0 µg/mL	--[a]	--[a]	--	--
		125 µg/mL	--[a]	--[a]	--	--
		180 µg/mL	--[a]	--[a]	--	--
		255 µg/mL	--[a]	--[a]	--	--
		365 µg/mL	--[a]	--[a]	--	--
		521 µg/mL	--[a]	--[a]	--	--
		745 µg/mL	--[a]	--[a]	--	--
		1070 µg/mL	--[a]	--[a]	--	--
		1520 µg/mL	11.2	9.8	10.5	4
		2170 µg/mL	10.6	11.3	11.0	0
		3100 µg/mL	9.8	10.4	10.1	7

[a] Not analyzed since non-toxic dose levels were achieved (≤15% mitotic index reduction).
DMSO = dimethylsulfoxide

Table 6: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8223263 Trial No.: B1 Date: 03/03/10 Test Article: (b) (4)

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
										simple					Totals[c]		
										gaps	breaks	chte	chre	mab	-g	+g	
Controls																	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		7	1				1	8	
			B	100		100	0	0		4	1				1	5	
			Total	200		200				11	2				2	13	
			Average	%	0		0.0	0.0		5.5	1.0				1.0	6.5	
Positive:	CP	25.0 µg/mL	A	50		100	0	0		9	17	4			18	22	
			B	50		100	0	0		5	18	1		1	20	21	
			Total	100		200				14	35	5		1	38	43	
			Average	%	--		0.0	0.0	-	14.0	35.0	5.0		1.0	38.0	43.0	+
Test Article		1520 µg/mL	A	100		100	0	0		5	3				3	8	
			B	100		100	0	0		3					0	3	
			Total	200		200				8	3				3	11	
			Average	%	4		0.0	0.0	-	4.0	1.5				1.5	5.5	-
		2170 µg/mL	A	100		100	0	0		3	1				1	4	
			B	100		100	0	0		3	1				1	4	
			Total	200		200				6	2				2	8	
			Average	%	0		0.0	0.0	-	3.0	1.0				1.0	4.0	-
		3100 µg/mL	A	100		100	0	0		3	1				1	4	
			B	100		100	0	0		6	1				1	7	
			Total	200		200				9	2				2	11	
			Average	%	7		0.0	0.0	-	4.5	1.0				1.0	5.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication
[a] % Mitotic index reduction as compared to the vehicle control.
[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide CP = Cyclophosphamide

Conclusion

(b) (4) did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes with or without an exogenous metabolic activation system in a valid assay.

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7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: An *In Vivo* Bone Marrow Micronucleus Test of SABER-Bupivacaine in Sprague Dawley Rats
- with SABER-Bupivacaine and SABER placebo

Study no:	(b) (4)-434056
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 1, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SABER-Bupivacaine - Lot no. K0059, 98.2%, liquid SABER placebo (SABER only) – Lot K0058

Key Study Findings

- SABER-Bupivacaine (12%) solution and SABER placebo were tested at the limits of solubility in the *in vivo* micronucleus assay.
- SABER-Bupivacaine (12%) solution and SABER placebo met the criteria for a negative response for bone marrow cytotoxicity and clastogenicity in rats under the conditions of a valid *in vivo* micronucleus assay.

Methods

Doses in definitive study:

Group Number	Treatment	Dose Level (mg/kg)	Concentration (%)
1	Placebo	0	0
2	SABER-Bupivacaine	26.4[a]	12[a]
3	SABER-Bupivacaine	79.2[a]	12[a]
4	SABER-Bupivacaine	264[a]	12[a]
5	Positive control (CPS)	60[b]	6 mg/mL[b]

[a] = 12% bupivacaine by weight, which, with a density of (b) (4) g/mL, translates to 132 mg/mL. The dose levels shown are mg/kg of bupivacaine.

[b] = Dose level and dose concentration listed applicable to the CPS dose level and CPS concentration for Group 5.

Frequency of dosing: Single dose
Route of administration: Subcutaneous (SC)
Dose volume: See table below
Formulation/Vehicle: None for test article and placebo, deionized water for positive control

Species/Strain: Crl:CD(SD) rats
Number/Sex/Group:

Group Number	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Animals[a]	
				Males	Females
1	Placebo	0	2	6	6
2	SABER-Bupivacaine	26.4[b]	0.2	6	6
3	SABER-Bupivacaine	79.2[b]	0.6	6	6
4	SABER-Bupivacaine	264[b]	2	6	6
5	Positive control[c]	60[c]	10	6	6

[a] = All animals were euthanized on study day 3; five animals/sex/group were utilized for bone marrow collection.

[b] = 12% bupivacaine by weight, which, with a density of (b) (4) g/mL, translates to 132 mg/mL.

[c] = A single dose of CPS was administered to the positive control group on study day 2; animals were euthanized approximately 24 hours following dosing.

Satellite groups: None
Basis of dose selection: Previous toxicity studies limiting dose volume to 2 mL/kg
Negative control: Placebo (SABER)
Positive control: Cyclophosphamide monohydrate (CPS), Lot no. 079K1569, 100.5% purity

Study Validity Valid – dose volume maximum and 1 death in high dose group (cause undetermined)

Observations and Results

Survival - All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. The male found dead was examined

macroscopically as soon as possible to ensure that tissues were not lost due to autolysis.

One male (no. 24525) in the 264 mg/kg group was found dead approximately 1 hour following dosing on study day 0. The only macroscopic finding at the gross examination was dark red area at the injection site. The cause of death for this male is undetermined.

Clinical observations - Clinical examinations were performed at the time of dose administration and approximately 1 to 2 hours following dose administration on the day of dosing (study day 0 for Groups 1-4 and study day 2 for Group 5). All animals were observed on the non-dosing day (study day 1).

There were no test article-related clinical observations.

Body weights - Individual body weights were recorded at the time of randomization and on study days 0, 2, and 3.

Body weights were unaffected by test article administration.

Food consumption - Individual food consumption was recorded at the time of randomization and on study days 0, 2, and 3.

Food consumption was unaffected by test article administration.

Macroscopic examination - A gross necropsy was conducted on the male found dead on study day 3. The necropsy included, but was not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera.

Male no. 24525 in the 264 mg/kg group was found dead on study day 0. The only macroscopic finding at the gross examination was dark red area at the injection site. The cause of death for this male is undetermined.

Micronucleus evaluation - Bone marrow was collected from the first 5 of 6 animals in each sex/group at the time of euthanasia from the right femur of animals.

SABER-Bupivacaine (12%) solution (proposed drug product concentration) and SABER placebo did not produce a statistically significant increase in the mean %MN-PCEs compared to the vehicle control group. No bone marrow cytotoxicity (decreases in the mean PCE:TE ratio) was noted in any test article-treated group. The %MN-PCEs in the positive control groups were statistically significantly higher than in the vehicle control group. The group mean values for both %MN-PCEs and PCE:TE ratios for the vehicle and positive control groups were within the respective historical control ranges.

Males, 24 Hours after Single Dose Released Over 3 Days [a]

TREATMENT	ANIMAL No.	MN PCEs/ 2000 PCEs	% MN PCEs	PCEs	NCEs	PCE:TE Ratio
SABER-Bupivacaine Placebo (Vehicle)	24512	0	0.00	775	225	0.78
	24519	0	0.00	637	363	0.64
	24521	2	0.10	458	542	0.46
	24524	1	0.05	312	688	0.31
	24531	1	0.05	450	550	0.45
Mean ± SD			**0.04 ± 0.04**			**0.53 ± 0.18**
SABER-Bupivacaine (12%) Solution (26.4 mg/kg)	24507	1	0.05	282	718	0.28
	24510	0	0.00	582	418	0.58
	24529	0	0.00	541	459	0.54
	24530	0	0.00	663	337	0.66
	24537	2	0.10	472	528	0.47
Mean ± SD			**0.03 ± 0.04**			**0.51 ± 0.14**
SABER-Bupivacaine (12%) Solution (79.2 mg/kg)	24509	1	0.05	620	380	0.62
	24511	0	0.00	332	668	0.33
	24517	2	0.10	464	536	0.46
	24522	0	0.00	393	607	0.39
	24527	0	0.00	634	366	0.63
Mean ± SD			**0.03 ± 0.04**			**0.49 ± 0.13**
SABER-Bupivacaine (12%) Solution (264 mg/kg)	24513	1	0.05	537	463	0.54
	24514	0	0.00	567	433	0.57
	24520	0	0.00	260	740	0.26
	24526	0	0.00	794	206	0.79
	24533	1	0.05	521	479	0.52
Mean ± SD			**0.02 ± 0.03**			**0.54 ± 0.19**
Cyclophosphamide (60 mg/kg)	24508	30	1.50	541	459	0.54
	24515	20	1.00	432	568	0.43
	24516	40	2.00	536	464	0.54
	24518	34	1.70	638	362	0.64
	24532	20	1.00	584	416	0.58
Mean ± SD			**1.44 ± 0.44***			**0.55 ± 0.08**

MN = Micronucleated
NCE = Normochromatic Erythrocyte
TE = Total erythrocytes (PCE + NCE)
PCE = Polychromatic Erythrocyte
*Statistically different than vehicle control $p \leq 0.05$
[a] Except for cyclophosphamide treatment: single dose with 24 hour postdose bone marrow harvest

Mean = $\frac{\sum x_n}{n}$ Where x = the individual values; n = the number of values

SD = Standard Deviation = $\sqrt{\frac{\sum (x-\bar{x})^2}{n-1}}$

Females, 24 Hours after Single Dose Released Over 3 Days [a]

TREATMENT	ANIMAL No.	MN PCEs/ 2000 PCEs	% MN PCEs	PCEs	NCEs	PCE:TE Ratio
SABER-Bupivacaine Placebo (Vehicle)	24543	0	0.00	792	208	0.79
	24546	1	0.05	594	406	0.59
	24558	0	0.00	520	480	0.52
	24559	2	0.10	506	494	0.51
	24566	0	0.00	501	499	0.50
Mean ± SD			**0.03 ± 0.04**			**0.58 ± 0.12**
SABER-Bupivacaine (12%) Solution (26.4 mg/kg)	24541	0	0.00	521	479	0.52
	24542	0	0.00	684	316	0.68
	24555	0	0.00	702	298	0.70
	24561	1	0.05	728	272	0.73
	24571	1	0.05	368	632	0.37
Mean ± SD			**0.02 ± 0.03**			**0.60 ± 0.15**
SABER-Bupivacaine (12%) Solution (79.2 mg/kg)	24544	0	0.00	816	184	0.82
	24545	0	0.00	524	476	0.52
	24549	0	0.00	783	217	0.78
	24562	0	0.00	523	477	0.52
	24564	1	0.05	643	357	0.64
Mean ± SD			**0.01 ± 0.02**			**0.66 ± 0.14**
SABER-Bupivacaine (12%) Solution (264 mg/kg)	24548	0	0.00	652	348	0.65
	24551	1	0.05	635	365	0.64
	24553	0	0.00	677	323	0.68
	24554	2	0.10	294	706	0.29
	24565	1	0.05	477	523	0.48
Mean ± SD			**0.04 ± 0.04**			**0.55 ± 0.16**
Cyclophosphamide (60 mg/kg)	24547	12	0.60	560	440	0.56
	24552	12	0.60	460	540	0.46
	24556	18	0.90	619	381	0.62
	24557	19	0.95	544	456	0.54
	24560	8	0.40	530	470	0.53
Mean ± SD			**0.69 ± 0.23***			**0.54 ± 0.06**

MN = Micronucleated
NCE = Normochromatic Erythrocyte
TE = Total erythrocytes (PCE + NCE)
PCE = Polychromatic Erythrocyte
*Statistically different than vehicle control $p \leq 0.05$
[a] Except for cyclophosphamide treatment: single dose with 24 hour postdose bone marrow harvest

Mean = $\frac{\sum x_n}{n}$ Where x = the individual values; n = the number of values

SD = Standard Deviation = $\sqrt{\frac{\sum (x-\bar{x})^2}{n-1}}$

Dosing solution - The test article, SABER-Bupivacaine (12%) Solution, and control article (placebo), SABER placebo, were physically and chemically stable throughout the duration of the study.

Conclusion - - SABER-Bupivacaine (12%) solution and SABER placebo met the criteria for a negative response for bone marrow cytotoxicity and clastogenicity in rats under the conditions of this valid assay. While a valid assay, it may not really be fully evaluating the potential genotoxicity of the drug substance, drug product, and it components as SAIB persists at the injection site for a prolonged period of time. This issue will be discussed in section 11.

7.4 Other Genetic Toxicity Studies

None conducted.

8 Carcinogenicity

Note: For the pharmacological active ingredient, bupivacaine, a 505(b)(2) reference is made to the approved Marcaine (bupivacaine hydrochloride - NDA 16-964) label for carcinogenicity data for bupivacaine. No other carcinogenicity studies were conducted.

9 Reproductive and Developmental Toxicology

Note: For the pharmacological active ingredient, bupivacaine, a 505(b)(2) reference is made to the approved Marcaine (bupivacaine hydrochloride - NDA 16-964) label for reproductive and developmental toxicology data for bupivacaine. The listed embryonic fetal development study reviewed is an assessment of SABER placebo (no active ingredient).

9.1 Fertility and Early Embryonic Development

No studies conducted.

9.2 Embryonic Fetal Development

Study title: Developmental Toxicity Study of SABER™-Bupivacaine Placebo in Rats

Study no.:	11-11-803-R-SC-TT
Study report location:	eCTD DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 28, 2011 (protocol approval)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Test article - SABER placebo, lot K0058 (DURECT; 721003F (b) (4)), 100% pure – 75%

Sucrose Acetate Isobutyrate
(SAIB), 25% Benzyl Alcohol (BA)
Vehicle – 0.9% NaCl, lot C829432

Key Study Findings

- Sperm positive female SD rats were treated with SABER placebo (75%:25% SAIB:BA) by subcutaneous injections of 0 (2 mL/kg 0.9% saline), 0.2, 0.6, & 2 mL/kg every 3 days of gestation starting on gestation day 7 until gestation day 16.
- All rats survived to sacrifice. Body weights/body weight gains, food consumption, Caesarean-sectioning parameters, fetal gross and visceral alterations, and ossifications site averages were unaffected by treatment.
- Injection site reactions (i.e., swelling, discoloration, and scabs) occurred at all dose levels of SABER™-Bupivacaine placebo tested, and the incidences were dose dependent. These observations generally occurred in the first few days of dosing and, in many cases, persisted through the end of the dose period and into the post-dose period.
- In the 2 mL/kg SABER placebo group, 6 fetuses in 1 litter had extra presacral vertebrae with or without extra ribs. While these findings were not previously identified in the testing lab's historical control data, the occurrence was considered of unknown toxicological relevance and a sporadic occurrence as it occurred in only a single litter. Both SAIB and BA are not known to cause such effects. This conclusion was confirmed in a consult to the FDA OND PTCC Reproductive and Developmental Subcommittee.
- There was no increase in embryo lethality, no effect on fetal body weight, and no fetal alterations (malformations or variations) attributed to SABER placebo at any dose tested. On the basis of these data, the developmental NOAEL was considered to be 2 mL/kg.

Methods

Doses:	0, 0.2, 0.6, & 2 mL/kg of test article
Frequency of dosing:	Every 3 days during major organogenesis Gestation Days (GD) 7, 10, 13, & 16
Dose volume:	2, 0.2, 0.6, & 2 mL/kg
Route of administration:	Subcutaneous to the shaved back
Formulation/Vehicle:	BA
Species/Strain:	Crl:CD(SD) Sprague Dawley rats (non-pregnant females and breeder males)
Number/Sex/Group:	25 mated females per group
Satellite groups:	None.
Study design:	Rotate injections sites each dose (4 total injection sites) High dose based on other toxicity studies
Deviation from study protocol:	Nothing affecting the outcome of the study

Observations and Results

Maternal Mortality

The rats were assessed for viability at least twice daily during the study.

All maternal rats survived the study period.

Maternal Clinical Signs

The rats were observed for general appearance at least weekly during the acclimation period; on GD 0; once daily during dose period, including before each dose was administered; and once daily during the post-dose period.

In maternal animals, a high incidence of injection site reactions (i.e., swelling, discoloration, and scabs) occurred at all dose levels of SABER™-Bupivacaine placebo tested, and the incidences were dose dependent. These observations generally occurred in the first few days of dosing and, in many cases, persisted through the end of the dose period and into the post-dose period.

GROUP	1	2	3	4
TEST MATERIAL	CONTROL ARTICLE	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a	2	0.2	0.6	2
MAXIMUM POSSIBLE INCIDENCE	375/ 25	375/ 25	375/ 25	375/ 25
MORTALITY	0	0	0	0
INJECTION SITES:				
INJECTION SITE(S): SWOLLEN	0/ 0	44/ 10	161/ 21**	304/ 25**
INJECTION SITE(S): DISCOLORATION b	5/ 1	118/ 18**	180/ 22**	253/ 23**
INJECTION SITE(S): SCAB(S)	0/ 0	88/ 15**	162/ 20**	131/ 22**
CLINICAL OBSERVATIONS:				
SPARSE HAIR COAT: LIMB(S)	0/ 0	22/ 2	15/ 1	0/ 0
SPARSE HAIR COAT: UNDERSIDE	0/ 0	10/ 1	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH THE OBSERVATION
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP
a. Dose administration occurred on Days 7, 10, 13 and 16 of presumed gestation.
b. Red, purple and/or color was not recorded.
** Significantly different from the control group value ($p \leq 0.01$).

