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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

Application number: NDA 209511

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Product: XARACOLL® (Bupivacaine Hydrochloride Collagen-Matrix Implants)

Indication: Surgical site placement to produce postsurgical analgesia following (b) (4)

Applicant: Innocoll Pharmaceuticals

Clinical Review Division: Division of Anesthesiology, Addiction Medicine, and Pain Medicine (DAAP)

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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY.....	3
1.1	INTRODUCTION AND REGULATORY HISTORY	3
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	4
1.3	RECOMMENDATIONS	6
2	DRUG INFORMATION.....	11
2.1	DRUG	11
2.2	RELEVANT INDs, NDAs, BLAs AND DMFs.....	11
2.3	DRUG FORMULATION	12
2.4	COMMENTS ON NOVEL EXCIPIENTS	12
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	13
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	14
2.7	REGULATORY BACKGROUND	15
3	STUDIES SUBMITTED	19
3.1	STUDIES REVIEWED	19
3.3	PREVIOUS REVIEWS REFERENCED.....	19
4	PHARMACOLOGY	20
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	20
5.1	PK/ADME	20
6	GENERAL TOXICOLOGY	20
6.1	SINGLE-DOSE TOXICITY	20
7	GENETIC TOXICOLOGY.....	21
8	CARCINOGENICITY.....	26
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	26
10	SPECIAL TOXICOLOGY STUDIES	26
10.1	OSTEOTOMY STUDIES.....	26
10.2	EXTRACTABLES AND LEACHABLES STUDIES.....	44
11	INTEGRATED SUMMARY AND SAFETY EVALUATION	52
12	APPENDIX/ATTACHMENTS.....	52

1 Executive Summary

1.1 Introduction and Regulatory History

NDA 209511 was submitted by Innocoll Pharmaceuticals for marketing approval of Xaracoll, which is an implantable combination product containing bupivacaine and purified Type I bovine collagen, for the proposed indication of placement into a surgical site to produce postsurgical analgesia following (b) (4). Each Xaracoll implant contains 100 mg bupivacaine HCl. The maximum recommended human dose (MRHD) is 300 mg (3 x 100 mg implants), which is equivalent to 266.4 mg bupivacaine base. The NDA was submitted via the 505(b)(2) pathway relying on the Agency's previous findings of safety of the listed drug Marcaine (NDA 16964; approved 1972) and literature.

NDA 209511 was originally submitted on 10/31/2016. However, the Division issued a Refuse-to-File letter on 12/23/2016 with issues from almost all disciplines. Of note, the Applicant changed the collagen component of the product during development and did not test the to-be-marketed (TBM) product in pivotal nonclinical toxicology studies and the comparative human bioavailability (BA) study. Only studies with the older formulation were submitted to the original NDA. In addition, adequate extractables/leachables data were not included with the original NDA. The resubmission dated 2/2/2018 (SDN 9) included a new GLP toxicology study with the TBM product, new human comparative BA study using the TBM product, and a new extractables/leachables evaluation. Also of note, when the Applicant originally submitted the NDA, they referenced both listed drugs Marcaine and (b) (4) as part of the 505(b)(2) NDA submission; however, only Marcaine was referenced with the resubmission. This was significant because the clinical BA study demonstrated that the AUC exposure level to bupivacaine following XARACOLL treatment at the MRHD exceeded the listed product Marcaine given at 175 mg by approximately 2X (e.g., $AUC_{0-\text{last}}$ (h•ng/mL) of 19493 vs 9708, respectively), which is not surprising given the bupivacaine HCl dose of 300 mg in XARACOLL vs 175 mg in Marcaine. Therefore, the referenced drug product did not support the systemic safety of the higher exposures obtained via this drug product formulation. In brief, the NDA was not approved in the first cycle review and the complete response letter (CRL) dated 11/30/2018 included four deficiencies, which are summarized at a high level below (see regulatory history section below to see nonclinical deficiencies verbatim as stated in the CRL):

- Inadequate characterization of the systemic safety of bupivacaine exposures via the drug product formulation
- In vivo micronucleus study for bupivacaine was not valid
- Inadequate extractables/leachables evaluation to support the safety of the proposed container closure system

- Inadequate justification for the proposed specification for (b) (4) in the drug product

The current submission (SDN 44; 2/26/2020) is in response to the Complete Response Letter (CRL) dated 11/30/2018. The Applicant provided information to address each of the four deficiencies summarized above. To address the systemic safety of bupivacaine, the Applicant provided clinical PK information to demonstrate that the systemic exposures achieved with Xaracoll at the MRHD are within the reference product, which has an MRHD of 400 mg based on the approved product labeling. Refer to the clinical pharmacology and clinical reviews regarding the adequacy of the information to address this deficiency. The Applicant provided new data to adequately address the remaining three deficiencies from a nonclinical perspective. In brief, a new in vivo micronucleus study was submitted that tested adequate doses. The test was considered valid and demonstrated that bupivacaine is negative for clastogenicity under the conditions of the test. New extractable/leachables data, including a sufficiently robust extractables study and sufficient leachables stability methodology and testing, were submitted to adequately justify the safety of the container closure system. The Applicant reduced the specification for (b) (4) so that the potential daily exposure would be within the acceptable daily intake as per ICH M7 for potentially genotoxic impurities.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical program for Xaracoll, which includes the totality of the data submitted in the previous and current review cycles, adequately supports the safety of the product for the proposed clinical use. Notable findings are discussed below.

There are no nonclinical concerns with the drug substance and drug product specifications as they are in compliance with the recommendations outlined in ICH M7 for mutagenic impurities, ICH Q3A(R2) for drug substance impurities, and ICH Q3B(R2) for drug product degradants. Notably, the Applicant lowered the specification for (b) (4) a potentially genotoxic compound, with this resubmission to NMT (b) (4) ppm, which results in a potential total daily intake of (b) (4) mcg/day. This is within the acceptable daily intake as per ICH M7 and therefore the nonclinical deficiency included in the CRL letter dated 11/30/2018 is adequately addressed.

The Applicant has provided new genetic toxicology data to demonstrate that bupivacaine is negative for mutagenic and clastogenic potential. During development, the Division noted that although not required for approval of the product, the standard battery of genetic toxicity studies for bupivacaine would ideally be completed to appropriately inform labeling. The Applicant submitted the full battery of studies, including an 1) in vitro reverse mutation assay in bacterial cells (Ames), 2) in vitro mammalian cell gene mutation test (L5178Y/TK+/- mouse lymphoma assay), and 3) in vivo clastogenicity assay in rodent (micronucleus assay), during the previous review cycle; however, the in vivo study was not considered valid because the high dose did not product frank toxicity. With this resubmission, new in vivo data were submitted, and

the study is concluded to be valid and it adequately addresses the deficiency in the CRL dated 11/30/2018.

The Applicant provided adequate extractables and leachables data to justify the safety of the container closure system for the proposed expiry of 2 years. Although insufficient data were provided during the previous review cycle, new data were submitted with this resubmission that demonstrated that no leachables were observed above the safety concern threshold of $10^{(b)}_{(4)}$ mcg/day from a sufficient number of drug product stability batches at multiple time points. The CMC reviewer confirmed that methodology and analytical limits employed were all acceptable. Therefore, the newly submitted data adequately addresses the deficiency outlined in the CRL dated 11/30/2018.

During the previous review cycle, the local safety of the drug product formulation was considered adequately characterized. The Applicant conducted an extensive literature review for bupivacaine and submitted a pivotal GLP 56-day rat toxicology study testing the local tissue effects of the to-be-marketed bupivacaine collagen sponge implant in a wound closure model. They also evaluated wound healing qualitatively in this study. The GLP toxicology study results suggest the potential for a clear local tissue response which includes microscopic evidence of marked necrosis of tissue surrounding the implant and a chronic-active inflammatory state consistent with the implantation of a foreign material. Mild to moderate necrosis was noted in the saline group, the bupivacaine injection control group, and the collagen matrix alone implant group. Only the bupivacaine collagen implant groups demonstrated marked local tissue necrosis. There was no clear impact on wound healing in this study based on clinical and gross macroscopic observations alone suggesting that the enhanced local tissue response did not translate into significant clinical impact. The existing human local safety data should be leveraged to confirm that these local changes are not considered clinically adverse. The implanted material was no longer clearly present in tissue somewhere between 35- and 56-days post implantation.