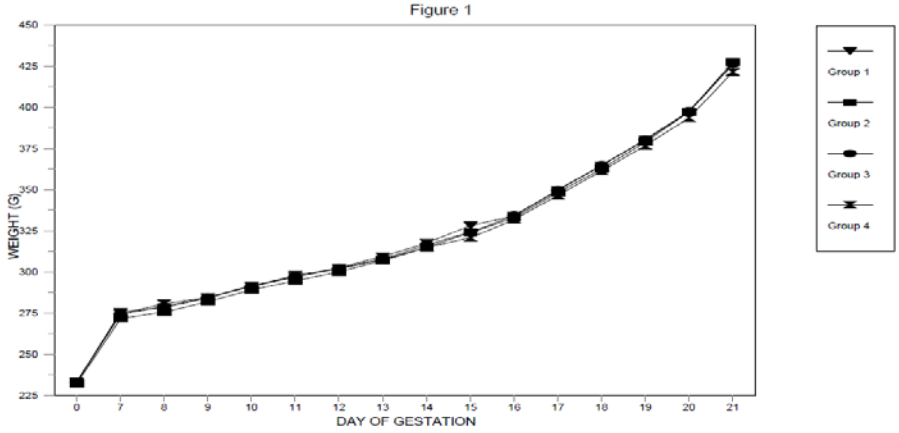
All other clinical observations (i.e., sparse hair coat on the limbs or underside) were considered unrelated to subcutaneous administration of SABER placebo as they were not dose-dependent.

Maternal Body Weight

Body weights were recorded at least weekly during the acclimation period, on GD 0, and daily during the dose and post-dose periods.

No treatment-related changes in maternal body weight were observed. While administration of SABER™-Bupivacaine placebo was associated with a transient but statistically significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in mean maternal body weight gains at 0.6 mL/kg on DGs 12 to 15 and at 2 mL/kg on DGs 8 to 9, 14 to 15, and 12 to 15, compared to the control article group values, there were no changes in body weight gains for the dose period (calculated as DGs 7 to 19) or gestation period (DGs 0 to 21) overall. Likewise, mean maternal body weights were unaffected by administration of SABER™-Bupivacaine placebo at any level tested. Se figure for control (group 1), 0.2 mL/kg (group 2), 0.6 mL/kg (group 3), and 2 mL/kg (group 4).

MATERNAL BODY WEIGHTS
Figure 1



Feed Consumption

Food consumption values were recorded on GDs 0, 7, 10, 12, 15, 18, and 21.

There were treatment-related changes in absolute (g/day) or relative (g/kg/day) food consumption at any dose level tested.

Toxicokinetics

None conducted (no active ingredient).

Dosing Solution Analysis

Material was used as supplied from sponsor in differing volumes on neat material. The pre-dose and returned post-dose test article samples were analyzed for sample appearance, degree of coloration, identity, and excipient related degradation products (b) (4). The results showed that the end of dosing (post-dose) test article was physically and chemically stable for the duration of use in the study.

Necropsy

On GD 21, all female rats were euthanized, caesarean-sectioned, and examined for gross lesions. All gross lesions/masses were collected and preserved. The cervix and uterus of one non-pregnant rat from the high dose (HD) was collected for possible future evaluation. The uterus of the one HD apparently non-pregnant animal was examined by being pressed between glass plates to confirm absence of implantation sites.

The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color, or shape; any abnormalities were recorded), live and dead fetuses, and early and late resorptions. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to stimuli. Non-responding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. Placentae were examined for size, color, and shape.

After sacrifice, fetuses were examined for sex and external, visceral and skeletal abnormalities. The body weight of each fetus was recorded. Fetuses were individually identified with the study number, litter number, uterine distribution, fetus number, and fixative. Approximately one-half of the fetuses in each litter were examined for visceral abnormalities by using a modification of the microdissection technique of Staples. Each fetus was fixed in Bouin’s solution and the heads were subsequently examined by free-hand sectioning; head sections were stored in alcohol. The decapitated carcasses were not retained.

The remaining fetuses (approximately one-half of the fetuses in each litter) were examined for skeletal abnormalities after staining with alizarin red S. Following examination, skeletal preparations were retained in glycerin, with thymol added as a preservative.

Fetal alterations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.

Maternal Necropsy

Clinical observations in the treated groups that persisted to the end of the gestation period as listed in the clinical sigh table previously (i.e., swelling, discoloration, scabs, and sparse hair coat) were confirmed at the time of necropsy. In addition, subcutaneous brown and red firm material corresponding to the clinical observation of swelling at the injection site was observed in one rat in the 2 mL/kg dose group and was attributed to administration of SABER™-Bupivacaine placebo. No other gross lesions were observed at necropsy.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

No treatment-related effects were observed. The litter averages for corpora lutea, implantations, percentage of preimplantation loss, litter size, live fetuses, resorptions, percentage of postimplantation loss, dams with any resorptions, percentage of live male fetuses, fetal body weights, and percentage of dead or resorbed conceptuses were comparable among the 4 dose groups and did not significantly differ. No dam had a litter consisting of only dead or resorbed conceptuses, and all placentae appeared normal.

There was 1 dead fetus in the 2 mL/kg dose group. However, this finding was not attributed to SABER™-Bupivacaine placebo by the sponsor because: 1) this was only a single event; 2) there was no overall increase in postimplantation loss in this dose group; and 3) fetal death occurs sporadically. Reviewer agrees.

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	25(100.0)	25(100.0)	25(100.0)	24(96.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	25	25	25	24
CORPORA LUTEA	MEAN±S.D.	15.4 ± 2.2	15.5 ± 1.8	15.8 ± 2.7	15.7 ± 2.2
IMPLANTATIONS	MEAN±S.D.	14.6 ± 1.8	15.2 ± 1.7	15.3 ± 2.1	15.3 ± 2.3
% PREIMPLANTATION LOSS	MEAN±S.D.	4.7 ± 7.1	2.0 ± 3.4	2.7 ± 6.2	2.4 ± 5.3
LITTER SIZES	MEAN±S.D.	13.8 ± 2.1	14.4 ± 1.6	14.4 ± 2.0	14.7 ± 2.2
LIVE FETUSES	N	346	361	359	351
	MEAN±S.D.	13.8 ± 2.1	14.4 ± 1.6	14.4 ± 2.0	14.6 ± 2.2
DEAD FETUSES	N	0	0	0	1
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2
RESORPTIONS	MEAN±S.D.	0.8 ± 1.2	0.8 ± 1.1	1.0 ± 1.2	0.7 ± 1.0
EARLY RESORPTIONS	N	20	19	22	16
	MEAN±S.D.	0.8 ± 1.2	0.8 ± 1.1	0.9 ± 1.0	0.7 ± 1.0
LATE RESORPTIONS	N	0	0	2	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
% POSTIMPLANTATION LOSS	MEAN±S.D.	5.5 ± 8.6	4.7 ± 6.9	6.0 ± 7.2	4.4 ± 6.1
DAMS WITH ANY RESORPTIONS	N(%)	14(56.0)	10(40.0)	13(52.0)	10(41.7)
DAMS WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dose administration occurred on Days 7, 10, 13 and 16 of gestation.

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	25(100.0)	25(100.0)	25(100.0)	24(96.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	25	25	25	24
DAMS WITH VIABLE FETUSES	N(%)	25(100.0)	25(100.0)	25(100.0)	24(100.0)
PLACENTAE APPEARED NORMAL	N(%)	25(100.0)	25(100.0)	25(100.0)	24(100.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dose administration occurred on Days 7, 10, 13 and 16 of gestation.

Offspring (Malformations, Variations, etc.)

No treatment-related overall fetal alterations were observed.

FETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EVALUATED	N	25	25	25	24
FETUSES EVALUATED	N	346	361	359	352
LIVE	N	346	361	359	351
DEAD b	N	0	0	0	1
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	6(24.0)	2(8.0)	8(32.0)	7(29.2)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	9(2.6)	3(0.8)	8(2.2)	12(3.4)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	2.7 ± 5.5	0.9 ± 3.5	2.3 ± 3.5	3.5 ± 8.0

a. Dose administration occurred on Days 7, 10, 13 and 16 of gestation.
b. Values for dead fetus were excluded from summarization and statistical analyses.

No treatment-related fetal gross external effects were observed.

FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SABER™- BUPIVACAINE PLACEBO	SABER™- BUPIVACAINE PLACEBO	SABER™- BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EVALUATED	N	25	25	25	24
LITTERS WITH LIVE FETUS(ES)	N	25	25	25	24
FETUSES EVALUATED	N	346	361	359	352
LIVE	N	346	361	359	351
DEAD b	N	0	0	0	1
EYE: BULGE DEPRESSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)	0(0.0)
TAIL: THREAD-LIKE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)

a. Dose administration occurred on Days 7, 10, 13 and 16 of gestation.
b. Dead fetus was excluded from summarization and statistical analyses

No treatment-related fetal soft tissue alterations were observed.

FETAL SOFT TISSUE ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SABER™- BUPIVACAINE PLACEBO	SABER™- BUPIVACAINE PLACEBO	SABER™- BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EVALUATED	N	25	25	25	24
LITTERS WITH LIVE FETUS(ES)	N	25	25	25	24
FETUSES EVALUATED	N	166b	174	173	169
LIVE	N	166	174	173	168
DEAD c	N	0	0	0	1
LUNGS: INTERMEDIATE LOBE ABSENT					
LITTER INCIDENCE	N(%)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	0(0.0)	0(0.0)
SITUS INVERSUS					
LITTER INCIDENCE	N(%)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	0(0.0)	0(0.0)

a. Dose administration occurred on Days 7, 10, 13 and 16 of gestation.
b. Excludes fetus 4603-12, which was intended for skeletal examination but was inadvertently fixed in Bouin's rather than alcohol.
c. Dead fetus was excluded from summarization and statistical analyses.
d. Fetus 4613-10 had other soft tissue alterations.

Overall, no treatment-related fetal skeletal alterations were observed. However, in 1 litter in the 2 mL/kg dose group, not the litter with the 1 dead fetus, some findings not in the other groups and not in the testing lab's historical control data were observed (highlighted in yellow in the following tables). Six (6) fetuses in 1 litter had extra presacral vertebrae with or without extra ribs. Four (4) of these fetuses had 15 thoracic

vertebrae and 6 lumbar vertebrae present. One (1) of these also had 15 ribs present on the right side, whereas there were 15 ribs bilaterally in the other 3 fetuses. Two (2) other fetuses from the same litter had 7 lumbar vertebrae present. While these findings were not previously identified in the testing lab's historical control data, the involvement of only a single litter makes the relationship to treatment unknown. This conclusion was confirmed in a consult to the FDA OND PTCC Reproductive and Developmental Subcommittee.

No other skeletal fetal alterations may have been attributable to administration of SABER™-Bupivacaine placebo or exceeded the historical control data.

FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP TEST MATERIAL		1 CONTROL ARTICLE	2 SABER™-BUPIVACAINE PLACEBO	3 SABER™-BUPIVACAINE PLACEBO	4 SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EVALUATED	N	25	25	25	24
LITTERS WITH LIVE FETUS(ES)	N	25	25	25	24
FETUSES EVALUATED	N	179	187	186	183
LIVE	N	179	187	186	183
SKULL: NASAL-FRONTAL SUTURE, LARGE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	2(8.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	2(1.1)g,h
SKULL: ZYGOMATIC, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.0)	0(0.0)	0(0.0)	2(8.3)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	2(1.1)g,h
SKULL: SQUAMOSAL, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	1(0.5)h
SKULL: EYE SOCKET, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA					
LITTER INCIDENCE	N(%)	1(4.0)	1(4.0)	4(16.0)	1(4.2)
FETAL INCIDENCE	N(%)	1(0.6)	1(0.5)	4(2.2)	1(0.5)f
CERVICAL VERTEBRAE: ARCH, 7TH CERVICAL ARCH HAS THE APPEARANCE OF THE 6TH					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)d	1(0.5)
CERVICAL VERTEBRAE: ARCH, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)d	0(0.0)
CERVICAL VERTEBRAE: ARCH, 6TH CERVICAL ARCH HAS THE APPEARANCE OF THE 7TH					
LITTER INCIDENCE	N(%)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)

FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP TEST MATERIAL		1 CONTROL ARTICLE	2 SABER™-BUPIVACAINE PLACEBO	3 SABER™-BUPIVACAINE PLACEBO	4 SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EVALUATED	N	25	25	25	24
LITTERS WITH LIVE FETUS(ES)	N	25	25	25	24
FETUSES EVALUATED	N	179	187	186	183
LIVE	N	179	187	186	183
THORACIC VERTEBRAE: ARCHES, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)d	0(0.0)
THORACIC VERTEBRAE: CENTRUM, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)d	0(0.0)
THORACIC VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	2(8.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	3(1.7)	0(0.0)	1(0.5)	0(0.0)
THORACIC VERTEBRAE: 15 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	4(2.2)**i,j,k,L
LUMBAR VERTEBRAE: ARCH AND CENTRUM, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)m
LUMBAR VERTEBRAE: 3 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)m
LUMBAR VERTEBRAE: ARCH, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)m
LUMBAR VERTEBRAE: CENTRUM, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)m

** Significantly different from the control group value ($p \leq 0.01$).

FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP TEST MATERIAL		1 CONTROL ARTICLE	2 SABER™-BUPIVACAINE PLACEBO	3 SABER™-BUPIVACAINE PLACEBO	4 SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EVALUATED	N	25	25	25	24
LITTERS WITH LIVE FETUS(ES)	N	25	25	25	24
FETUSES EVALUATED	N	179	187	186	183
LIVE	N	179	187	186	183
LUMBAR VERTEBRAE: 6 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	4(2.2)i,j,k,L
LUMBAR VERTEBRAE: 7 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	2(1.1)
SACRAL VERTEBRAE: 0 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)m
CAUDAL VERTEBRAE: 0 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)m
RIBS: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)d	0(0.0)
RIBS: SHORT					
LITTER INCIDENCE	N(%)	1(4.0)	1(4.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	1(0.6)	2(1.1)	0(0.0)	1(0.5)
RIBS: INCOMPLETELY OSSIFIED (HYPOPLASTIC)					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)h
RIBS: THICKENED					
LITTER INCIDENCE	N(%)	1(4.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	1(0.5)e	0(0.0)

FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP TEST MATERIAL		1 CONTROL ARTICLE	2 SABER™-BUPIVACAINE PLACEBO	3 SABER™-BUPIVACAINE PLACEBO	4 SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EVALUATED	N	25	25	25	24
LITTERS WITH LIVE FETUS(ES)	N	25	25	25	24
FETUSES EVALUATED	N	179	187	186	183
LIVE	N	179	187	186	183
RIBS: WAVY					
LITTER INCIDENCE	N(%)	1(4.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	1(0.5)e	0(0.0)
RIBS: 15 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	4(2.2)i,j,k,L
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	1(0.5)f
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)f
STERNAL CENTRA: NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)d	0(0.0)
PELVIS: PUBIS, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)g
PELVIS: CLOSE SET					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)m

All fetal ossification site averages were comparable among the 4 dose groups.

FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

GROUP TEST MATERIAL		1 CONTROL ARTICLE	2 SABER™-BUPIVACAINE PLACEBO	3 SABER™-BUPIVACAINE PLACEBO	4 SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EXAMINED	N	25	25	25	24
FETUSES EXAMINED	N	179	187	186	183
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	0.96 ± 0.08	0.96 ± 0.10	0.97 ± 0.12	0.97 ± 0.08
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	13.06 ± 0.11	13.07 ± 0.10	13.10 ± 0.16	13.12 ± 0.17
LUMBAR	MEAN±S.D.	5.94 ± 0.11	5.92 ± 0.10	5.90 ± 0.16	5.89 ± 0.16
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	7.23 ± 0.77	7.50 ± 0.67	7.52 ± 0.65	7.44 ± 0.81
RIBS (PAIRS)	MEAN±S.D.	13.05 ± 0.08	13.04 ± 0.06	13.07 ± 0.13	13.10 ± 0.15
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.98 ± 0.06	4.00 ± 0.00	3.98 ± 0.10	4.02 ± 0.17
XIPHOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.00 ± 0.02	4.00 ± 0.00	4.00 ± 0.02	4.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	7.57 ± 0.94	8.15 ± 0.67	7.98 ± 0.78	7.81 ± 0.92
HINDLIMB b					
TARSALS	MEAN±S.D.	0.03 ± 0.07	0.02 ± 0.05	0.01 ± 0.04	0.02 ± 0.05
METATARSALS	MEAN±S.D.	4.82 ± 0.24	4.89 ± 0.19	4.87 ± 0.19	4.77 ± 0.27
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	5.62 ± 0.83	5.95 ± 0.77	5.74 ± 0.78	5.84 ± 0.92

a. Dose administration occurred on Days 7, 10, 13 and 16 of gestation.
b. Calculated as average per limb.

FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO	SABER™-BUPIVACAINE PLACEBO
DOSE VOLUME (ML/KG)a		2	0.2	0.6	2
LITTERS EXAMINED	N	25	25	25	24
FETUSES EXAMINED	N	179	187	186	183
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	0.96 ± 0.08	0.96 ± 0.10	0.97 ± 0.12	0.97 ± 0.08
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	13.06 ± 0.11	13.07 ± 0.10	13.10 ± 0.16	13.12 ± 0.17
LUMBAR	MEAN±S.D.	5.94 ± 0.11	5.92 ± 0.10	5.90 ± 0.16	5.89 ± 0.16
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	7.23 ± 0.77	7.50 ± 0.67	7.52 ± 0.65	7.44 ± 0.81
RIBS (PAIRS)	MEAN±S.D.	13.05 ± 0.08	13.04 ± 0.06	13.07 ± 0.13	13.10 ± 0.15
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.98 ± 0.06	4.00 ± 0.00	3.98 ± 0.10	4.02 ± 0.17
XIPHOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.00 ± 0.02	4.00 ± 0.00	4.00 ± 0.02	4.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	7.57 ± 0.94	8.15 ± 0.67	7.98 ± 0.78	7.81 ± 0.92
HINDLIMB b					
TARSALS	MEAN±S.D.	0.03 ± 0.07	0.02 ± 0.05	0.01 ± 0.04	0.02 ± 0.05
METATARSALS	MEAN±S.D.	4.82 ± 0.24	4.89 ± 0.19	4.87 ± 0.19	4.77 ± 0.27
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	5.62 ± 0.83	5.95 ± 0.77	5.74 ± 0.78	5.84 ± 0.92

a. Dose administration occurred on Days 7, 10, 13 and 16 of gestation.
b. Calculated as average per limb.

9.3 Prenatal and Postnatal Development

No studies conducted.

10 Special Toxicology Studies

10. 1 Wound Healing

Study title: A study of the effects of Sucrose Acetate isobutyrate (SAIB)-based formulations on wound healing using the rat linear incision model

Study no.: DUR2
Study report location: eCTD in DARRTS
Conducting laboratory and location: (b) (4)
Date of study initiation: February, 10, 2004
GLP compliance: No
QA statement: No
Drug, lot #, and % purity:

Key Study Findings

- Male Sprague Dawley rats received intended wounds on day 0 and were treated with various SAIB-containing formulations (0.125 mL in a 2.5 cm incision), two of which were similar to SABER-Bupivacaine and SABER placebo, by wound instillation or SC injection.
- A mechanical determination of wound strength was measured on day 7 after the treatment and histology conducted on the SABER-Bupivacaine similar formulation and untreated wound (negative control).
- No gross differences were noted in the wound sites as well as no difference in wound strength compared to the negative control.
- The SABER-Bupivacaine similar formulation caused more severe histological effects than the negative control, notably inflammation, granulation, angiogenesis, epithelialization, and gaps. The SABER placebo group was not evaluated histologically.
- In summary, under the conditions of the assay, formulations similar to SABER-Bupivacaine and SABER placebo did not adversely effects wound healing over 7 days and the SABER-Bupivacaine similar formulation ~~cause~~ was associated with increased histological effects compared to the negative control consistent with a foreign body response.

Methods

Doses:

Study Group	Formulation	Treatment	Bupivacaine		Minimum No. Wounds/ Group	Post-op Evaluation Time Points
			Dose	mg/kg*		
A	#1	"in wound" 0.05 ml/cm (0.125 ml total)	15	50	15	Day 7
B	#2	"in wound" 0.05 ml/cm (0.125 ml total)	0	0	10	Day 7
C	#5	Paired Trailing S.C, 0.05 ml/cm x 2 (0.25 ml total)	28	92	10	Day 7
D	#6	Paired Trailing S.C, 0.05 ml/cm x 2 (0.25 ml total)	0	0	10	Day 7
E	#1	Paired Trailing S.C, 0.05 ml/cm x 2 (0.25 ml total)	30	100	10	Day 7
F	#2	Paired Trailing S.C, 0.05 ml/cm x 2 (0.25 ml total)	0	0	10	Day 7
G	#3	"in wound" 0.05 ml/cm (0.125 ml total)	15	50	15	Day 7
H	#4	"in wound" 0.05 ml/cm (0.125 ml total)	0	0	10	Day 7
I	#5	"in wound" 0.05 ml/cm (0.125 ml total)	14	46	15	Day 7
J	#6	"in wound" 0.05 ml/cm (0.125 ml total)	0	0	10	Day 7
K	#7	"in wound" 0.05 ml/wound	6.5	21	15	Day 7
L	#8	"in wound" 0.05 ml/wound	0	0	10	Day 7
M		Control (Incision only)	0	0	15	Day 7

Formulations 1 (SABER-Bupivacaine), 2 (SABER placebo) administered by wound instillation (group A & B) or injected SC (groups E & F) and incision control most relevant groups to proposed drug product SABER-Bupivacaine (see formulation table below)

Frequency of dosing: Single dose (may be split in ½)

Route of administration: On the day (Day 0) of surgery, under anesthesia,

symmetric 2.5cm full thickness linear incisions wounds were created over each dorsolateral flank of the animals. The materials were administered directly into the wound prior to incision closure, or by paired subcutaneous trailing injection immediately following wound closure.

Dose volume: See table

Formulation/Vehicle: Varied (see formulations – groups 1, 2, & 8 most relevant for SABER-Bupivacaine proposed drug product containing SAIB and BA)

#	Formulation	% Bupivacaine
1	SAIB/BA (75:25) (Solution in vial)	12% (wt/wt)
2	SAIB/BA (75:25) (Solution in vial)	0%
3	SAIB/Mig/BA (50/25/25) (Solution in vial)	12% (wt/wt)
4	SAIB/Mig/BA (50/25/25) (Solution in vial)	0%
5	Emulsion F12A (Pre heat vial to 45°C)	11% (wt/vol)
6	Emulsion F12AB (Pre heat vial to 45°C)	0
7	Paste F12B (Pre-loaded syringe)	13% (wt/vol)
8	Paste F12BB (Pre-loaded syringe)	0

Note: SAIB = Sucrose Acetate isoButyrate
Mig = Miglyol 810
BA = Benzyl Alcohol

Note: proposed drug product is 12% Bupivacaine, 66% SAIB and 22% BA (similar to #1 (b) (4) (b) (4)

Species/Strain: SPF Sprague Dawley rats

Number/Sex/Group: 5 males/group

Age: 8 weeks

Weight: 250-300 g

Satellite groups: none

Unique study design: Assessment of wound healing process over 7 days using biomechanical measurements of wound strength and histological evaluation

Deviation from study protocol: Nothing significant

Observations and Results

Mortality

Daily observations conducted from day -7 until termination (day 7 after incision).

No deaths observed.

Clinical Signs

Daily observations conducted from day -7 until termination (day 7 after incision).

No abnormal clinical signs observed.

Body Weights

Body weights measured on days 0 & 7.

Animals increased in body weight with no differences among groups.

Feed Consumption, Ophthalmoscopy, ECG, Hematology, Clinical Chemistry, Urinalysis, Organ Weights, Toxicokinetics, and Dosing Solution Analysis were not evaluated.

Gross Pathology

Wounds were grossly observed on day 7.

There were no visual differences reported in the appearance of the wounds on day 7.

Histopathology

The wounds of groups A (SABER-Bupivacaine equivalent), G, I, K, & M (wound control) were evaluated on day 7. Indices scored and severity grading system was as follows:

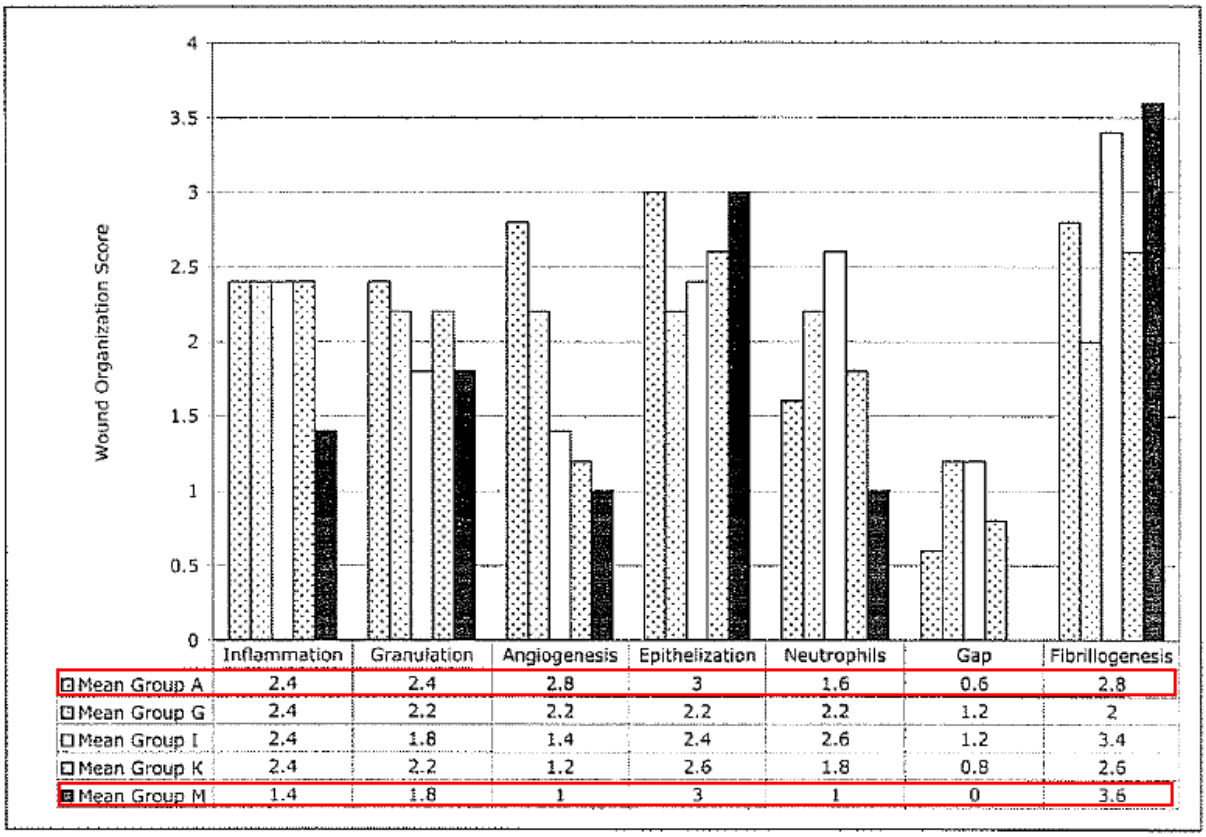
- Inflammation (1-5) Based on the general infiltration of inflammatory cells, including lymphocytes, eosinophils, macrophages, and neutrophils.
- Granulation (1-5) Extent of fibroblast accumulation as part of granulation tissue.
- Angiogenesis (1-5) Development of prominent neovasculature as part of granulation tissue formation
- Epithelization (1-5) Extent of wound resurfacing and degree of differentiation of keratinocytes.
- PMN/Neutrophils (1-5) Index of acute inflammation and consistent with presence of foreign material or infectious organisms
- Gap (5-1) Degree of separation of wound margins
- Fibrillogenesis (1-5) Organization of collagen fiber bundles and intensity of collagen staining

Adequate Battery – no (wound only)

Peer Review - no

Histological Findings – Group A exhibited increased inflammation, granulation, angiogenesis, neutrophils, and gaps, compared to wound only (group M). This appears to be a typical foreign body reaction to the injection.

SUMMARY OF HISTOLOGICAL TISSUE RESPONSE



	Inflammation	Granulation	Angiogenesis	Epithelization	Neutrophils	Gap	Fibrillogenesis
Mean Group A	2.4	2.4	2.8	3	1.6	0.6	2.8
Mean Group G	2.4	2.2	2.2	2.2	2.2	1.2	2
Mean Group I	2.4	1.8	1.4	2.4	2.6	1.2	3.4
Mean Group K	2.4	2.2	1.2	2.6	1.8	0.8	2.6
Mean Group M	1.4	1.8	1	3	1	0	3.6

Raw Data for Histology

	Group Description	Inflammation	Granulation	Angiogenesis	Epithelization	Neutrophils	Gap	Fibrillogenesis	Comments
Group A	Formulation #1 - "in wound" 0.05ml/cm (0.125 ml total)	2	2	3	4	1	0	3	
		1	1	2	4	0	2	3	cyst
		3	3	3	2	2	0	3	cyst
		4	3	3	2	3	1	2	lg cyst
		2	3	3	3	2	0	3	

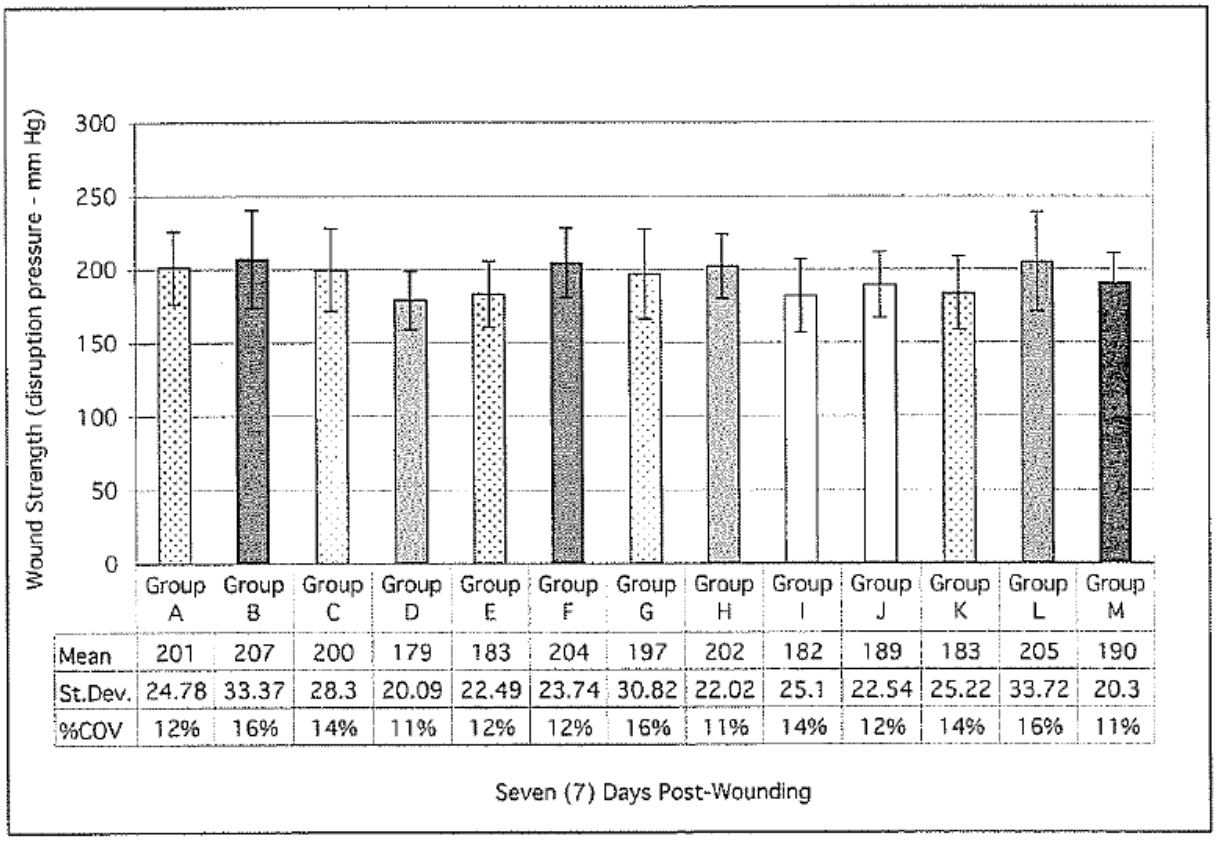
		Inflammation	Granulation	Angiogenesis	Epithelization	Neutrophils	Gap	Fibrillogenesis	Comments
Group M	Control (incision only)	2	3	2	2	1	0	2	
		0	1	1	3	0	0	4	
		3	2	1	4	3	0	4	superf abcess
		1	2	0	3	0	0	4	
		1	1	1	3	1	0	4	

Special Evaluation – *In Vivo* Biomechanical Testing

On Day 7 post-wounding, the respective groups were prepared for biomechanical testing. The sutures were removed. A disposable acrylic test ring (ID 2.5cm) was placed around the wound and secured to the skin using cyanoacrylate glue. A small amount of

perfluorinated grease was applied to the top of the ring interface to assure a tight vacuum seal. The BTC-2000TM test chamber (2.5 cm I.D.) was integrated with the test ring until the chamber and ring were securely interconnected. The test chamber was held by hand comfortably to assure that no positive force was being exerted on the wound. The BTC-2000™ test-start button was triggered. A constant negative pressure was applied to the wound at a rate of 10mmHg/second, producing a multi-axial stress on the wound. Abrupt displacement of wound margins (wound Failure) was captured by a displacement laser. The time synchronized data of pressure (mmHg) and wound deformation (mm) were graphically displayed, logged to disk, and analyzed by the BTC.2000™ in real-time.