During the previous review cycle, the systemic safety of the drug product formulation had not been adequately qualified. To establish a scientific bridge to the systemic safety of the reference product, a clinical bioavailability (BA) study was performed and demonstrated that the AUC exposure level to bupivacaine following XARACOLL treatment at the maximum recommended human dose (MRHD) exceeded the listed product Marcaine, which was given at 175 mg by approximately 2X (e.g., AUC_{0-last} (h•ng/mL) of 19493 vs 9708, respectively). The CRL dated 11/30 noted that the systemic safety of bupivacaine could be qualified via the conduct of general toxicology studies in two species that demonstrate systemic exposure coverage for the human exposures at the MRHD. Alternatively, this could be addressed with human data. With this resubmission, the Applicant provided new human PK data to demonstrate that the exposure levels with XARACOLL are within the referenced product, which has an MRHD of 400 mg according to the label. PK modeling data were provided to predict that the Marcaine exposure data would actually exceed Xaracoll. Refer to the clinical pharmacology and clinical reviews for details regarding the adequacy of these data to address the deficiency.

During the previous review cycle, the Applicant submitted a literature review of the published studies to address the requirements outlined in the Pregnancy Labeling and Lactation Rule (PLLR). Review of the published studies submitted did not identify data that substantively changed the overall conclusions of the referenced reproductive and developmental toxicity studies in the Marcaine labeling. As such, no additional labeling recommendations are provided at this time.

Based on the agreed initial Pediatric Study Plan (iPSP), no juvenile animal toxicology studies were required to support the safety of the two pediatric studies that will evaluate the safety, tolerability, pharmacokinetics, and analgesic effects of the drug product in children from 2 to <17 and 0 to <2 years of age based on the vast human experience with bupivacaine in children down to birth.

Although not required for the proposed indication and not discussed with the Agency previously, the Applicant included bone healing studies in sheep and rat with this resubmission. The sheep study was a non-GLP pilot study to demonstrate the feasibility of an implantation study in an osteotomy model, but it did not include adequate endpoints to evaluate bone healing. In the GLP rat study, animals were subjected to an osteotomy procedure and then treated with saline, IR bupivacaine, a placebo matrix implant, or two different doses of the bupivacaine collagen implant. Based on torsional strength testing, radiologic evaluations, microCT data, and histopathological evaluations, the study results suggest that the bupivacaine collagen-matrix implant inhibited bone healing following osteotomy in this rat model compared to controls, including standard of care bupivacaine alone. The clinical significance of these findings is not clear.

Because there currently are no clinical studies testing the effect of this drug product on bone healing, if there is concern that the drug product may be used in procedures that involve placement near bone, the following labeling language could be included in Section 13.2 and cross referenced from warnings and precautions.

Bupivacaine collagen-matrix implants delayed bone healing in a rat osteotomy model compared to saline, bupivacaine, or placebo-collagen matrix alone. The clinical significance of these delays is not known.

1.3 Recommendations

1.3.1 Approvability

From a pharmacology toxicology perspective, there are adequate nonclinical data to justify the safety of XARACOLL for the proposed clinical use and therefore we recommend approval with the recommended labeling changes.

1.3.2 Additional Non-Clinical Recommendations

None at this time.

1.3.3 Labeling

Formal labeling negotiations have not been completed to date given the complete response recommendation. The labeling suggestions below have not been fully discussed with the review team or the Applicant. The reader is referred to the second cycle review and ultimately an approval letter for final labeling content. Final labeling recommendations should be consistent with the most recently approved Marcaine labeling, adjusted as appropriate.

Marcaine label (2012)	Applicant's Proposed Labeling (April 2018, Version 1.2)	Recommended Labeling	Rationale/Comment
	HIGHLIGHTS OF PRESCRIBING INFORMATION INDICATIONS AND USAGE XARACOLL (b) (4) [REDACTED] for placement into the surgical site to produce postsurgical analgesia following (b) (4) (1). USE IN SPECIFIC POPULATIONS • Pregnancy: based on animal data, may cause fetal harm (8.1).	HIGHLIGHTS OF PRESCRIBING INFORMATION INDICATIONS AND USAGE XARACOLL is an implantable combination product containing bupivacaine, an amide local anesthetic, and purified Type I bovine collagen, that is indicated for placement into the surgical site to produce postsurgical analgesia following (b) (4) (1). USE IN SPECIFIC POPULATIONS • Pregnancy: May cause fetal harm (8.1).	Established pharmacological class needs to be included here in the Highlights. See clinical review for final indication. This has been deleted in recent labels for other drugs and is appropriate here – Maternal Health call.
Pregnancy Category C: 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.	8.1 Pregnancy Risk Summary There are no studies conducted with XARACOLL in pregnant women. Bupivacaine hydrochloride produced developmental toxicity when administered subcutaneously to pregnant rats and rabbits at clinically	8.1 Pregnancy Risk Summary There are no studies conducted with XARACOLL in pregnant women. In animal studies, embryo-fetal lethality was noted when bupivacaine was administered subcutaneously to pregnant rabbits during	Information from current Marcaine label (2020) formatted per the Pregnancy and Lactation Labeling Rule (PLLR), amended by adding appropriate dose ratios and dosing periods to comply with PLLR requirements.

Bupivacaine hydrochloride produced developmental toxicity when administered subcutaneously to pregnant rats and rabbits at clinically relevant doses.	relevant doses. Based on animal data, advise pregnant women of the potential risks to a fetus.	organogenesis at clinically relevant doses. Decreased pup survival was observed in a rat pre- and post-natal developmental study (dosing from implantation through weaning) at a dose level comparable to the daily maximum recommended human dose (MRHD). Based on animal data, advise pregnant women of the potential risks to a fetus.	The literature was reviewed and there was no information that was deemed warranted for the label at this time.
This does not exclude the use of MARCAINE at term for obstetrical anesthesia or analgesia. (See Labor and Delivery)	The background risk of major birth defects and 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.	The background risk of major birth defects and 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.	Standard PLLR label language. We defer to the Maternal Health and clinical review teams on clinical labeling language.
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	<u>Data</u> <i>Animal Data</i> Bupivacaine hydrochloride was administered subcutaneously to rats at doses of 4.4, 13.3 and 40 mg/kg and to rabbits at doses of 1.3, 5.8 and 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). No embryo-fetal effects were observed in rats at up to 40 mg/kg, a dose	<u>Data</u> <i>Animal Data</i> Bupivacaine HCl produced developmental toxicity when administered subcutaneously to pregnant rats and rabbits at clinically relevant doses. Bupivacaine HCl was administered subcutaneously to rats at doses of 4.4, 13.3 and 40 mg/kg and to rabbits at doses of 1.3, 5.8 and 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). No embryo-fetal effects were observed in rats at up to 40 mg/kg, a dose that caused increased	The Applicant included appropriate exposure margins.
			Language consistent with Marcaine label.

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. 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/day, 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.	that caused increased maternal lethality. This dose is approximately 1.3 times the daily maximum recommended human dose (MRHD) of 300 mg when calculated on a mg/m² body surface area (BSA) for a 60 kg woman. An increase in embryo-fetal deaths was observed in rabbits at the high dose (1.4 times the MHRD based on BSA) in the absence of maternal toxicity. (b) (4) No Observed Adverse Effect Level (b) (4) approximately 0.4 times the MRHD on a BSA basis. In a rat pre- and post-natal development study (dosing from implantation through weaning) conducted at subcutaneous doses of 4.4, 13.3, and 40 mg/kg/day, decreased pup survival was observed at the high dose (approximately 1.3 times the daily MRHD on a BSA basis).	maternal lethality. This dose is approximately 1.3 times the daily maximum recommended human dose (MRHD) of 300 mg when calculated on a mg/m² body surface area (BSA) for a 60 kg woman. An increase in embryo-fetal deaths was observed in rabbits at the high dose (approximately 1.4 times the MHRD on a BSA basis) in the absence of maternal toxicity. The developmental No Observed Adverse Effect Level in rabbits is approximately 0.4 times the MRHD on a BSA basis. In a rat pre- and post-natal development study (dosing from implantation through weaning) conducted at subcutaneous doses of 4.4, 13.3, and 40 mg/kg/day, decreased pup survival was observed at the high dose (approximately 1.3 times the daily MRHD on a BSA basis).	
Nursing Mothers: Bupivacaine has been reported to be excreted in human milk suggesting that the nursing infant could be theoretically exposed to a dose of the drug. Because of the potential for serious adverse reactions in nursing infants from bupivacaine, a decision should be made whether to discontinue nursing or not	8.2 Lactation Risk Summary Bupivacaine has been reported to be excreted in human milk suggesting that the nursing infant could be theoretically exposed to a dose of the drug. There is no available information on effects of the drug in the breastfed infant or effects of the drug on milk production. The developmental and health benefits of	8.2 Lactation Risk Summary Bupivacaine has been reported to be excreted in human milk suggesting that the nursing infant could be theoretically exposed to a dose of the drug. There is no available information on effects of the drug in the breastfed infant or effects of the drug on milk production. The developmental and health benefits of	We defer to the Maternal Health and clinical review teams on clinical labeling language.

administer bupivacaine, taking into account the importance of the drug to the mother.	breastfeeding should be considered along with the mother's clinical need for XARACOLL and any potential adverse effects on the breastfed infant from XARACOLL or from the underlying maternal condition.	breastfeeding should be considered along with the mother's clinical need for XARACOLL and any potential adverse effects on the breastfed infant from XARACOLL or from the underlying maternal condition.	
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. In general, the progression of anesthesia is related to the diameter, myelination, and conduction velocity of affected nerve fibers. Clinically, the order of loss of nerve function is as follows: (1) pain, (2) temperature, (3) touch, (4) proprioception, and (5) skeletal muscle tone.	12.1 Mechanism of Action (b) (4) bupivacaine 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. Clinically, the order of loss of nerve function is (1) pain, (2) temperature, (3) touch, (4) proprioception, and (5) skeletal muscle tone.	12.1 Mechanism of Action Local anesthetics such as bupivacaine 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. In general, the progression of anesthesia is related to the diameter, myelination, and conduction velocity of affected nerve fibers. Clinically, the order of loss of nerve function is (1) pain, (2) temperature, (3) touch, (4) proprioception, and (5) skeletal muscle tone.	Applicant has deleted the bolded sentence from the Marcaine label without an explanation. It should be retained.
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.	13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenesis</u> Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine have not been conducted. <u>Mutagenesis</u> Bupivacaine was not mutagenic or clastogenic in a bacterial reverse mutation assay, an in	13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenesis</u> Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine have not been conducted. <u>Mutagenesis</u> Bupivacaine was not mutagenic or clastogenic in a bacterial reverse mutation assay, an in	From Marcaine label. The Applicant conducted new genotox studies with bupivacaine (all three) and XARACOLL

	vitro mammalian cell gene mutation test, and an in vivo mammalian erythrocyte micronucleus assay. <u>Impairment of Fertility</u> The effect of bupivacaine on fertility has not been determined.	vitro mammalian cell gene mutation test, and an in vivo mammalian erythrocyte micronucleus assay. <u>Impairment of Fertility</u> The effect of bupivacaine on fertility has not been determined.	extracts (first two) for this NDA. Consistent with Marcaine. No reliable PLLR-related literature search data was identified.
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2 Drug Information

2.1 Drug

CAS Registry Number - 18010-40-7 (anhydrous), 14252-80-3 (monohydrate)

Generic Name - Bupivacaine hydrochloride

Code Name - NR

Chemical Name - 1-Butyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide hydrochloride monohydrate

Molecular Formula/Molecular Weight - $C_{18}H_{28}N_2O \cdot HCl \cdot H_2O$

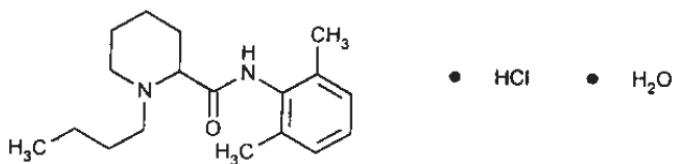
Molecular Weights

342.90 monohydrate

324.89 anhydrous

288.43 free base anhydrous

Structure or Biochemical Description



Pharmacologic Class - amide local anesthetic

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
16964	Marcaine	DAAAP	0.25% injection	Approved	10/3/1972	Local anesthesia	Hospira

IND#	Drug	Status	Division	Indication	Stamp Date	Sponsor
77127	Bupivacaine Collagen Matrix	Active	DAAAP	Post-surgical pain	3-1-2007	Innocoll Pharmaceuticals

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
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(b) (4)

2.3 Drug Formulation

Composition - Xaracoll is a white to off-white drug/device combination product composed of a (b) (4) purified collagen matrix as the device component containing the amide local anesthetic bupivacaine HCl as the drug substance component. The product configuration is a 5 × 5 × 0.5 cm matrix implant consisting of purified type I bovine collagen prepared as a (b) (4). The final collagen/bupivacaine dispersion is a (b) (4).

(b) (4)

Each final (b) (4) drug

product matrix contains 75 mg of collagen and 100 mg of bupivacaine HCl. The composition of each product matrix is shown in the table below.

Xaracoll Composition

Component	Quality standard	Quantity per matrix	Function
Bovine type I collagen, purified	(b) (4)	75 mg	Excipient
Bupivacaine HCl	USP	100 mg	Drug substance

(b) (4)

(b) (4)

2.4 Comments on Novel Excipients

Technically, bovine Type I collagen is a novel excipient because it has not been previously used in an FDA-approved drug product for subcutaneous administration or in a wound closure environment as per the FDA Inactive Ingredients Database (IID). As such, the existing toxicology studies, in conjunction with the ISO studies reviewed by CDRH were submitted to justify the safety of this excipient. Because bovine collagen is comparable to human collagen (almost identical amino acid sequences and antigenic

determinants) and exposure to this excipient is expected to be comparable to collagen levels found in the body, genetic toxicology studies and reproductive and developmental toxicology studies were not required to support this NDA.

The collagen used in this product is derived from bovine Achilles tendons obtained from (b) (4) closed herds and certified bovine spongiform encephalopathy free. The reader is referred to the CMC quality review for details and the CDRH review for biocompatibility assessments, which included genetic toxicology studies and an evaluation of extractables and leachables from the collagen matrix.

Because bovine collagen is comparable to human collagen (almost identical amino acid sequences and antigenic determinants) and exposure to this excipient and its degradation products is expected to be comparable to human collagen found ubiquitously in the body, genetic toxicology studies and reproductive and developmental toxicology studies were not required to support this NDA. Although collagen will likely take up to 56 days to degrade and be removed from the implantation site, the lack of genetic toxicity concern and relatively short duration of exposure for this indication preclude the need for carcinogenicity studies, consistent with recommendations in ICH S1A.

2.5 Comments on Impurities/Degradants of Concern

The drug substance and drug product specifications proposed during the previous review cycle were acceptable with the exception of the drug product specification for (b) (4). Refer to the NDA 209511 nonclinical review dated 11/5/2018 for details.

In brief, the proposed specification for (b) (4) during the previous review cycle was NMT (b) (4) ppm. Based on this specification, the potential total daily intake (TDI) is (b) (4) mcg for a 300 mg maximum recommended human dose (MRHD). The Applicant did not provide genotox data for this compound, but a review of toxicological resources, including TOXNET, suggest that it does test positive in the Ames assay as a mutagen. The TDI exceeds the acceptable daily intake of (b) (4) mcg for a mutagenic impurity in a drug administered for (b) (4) as per ICH M7. Actual levels of (b) (4) in drug product batches were well within the proposed specification of NMT (b) (4) ppm. The complete response letter dated 11/30/2018 included the following deficiency related to this degradant:

You have not provided adequate justification for the proposed specification for (b) (4) in the drug product formulation.