The SABER-Bupivacaine similar and SABER placebo similar groups (A & E and B & F, respectively) exhibited no significant differences after instillation to wound or SC injection, respectively, from the wound control (group M). Only group D exhibited a reduced wound strength at $p<0.05$. (see figure)



	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H	Group I	Group J	Group K	Group L	Group M
Mean	201	207	200	179	183	204	197	202	182	189	183	205	190
St.Dev.	24.78	33.37	28.3	20.09	22.49	23.74	30.82	22.02	25.1	22.54	25.22	33.72	20.3
%COV	12%	16%	14%	11%	12%	12%	16%	11%	14%	12%	14%	16%	11%

Results from In-vivo Biomechanical Testing, 7 Days Post-Wounding.

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Study title: A single dose wound healing study in full-thickness wounds in minipigs

Study no.:	60111
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 16, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SABER Bupivacaine (12:66:22% bupivacaine:SAIB:BA),Lot 015-04). 96.9-100% purity (post-dose – pre-dose) SABER Bupivacaine placebo (Lot GLP-803), 75:25% SAIB:BA, purity NA Negative control, 5% CMC gel (batch I.385091), NA

Key Study Findings

- Three (3) female Gottingen SPF minipigs received eight full-thickness wounds (diameter 20 mm) on day 1 and were observed for 15 days. Immediately after surgery, the wounds were treated topically within the wound tissue with 5% CMC gel (carboxymethylcellulose negative control), SABER placebo (vehicle), or SABER-Bupivacaine in a dose volume of 0.5 ml per wound. Observations of the wounds, included planimetric measurement of wound contraction, was performed on a daily basis. On Day 15 necropsy, each wound was sampled, fixed in neutral buffered formalin and processed for histopathological examination.

- No treatment-related clinical signs were seen during the study. The body weights were within a normal range of body weight for fully grown Gottingen minipigs.

- The macroscopic wound observations revealed no treatment-related differences regarding inflammation of the wounds edges, the skin surrounding the wounds, grade of hemorrhage, or grade of exudation. Necrotic tissue was not observed in any of the wounds during the study and hypergranulation was only seen in a few wounds on a single day during the study.

- Planimetric-based, wound contraction was identified on Day 2 for the wounds of all treatments. All wounds contracted to a comparable amount (~60%) by the end of the study with a greater rate of contraction for the CMC-treated wounds on certain days during the study. Other observations (growth of granulation tissue, area of granulation tissue, re-epithelialization) were generally comparable with some increased severity in the SABER-Bupivacaine group commenced on Day 9 for the wounds of all treatments. No differences in the re-epithelialized wound areas between the different treatments were seen during the study. The re-epithelialized

area covered ~90% of the total wound area on Day 15 for the treated wounds of all groups.

- Microscopically, the wound healing process was advanced comparably in all treated wounds. SABER-Bupivacaine and SABER placebo had no significant adverse effects on the wound healing in the minipigs compared to a CMC control. No wound only group was included so no absolute effect on wound healing could be determined.

Methods

Doses:

	Animal No					
	1		2		3	
Localisation	L	R	L	R	L	R
Cranial	1	2	3	1	2	3
	2	3	1	2	3	1
	3	1	2	3	1	2
Caudal	1	2	3	1	2	3

Treatment 1: SABER-Bupivacaine; Treatment 2: SABER placebo; Treatment 3: negative control (CMC)

Frequency of dosing: Single application
Route of administration: Topical to wound
Dose volume: 0.5 mL per wound
Formulation/Vehicle: CMC for negative control
Species/Strain: Gottingen SPF minipigs
Number/Sex/Group: 3 females
Age: Not reported
Weight: 29.5-34.8 kg
Satellite groups: None
Unique study design: The wounds were established on Day 1. Eight circular full-thickness wounds (diameter 20 mm) were made on the prepared area (dorso-lateral area of either side of the back). The wounds were numbered 1 (most cranial) to 4 (most caudal) on the left side on the animal, and 5 (most cranial) to 8 (most caudal) on the right side of the animal. Wound contraction was measured for 15 days.
Deviation from study protocol: Nothing significant

Observations and Results

Mortality

Mortality was observed daily. No mortality was observed.

Clinical Signs

All visible signs of ill health and any behavioral changes were recorded daily.

No treatment-related clinical signs were seen in any of the animals during the study.

Body Weights

All animals were weighed on allocation to the study, one week before start of treatment (day -7), on the first day of treatment (day 1) and on day 8. Also the weight at necropsy was recorded (day 15).

The body weights were within a normal range of body weight for fully grown Gottingen minipigs.

Feed Consumption

From day -7, the consumption of food was estimated daily for each animal by weighing unconsumed diet.

No treatment-related effects were observed.

Ophthalmoscopy, ECG, Hematology, Clinical Chemistry, Urinalysis, Organ Weights, and Toxicokinetics not evaluated.

Gross Pathology

Macroscopic Wound Observation

Each wound was photographed and evaluated macroscopically on days 2-15 for the following parameters:

- Appearance of wound and wound edge (inflammation)
- Condition of skin surrounding the wound (inflammation)
- Hemorrhage
- Amount of exudate present
- Presence of slough/necrotic tissue
- Presence of granulation tissue
- Hypergranulation

The parameters were scored according to the following grading system:

0 not present
1 minimal
2 slight
3 moderate
4 marked

Minimal to slight inflammation of the wound edges and skin surrounding the wounds was observed in a majority of all wounds through day 15, with no differences between the individual treatments. From day 10, no inflammatory reaction of the wound edges was seen in any of the wounds including CMC negative control (no wound only, sham control included). Some inflammation of the skin was observed on day 15.

Slight to marked hemorrhage was observed for all wounds on day 2. Minimal to moderate hemorrhage was observed on days 3-7 with a decreasing severity to minimal for the majority of all wounds by day 8. No hemorrhage was observed by day 12 except for minimal hemorrhage in a few CMC-treated wounds on days 14 and 15. No real differences in the grade of hemorrhage were observed for the different treatments during the study.

Slight to moderate amounts of exudate were observed in all wounds on day 2, thereafter decreasing in severity to minimal to slight by day. The amount of exudation generally further decreased during the remaining part of the study, being minimal or absent for all wounds on day 15. No significant differences in the grade of exudation were observed between the different treatments during the study.
No necrotic tissue was observed in any of the wounds during the study.

Minimal to slight growth of granulation tissue was observed starting on day 6 for the wounds of all treatments for all groups. On day 7, slight to moderate amounts of granulation tissue were present in all wounds. Up to marked amounts of granulation tissue were observed by day 8. From day 9 and thereafter, marked amounts of granulation tissue were observed in all groups. The amount of granulation tissue was generally the same for all test groups.

Minimal hypergranulation was observed in a single vehicle treated wound site (minimal) and in a single CMC-treated wound site (slight) on Day 11. Besides these findings, no hypergranulation was observed during the study.

Wound Areas

Wound Contraction, Area of Granulation Tissue, and Area of Re-Epithelialization were measured for 15 days.

Wound Contraction - Wound contraction (decrease of wound areas compared to day 1) was identified starting on day 2 for the wounds of all treatment groups. All wounds

further contracted during the study, reaching comparable levels of 58.6, 59.6 and 61.7% on day 15 for the wounds treated with SABER-Bupivacaine, SABER placebo (vehicle), and CMC gel, respectively. Wound contraction was greater for the CMC-treated wounds compared with the other group wounds during the course of the study (15 days), but total wound contraction was essentially the same by day 15 for all groups. No differences were seen between the SABER-Bupivacaine wounds and the SABER placebo wounds. While the time course of wound contraction was different, the 15-day end result was comparable for all groups (see tables). Of note is that there was no "wound only" group for absolute comparison purposes.

A Single Dose Wound Healing Study in Full-Thickness Wounds in Minipigs

Wound areas in % of wound area day 1

Mean values

TREATMENT	% AREA DAY 2				% AREA DAY 3				% AREA DAY 4				% AREA DAY 5				% AREA DAY 6			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	96.6	2.6	8		88.0	7.0	8		95.6	6.0	8		95.9	7.2	8		95.9	6.6	8	
2	97.0	8.2	8		90.4	8.4	8		97.3	9.5	8		94.3	10.1	8		96.7	10.3	8	
3	88.4	8.8	8	*	84.6	13.0	8		90.8	11.7	8		89.5	15.4	8		88.6	15.3	8	

TREATMENT	% AREA DAY 7				% AREA DAY 8				% AREA DAY 9				% AREA DAY 10				% AREA DAY 11			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	85.5	10.3	8		76.8	12.1	8		63.7	12.8	8		58.9	10.9	8		50.6	11.6	8	
2	84.4	8.2	8		70.6	9.7	8		61.5	9.4	8		57.0	9.0	8		48.2	9.4	8	
3	74.8	9.5	8	*	64.6	10.1	8		56.5	8.7	8		49.8	8.9	8	*	46.6	9.5	8	

TREATMENT	% AREA DAY 12				% AREA DAY 13				% AREA DAY 14				% AREA DAY 15			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	50.5	14.8	8		48.1	15.1	8		44.9	10.7	8		41.4	10.6	8	
2	48.2	11.2	8		44.7	10.7	8		42.9	8.4	8		40.4	9.5	8	
3	43.0	13.0	8		38.6	13.5	8	*	40.5	8.9	8		38.3	10.2	8	

Area of Granulation - Growth of granulation tissue was identified by day 6 for the wounds of all treatments. The area of granulation tissue for the CMC-treated wounds was smaller on days 7 and 9 as compared with the vehicle-treated wounds (differences seen in absolute values only; no differences seen when comparing relative values). This was considered an indirect effect of the generally smaller wound area for the CMC-

treated wounds in comparison with the other group wounds on those days. By day 15, the percent of wound area with granulation was comparable for all groups at 10, 12.6 and 8.4% for wounds treated with SABER-Bupivacaine, SABER placebo (vehicle), and CMC gel, respectively No differences were observed between the SABER-Bupivacaine and SABER placebo (vehicle) treated wounds. Again, there is no “wound only” group for absolute comparison purposes.

Area of granulation tissue in % of wound area from the same day

Mean values

TREATMENT	% AREA DAY 6				% AREA DAY 7				% AREA DAY 8				% AREA DAY 9				% AREA DAY 10			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	69.9	36.6	8		100.0	0.0	8		100.0	0.0	8		88.3	13.0	8		55.3	12.6	8	
2	61.4	38.0	8		100.0	0.0	8		100.0	0.0	8		88.2	10.9	8		55.6	11.6	8	
3	72.1	31.9	8		100.0	0.0	8		100.0	0.0	8		77.7	12.4	8		50.6	9.6	8	

TREATMENT	% AREA DAY 11				% AREA DAY 12				% AREA DAY 13				% AREA DAY 14				% AREA DAY 15			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	35.3	11.2	8		44.4	8.1	8		26.4	18.5	8		10.0	9.8	8		10.0	7.5	8	
2	36.6	16.6	8		45.0	14.3	8		26.5	17.4	8		14.1	10.2	8		12.6	13.1	8	
3	36.0	11.1	8		43.7	25.5	8		21.0	19.5	8		13.8	8.6	8		8.4	8.9	8	

Area of Re-Epithelialization - Re-epithelialization commenced on day 9 for the wounds of all treatments. No differences in the re-epithelialized wound areas between the different treatments were seen during the study. The re-epithelialized area covered about 90, 87.3 and 91.6% of the total wound area on Day 15 for the wounds treated with SABER-Bupivacaine, SABER placebo (vehicle) and CMC gel groups, respectively. Again, there is no “wound only” group for absolute comparison purposes.

Area of granulation tissue in % of wound area from the same day

Mean values

TREATMENT	% AREA DAY 6				% AREA DAY 7				% AREA DAY 8				% AREA DAY 9				% AREA DAY 10			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	69.9	36.6	8		100.0	0.0	8		100.0	0.0	8		88.3	13.0	8		55.3	12.6	8	
2	61.4	38.0	8		100.0	0.0	8		100.0	0.0	8		88.2	10.9	8		55.6	11.6	8	
3	72.1	31.9	8		100.0	0.0	8		100.0	0.0	8		77.7	12.4	8		50.6	9.6	8	

TREATMENT	% AREA DAY 11				% AREA DAY 12				% AREA DAY 13				% AREA DAY 14				% AREA DAY 15			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	35.3	11.2	8		44.4	8.1	8		26.4	18.5	8		10.0	9.8	8		10.0	7.5	8	
2	36.6	16.6	8		45.0	14.3	8		26.5	17.4	8		14.1	10.2	8		12.6	13.1	8	
3	36.0	11.1	8		43.7	25.5	8		21.0	19.5	8		13.8	8.6	8		8.4	8.9	8	

In summary, there were no notable differences Wound Contraction, Area of Granulation Tissue, and Area of Re-Epithelialization over the 15-day observation period other than the increased rate but not final total wound contraction in the CMC-treated group.

Histopathology

Each wound was cut free as a block separated from skeletal muscle tissue. If any adherence to the underlying skeletal muscle occurred, part of the muscle was included. After fixation, three representative samples from each wound were embedded in paraffin and sectioned (nominal thickness 5 µm). One section was stained with hematoxylin and eosin and another section was subjected to immunohistostaining for angiogenesis (Streptavidin/Biotin immunoperoxidase technique to demonstrate von Willebrand factor – blood glycoprotein involved in hemostasis). The third section was stained with Masson's Trichrome for valuation of newly formed collagen. After staining, the slides were observed under the light microscope.

Adequate Battery – No, wound only, but that is the study focus.

Peer Review – No.

Histological Findings - Marked amounts of granulation tissue developed in all treated group wounds, consisting mainly of large numbers of thin-walled blood vessels and fibrocytes/fibroblasts (fibrovascular connective tissue).

A crust had formed on top of the granulation tissue in most wounds for all treatment groups. The extent of these crusts ranged from minimal to slight. The crusts consisted mainly of necrotic cell debris and exudate.

The re-epithelialization was complete (massive) in most wounds in the treated groups. Re-epithelialization was moderate to marked for the other wounds (e.g., placebo and/or negative control). The thickness of the epithelium was moderate to marked in most cases with rete-ridge formation.

In the superficial parts of all treated wounds for all treatment groups, a minimal to moderate inflammation was observed. In the deeper parts of the wounds a minimal to moderate inflammation was observed for the CMC and SABER placebo (vehicle) groups. For the SABER-Bupivacaine group, the deeper inflammation was minimal to moderate.

Minimal to slight numbers of cystic spaces were observed in the granulation tissue of three of eight treated wounds (CMC group), one of eight treated wounds (vehicle), and three of eight treated wounds (SABER-Bupivacaine).

Clear vacuoles were observed in the profound granulation tissue of all CMC treated wounds (minimal to slight), vehicle wounds (minimal to moderate), and SABER-Bupivacaine treated wounds (minimal to marked). Giant cells associated with the clear vacuoles were observed in CMC treated (minimal to slight), vehicle treated (minimal to moderate), and SABER-Bupivacaine treated (minimal to moderate) groups.
Newly formed collagen was observed in CMC treated (marked), vehicle treated (moderate to marked), and SABER-Bupivacaine treated (marked) groups. Moderate angiogenesis was observed in all treated wounds.

In summary, No marked differences were observed in wound healing between the treatment groups. No macroscopic differences at wound injection sites were noted among negative control (CMC), vehicle control (SABER placebo, and SABER-Bupivacaine. The CMC-treated wounds exhibited slightly more pronounced wound contraction than the other two groups. Microscopically, a slight tendency towards less advanced re-epithelialisation and more inflammation and clear vacuoles occurred in the SABER-Bupivacaine animals compared to the CMC animals. SABER placebo animals fell somewhere in between these observations with no marked differences in overall reaction/wound healing among the three treatment groups.

Special Evaluation – see Wound Contraction, Area of Granulation Tissue, and Area of Re-Epithelialization discussed previously.

Dosing Solution Analysis

Both pre-dose and post-dose active samples were analyzed for appearance, potency, and degradation products. The results showed that the active formulation remained clear, light yellow-brown in color, and percentage of label strength assays showed that

the product was chemically stable during the animal dosing period. Total degradation of pre-dose samples (by area normalization) was 0.51% and that of post-dose samples was 0.74%. Both pre-dose and post-dose placebo samples were analyzed for appearance. The placebo (vehicle) formulation remained a clear, colorless solution.

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A Study of the Effects of Sucrose Acetate Isobutyrate (SAIB)-based Formulations on Wound Healing Using the Rat Linear Incision Model (study DUR 1 – non-GLP) – This pilot study used the (b) (4) for the SAIB (SABER placebo) . The objective was to evaluate the effects of SAIB/(b) (4) (70%/30%, lot 263-85-1-5) at differing volumes on the wound healing process after 7 days using biomechanical measurements. On day 0, symmetric, 2.5 cm full thickness linear incisions wounds were created over each dorsolateral flank of the shaved animals. The wound margins were approximated and closed with surgical skin sutures. Immediately after wound closure, at a distance of 1 cm away from the opposed wound margins, a trailing subcutaneous injection (2.5 cm in length) of SAIB/(b) (4) (70%/30%) or saline was administered parallel to the surgical wound.