To address this deficiency:

Either reduce the specification for (b) (4) to NMT (b) (4) mcg/day or provide an adequate toxicological risk assessment for this compound to justify the proposed specification in accordance with the ICH guidance document: *M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*, available at <https://www.fda.gov/downloads/Drugs/Guidance/ComplianceRegulatoryInformation/Guidances/UCM347725.pdf>.

With this resubmission, the Applicant has tightened the specification for (b) (4) to NMT (b) (4) ppm as shown in the table below. Note, all other specifications remain the same and were considered acceptable during the previous review cycle. Based on the proposed specification of NMT (b) (4) ppm, the potential TDI is (b) (4) mcg/day, which is with the appropriate ADI as per ICH M7. Therefore, the Applicant has adequately addressed this deficiency.

Final testing	Acceptance criteria	SOP
Characteristics/identity		
Appearance	White to off-white porous sponge	QC6-008
Identity bupivacaine (HPLC)	Sample RT (b) (4) of reference standard RT	QC6-009
DSC - Peak Denaturation Temperature	(b) (4)	QC6-059
Identity collagen (SDS-PAGE)	Identification of Collagen (b) (4)	QC6-061
Purity testing		
(b) (4)	NMT (b) (4) ppm	QC6-057
	NMT (b) (4) ppm	QC6-012
	NMT (b) (4) ppm	QC6-012
Related substances by GC	Any single impurity < (b) (4) Total impurities ≤ (b) (4)	QC6-010
Related substance (b) (4) (HPLC)	NMT (b) (4) %	QC6-009
Assay testing		
(b) (4)		QC6-008
		QC6-008 USP (b) (4)
Assay bupivacaine HCl (HPLC)	(b) (4)	QC6-009
Content uniformity (HPLC)	(b) (4) NMT (b) (4) Complies to USP <905>	QC6-009 USP <905>
Dissolution (HPLC)	30 min: (b) (4) 120 min: (b) (4) 360 min: NLT (b) (4)	QC6-011 QC6-009 USP <711>
Microbiological testing		
Bacterial endotoxins	NMT (b) (4) EU/implant	QC8-020 USP <85>
Sterility	Sterile	QC8-014 USP <71>

NMT = not more than; NLT = not less than; AV = acceptance value; EU = endotoxin units; RT = retention time

2.6 Proposed Clinical Population and Dosing Regimen

Xaracoll is proposed for preemptive treatment of postsurgical pain following (b) (4). Each bupivacaine HCl collagen-matrix contains 100 mg bupivacaine HCl (equivalent to 88.8 mg of bupivacaine) (b) (4) (b) (4) in 75 mg of collagen

in a matrix measuring approximately 5 cm x 5 cm x 0.5 cm. The proposed dose of Xaracoll is 300 mg (equivalent to a total of 266.4 mg of bupivacaine), achieved by implanting 3 x 100 mg collagen matrices during soft tissue surgery at multiple layers in the soft tissue (e.g., between the fascia/muscle closure and in the layers below the skin closure). Xaracoll was studied primarily in hernioplasty and hysterectomy, but, from a nonclinical perspective, these two surgeries are representative models of different types of soft tissue surgeries, given their unique anatomic considerations, surgical approaches and placement of Xaracoll in the surgical wound.

2.7 Regulatory Background

- Bupivacaine has been marketed in the United States for over 20 years as Marcaine. This New Drug Application is being submitted using a 505(b)(2) regulatory pathway with Marcaine (NDA 016964 – approved 10/3/1972) as the listed drug.
- NDA 209511 was originally submitted on 10/31/2016. However, the Division issued a Refuse-to-File (RTF) letter on 12/23/2016 with issues from almost all disciplines. The following nonclinical RTF issues were included in the letter:
 1. There are inadequate nonclinical data in the NDA to adequately qualify the safety of the to-be-marketed drug product. We note that the pivotal nonclinical 56-day bupivacaine collagen implant study (e.g., (b) (4)-134502) did not test the to-be-marketed (b) (4) collagen (b) (4) containing drug product, but rather an earlier development product. To address this issue, you must repeat your pivotal toxicology study testing with the to-be-marketed drug product, or provide justification for how the findings of the conducted study can be extrapolated to support the safety of the to-be-marketed product with respect to the endpoints tested (e.g., bupivacaine release, local toxicity, duration and fate of the inserted sponge).
 2. The NDA did not include an adequate extractables/leachables evaluation per our discussions during development (refer to Meeting Minutes dated January 12, 2012, and Written Responses dated August 10, 2015). We acknowledge your proposal to provide an extractables/leachables evaluation just prior to commercialization since your product is a (b) (4) matrix, which would typically be associated with a low risk of leaching of container closure system components into the drug product. However, an extractables/leachables evaluation is required to file the NDA as it is critical to support the safety of your product.
- NDA 209511 was resubmitted 2/2/2018 (SDN 9). However, the NDA was not approved and the complete response letter dated 11/30/2018 included the following deficiencies from a nonclinical perspective.
 1. You have not provided an adequate characterization of the systemic safety of bupivacaine exposures via your drug product formulation. Specifically, based on the existing human pharmacokinetic data, your product results in an AUC(0-last) that is twice that of the referenced product. Your existing toxicology data in the rat model do not test exposures that provide coverage for the human exposures via your product.

To address this deficiency:

Conduct adequate toxicology studies in two species that provide adequate coverage for the proposed human exposures via your drug product (AUC and C_{max}).

2. You have not provided a valid in vivo micronucleus assay for bupivacaine. Specifically, the high dose selected for the assay did not result in frank toxicity.

To address this deficiency:

Repeat the in vivo micronucleus assay for bupivacaine testing doses that result in frank toxicity in accordance with the ICH guidance document: S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, available at <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074931.pdf>.

3. You have not provided an adequate extractables/leachables evaluation to support the safety of your proposed container closure system. Specifically, although your extraction study, submitted September 20, 2018 (SDN 33), was capable of detecting compounds at the requested safety concern threshold of (b) (4) mcg/day, you did not identify many of the compounds detected above this safety threshold. Further, you did not provide adequate leachables data from multiple batches at release where compounds identified in the extraction study were targeted using validated methods.

To address this deficiency:

Identify the compounds detected in the extraction studies completed to date and evaluate the drug product stability batches for the presence of any extractable detected at (b) (4) mcg/day or higher. Provide a toxicological risk assessment for any leachable compound present in the drug product at (b) (4) mcg/day or greater.

4. You have not provided adequate justification for the proposed specification for (b) (4) (b) (4) in the drug product formulation.

To address this deficiency:

Either reduce the specification for (b) (4) to NMT (b) (4) mcg/day or provide an adequate toxicological risk assessment for this compound to justify the proposed specification in accordance with the ICH guidance document: M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, available at <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM347725.pdf>.

- Type A Post Action meeting was held with Applicant on 5/28/2019 to discuss resubmission (Meeting minutes dated 6/28/2019).
- Type C guidance provided to Applicant regarding extractable leachable plan via written responses dated 10/19/2019.
- The current submission (February 26, 2020) is in response to the Complete Response letter (CRL) dated November 30, 2018. The purpose of this amendment (Resubmission) is to provide a complete response that addresses all deficiencies in the CRL.

2.8 Container Closure System

The container closure system for XARACOLL consists of primary container closure packaging components (see table below), an implant carrier frame (triplet carrier for single blisters), and a secondary packaging pouch.

Primary Container Closure System Components

Component	Description	Regulatory reference	Supplier
Primary container - blister	<u>Dimensions:</u> 63.2 mm × 63.2 mm × 15 mm (outer size) 48–54 mm × 48–54 mm conical (inner size) <u>Material:</u> (b) (4)	21 CFR 177.1315 178.3297 178.3770	(b) (4)
Primary closure - (b) (4)	<u>Dimensions:</u> 289 mm × 289 mm (b) (4) 64.5 mm × 74.0 mm (b) (4) <u>Material:</u> (b) (4)	21 CFR 177.1520	

The following figures show pictures of the primary container closure components.



Although the final XARACOLL drug product is a (b) (4) implantable product, the final collagen/bupivacaine (b) (4)

To justify the safety of the container closure system, the Applicant submitted extractables and leachables studies, which are reviewed in Section 10 of this review.

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3 Studies Submitted

- includes submissions of 10/31/2016, 2/2/2018, and 2/26/2020

3.1 Studies Reviewed

Study Number	Study Title	GLP/nonGLP
INNO-1701	GLP Study to Evaluate the Effects of the Bupivacaine HCl Sponge on Bone Healing in a Rat Femur Osteotomy Model	GLP
S16090	14-Day Tolerability Study of INL-001 in a Sheep Tibial Osteotomy Model	Non-GLP
S16090 TK	14-Day Tolerability Study of INL-001 in a Sheep Tibial Osteotomy Model	Non-GLP
AE32HH.125M012RICH (b) (4)	In Vivo Mammalian Erythrocyte Micronucleus Assay Following Subcutaneous Administration of 0.5% Bupivacaine HCl in Sprague-Dawley Rats	GLP
	Evaluation of Xaracoll Implant Packaging Components: Leachable Compounds Toxicological Assessment	N/A
	Toxicological Safety Qualification of Leachables Above the Safety Concern Threshold Associated with the Xaracoll Bupivacaine HCl Collagen-Matrix Implant	N/A

3.2 Studies Not Reviewed

Study Number	Study Title	GLP/nonGLP
P1748	Quantitation of Bupivacaine in Sheep Plasma via LC-MS/MS Detection	Non-GLP

3.3 Previous Reviews Referenced

Refer to the pharmacology toxicology review completed in the first review cycle by Dr. Bond dated 11/2/2018.

4 Pharmacology

No new pharmacology studies were submitted with this NDA. The pharmacology of bupivacaine is well characterized. According to the reference product Marcaine label:

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. In general, the progression of anesthesia is related to the diameter, myelination, and conduction velocity of affected nerve fibers. Clinically, the order of loss of nerve function is as follows: (1) pain, (2) temperature, (3) touch, (4) proprioception, and (5) skeletal muscle tone.

1.2.1.2 Secondary Pharmacodynamics (Current CR Submission)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No new PK/ADME studies were included with this resubmission.

6 General Toxicology

6.1 Single-Dose Toxicity

No new general toxicology studies were included with this resubmission. During the previous review cycle, a clinical BA study demonstrated that the AUC exposure level to bupivacaine following XARACOLL treatment at the maximum recommended human dose (MRHD) exceeded the listed product Marcaine given at 175 mg by approximately 2X (e.g., AUC_{0-last} (h•ng/mL) of 19493 vs 9708, respectively). The complete response letter dated 11/30/2018 included the following deficiency:

You have not provided an adequate characterization of the systemic safety of bupivacaine exposures via your drug product formulation. Specifically, based on the existing human pharmacokinetic data, your product results in an AUC(0-last) that is twice that of the referenced product. Your existing toxicology data in the rat model do not test exposures that provide coverage for the human exposures via your product.

To address this deficiency:

Conduct adequate toxicology studies in two species that provide adequate coverage for the proposed human exposures via your drug product (AUC and C_{max}) or provide clinical data to support the safety of the proposed exposure.

With this resubmission, rather than conduct general toxicology studies in two species to qualify the safety of systemic exposures to bupivacaine achieved in patients at the (MRHD), the Applicant provided new clinical information to demonstrate that the systemic exposure levels (AUC and C_{max}) to bupivacaine with XARACOLL are within the reference drug Marcaine, which has a MRHD of 400 mg in the label. We defer to the clinical and clinical pharmacology review teams as to the adequacy of the information to

qualify the systemic safety of bupivacaine based on the proposed clinical use.

7 Genetic Toxicology

During the previous review cycle, a full battery of genetic toxicology studies for bupivacaine was submitted. Although the studies demonstrated bupivacaine was negative in the test battery, the high dose tested in the in vivo study did not result in frank toxicity and therefore the study was not considered valid. The complete response letter dated 11/30/2018 included the following deficiency related to the genetic toxicology study:

You have not provided a valid in vivo micronucleus assay for bupivacaine. Specifically, the high dose selected for the assay did not result in frank toxicity.

To address this deficiency:

Repeat the in vivo micronucleus assay for bupivacaine testing doses that result in frank toxicity in accordance with the ICH guidance document: S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, available at <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074931.pdf>.

With this resubmission, the Applicant included a new in vivo micronucleus study (Study AE32HH.125M012RICH (b) (4)). The highest dose tested produced adverse clinical signs, including piloerection, decreased motor activity, hunched posture, prostration, and labored breathing and resulted in lethality, which contributed to the study being considered valid. This adequately addresses the nonclinical deficiency.

Study title: In Vivo Mammalian Erythrocyte Micronucleus Assay Following Subcutaneous Administration of 0.5% Bupivacaine HCl in Sprague-Dawley Rats

Study no:	AE32HH.125M012RICH (b) (4)
Study report location:	EDR Module 4.2.3.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 12, 2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- 0.9% saline, 80-081-JT & 75-204-DK - Bupivacaine HCl Injection (USP, 0.6%), Lot 89-390-DK, 100.8%

Key Study Findings:

- No notable reductions in the PCEs/EC ratio were observed in the test article (bupivacaine HCl) groups compared to the vehicle control group at either time point (24 or 48 hours), indicating the test article did not induce cytotoxicity as there was no significant difference in the group variance ($p > 0.05$) for mean of the micronucleus frequency in the vehicle and test article groups. The positive control, CP, induced a statistically significant increase in the incidence of MnPCEs (Student's t-test, $p \leq 0.05$) relative to vehicle control. The number of MnPCEs in the vehicle control group did not exceed the historical control range.
- While there was a narrow testing dose range (30, 35, & 40 mg/kg), toxicity including clinical symptoms in all groups and lethality in the low and high dose groups were observed making this a valid test.
- Under the conditions of this study, the administration of bupivacaine HCl at doses up to and including a dose of 40 mg/kg was concluded to be negative in the in vivo mammalian erythrocyte micronucleus assay in rats with 24- and 48-hour evaluations.

Methods

Doses in definitive study:

Euthanasia Time (hours after treatment) ^A				24	48
Group	Treatment	Dose Level (mg/kg/dose)	Dose Volume ^B (mL/kg/dose)	Number of Male Animals	
1	Vehicle control	0	8	6	6
2	Bupivacaine HCl ^C	30	6	6	0
3	Bupivacaine HCl ^C	35	7	6	0
4	Bupivacaine HCl ^C	40	8	6+3 ^D	6

^ARange(s): 24-27 hours and 45-48 hours, respectively

^BBased upon individual body weight

^C0.5% Bupivacaine HCl

^DAn additional three animals were added to the high dose group to account for potential mortality.

Frequency of dosing: Single dose. Animals were treated (on Day 1 at t = 0) with the test or control article as described below.

Route of administration: Subcutaneous

Dose volume: See table above

Formulation/Vehicle: 0.9% saline

Species/Strain: Sprague-Dawley (Hsd:SD) rats

Number/Sex/Group: 6

Satellite groups: No

Basis of dose selection: Dose range-finding study

Negative control: 0.9% saline

Positive control: cyclophosphamide

Body Weights and Animal Observation - Body weights were recorded prior to the first dose for the purpose of dose volume calculations and once on the day of euthanasia. Prior to the first day of dose administration, animals were observed once daily for signs

of illness and poor health. Animals were observed for clinical signs of toxicity prior to dose; approximately 1, 2, and 4 hours following each dose administration; at least once daily thereafter.

Bone Marrow Collection and Slide Preparation - Femoral bone marrow was collected at approximately 24 and 48 hours after dose administration, as indicated in the table above.

Scoring - Bone marrow was evaluated by fluorescent microscopy. The staining procedure permitted the differentiation by color of polychromatic and normochromatic erythrocytes (bright orange PCEs and ghost-like, dark green NCEs, respectively).