Study Group	Treatment	Minimum No. Wounds/ Group	Post-op Evaluation Time Points
A	Control (no treatment)	10	Day 7
B	Control-saline (0.25 ml/cm; 0.625 ml/injection; 1.25 ml total per incision)	10	Day 7
C	SABER-placebo (0.1 ml/cm; 0.25 ml/injection; 0.5 ml total per incision)	10	Day 7
D	SABER-placebo (0.25 ml/cm; 0.625 ml/injection; 1.25 ml total per incision)	10	Day 7

There were no visual differences observed in the appearance of the different group's wounds. Biomechanical testing (wound healing/incision strength), performed on day 7 post-wounding, identified no differences between the groups.

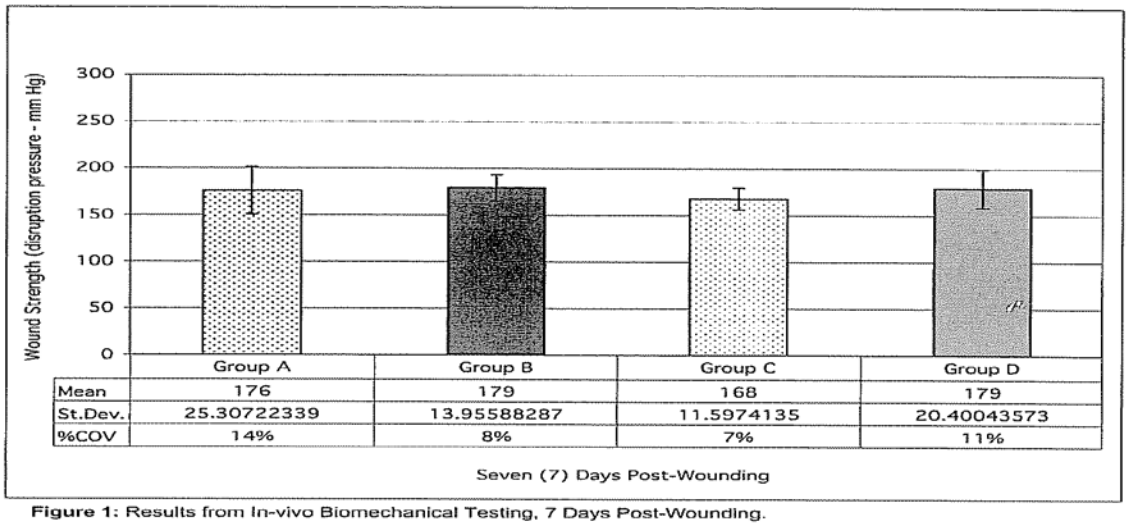


Figure 1: Results from In-vivo Biomechanical Testing, 7 Days Post-Wounding.

In summary, this pilot study included the *in vivo* biomechanical evaluation of subcutaneous SAIB/[redacted] (b) (4) (70%/30%) in the linear incision wound healing model. The measured parameter of *in vivo* wound strength revealed there were no significant ($p>0.05$) differences between the groups at 7 Days post-wounding. Moreover, there were no differences observed in mechanical wound strength between the two volumes of SAIB/[redacted] (b) (4) (70%/30%) tested. In-vivo mechanical measurements of wound strength provided reliable and reproducible means for quantifying the effects of the various treatments.

10.2 Hemolysis

Study title: Hemolytic potential and plasma compatability study with five test articles in human blood

Study no.:	01-11-803-X-VO-ATX
Study report location:	eCTD in DARRTS
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	April 15, 2011
GLP compliance:	Yes.
QA statement:	Yes.

Drug, lot #, and % purity:

Test Article	Manufacturer	Lot No.	Storage	Purity[a]
POSIDUR (SABER-Bupivacaine), provided at 132 mg/mL of bupivacaine (Test Article 1)	DURECT	K0059	At room temperature	98.2%
SABER-Bupivacaine vehicle (placebo); 0 mg/mL (Test Article 2)	DURECT	K0058	At room temperature	MS
Sucrose acetate isobutyrate (SAIB), provided at 100% (undiluted) (Test Article 3)	DURECT	001-11	At room temperature	100.0% (HPLC)
0.75% Sensorcaine-MPF (Bupivacaine HCl Injection, USP) (Test Article 4)	(b) (4)	WH2115	At room temperature	98.4%
0.9% Sodium Chloride for Injection, USP (Test Article 5)		C813600	At room temperature	MS

MS = Meets specifications.
a See Certificates of Analysis.

Test group 1 – SABER-Bupivacaine
Test group 2 – SABER placebo
Test group 3 – SAIB
Test group 4 – Sensorcaine (bupivacaine HCl)
Test group 5 – 0.9% sodium chloride

Key Study Findings

- The purpose of this study was to assess the hemolytic potential of the test articles POSIDUR (SABER-Bupivacaine), SABER-Bupivacaine vehicle (placebo), sucrose acetate isobutyrate (SAIB), 0.75% Sensorcaine-MPF (Bupivacaine HCl Injection, USP), and 0.9% Sodium Chloride for Injection (negative control), USP, in human whole blood and the compatibility of these test articles with human plasma.
- Supernatant of hemolytic test samples were evaluated macroscopically for changes in color or clarity and the presence of flocculation, precipitation, or coagulation relative to the homologous plasma sample.
- SABER-Bupivacaine and SABER-Bupivacaine vehicle both caused hemolysis when added to human whole blood and resulted in a viscous (and, for the vehicle, cloudy) macroscopic appearance, with globules resembling bubbles visible microscopically, when added to human plasma.
- In summary, it appears that the benzyl alcohol component of SABER-Bupivacaine and SABER placebo may be involved in the hemolysis as bupivacaine and SAIB alone did not cause hemolysis.

Methods and Results

Hemolytic Potential

Hemolytic Potential Testing Design Table

Tube Number	Human		Test Article	Saponin 1%[a]
	Blood	Plasma		
1	+	-	+ (Test Article 1)	-
2	+	-	+ (Test Article 2)	-
3	+	-	+ (Test Article 3)	-
4	+	-	+ (Test Article 4)	-
5	+	-	+ (Test Article 5)	-
6[b]	+	+	-	-
7[c]	+	-	-	+

+ = Approximately 0.5 mL added to tube (see Protocol Deviations).
- = Not added to tube.
a Saponin is a hemolytic agent used to lyse erythrocytes.
b Analysis control sample/negative for hemolysis.
c Analysis control sample/positive for hemolysis.

Each tube was mixed on a rocker plate or other mixing apparatus for at least 5 minutes and then incubated for 40 to 45 minutes at approximately 37°C. During incubation, tubes were mixed by inversion every 5 to 10 minutes. After incubation, the tubes were centrifuged (centrifuge set to maintain 2 to 8°C), and the supernatant was harvested. A hemoglobin index was determined for the supernatant of each tube. The hemoglobin index approximates the amount of hemoglobin in mg/dL. The hemolytic index, approximating the amount of hemoglobin present in the supernatant of the test article mixtures, was compared with that of the negative control. Hemolysis was present (recorded as a positive test result) if the hemoglobin index was >500 (i.e., approximately 500 mg/dL) more than that of the negative control.

POSIDUR and SABER-Bupivacaine vehicle both caused hemolysis when added to human whole blood. Hemoglobin indices corresponded to approximate concentrations of 11788 and 5357 mg/dL, respectively. No hemolysis was associated with SAIB; 0.75% Sensorcaine-MPF; or 0.9% Sodium Chloride for Injection, USP. Hemoglobin indices corresponded to approximate concentrations of 169, 62, and 20 mg/dL, respectively.

Hemolytic Potential Test Results

Mixture	Hemoglobin[a] (mg/dL)	Test Result	Tube No.
Human blood plus:			
Test Article 1	11788[b]	Positive	1
Test Article 2	5357[b]	Positive	2
Test Article 3	169	Negative	3
Test Article 4	62	Negative	4
Test Article 5	20	Negative	5
Human plasma	28	Negative	6
1% Saponin	6748	Positive	7

Negative = No hemolysis.
a Hemoglobin concentration of the mixture supernatants.
b Results had ABS comment.

Plasma Compatibility:

Tube Number	Human Plasma	Test Article
1	+[a]	-
2	+	+ (Test Article 1)
3	+	+ (Test Article 2)
4	+	+ (Test Article 3)
5	+	+ (Test Article 4)
6	+	+ (Test Article 5)

+ = Approximately 0.5 mL added to tube (see Protocol Deviations).
- = Not added to tube.
a Tubes with homologous plasma were used for comparison purposes.

Tubes were placed on a rocker plate for at least 5 minutes, and the contents of each tube were examined macroscopically. Changes in color or clarity relative to the homologous plasma sample and the presence of flocculation, precipitation, or coagulation were recorded. When macroscopic evaluation of a mixture revealed any differences relative to the homologous plasma sample, microscopic examinations were done on that mixture and the homologous plasma sample for comparison purposes. When no changes or differences relative to the homologous plasma sample were observed, a microscopic examination was not done on that mixture.

The 0.75% Sensorcaine-MPF resulted in a cloudy macroscopic appearance, with particles visible microscopically, when added to human plasma. POSIDUR and SABER-Bupivacaine vehicle resulted in a viscous (and, for the vehicle, cloudy) macroscopic appearance, with globules resembling bubbles visible microscopically, when added to human plasma. SAIB and 0.9% Sodium Chloride for Injection, USP, did not result in macroscopic changes in appearance when added to human plasma.

Plasma Compatibility Test Results

Mixture	Macroscopic Observation	Microscopic Observation	Tube No.
Human plasma and:			
Human plasma used for comparisons			1
Test Article 1	Viscous	Globules that resemble bubbles	2
Test Article 2	Cloudy/viscous	Globules that resemble bubbles	3
Test Article 3	NC	NA	4
Test Article 4	Cloudy	Particle seen	5
Test Article 5	NC	NA	6

NA = Not applicable.
NC = No change.

Dosing Solution Analysis

POSIDUR (SABER-Bupivacaine – test article 1) and SABER-Bupivacaine vehicle (placebo – test article 2), were analyzed for identity, potency, drug-related degradants, and excipient-related degradants. Pre-dose and post-dose results indicated that these test articles were chemically stable and suitable for use for the duration of this study.

Sucrose acetate isobutyrate (SAIB); 0.75% Sensorcaine-MPF (Bupivacaine HCl Injection, USP); and 0.9% Sodium Chloride for Injection, USP; were used within manufacturer-supplied retest or expiration dates and were therefore considered suitable for use for the duration of this study.

11 Integrated Summary and Safety Evaluation

Introduction and Overview - SABER®-Bupivacaine (POSIMIR™ - NDA 204803), is an extended-release bupivacaine depot drug product for surgical instillation into wound sites. It has been submitted by DURECT Corporation pursuant to Section 505(b)(2) of the FD&C Act, relying on FDA's general findings of safety and efficacy for Marcaine (bupivacaine hydrochloride – NDA 16964) injection, the referenced NDA approved October 3, 1972. Marcaine is a fast acting local anesthetic consisting of a bupivacaine hydrochloride solution. Exparel® (NDA 22496), another extended-release bupivacaine depot drug product which has been recently approved (October 28, 2011) using Marcaine as its reference NDA, is also referred to by the applicant, but it is not the reference NDA. The excipients in SABER-Bupivacaine are sucrose acetate isobutyrate (SAIB) and benzyl alcohol (BA). They, have been used in other approved drug products but not by the proposed injection route (SAIB) or at as high a dose level as proposed (BA).

The bupivacaine in SABER-Bupivacaine is an amide-type local anesthetic indicated for administration into the surgical incision which as a depot is intended to produce prolonged post-surgical analgesia. SABER-Bupivacaine is a sterile nonpyrogenic, clear, light yellow to amber solution that contains bupivacaine (12%, 132 mg/mL), BA (22%, 242 mg/mL), and SAIB (66%, 726 mg/mL). Upon the single instillation the maximum proposed dose of 5 mL total (single dose (b) (4)), the extended-release biodegradable matrix (SAIB depot) is purported to continuously release bupivacaine over 24-72 hours. The SAIB forms a depot for bupivacaine as the solvent BA diffuses away from the injection site thereby resulting in formation of the SAIB-based depot and the extended release of bupivacaine over time compared to Marcaine.

Nonclinical Testing – The nonclinical program was designed to support the single administration of SABER-Bupivacaine by instillation around a surgical incision. Nonclinical batches of SABER-Bupivacaine (with BA as solvent) used in testing were comparable in composition at release and over time (stability) to the proposed drug product. Nonclinical findings address the issues of systemic toxicity and exposure to SABER-Bupivacaine and SABER placebo (no bupivacaine), their local toxicity, and a comparison of nonclinical exposure levels to proposed clinical exposure for the drug

product (risk assessment – dose ratios). Nonclinical levels of bupivacaine exposure are primary relative to drug approval as human bupivacaine exposure from SABER-Bupivacaine is greater than the reference NDA bupivacaine exposure as part of this 505(b)(2) submission. Excipients, degradants, and extractables/leachables (E/Ls), and effects on nerves, wound healing, and potential hemolysis were also evaluated relative to human safety under the proposed use condition.

Systemic Toxicity

The systemic safety of SABER-Bupivacaine is acceptable because the proposed bupivacaine exposure is supported by nonclinical:clinical safety margins, acceptable product quality specifications and stability, and valid nonclinical studies with an acceptable compositional comparability between the nonclinical test product and the proposed drug product. Apparently, only labeling is supported as a 505(b)(2) submission using the approved, referenced Marcaine as the proposed human exposure to bupivacaine is greater than that for Marcaine. The nonclinical support also includes submitted pivotal nonclinical studies, most notably single dose studies and a repeat dose study with prolonged observation periods and toxicokinetic (TK) measurements. In addition, genotoxicity testing results for bupivacaine, SABER-Bupivacaine, and SABER placebo support human safety. Potential carcinogenicity and reproductive toxicity of bupivacaine are addressed using a 505(b)(2) reference to the approved Marcaine (NDA 16964).

The systemic safety of Inactive Ingredients SAIB and BA for clinical use in SABER-bupivacaine is generally supported by their recognition as Generally Recognized as Safe (GRAS) by the FDA when used by oral routes, their use in approved and marketed products (oral only for SAIB), and extensive nonclinical databases on both ingredients as included in World Health Organization (WHO) data reports. However, for purposes of this application, as the amount of BA and SAIB in the proposed drug product exceeds that as listed for the above references and only the oral route of SAIB has been previously tested, the primary support for human safety is provided by the submitted nonclinical studies, most notably a repeat dose study for the proposed single dose indication. Nonclinical testing with SABER-Bupivacaine and/or SABER placebo includes that as listed in an earlier paragraph plus embryo-fetal testing of SABER placebo. This submitted nonclinical testing is consistent with the FDA *Guidance for Industry: Nonclinical Safety Studies for the Safety Evaluations of Pharmaceutical Excipients* (May 2005) noting that a full reproductive toxicology test battery data exists for both ingredients in the literature. See section 2.4 (Comments on Novel Excipients) for a detailed evaluation.

The systemic safety is generally supported for the degradation products of bupivacaine (b) (4) – see local toxicity section regarding unacceptable specification for this degradant) and SABER-Bupivacaine (b) (4) and for the extractables/leachables (E/Ls) from the container/closure system (E/Ls and (b) (4) from the rubber stopper. This support is based on the use of the source drug substance (i.e., specification limits allowable or qualified in testing), inactive ingredients in FDA-approved and marketed products, and risk assessments of observed extractable levels

in the drug product in excess of known safe levels (see section 2.5). But, again, the primary nonclinical support is the submitted nonclinical studies. Most notable is a repeat dose toxicity study with aged drug product containing increased levels of degradants that supports or identifies human safety levels at proposed human dosing levels for the proposed drug product. In addition, (b) (4) is controlled by appropriate, stringent specification. (b) (4) were tested for genotoxicity and were not genotoxic. (b) (4) and E/Ls also have literature data supporting the safety of levels in the proposed drug product. No (b) (4) were detected in the rubber stopper after attempted extraction. The submitted information is consistent with FDA/ICH *Guidances for Industry: Q3A Impurities in New Drug Substances* (February 2003) and *Q3B(R2) Impurities in New Drug Products* (August 2006)), *Safety Practices and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products*. Product Quality Research Institute, (September 8, 2006), and current FDA thinking supporting human exposures (b) (4) µg/day for genotoxins and (b) (4) µg/day for nongenotoxins. See section 2.5 (Comments on Impurities/Degradants and Extractables/Leachables of Concern) for a detailed evaluation.

Local Toxicity

The local toxicity associated with SABER-Bupivacaine and SABER placebo (SABER depot – SAIB and BA) were not unexpected as only the anticipated effects comprising local inflammation and a foreign body reaction were observed in the absence of systemic toxicity. Literature data was also submitted to support the human safety of SAIB and BA. The submitted nonclinical testing satisfies testing needs as listed in the FDA *Guidance for Industry and Review Staff: Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route* (March 2008).