Micronuclei are brightly stained bodies that generally are round and that generally are between 1/20 and 1/5 the size of the PCE. Scoring was based upon the micronucleated cell, not the micronucleus. Therefore, occasional cells with more than one micronucleus were counted as one micronucleated PCE (MnPCE), not two (or more) micronuclei.

At least 4000 PCEs/animal were scored for the presence of micronuclei (MnPCEs). In addition, at least 500 total erythrocytes (PCEs + NCEs) were scored per animal to determine the proportion of PCEs as an index of bone marrow cytotoxicity.

Study Validity

The group mean frequency of MnPCEs for the vehicle control group should ideally be within the 95% control limits of the distribution of the historical negative control database. If the concurrent negative control data fall outside the 95% control limits, they may be acceptable as long as these data are not extreme outliers (indicative of experimental or human error).

The frequency of MnPCEs for the scoring positive controls must be significantly greater than the concurrent vehicle control ($p \leq 0.05$) and should be compatible with those observed in the historical positive control data base.

At least three doses were tested for at least one sampling time. Up to six animals/group were available for analysis.

The maximum dose evaluated for micronucleus induction must:

- a. Be the MTD or MFD, or
- b. Demonstrate cytotoxicity in the bone marrow (reduction in the PCE/NCE ratio) of more than 50% but not less than 20% of the control value), or
- c. In the absence of cytotoxicity or MFD, a dose (limit dose) was used

Evaluation of Test Results

A test article was considered to have induced a positive response if

- a. At least one of the test article doses exhibits a statistically significant increase when compared with the concurrent negative control ($p \leq 0.05$), and

- b. When multiple doses are examined at a particular sampling time, the increase is dose-related ($p \leq 0.01$ and $R^2 \geq 70\%$), and
- c. Results of the group mean in at least one group is outside the 95% control limit of the historical negative control data.

A test article was considered to have induced a clear negative response if none of the criteria for a positive response were met and there was evidence that the bone marrow was exposed to the test article (unless intravenous administration was used).

Results

Clinical Signs - Due to the mortality and observations seen during the dose range-finding assay (males and females), a dose level considered slightly above the maximum tolerated dose for the definitive micronucleus assay was set at 40 mg/kg. The definitive phase was conducted in male rats only due to an apparent absence in sex-related toxicity in the dose range-finding phase. The following clinical signs were observed in the definitive study:

Dose Level	Males
0 mg/kg/dose (vehicle control)	Piloerection
30 mg/kg/dose bupivacaine HCl	Piloerection
35 mg/kg/dose bupivacaine HCl	Piloerection
40 mg/kg/dose bupivacaine HCl	Piloerection, decreased motor activity, hunched posture, prostrate, & labored breathing

Mortality – Mortality was observed in the low and high dose groups.

Dose Level	Males
30 mg/kg/dose bupivacaine HCl	1/6
35 mg/kg/dose bupivacaine HCl	0/6
40 mg/kg/dose bupivacaine HCl	3*/15

*Animal 475 was observed to have convulsions prior to declaring death.

Body Weights – Body weights were only measured prior to dosing.

Bone Marrow Analysis

The incidence of polychromatic erythrocytes (PCEs) containing micronuclei (MnPCEs) per 24,000 PCEs scored (4000 PCEs/animal) and the proportion of PCEs per total erythrocytes are summarized and presented by treatment group and by sacrifice time in the following table.

Summary of Bone Marrow Micronucleus Analysis

Treatment	Gender	Time (Hrs)	Animals	%PCE (Mean +/- SD)	Toxicity (%)	% MnPCE (Mean +/- SD)	Number of MnPCE/PCE Scored
Vehicle							
0 mg/kg/dose	M	24	6	54.6 ± 2.1	---	0.10 ± 0.04	23 /24000
Bupivacaine HCl							
30 mg/kg/dose	M	24	5	53.4 ± 4.3	-2	0.10 ± 0.04	19 /20000
35 mg/kg/dose	M	24	6	52.5 ± 1.8	-4	0.13 ± 0.04	30 /24000
40 mg/kg/dose	M	24	6	54.8 ± 3.5	0	0.08 ± 0.02	19 /24000
CP							
40 mg/kg/dose	M	24	5	47.1 ± 3.6**	-14	2.37 ± 0.56**	474 /20000
Vehicle							
0 mg/kg/dose	M	48	6	51.9 ± 3.9	---	0.09 ± 0.05	20 /24000
Bupivacaine HCl							
40 mg/kg/dose	M	48	6	52.0 ± 1.5	0	0.10 ± 0.04	23 /24000

**p < 0.01, One-Way ANOVA with Post-Hoc Dunnett's Test or T-Test
 24 Hrs MnPCE Male GLM P-value = 0.282, R-sqr = 17.80%
 Vehicle = 0.9% saline for injection

The scoring results and a statistical analysis of data indicated the following:

- No appreciable reductions in the PCEs/EC ratio were observed in the test article (bupivacaine HCl) groups compared to the vehicle control group at either time point (24 or 48 hours), indicating the test article did not induce cytotoxicity.
- There was no significant difference in the group variance ($p > 0.05$) for mean of the micronucleus frequency in the vehicle and test article groups.
- No statistically significant increase in the incidence of MnPCEs was observed in the test article (bupivacaine HCl) treated groups relative to the vehicle control group at either time point (24 or 48 hours) ($p > 0.05$).
- The positive control, CP, induced a statistically significant increase in the incidence of MnPCEs (Student's t-test, $p \leq 0.05$) relative to vehicle control.
- The number of MnPCEs in the vehicle control group did not exceed the historical control range.

Conclusion

Prior to the conduct of the definitive micronucleus study, a dose range-finding micronucleus study demonstrated that a dose level of 40 mg/kg bupivacaine hydrochloride injection, USP 0.5% (bupivacaine HCl) was frankly toxic to rats as evidenced by adverse clinical observations and mortality. Under the conditions of this study, the administration of bupivacaine HCl at doses up to and including a dose of 40 mg/kg was concluded to be negative in the in vivo mammalian erythrocyte micronucleus assay in rats.

Dose Formulation Analysis

The different dose levels necessary for this study were achieved based on administration of differing volumes of the undiluted test article. Consequently, since no study specific formulations were prepared, the assessment of achieved concentration and homogeneity (formulations analysis) was not required in this study.

8 Carcinogenicity

No carcinogenicity studies were conducted or required for this single dose drug product.

9 Reproductive and Developmental Toxicology

No new reproductive and developmental toxicology studies were included with this resubmission and none were required for the NDA. During the previous review cycle, the label was updated to be in accordance with the Pregnancy Lactation and Labeling Rule (PLLR). The Applicant provided a literature review, which was evaluated by this reviewer, and we agreed that no new information based on the published literature should be added to labeling. Refer to the NDA 209511 nonclinical review dated 11/5/2018 for more information.

10 Special Toxicology Studies

10.1 Osteotomy Studies

Study Title: 14-Day Tolerability Study of INL-001 in a Sheep Tibial Osteotomy Model

Study no.: S16090
Study report location: SDN 44; 2/26/2020; ECTD 4.2.1.2; [inno-1701-report](#)
Study initiation date: November 15, 2018
Conducting laboratory and location: (b) (4)
Duration: Single dose 14 days
Duration Units: days
GLP compliance: N
Drug, lot #, and % purity:

- Placebo Collagen matrix, Batch 18030907
- Reference article - Bupivacaine HCl, 7.5 mg/mL, Batch CBU180097
- Reference article - INL-001 [bupivacaine hydrochloride (HCl) collagen-matrix implants], Batch 17011108, 100 mg bupivacaine HCl, 5 cm x 5 cm x 0.5 cm collagen matrix/white sponges

Target Organ⁺: BONE

Key Findings:

The objectives of this pilot study were to evaluate an implantation approach and tolerability of INL-001 [bupivacaine hydrochloride (HCl) collagen matrix implants] in a range of doses in a sheep osteotomy model for a 14-day period following surgery and provide data to support dose selection for a subsequent study. Additionally, the toxicokinetics (TK) of INL-001 were evaluated.