The local reaction to SABER-Bupivacaine was similar to the local reactions (i.e., foreign body reaction) in other approved drugs with different injectable depots such as Risperdal Consta (NDA 21346, polylactide-co-glycolide microspheres, chronic biweekly intramuscular dosing) , Eligard (multiple NDAs, polylactide-co-glycolide polymer, SC dosing q 1 to 6 months depending on dose level), Lupron Depot (multiple NDAs, microspheres containing carboxymethylcellulose and mannitol, intramuscular dosing q 1 and 3 months), and Zoladex (NDAs 19726 and 20578, polylactide-co-glycolide copolymer, SC implant q 3 or 6 months).

Of note is the persistence of the depot material SAIB for at least 12 months after a single injection of SABER-Bupivacaine in rabbits. At 12 months (last time point examined), the SAIB also demonstrates comparable local toxicity between SABER-Bupivacaine and SABER placebo and is compositionally the same material as injected 12 months earlier (i.e., unchanged). In ^{14}C-SAIB distribution studies in rats, ~40-60% of the SAIB radiolabel was shown to persist at the injection site at 10 weeks post-dosing (last time point evaluated) whether administered SC or into a surgical wound. The radiolabel of ^{14}C-SAIB administered in solvent (BA (b) (4))

persisted at the injection site at a level of ~40% at 6 week after dosing (BA solvent, last time point evaluated) and at ~50% at 10 weeks (b) (4), last time point evaluated). Local toxicity is observed in animals immediately after dosing (e.g., marked inflammation) and decreases over time. The biological or toxicological significance of this persistent depot material to humans is unknown. The relevance of this nonclinical data to human safety is to be determined by the medical review team in conjunction with consideration of the overall human benefit of the proposed drug.

Injection site necrosis was observed in dogs after intra-articular (IA) dosing with SABER-Bupivacaine and SABER placebo. While the proposed dose route for this NDA submission is instillation, this observed local effect should be referenced in the event of any proposed IA dosing in the future. Notation of this potential adverse event after IA dosing is noted in the product label precaution section, referencing potential chrondrolysis.

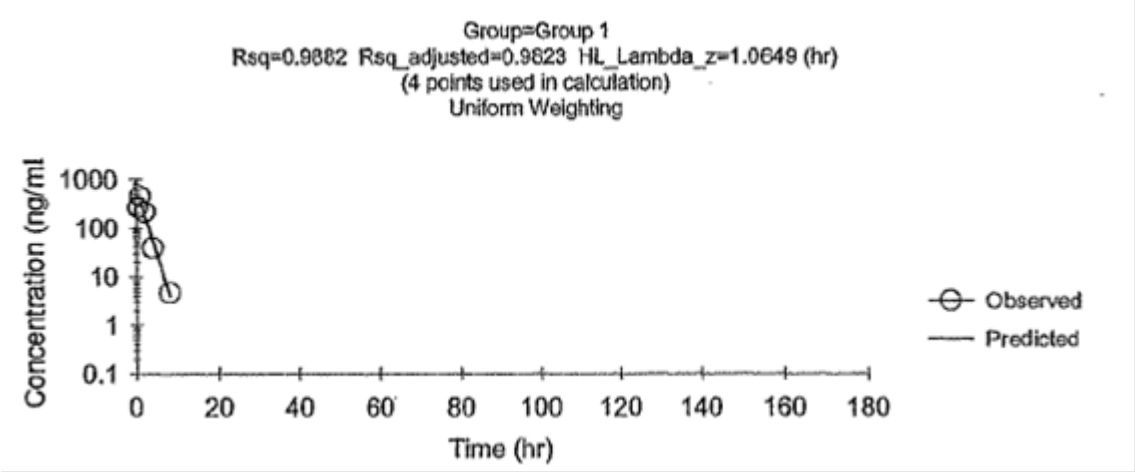
In summary, potential systemic toxicity regarding human safety appears to have been supported in this submission. Local toxicity occurs related to SABER-Bupivacaine at a degree no greater than for SABER placebo (no bupivacaine, only excipients SAIB and BA). In addition, the depot SAIB in SABER-Bupivacaine is present for a prolonged period of time. The importance of this local toxicity related to the persistence of SAIB for at least 12 months after instillation needs to be addressed regarding human safety. An unacceptable specification for degradant (b) (4) is being addressed with the Applicant. Should this not be resolved, a CR may be recommended.

Systemic exposure to bupivacaine

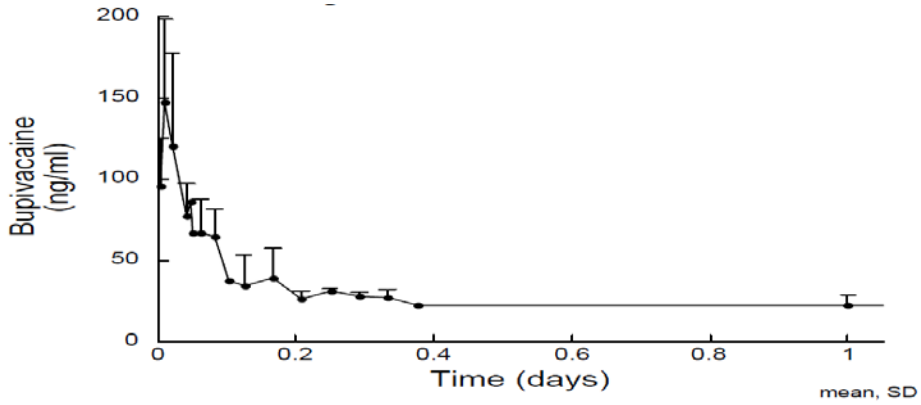
For illustration purposes only, not for quantitative purposes, following are representative bupivacaine release figures in rats (study 11519.01.08) and humans (study SABER01-01 & CLIN005-0008) using a nonclinical test article equivalent to the proposed drug product (bupivacaine at 12% - 132 mg/mL; BA at 22% - 242 mg/mL; and SAIB at 66% - 726 mg/mL). The ability to accurately measure bupivacaine in mouse, rat, rabbit, dog, and minipig plasma was validated using an HPLC/MS/MS method (study BAS-0100).

The time course of release of bupivacaine from a single SC injection of bupivacaine HCl in rats (group 1) and humans exhibit rapid distribution-elimination kinetics.

Rats

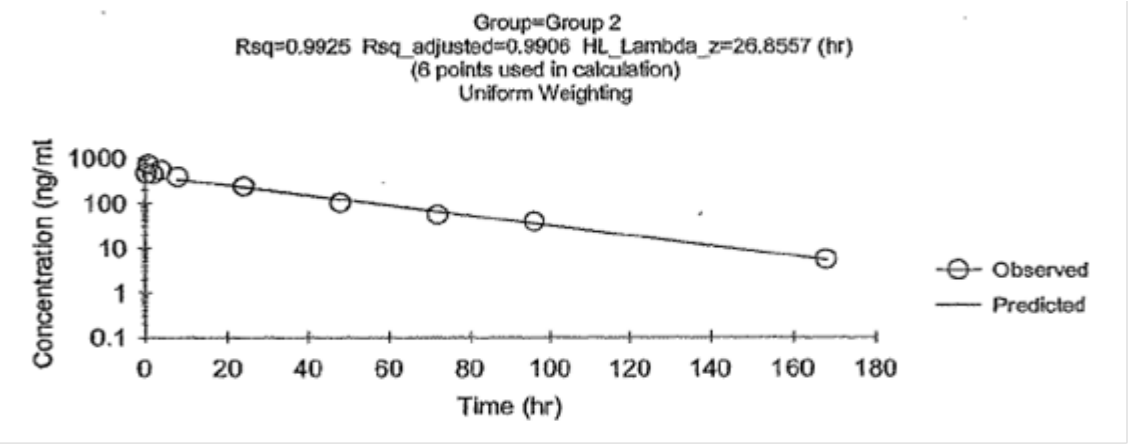


Humans

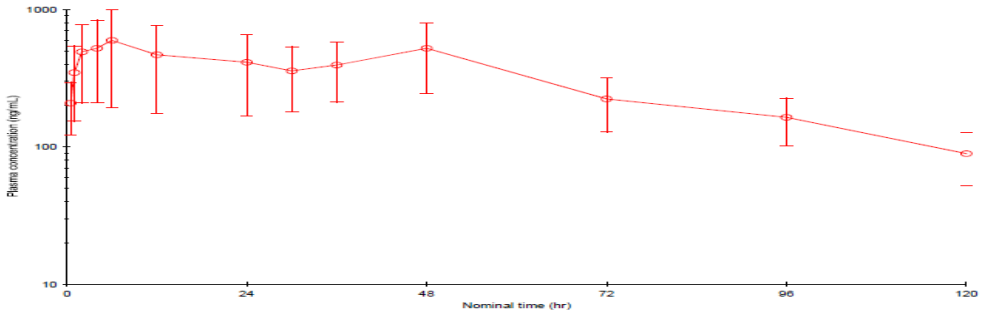


SABER-bupivacaine SC injection exhibits differing kinetics to injected bupivacaine HCl in rats (group 2) and humans with delayed distribution and elimination, the main advantage in prolonged local anesthesia purported by the Applicant.

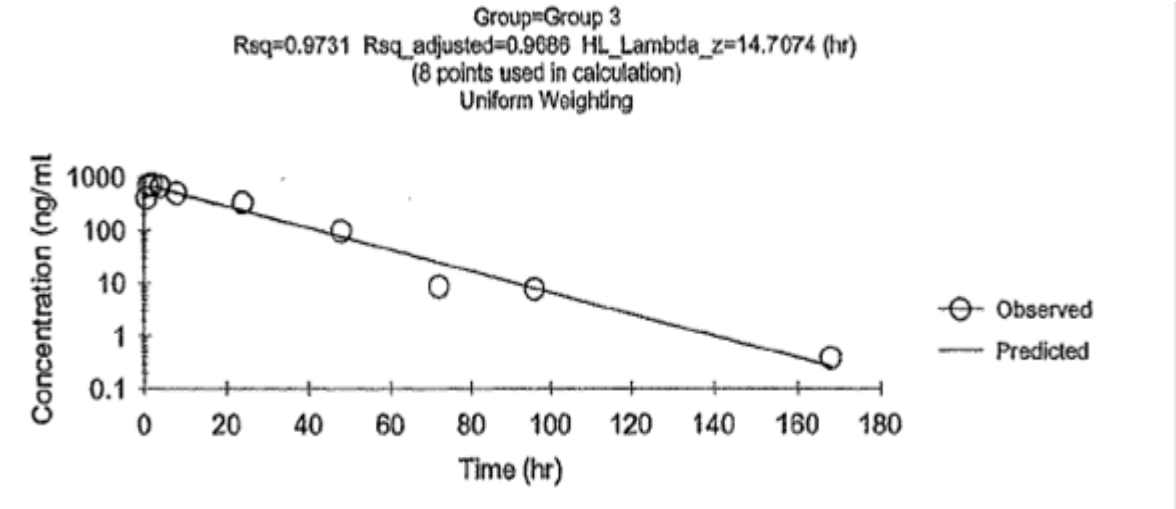
Rats



Humans



Of note is that kinetics of bupivacaine is similar after being dripped over an incision in rats (group 3) compared to after a SC injection (group 2 above) with a slightly slower release after SC injection) making SC rat studies relevant to proposed human wound injection use in comparison of systemic exposure.



In summary, nonclinical test material of similar to identical composition to the proposed drug product exhibited comparable kinetics making the nonclinical studies with SABER-Bupivacaine appropriate for evaluating the human safety of SABER-Bupivacaine.

Systemic and Local Safety of SABER-Bupivacaine and SABER placebo

The nonclinical testing program was designed to evaluate the systemic and local toxicity of the drug product and related issues and be consistent with FDA Guidances and current FDA thinking for reformulated drug products, excipients, degradants, impurities, and extractables/leachables. Available literature data on some of the materials was used to eliminate the need for some of the FDA-required testing. Nonclinical testing and the human safety assessment focused on the evaluation of the following, each of which will be evaluated individually:

1. Systemic exposure to bupivacaine after nonclinical SABER-Bupivacaine treatment in comparison to proposed human systemic exposures and approved exposure levels to bupivacaine as part of a 505(b)(2) submission.
2. Potential systemic and local toxicity of SABER-Bupivacaine and SABER placebo (excipients BA and SAIB only, no bupivacaine) and local toxicity of SAIB.
3. Safety Assessment of "aged, yellow" SABER-Bupivacaine with increased levels of degradants at release levels of bupivacaine in a degradant qualification study for proposed drug product specifications.
4. Determination of acceptable levels related to human safety for excipients (BA and SAIB), degradants (b) (4) and Extractables/Leachables (numerous).
5. Local effects of SABER-Bupivacaine and SABER placebo on sciatic nerve, wound healing, and hemolysis.
6. Persistence, disposition, and local toxicity of SAIB at the injection site.
7. Potential for BA to be distributed to mother's milk with resulting fetal/neonate exposure.
8. *In vitro* genotoxicity of the drug substance (bupivacaine) and SABER-Bupivacaine degradation products (b) (4) .
9. *In vivo* genotoxicity of the drug product SABER-Bupivacaine and SABER placebo.
10. Embryo-fetal developmental toxicity of SABER placebo.
11. Nonclinical data evaluating the local toxicity by a dose route other than the proposed injection route, Intra-articular (IA) dosing.

1. Systemic exposure to bupivacaine after nonclinical SABER-Bupivacaine treatment in comparison to proposed human systemic exposures and approved exposure levels to bupivacaine as part of a 505(b)(2) submission.

1) The Maximum Recommended Human Dose (MRHD) for local tissue infiltration of the approved bupivacaine drug, Marcaine, is 400 mg bupivacaine/day.

    a. In one of the clinical trials (BU-002-IM), treatment at 50 mg bupivacaine resulted in pharmacokinetic (PK) values for Cmax of 90 ng/mL and for AUC_∞ of 960 ng*h/mL. Extrapolated to the MRHD of 400 mg/day results in a Cmax of 720 ng/mL and for AUC_∞ of 7,680 ng*h/mL
    b. In another clinical trial (BU-001-IM), treatment at 100 mg bupivacaine resulted in pharmacokinetic (PK) values for Cmax of 342 ng/mL and for AUC_t of 5650 ng*h/mL. Extrapolated to the MRHD of 400 mg/day results in a Cmax of 1,368 ng/mL and for AUC_∞ of 22,60 ng*h/mL.
    c. The extrapolated values are used only for qualitative, comparative purposes, not for determination of human safety. Variability in the human data for Marcaine injection is great with standard deviations ranging from 2 to 3-fold the mean value so extrapolation of mean PK values to supposed MRHD levels should be kept in perspective.

2) Bupivacaine exposures after dosing with 5 mL of SABER-Bupivacaine in clinical trials were determined:

    a. Inguinal hernia repair (CLIN-803-006-0006) - 867 ng/mL (Cmax) and 41,461 ng*h/mL (AUC_∞).
    b. Subacromial decompression (BU-002-IM). 593 ng/mL (Cmax) and 19,960 ng*h/mL (AUC_∞).
    c. For the extrapolated MRHD values from clinical trial CLIN-803-006-0006, the 4-fold extrapolated reference NDA PK values support safety of proposed human exposures for Cmax but not AUC.
    d. Considering the unacceptable use of data extrapolation in determining human safety in this case and only partial support of proposed human exposure levels, the nonclinical bupivacaine levels will be used to calculate human safety margins.

3) The relevant nonclinical studies in rats and rabbits demonstrated only local toxicity with the high doses being systemic No Observed Adverse Effect Levels (NOAELs) for bupivacaine exposures. SABER-Bupivacaine and SABER placebo groups exhibited similar local toxicity, an issue to be addressed in another section. Toxicokinetic (TK) values were as follows:

    a. Single SC dose in rats (study 11519.01.04) with the highest bupivacaine exposure levels of 1432 ng/mL (Cmax) and 77,423 ng*h/mL (AUC_∞).

b. Single SC dose in rabbits (study A784.6.1) with the highest bupivacaine exposure level of 3,033 ng/mL (Cmax) and 48,465 ng*h/mL (AUC_{last})
c. Repeat (1/week for 4 weeks) SC doses in rats (study BR1265) with the highest bupivacaine exposure level of 731 ng/mL (blood level, only, no toxicokinetic analysis).

4) In summary, MRHD bupivacaine exposure levels for the 505(b)(2) referenced, approved NDA have not been adequately demonstrated to be comparable or larger than those proposed for the SABER-Bupivacaine human indication and therefore not adequate to be used to support human safety at proposed dosing with SABER-Bupivacaine. Therefore, the nonclinical studies are needed to support human safety at the proposed doses of SABER-Bupivacaine, most notably at the highest proposed doses of 5 mL in treating hernia. While the repeat dose rat study exhibited its highest blood levels (no AUC) that were only comparable to that for the human inguinal hernia clinical trial Cmax, the combined nonclinical data is considered adequate to support the largest proposed human bupivacaine exposure levels after a single dose with animal:human safety margins for systemic exposure of 0.8 & 1.7 (rats) and 3.5 (rabbits) for the Cmax and 1.9 (rats) and 1.2 (rabbits) for the AUC (see table). Based on these comparisons, human dosing is supported by nonclinical data at the largest proposed bupivacaine single exposure level after dosing with SABER-Bupivacaine.