On Dosing Day 1, fourteen female animals underwent surgical procedures to create an osteotomy defect, stabilized by a neutralization plate, and were administered liquid bupivacaine HCl (2 mg/kg; Group 1) or implanted with three placebo collagen-matrices (Group 2), or one (Group 3), two (Group 4), or three (Group 5) collagen matrices of INL-001, each containing 100 mg of bupivacaine HCl. The group sizes were two sheep for the bupivacaine liquid (Group 1) and three sheep for all other treatment groups (Groups 2-5). Blood samples for bupivacaine toxicokinetic analysis were collected from all study animals through 120 hours post-surgery.

All animals recovered from surgery, but three of the 14 animals (one animal each in Groups 2, 3, and 4) were euthanized at an unscheduled time point (Dosing Day 3, 5, or 14). These three unscheduled deaths were undertaken for animal welfare due to non-weight bearing on the contralateral (non-surgical) hind limb and were not considered test article related. The remaining 11 animals did successfully recover and were observed through 14-days post-surgery.

There were no effects on body weights or food consumption across all treatment groups post-surgery. For animals surviving to scheduled euthanasia, there were no drug-related clinical findings with the only post-operative clinical findings consisting of limited weight bearing of the surgical limb (lameness) that resolved between 10 to 14 days in all treatment groups. There were no collagen-matrix or INL-001-related clinical findings.

For all animals, the surgical site demonstrated healing with no observable differences between treatment groups. Several animals that received collagen-matrix implants (Groups 2-5) were observed with red fibrous material in the incision area and/or along the neutralization plate and fluid was noted in some animals in the incision area.

As it was not possible to generate group mean bupivacaine half-life data due to lack of measurable concentrations for some animals across the bupivacaine-treated groups, individual bupivacaine half-life data were evaluated. As a result, the individual animal bupivacaine half-life was determined to be 6.55 hours for one Group 1 animal receiving liquid bupivacaine at 2 mg/kg. For Groups 3 to 5, individual animal bupivacaine half-life was independent of dose with values between 7.51 and 17.5 hours. The bupivacaine exposure-INL-001 dose relationship in this study was not linear. Individual animals showed variability in exposure and clearance values.

In summary, this pilot study demonstrated the feasibility of the implantation approach for INL-001 in the sheep osteotomy model and the acceptable tolerability of INL-001 [up to three matrix implants] during a 14-day post-surgical recovery period for up to 3 x 100 mg bupivacaine sponges. For animals with measurable bupivacaine levels, the bupivacaine half-life appeared independent of dose and ranged between 7.51 to 17.5 hours. The bupivacaine exposure-INL-001 dose relationship in this study was not linear; however, this was in the context of a small number of animals per group.

Methods

Doses:

Group	Treatment	Number of Animals	Dose	Number of Collagen Matrices Implanted
1	Reference (Liquid Bupivacaine)	2 Female	Bupivacaine HCl (0.75%) at 2 mg/kg	Not Applicable
2	Collagen (Placebo Collagen-Matrix)	3 Female	0 mg	3
3	Test Article (Low Dose)-INL-001	3 female	100 mg bupivacaine HCl	1
4	Test Article (Mid Dose)-INL-001	3 Female	200 mg bupivacaine HCl	2
5	Test Article (High Dose)-INL-001	3 Female	300 mg bupivacaine HCl	3

Notes: Prior to surgical procedures on designated study animals, a practice surgery procedure was performed on one animal to refine technique. Each collagen matrix was cut-down to ~2.5 cm x 2.5 cm x 0.5 cm to allow for equal implant on each side of the osteotomy site. The amount of bupivacaine HCl in each collagen matrix was 0 mg for the placebo article and 100 mg for the test article groups.

Frequency of dosing: Single implantation time
 Number/Sex/Group: See table
 Dose volume: 7.5 mg/mL for reference Bupivacaine HCl, NA for sponge/sponges

Formulation/Vehicle: Water for Bupivacaine HCl for reference group
Route of administration: UNASSIGNED
Species: SHEEP
Strain: Dorset Cross Sheep
Age / Sexual Maturity: Female - Estimated 4.7 to 5.8 years on Dosing Day 1
Comment on Study Design and Conduct: The dose levels of the test article were selected by the Sponsor and provided an equivalent range of implants that may be used in humans (one to three INL-001) and were administered in a dose-escalation paradigm to ensure tolerability before proceeding to the next higher level.

The tibial osteotomy model in sheep is a common surgical model to evaluate effects of drug-device test articles on bone healing since the size of the animal more closely approximates humans.

A curved incision was made over the medial aspect of the tibia. An osteotomy of the bone was made at its midsection and treatment and closure applied, including bone plate.

All animals successfully underwent and initially-recovered from the surgical procedures. The test, control, and reference material was administered/implanted to the osteotomy site of all animals on Dosing Day 1 only.

Dosing Solution Analysis: None

Observations and Results

Mortality

In-cage observations were made at least twice daily (AM and PM) during the study period to assess mortality.

No drug product-related mortality occurred.

Three animals were euthanized at an unscheduled event (Dosing Day 3, 5, or 14). These animals were euthanized due to non-weight bearing on the contralateral (non-surgical) hind limb. The finding occurred in one animal each in Groups 2, 3, and 4, and was not attributed to control or test article. Instead, these observations were likely limitations of the surgical and/or recovery procedures employed in this study. The

remaining 11 animals did successfully recover and were observed through 14-days post-surgery.

Clinical Signs

In-cage observations were made at least twice daily (AM and PM) during the study period to assess general health and morbidity. Detailed clinical observations were performed prior to surgery and daily through study termination. Clinical observations were also performed at target times of 1, 2, 4, 8, and 12 hours post-surgical procedures to monitor tolerability of the test implant(s). The animals were observed for clinical signs of illness or reaction to test article administration.

Clinical observations included minimal to mild decreased weight bearing, toe touching, laying down, swelling at the dose site, and/or nasal discharge. These findings were observed in animals from all groups beginning post-surgery. Findings of decreased weight bearing for surgical limb continued through Dosing Days 10 to 14 for animals in all treatment groups that completed the study.

Body Weights

During the study, each animal was weighed weekly, including the day of scheduled termination.

There were no treatment-related body weight effects due to bupivacaine HCl, placebo-collagen matrix, or INL-001. As expected, all animals lost a slight to moderate amount of body weight between Acclimation Day 12 and Dosing Day 1 due to the fasted state for surgical procedures. At Dosing Days 8 and 15, all available animals were observed to maintain or gain a slight amount of body weight. These changes in body weight were similar between all treatment groups.

Group Summary Body Weight Results (kg)

Gender		Female				
Group		1	2	3	4	5
Dose		Reference (Liquid Bupivacaine)	Control (Placebo Collagen- matrix)	Low Dose (100 mg bupivacaine HC1)	Mid Dose (200 mg bupivacaine HC1)	High Dose (300 mg bupivacaine HC1)
N		2	3	3	3	3
Acclimation Day 2	Mean	57.5	64.0	65.3	59.3	67.3
	SD	23.3	6.2	2.1	13.0	14.3
Acclimation Day 9	Mean	73.5	71.3	74.0	69.7	73.0
	SD	3.5	7.0	5.6	4.9	12.5
Acclimation Day 12	Mean	72.0	68.7	72.0	72.0	74.3
	SD	2.8	8.6	8.2	2.6	11.0
Dosing Day 1	Mean	67.5	67.0	69.0	67.0	67.3
	SD	2.1	7.2	8.2	4.6	11.0
Dosing Day 8	Mean	69.5	61.0	67.0	68.5	69.0
	SD	0.7	5.7	5.0	6.4	12.2
Dosing Day 15	Mean	70.0	59.5	68.0	65.5	69.0
	SD	1.4	4.9	5.0	6.4	10.4

Food Consumption

Daily food availability was monitored to ensure each animal consumed an expected amount of food, specifically following surgery.