Nonclinical:Clinical Bupivacaine Exposure Comparisons with Safety Margins – SM) after a Single Subcutaneous Dose with SABER Bupivacaine

species	drug	Cmax (ng/mL)	AUC (ng*h/mL)	SM[d] Cmax	SM[d] AUC
Human[a]	Marcaine	90	960	--	--
		720	7,680	--	--
Human[b]	Marcaine	342	5,650	--	--
		1368	22,600	--	--
Human[c]	SABER-Bupivacaine	593	19,960	--	--
		867	41,461	--	--
Rat	SABER-Bupivacaine	1,432	74,423	1.7	1.8
Rabbit		3,033	48,645	3.5	1.2
Rat (multiple dosing)		737	NR[e]	0.8	NR[e]

a – study BU-002-IM (sub acromial decompression study) – 50 mg bupivacaine or 1/8th the MRHD for referenced, approved NDA (Marcaine) and also extrapolated 8-fold to MRHD levels assuming linearity (second line)
b – study BU-001-IM (abdominal hysterectomy study) – 100 mg bupivacaine or 1/4th the MRHD for referenced, approved NDA (Marcaine) and

also extrapolated 4-fold to MRHD levels assuming linearity (second line)
c - bupivacaine exposure from SABER-Bupivacaine for proposed indications from clinical studies BU-002-IM (Subacromial Decompression) and CLIN-803-006-0006 (inguinal hernia repair)
d - animal:human exposure Safety Margins (SM) at nonclinical systemic No Observed Adverse Effect Level (NOAEL) using the larger human SABER-Bupivacaine blood levels
e - not reported

2. Potential systemic and local toxicity of SABER-Bupivacaine and SABER placebo (BA and SAIB only, no bupivacaine) and local toxicity of SAIB.

Three nonclinical studies, single dose studies in rat and rabbit and a repeated-dose study in rat were conducted to assess the systemic and local toxicity of the proposed SABER-Bupivacaine formulation when administered via an SC injection.

1) Groups of male and females SD rats received single subcutaneous injections of vehicle (SABER placebo, 2 mL/kg), saline (2 mL/kg), 24 mg/kg SABER-Bupivacaine (0.2 mL/kg), 72 mg/kg SABER-Bupivacaine (0.6 mL/kg), or 240 mg/kg SABER-Bupivacaine (2 mL/kg) (study 11519.01.04). Animals were sacrificed on day 15 and 43 (6 weeks).

One high dose female was found dead on the day of dosing from unknown cause. All other animals survived until their scheduled sacrifice. Only clinical observations associated with treatment included discoloration, swelling, sore/ulcer, discharge, scab, and discolored scabrous areas at the injection site. The saline group was unaffected by treatment.

No treatment related effects on body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, and macroscopic and microscopic pathology (other than injection sites). All injection site effects were in vehicle control (SABER placebo) and SABER-Bupivacaine groups with no effects in saline control group.

Subcutaneous injections of either the vehicle or of any of the three concentrations of bupivacaine (in the same vehicle) produced localized inflammatory reactions characterized primarily by panniculitis with subcutaneous cyst formation and reactive fibrosis. However, all of the injection site reactions were qualitatively and quantitatively similar between the sites injected with the vehicle and those injected with similar volumes of the vehicle and bupivacaine. Moderate to marked cutaneous necrosis was observed on day 15 in 1 mid dose male, 3 high dose males, and 4 vehicle (SABER placebo) females but none was observed on day 43.

There was evidence of reduction of the various inflammatory and reactive processes between the Day 15 and Day 43 necropsy time points, although marked subcutaneous cysts persisted in most males and females of the vehicle control (SABER placebo) and high dose groups. These dose groups were administered the same, largest dose volume (2 mL/kg). The SABER-Bupivacaine

test article is the same composition as the proposed drug product. The low SABER-Bupivacaine dose of 0.2 mL/kg (~0.1 mL) was not a local toxicity NOAEL for injection site effects.

Toxicokinetic (TK) data collected identified exposure levels that caused no systemic toxicity, but local toxicity similar to the vehicle (SABER placebo). Peak blood levels were within 1-2 hours with levels maintaining similar, higher exposure to bupivacaine for 8-24 hours. Regarding the lack of systemic toxicity, TK values at the high dose were a gender averaged Cmax of 1,432 ng/mL and $AUC_{0-\infty}$ of 74,423 ng•h/mL.

2) Male and female New Zealand White rabbits received single subcutaneous doses (split to each side of the back) of SABER-Bupivacaine or SABER placebo at bupivacaine doses of 0 mg/kg (0.25 mL/kg placebo), 0 mg/kg (1.0 mL/kg placebo), 30 mg/kg (0.25 mL/kg), or 120 mg/kg (1.0 mL/kg) (study A784.6.1). Animals were sacrificed on day 15/18 and 44 post-dose.

Two male rabbits in the high dose group were sacrificed as moribund 3-4 hours after dosing on Day 1. The sacrificed animals had apparently aspirated stomach contents. Bupivacaine treatment-related mortality cannot be ruled out.
No treatment-related or toxicologically relevant effects were observed with regard to clinical signs, body weights, food consumption, ophthalmoscopy, hematology, and clinical chemistry.

Injection site effects were most common in animals sacrificed 15 days after dosing (interim sacrifice). The most common findings in the injection sites of animals from all groups were chronic inflammation and the presence of clear empty spaces (space, vacant, presumed to contain the test article). In all groups, the inflammation was usually of trace or mild severity and consisted of variable degrees of early fibrosis with infiltration of histiocytes and small mononuclear cells.

Observations were generally less common and severe after the 44 day terminal sacrifice, suggesting healing, recovery, and/or reversibility of local effects.
In a separate biocompatibility evaluation of injection sties, the test and control articles elicited an expected inflammatory and wound healing sequence of events with a foreign body reaction consistent with a 6-week implant time. Both control and treated groups were considered to be biocompatible (i.e., comparable responses).

This study demonstrated a prolonged release of Bupivacaine up to 2 days for the 30 mg/kg dose level and up to 4 days for the 120 mg/kg dose level with no efficacy implied.

Toxicokinetic (TK) data collected identified exposure levels that caused no systemic toxicity (NOAEL), but local toxicity similar to the vehicle (SABER

placebo). Peak blood levels were within 1-2 hours with levels maintaining similar exposure to bupivacaine for 8-24 hours. Regarding the lack of systemic toxicity, TK values at the high dose were a gender averaged Cmax of 3,033 ng/mL and $AUC_{0-\infty}$ of 48,645 ng•h/mL.

3) Groups of male and female SD rats received weekly subcutaneous injections of 0 (2.0 mL/kg), 102 (0.75 mL/kg), or 240 (2.0 mL/kg) mg/kg SABER-Bupivacaine for 4 weeks (days 1, 8, 15, & 22) followed by sacrifice on days 29-30 (1 week after last dose) and day 85 (8 weeks after day 28 sacrifice - recovery group) (study BR1265). The test material was an “aged” material (54 months) that exceeded acceptable yellow color specifications and had increased levels of impurities. At release, the test material met recipe specifications of the proposed drug product (12% bupivacaine, 66% Sucrose Acetate Isobutyrate, & 22% Benzyl Alcohol). At testing, the aged test material contained (b)(4)% bupivacaine label strength and (b)(4)% of label strength for SAIB. See number 3 of this section for a safety assessment/qualification of the degradant levels related to their drug product specifications.

No bupivacaine-related deaths or clinical symptoms were observed. The only notable treatment-related effect was at the site of injection where all dose groups were affected. Weekly injections of the placebo or the SABER-Bupivacaine solutions resulted in the macroscopic findings of small wounds, cysts, or nodules at the injection sites. These findings were diagnosed as chronic granulomatous inflammations. After an 8-week recovery period, the incidence and severity of these findings were reduced to a maximum of moderate severity compared to the animals autopsied in week 5 which exhibited up to a severe severity. Injections site effects were reversing but were still present 8 weeks after the initial sacrifice. The sponsor described this as a normal wound healing/recovery process.

The systemic NOAEL is 240 mg/kg and there was no local toxicity/injection site NOAEL. The low dose of 102 mg/kg could be a LOAEL with reversal of local toxicity, but local toxicity was not fully reversed at 8 weeks after the last dose. Based on reported blood levels with no toxicokinetic value calculations, blood levels are no greater than 441 & 695 ng/mL in males and 491 & 768 ng/mL in females for the low and high dose groups, respectively. The high dose mean value was 731 ng/mL and will be considered comparable to a Cmax value.

SAIB only (local toxicity)

SAIB was associated with apparently reversible local toxicity (see issue 6 of this section for a more detailed discussion).

In summary, nonclinical evaluations of potential local and systemic toxicity identified that the primary effect from a single subcutaneous dose of SABER-Bupivacaine is reversible local toxicity as no notable systemic toxicity was observed. The observed local toxicity is consistent with a foreign body reaction for an injected or inserted material including

progressing acute to chronic inflammation and associated fibrosis. Subcutaneous administration of SABER-Bupivacaine caused local toxicity comparable to that for SABER placebo (no bupivacaine), suggesting that bupivacaine was, at most, a minor contributor to the observed local effects. Of note is the persistence of the excipient depot material, Sucrose Acetate Isobutyrate (SAIB), which in the rabbit could be found at the injection site for up to 1 year after a single injection with continued local effects/toxicity (see issue 6 of this section for discussion of SAIB persistence). The medical review team is assessing the clinical significance of this local toxicity and persistence of the depot material.

This submitted nonclinical testing is consistent with the FDA *Guidance for Industry: Nonclinical Safety Studies for the Safety Evaluations of Pharmaceutical Excipients* (May 2005).

3. Safety Assessment of "aged, yellow" SABER-Bupivacaine with increased levels of degradants at release levels of bupivacaine in a degradant qualification study for proposed drug product specifications.

This repeat-dose study (4 weekly SC doses for 4 weeks with SABER-Bupivacaine in SD rats – study BR1265) can be considered the pivotal nonclinical study for assessing potential human toxicity as the tested 54-month old drug product has increased levels of degradants, levels which will be used to set drug product specifications, while maintaining a release-equivalent level of bupivacaine (see number 1. of this section for a Cmax comparison to the proposed human exposure to bupivacaine). See Product Quality review for a discussion of this "aged, yellow" drug product compared to the release drug product which is not yellow.

The issue to be discussed here is qualification of drug substance and degradants with the proposed drug product specifications. As noted in number 2 of this section, the systemic NOAEL was the high dose for which drug substance and other components were as follows:

SABER-Bupivacaine composition at release and at 54 months
- 12% bupivacaine, 66% SAIB, and 22% BA at release
  – proposed drug composition
- 54 months
  Bupivacaine - (b) (4)% of label strength
  SAIB (sucrose acetate isobutyrate) – (b) (4)% by weight (b) (4)% of Label strength)
  Benzyl alcohol (BA) – apparently not analyzed
- Degradation products

(b) (4)

SABER placebo
- Release composition of 75% SAIB and 25% BA

Drug product specifications, consistent with the tested levels above are as follows:

In summary, the 4-week, repeat dose study with “aged, yellow” SABER-Bupivacaine is considered adequate to support the proposed drug product specifications and satisfy repeat dosed testing for reformulated drug products.

4. Determination of acceptable levels related to human safety for excipients (BA and SAIB), degradants (b) (4) and Extractables/Leachables (numerous).

In addition to data presented previously in this section, more specific, detailed, information is presented in sections 2.3 (Drug Formulation), 2.4 (Comments on Novel Excipients), and 2.5 (Comments on Impurities/Degradants and Extractables/Leachables) of this NDA review. Firstly, nonclinical data, literature data, and subsequent risk assessments for all these chemicals have been determined to support human safety under the proposed conditions for the use of SABER-Bupivacaine.

Excipients –Complete literature data sets that include carcinogenicity exist for SAIB and BA by oral administration (see section 2.4 for specifics).

Submitted nonclinical data (4-week repeat SC dose study in rats - study BR1265) also supports proposed dosing with SAIB and BA as follows using Human Equivalent Dose (HED) comparisons:

Systemic Safety Margins (based on high dose systemic NOAEL)

1) (b) (4) mg/kg SAIB in rat high dose group x (b) (4) (rat BSA factor)
= (b) (4) mg/kg HED
(b) (4) mg/kg HED in rat for SAIB ÷ (b) (4) mg/kg maximum human dose
= SM of (b) (4)

2) (b) (4) mg/kg BA in rat high dose group x (b) (4) (rat BSA factor)
= (b) (4) mg/kg HED
(b) (4) mg/kg HED in rat for BA ÷ (b) (4) mg/kg maximum human dose
= SM of (b) (4)

Local toxicity was no different between SABER-Bupivacaine and SABER placebo (SAIB plus BA only).

Degradants – (b) (4) are supported for potential human exposure.

(b) (4)

Extractables/Leachables (E/Ls) – Support for potential human dosing for more than 30 materials (extractables, leachables, (b) (4) – see section 2.5 for detailed evaluation) was determined using several methods that included FDA draft guidance acceptable levels of ≤ 5 µg/day for nonstructural alerts, None of the E/Ls were identified as potentially genotoxic structural alerts by the Product Quality reviewer.

For the other identified compounds, risk assessments were conducted based on ISO 10993-12 (Establishment of allowable limits for leachable substances). This risk assessment technique utilizes animal or human data NOAELs/LOAELs divided by uncertainty factors (e.g., factors of 10 for animal to human data extrapolation and for inter-individual differences between humans), and exposure factors (e.g., bioavailability for an oral drug compared to the subcutaneous dose to be used for SABER-Bupivacaine) to determine the Tolerable Intake Level (TI – mg/kg/day) and Tolerable Exposure Level (TE - mg/day), the maximum predicted dose at which no adverse effects are predicted. This risk assessment technique is very similar to what the US EPA's Integrated Risk Information System (IRIS) uses and is widely accepted and considered appropriate by this reviewer.

Based on these assessments, all extractables/leachables as listed in section 2.5 are supported for potential human exposure at listed levels.

In summary, human safety of maximum potential exposure levels from use of SABER-Bupivacaine was supported for excipients (BA and SAIB), degradants (b) (4) and Extractables/Leachables (numerous).

5. Local effects of SABER-Bupivacaine and SABER placebo on sciatic nerve, wound healing, and hemolysis.

Sciatic nerve

Male Sprague Dawley rats were injected near the area of the sciatic nerve in the left thigh (right thigh as control) with either saline (250 µL), bupivacaine HCl (250 µL), SABER-Bupivacaine (24 mg – 200 µL; 48 mg – 400 µL), or SABER placebo (study 022-010). Body weights were recorded on the day of dosing and at necropsy. Food consumption values were recorded 5 days prior to dosing and for the week following test article administration. Clinical signs and a neurological examination were recorded daily. The neurological evaluation included evaluation of toe pinch, proprioception, and flexor withdrawal reflex as well as an observation of gait. All the animals were sacrificed 7 days (Day 8) after dose administration and the muscles and sciatic nerves from both hind legs were preserved. The treated legs were analyzed histopathologically. One control leg from each group was also evaluated.

The administration of bupivacaine produced a nerve block 3 hours post administration. The neurological signs appeared to resolve in most animals by Day 2. The gait of animals that received 24 mg or 48 mg SABER-Bupivacaine appeared to be the most severely affected with 3 of 5 animals (24 mg) and 1of 5 animals (48 mg) displaying an abnormal gait through Day 8.

Microscopically, there was an increased frequency of neuronal inflammation and axonal degeneration observed compared to untreated (right leg), saline treated, or bupivacaine HCl-treated groups. This effect appeared to result from both the presence of the vehicle as well as the bupivacaine since the frequency and/or severity of axonal changes showed incremental increases from control (right leg), to saline, to bupivacaine HCl, to bupivacaine-treated groups. Inflammation within the intermuscular fasciae and perineural connective tissues and myofiber degeneration and regeneration also showed incremental increases in frequency and severity after SABER-Bupivacaine administration.

In summary, relative to the proposed drug product, injected liquids SABER-Bupivacaine and SABER placebo produced increased local effects to the sciatic nerve compared to the saline, Bupivacaine HCl, and negative control (untreated nerve) groups. This has been described in other studies as an anticipated foreign body reaction due to the SAIB,

but in this case, the bupivacaine combined with SAIB and BA (SABER-Bupivacaine) appears to contribute to a more severe local reaction.

Wound healing

1) Male Sprague Dawley rats received intended wounds on day 0 and were treated with various SAIB-containing formulations (0.125 mL in a 2.5 cm incision), two of which were similar to SABER-Bupivacaine and SABER placebo (study DUR2). A mechanical determination of wound strength was measured on day 7 after the treatment and histology conducted on the SABER-Bupivacaine similar formulation and untreated wound (negative control).

No gross differences were noted in the wound sites as well as no difference in wound strength compared to the negative control. However, the SABER-Bupivacaine similar formulation caused more severe histological effects than the negative control, notably inflammation, granulation, and gaps.

In summary, under the conditions of the assay, formulations similar to SABER-Bupivacaine and SABER placebo did not adversely affect wound healing over 7 days although the SABER-Bupivacaine similar formulation was associated with increased histological effects consistent with a foreign body injection reaction compared to the negative control (incision only).

2) Female Gottingen SPF minipigs received eight full-thickness wounds on day 1 and were observed for 15 days (study 60111). Immediately after surgery, the wounds were treated topically within the wound tissue with 5% CMC gel (carboxymethylcellulose negative control), SABER placebo (vehicle), or SABER-Bupivacaine in a dose volume of 0.5 ml per wound. Observations of the wounds, included planimetric measurement of wound contraction, was performed on a daily basis. On Day 15 necropsy, each wound was sampled, fixed in neutral buffered formalin, and processed for histopathological examination.

No treatment-related clinical signs were seen during the study. The body weights were within a normal range of body weight for fully grown Gottingen minipigs. The macroscopic wound observations revealed no treatment-related differences regarding inflammation of the wounds edges, the skin surrounding the wounds, grade of hemorrhage, or grade of exudation. Necrotic tissue was not observed in any of the wounds during the study and hypergranulation was only seen in a few wounds on a single day during the study. Microscopically, the wound healing process was advanced comparably in all treated wounds. SABER-Bupivacaine and SABER placebo had no significant adverse effects on the wound healing in the minipigs compared to a CMC control. No wound only group was included so no absolute effect on wound healing could be determined.

Planimetric-based, wound contraction was identified on Day 2 for the wounds of all treatments. All wounds contracted to a comparable amount (~60%) by the end of the

study with a greater rate of contraction for the CMC-treated wounds on certain days during the study. Other observations (growth of granulation tissue, area of granulation tissue, re-epithelialization) were generally comparable commencing on day 9 for the wounds of all treatment groups with some increased severity in the SABER-Bupivacaine group. No differences in the re-epithelialized wound areas between the different treatments were seen during the study. The re-epithelialized area covered ~90% of the total wound area on Day 15 for the treated wounds of all groups.

In summary, injected SABER-Bupivacaine or SABER placebo caused no significant adverse effects on the wound healing process with only the expected local irritation effect of injection being observed which was comparable to the CMC negative control.

Hemolysis

The purpose of this study was to assess the hemolytic potential of the test articles SABER-Bupivacaine, SABER placebo (vehicle), sucrose acetate isobutyrate (SAIB), 0.75% Sensorcaine-MPF (Bupivacaine HCl Injection, USP), and 0.9% Sodium Chloride for Injection (negative control), USP, in human whole blood and the compatibility of these test articles with human plasma (study 01-11-803-X-VO-ATX). Supernatant of hemolytic test samples were evaluated macroscopically for changes in color or clarity and the presence of flocculation, precipitation, or coagulation relative to the homologous plasma sample.

SABER-Bupivacaine and SABER placebo both caused hemolysis when added to human whole blood. They also resulted in a viscous (and, for the vehicle, cloudy) macroscopic appearance, with globules resembling bubbles visible microscopically, when added to human plasma.

In summary, it appears that the benzyl alcohol component of SABER-Bupivacaine and SABER placebo may be involved in the hemolysis as bupivacaine and SAIB alone did not cause hemolysis. The toxicological significance of this hemolytic potential is unknown for this proposed SC drug.

6. Disposition, persistence, and local toxicity of SAIB at the injection site.

This issue is addressed as SAIB, the depot material allowing the prolonged release of bupivacaine, remains at the injection site for a prolonged period of time after the single injection of SABER-Bupivacaine. The local toxicity has been described in detail previously. What is attempted to be described in this section is the actual amount and physical disposition of the SAIB depot evaluated all or in part in 4 studies.

1) In a pharmacokinetics (PK) study of SAIB, the PK of ^{14}C-SAIB was evaluated after a single subcutaneous (SC) dose to male Sprague Dawley (SD) rats (study 7116-109). SAIB is the depot component of SABER-Bupivacaine, the proposed drug product. SC dose site histopathology was also evaluated. Determinations for PK and radioactivity were 6 weeks post dose. The test article was SAIB in a

[redacted] The proposed solvent for NDA 204803 (SABER-Bupivacaine) is Benzyl Alcohol (BA). This difference is not anticipated to affect SAIB radiolabel ADME to any significant degree [redacted]. The elimination half-life of radiolabel was prolonged over the 6 week study period (~21 days urine, ~18 days feces, ~23 days expired air). Elimination was 21.6% in urine, 13.7% in expired air, and 3.7% in feces with the remainder of the radioactivity at the dose site (61% of SAIB radiolabel largely remains at the dose site past 6 weeks after dosing). At 4 and 6 weeks, the SC dosing sites were surrounded by chronic inflammation, characterized by fibrosis and minimal lymphohistiocytic inflammation. Reversibility was not evident while inflammation persisted with severity similar at 4 and 6 weeks after dosing.

2) In another study (study B167-05), elimination from a single subcutaneous (SC) injection site was evaluated for differing formulation of SAIB. ^{14}C-SAIB formulations were injected in order to assess the disappearance of radioactivity after a single SC injection in male SD rats with determinations at 0, 2, 6 and 10 weeks post-dose. For an SAIB-Benzyl Alcohol (SAIB:BA formulation), the excipients used in the proposed drug product, radioactivity was measured at 98% (week 0), 57% (week 2), and 39% (week 6) of total administered. While 10 weeks was not evaluated for SAIB:BA, data from other formulations (e.g., SAIB: [redacted]) indicate that a significant amount of the SAIB would still be present at the injection site at 10 weeks post-dosing. Local toxicity was not evaluated.

3) The persistence of ^{14}C-SAIB was also determined in skin and SC tissue of male SD rats following injection SC or into a surgical wound (study 8255730) at 0, 1, 2, 6, & 10 weeks after dosing. After SC dosing, SAIB persistence was 93% (week 0), 83% (week 1), 77% (week 2), 64% (week 6), and 19% (week 10). After surgical wound dosing, SAIB persistence was 88% (week 0), 79% (week 1), 74% (week 2), 61% (week 6), and 20% (week 10), indicating the elimination or persistence of SAIB is similar whether injected SC or into a surgical wound. Local toxicity was not evaluated.

4) A 12-month observation period was employed to determine protracted irritation, the physical composition of SAIB, and the presence of the SAIB solvent after a single SC injection of SABER-Bupivacaine (not radiolabelled) in New Zealand Whit rabbits (study [redacted]-434007, Appendix F for SAIB structure analysis and presence of solvent). Histological observations of the injection site were conducted at week 2, 4, 6, 12, 26, 39, & 52 with analysis of the SAIB at week 39 & 52. The test article was SAIB in a solvent of [redacted] not BA as for the proposed drug. This difference is not anticipated to effect SAIB disposition to any significant degree as noted previously in this section, but may affect the severity of local toxicity. Single doses of SABER-Bupivacaine did not result in test article-related injection site toxicity up to 52 weeks post-injection if observations were based on comparison to SABER placebo, which resulted in local toxicity in its

own right. Early inflammation was predominantly granulomatous, of mild to moderate severity, and characterized by a mixture of mononuclear cells and multinucleated giant cells. Granulomatous inflammation was present in animals throughout the study but decreased in severity and incidence as the months progressed. Chronic inflammation, consisting of mononuclear cells, lymphocytes and often associated with fibrosis, was present starting at the 4-week post-injection evaluation and was noted in only one animal each at the 39- and 52-week post-injection valuations. At 52 weeks, minimal inflammation was observed in 1 of 3 placebo animals. Fibrosis was observed in 2 of 3 placebo and treated animals with the severity of mild (treated) and minimal and severe (placebo). SAIB was found to be essentially physically unchanged and still present 12 months after injection described as a viscous material with essentially no (b) (4) solvent present ((b) (4)%).

In summary - Of note regarding the SAIB depot is the persistence of this depot for up to 12 months after a single injection while also demonstrating local toxicity consistent with SABER-Bupivacaine and SABER placebo. To this end, the SAIB with some associated local toxicity persisted until at least 12 months after injection.

7. Potential for BA to be distributed to mother's milk with resulting fetal/neonate exposure.

The distribution and quantification of benzyl alcohol (BA) in selected tissues of the rat over time and the excretion of radioactivity into the milk of lactating rats after subcutaneous (SC) administration of SABER-Bupivacaine containing [^{14}C]-labeled benzyl alcohol was evaluated (study RPOS/FKM/204). Single SC doses of 0.2 mL/kg or 0.6 mL/kg were administered to female Wistar 4 days after parturition. Suckling pups were sampled at different time points post dose (1, 4, 8, & 24 hours) with the milk in each pup stomach being sampled and the radioactivity level being determined. In addition, radioactivity was determined in pooled plasma from each time point and in liver of the pups and in plasma of the dams at those time points.

Peak concentrations of benzyl alcohol-associated radioactivity (^{14}C BA) in plasma of dams were attained 1 hour after dosing. Following maternal SC administration of 0.2 mL/kg, concentration of radioactivity in plasma and liver of pups were generally below the lower limit of quantitation (LLOQ). Excretion of radioactivity into the milk was highest 4 hours after suckling. Following SC administration of 0.6 ml/kg, concentrations of radioactivity in plasma and liver of pups were highest 1 hour post-dose in plasma and in liver.

Concentrations of radioactivity were lower in pup plasma than in dam plasma. Concentrations of radioactivity in pup livers were similar to or slightly higher than plasma concentrations in pups. Radioactivity in milk was observed at both dose levels but never exceeded 0.4%of the dose of benzyl alcohol, with peaks occurring in suckling pups at 4 hours post maternal dosing with the amount excreted being higher at the dose of 0.6 mL/kg. If we assume comparable transfer in humans, ~ 5 mg BA would be

consumed orally at the human SABER-Bupivacaine dose containing 1210 mg BA in a suckling human neonate (0.4% x 1210 mg BA).

Benzyl alcohol has been associated with serious adverse reactions and death, particularly in pediatric patients. The "gasping syndrome," (characterized by central nervous system depression, metabolic acidosis, gasping respirations, and high levels of benzyl alcohol and its metabolites found in the blood and urine) has been associated with benzyl alcohol dosages >99 mg/kg/day in neonates and low-birth weight neonates. Additional symptoms may include gradual neurological deterioration, seizures, intracranial hemorrhage, hematologic abnormalities, skin breakdown, hepatic and renal failure, hypotension, bradycardia, and cardiovascular collapse [Benzyl Alcohol. Cosmetic Ingredient Review; International Journal of Toxicology; 20(suppl 3):23-50 (2001)].

Assuming 100% oral absorption of BA in human neonates after suckling, at the lower 5% level for birth weights (~1 kg), the neonate dose would be 5 mg/kg (0.4% of the administered dose). On this basis, the neonate risk to BA toxicity at least 20-fold below the observed effects level with the assumption for 100% oral absorption of BA which is not likely.

The actual human toxicological relevance of this is unknown but available data suggest that a single dose of SABER-Bupivacaine presents minimal risk of neonatal toxicity through suckling.

8. *In vitro* genotoxicity of the drug substance (bupivacaine) and SABER-Bupivacaine degradation products (b) (4)

Genotoxic degradant (b) (4) has been controlled and addressed by specification limits in the drug substance bupivacaine (see section 2.5). The potential genotoxicity for degradant (b) (4) has been addressed as it is not genotoxic based on literature data (see section 2.5).

The drug substance bupivacaine, bupivacaine degradant (b) (4), and SABER-Bupivacaine degradant (b) (4) were not mutagenic in *Salmonella* and *E. coli* in valid *in vitro* Ames assays and were not clastogenic in valid *in vitro* chromosomal aberration studies using human peripheral blood lymphocytes studies submitted with this NDA.

The submitted information is consistent with *FDA/ICH Guidances for Industry: Q3A Impurities in New Drug Substances (February 2003) and Q3B(R2) Impurities in New Drug Products (August 2006).*

9. *In vivo* genotoxicity of the drug product SABER-Bupivacaine and SABER placebo.

SABER-Bupivacaine and SABER placebo were not genotoxic *in vivo* in a rat micronucleus test. The absolute value of this information is unknown as the rats received a single SC dose and were sacrificed 3 days after dosing. This may be a point of discussion/comment because, as noted previously in section 4, SAIB persists for at least six months resulting in prolonged exposure and local irritation. These time periods were not evaluated for micronuclei. Of note is that bupivacaine was not genotoxic *in vitro* and the excipients BA and SAIB have been determined to not be genotoxic or carcinogenic based on submitted literature data.

While a valid assay, it may not really be full evaluating the potential genotoxicity of the drug substance, drug product, and it components as SAIB persists at the injection site for a prolonged period of time. A more appropriate study would be a SC injection followed by sampling for micronuclei after 28 days.

The submitted information is consistent with FDA/ICH Guidances for Industry: Q3A Impurities in New Drug Substances (February 2003) and Q3B(R2) Impurities in New Drug Products (August 2006).

10. Embryo-fetal developmental toxicity of SABER placebo.

Sperm positive female SD rats were treated with SABER placebo (75%:25% SAIB:BA) by subcutaneous injections every 3 days of gestation starting on gestation day 7 until gestation day 16 (study 11-11-803-R-SC-TT). All rats survived to sacrifice. Body weights/body weight gains, food consumption, Caesarean-sectioning parameters, fetal gross and visceral alterations, and ossifications site averages were unaffected by treatment.

Injection site reactions (i.e., swelling, discoloration, and scabs) occurred at all dose levels of SABER placebo tested, and the incidences were dose dependent. These observations generally occurred in the first few days of dosing and, in many cases, persisted through the end of the dose period and into the post-dose period.

In the high dose group, 6 fetuses in 1 litter had extra presacral vertebrae with or without extra ribs. While these findings were not previously identified in the testing lab's historical control data, the occurrence was considered of unknown toxicological relevance and a sporadic occurrence as it occurred in only a single litter. In addition, both SAIB and BA are not known to cause such effects. This conclusion was confirmed in a consult to the FDA OND PTCC Reproductive and Developmental Subcommittee.

In summary, there was no increase in embryo lethality, no effect on fetal body weight, and no fetal alterations (malformations or variations) attributed to SABER placebo at any dose tested. This is considered consistent with the lack of any such effects with either material tested separately.

11. Nonclinical data evaluating the local toxicity by a dose route other than the proposed injection route, Intra-articular (IA) dosing.

This nonclinical data summary is provided, even though it is not by the proposed drug product's route of administration, as the observed local toxicity is noteworthy. Future applications may be for the IA dosing route and the observed effects should be more specifically evaluated.

Single IA doses were administered to New Zealand White rabbits and Beagle dogs (studies 02-06-803-B-IJ-TX and 03-07-803-D-IJ-TX, respectively). An objective of these studies was to investigate the potential local toxicity after intra-articular dosing. Treatment was with saline (negative control), SABER placebo (vehicle control) or bupivacaine in SABER-Bupivacaine (3 dose levels). After the single IA dose, animals were sacrificed on days 14 and 42 post-dose.

No remarkable treatment-related effects were observed for mortality/moribundity, clinical signs, body weights, food consumption, ophthalmic examinations, hematology, clinical chemistry, coagulation, macroscopic pathology, ands absolute and relative organ weights. Recoverable clinical signs (e.g. scabs, inflammation, sore/ulcer, and injection site discoloration in rats; edema, swelling, limping in left hindlimb, and loose stool in dogs) were reported in both males and females.

Synovial hyperplasia, fatty degeneration, inflammation, and fibrosis were observed in both species for SABER-Bupivacaine and SABER placebo groups after histological examination of the dose sites. No microscopic lesions were present in the negative control joint. The incidence and the severity of the observed lesions were greatest in the high dose groups at Days 14 and 42 of euthanasia.

At day 14, joint cartilage necrosis was present in one vehicle control animal (moderate) and one high dose animal (marked), but not in the other two test article groups. Joint cartilage necrosis of marked severity was observed in all vehicle and high dose test article dogs at 42 days after dosing. Peer review confirmed original histopathology observations. No necrosis was observed in the joints of any rabbit treatment groups.

In summary, it should be noted that there is potential for progressive joint necrosis after IA administration of SABER-Bupivacaine which may be due to the vehicle (SABER placebo) with some enhancement of the severity by the presence of the bupivacaine. A warning for the possibility of chrondrolysis is contained in the product label.

Overall Nonclinical Conclusion

Human safety is supported at the maximum proposed single dose of SABER-Bupivacaine which is intended to provide sustained delivery of the bupivacaine local anesthetic for up to 3-4 days after surgery. As proposed exposure to bupivacaine is larger than for the approved, referenced NDA, this support for SABER-Bupivacaine is

based on acceptable product quality specifications and stability and valid nonclinical studies demonstrating acceptable systemic exposure and local tolerability with an acceptable clinical pharmacology relationship between nonclinical test product and the proposed drug product. Of note is the persistence of the excipient depot material, Sucrose Acetate Isobutyrate, which could be found at the injection site for up to 1 year after a single injection with continued local effects/toxicity. The clinical toxicological significance of this persistence and local toxicity from the depot material is unknown.

12 Appendix/Attachments

None.

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

/s/

GARY P BOND
01/08/2014

ADAM M WASSERMAN
01/08/2014
Review accepted. Please see my Supervisory memorandum.