On Dosing Day 3, all animals in Group 2 (collagen control) were observed with decreased appetite. On Dosing Days 4 and 5, one Group 4 (mid dose drug product – 2 sponges) animal was observed with no appetite; however, the animal was observed with a normal appetite by Dosing Day 6. All other animals were observed with expected food consumption during the study.

Individual Animal Food Consumption

Study ID	Animal ID	Dosing Day	Food Consumption Exception
2F1	5866	3	An attempt was made for grain, the grain remaining approx. 1200 g
2F1	5866	3	Approx. 1200g feed remaining in trough 30 minutes after offering
2F2	5870	3	An attempt was made for grain, the grain remaining approx. 1200 g
2F2	5870	3	Approx. 1200g feed remaining in trough 30 minutes after offering
2F3	5979	3	An attempt was made for grain, the grain remaining approx. 1200 g
2F3	5979	3	Approx. 1200g feed remaining in trough 30 minutes after offering
4F1	0962	4	Animal observed laying down under heat lamp while pen mate ate grain. An attempt was made to get animal to eat grain, but animal remained uninterested.
4F1	0962	5	Did not consume expected amount

Ophthalmoscopy – None.

ECG – None.

Clinical Pathology (Hematology, Clinical Chemistry, Urinalysis)

These endpoints were not evaluated following treatment. However, during acclimation, blood samples were collected for clinical pathology evaluation from non-fasted animals via direct venipuncture of the jugular vein to determine acceptability of animals for study inclusion. Evaluation of clinical pathology parameters prior to treatment indicated that all animals were in good general health for study inclusion (as stated in the report; however, the data was not reported.).

Gross Pathology

All animals were euthanized at the designated time point (Dosing Day 15) except for three animals which were euthanized early.

Euthanasia events following recovery from surgical procedures generally due to non-weight bearing on non-surgical leg which were not considered treatment related:

- Animal Placebo Collagen-Matrix, 0 mg bupivacaine (Day 3)
- Animal INL-001 [low-dose], 100 mg bupivacaine HCl (Day 5)
- Animal INL-001 [mid-dose], 200 mg bupivacaine (Day 14)

Organ Weights – None.

Histopathology

Adequate Battery: Not for a full toxicology study. Tissues evaluated were limited to the surgical site.

Peer Review: N/A

Special Evaluation

Following euthanasia of each animal, at least one radiograph of the surgical site was performed. For all animals at unscheduled and scheduled termination events, the closed surgical site was assessed for healing and then was opened for visual-assessment by an SRC veterinarian. The entire tibia of the surgical limb with plate and screws intact were collected, fixed in 10% neutral buffered formalin (NBF), and stored for possible future evaluation.

The surgical site was determined by a veterinarian to be healing, with no observable differences between treatment groups. Upon visual inspection of the opened surgical site, several animals evaluated at the scheduled necropsy in Groups 2-5 (8 of 12) and implanted with placebo collagen matrices or INL-001, were observed with red fibrous material in the incision area and/or along the neutralization plate. Fluid was also observed in the incision area for some of these animals. For one Group 5 animal (300 mg bupivacaine – 3 sponges), the tissue was observed to be less adhered to the bone than in other animals.

Study ID	Animal ID	Study Day	Necropsy Findings
1F1	5873	15	Plate and screws appear intact
1F2	5889	15	Plate and screws appear intact
2F1	5866	15	Leg and incision are healing normally. Plate looks good. No fluid or extra red material in incision
2F2	5870	5	Unscheduled euthanasia: Lower section of plate had broken off bone. Found one screw that was broken in half. Distal end of incision opened at necropsy.
2F3	5979	15	Leg and incision are healing normally. Very small amount of red fibrous material in cranial incision
3F1	0968	15	Leg and incision appear to be healing normally. Small amount of red shiny material seen over plate
3F2	0970	15	Leg and incision appear to be healing normally. Small amount of red shiny material seen over plate
3F3	5683	14	Unscheduled euthanasia: Soft tissues were warm and slightly swollen as compared to controls. Small amount of clear fluid in soft tissues. Soft red material found over the plate and bone. Plate and screws appear intact
4F1	0962	15	Leg appears to be healing well. Slight gap in cranial incision that may have test material adhered in incision. Red tissue and fibrous material in cranial incision
4F2	5871	15	Leg appeared to be healing well. Red fluid found all along length of plate. Red fibrous material also present all along length of plate
4F3	5974	3	Unscheduled euthanasia: Radiograph of surgical limb was performed, no visible observation of remaining test article devices; the plate and screws were removed from the surgical limb
5F1	5456	15	Leg and incision appear to be healing well. Red fibrous material and some fluid primarily in cranial incision
5F2	5688	15	Leg and incision appear to be healing well. Red fibrous material and small amount of fluid along length of plate but mostly in cranial incision
5F3	5986	15	Leg and incision appear to be healing well. More dried discharge evident on skin. Plate looks good but very little red fibrous material seen. Tissue is less adhered to bone than other animals

Toxicokinetics

On Dosing Days 1 through 6, toxicokinetic samples were collected for determination of bupivacaine concentrations from all study animals.

Number of Animals	Target Time Points
14	Pre-dose, 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 hours post administration

It was not possible to generate group mean bupivacaine half-life data due to lack of measurable concentrations for some animals across the bupivacaine-treated groups. However, individual bupivacaine half-life data were evaluated. The bupivacaine half-life after administration of the reference article (bupivacaine at 2 mg/kg) around the surgical site in the osteotomy model was 6.55 hours in one animal.

Individual animal bupivacaine half-life after administration of INL-001 was independent of dose (one to three implants; 100 to 300 mg of bupivacaine HCl) with individual half-

life values ranging from 7.51 to 17.5 hours.

The bupivacaine exposure-INL-001 dose relationship in this study was not linear, however, this was in the context of low number of animals per group.

Individual animals showed variability in exposure and clearance values.

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Study 2 (GLP)

Study Title: GLP Study to Evaluate the Effects of the Bupivacaine HCl Sponge on Bone Healing in a Rat Femur Osteotomy Model

Study no.:	INNO-1701
Study report location:	SDN 44; 2/26/2020; ECTD 4.2.1.2; Application 209511 - Sequence 0043 - Study Report
Study initiation date:	September 22, 2017
Conducting laboratory and location:	 (b) (4)
Duration:	28 and 56 days
Duration Units:	days
GLP compliance:	Y
Drug, lot #, and % purity:	- 100 mg Bupivacaine collagen-matrix implant, Lot Number 15041002 - Placebo collagen-matrix implant Lot Number: 15020502 Sterile Saline, Lot Numbers: B1609032, B1612171 Sterile Saline Lot Numbers: B1609032, B1612171 Bupivacaine HCl (0.75%), Lot Number: LBU170022
Target Organ ⁺ :	BONE

Key Findings:

Key findings from the GLP rat bone healing study that compared saline, bupivacaine HCl (4 mg), and two doses of the bupivacaine collagen sponge (4 mg and 8 mg) are as follows:

1. Bupivacaine collage-matrix implant groups tended to have lower torsional strength than control groups (saline, placebo collagen matrix, bupivacaine HCl); however, the effect was not statistically significant.

2. Bupivacaine collagen-matrix implant groups had statistically significantly less healing compared to than control groups (saline, placebo collagen matrix, bupivacaine HCl) based on radiography with males demonstrating a difference earlier than females.
3. MicroCT data demonstrated significantly less bone within the defect in animals treated with bupivacaine collagen-matrix implant compared to saline, placebo collagen matrix, and bupivacaine alone with males demonstrating a difference earlier than females.
4. Histopathologic evaluation of bone indicated that the Bupivacaine collagen-matrix implant animals demonstrated inhibition of bone healing compared to controls.
5. Collectively, the results suggest that the bupivacaine collagen-matrix implant inhibited bone healing following osteotomy in this rat model compared to controls, including standard of care bupivacaine alone. The clinical significance of these findings is not clear.

Methods

Doses:

Group	#/sex	Surgery Day	Treatment	Bupivacaine HCl Dose	Volume of solution or collagen-matrix implant dimension	Necropsy
1	24/M 24/F	0	Saline	NA	0.53 ml	12M/12F rats/time point on Days 28 ± 3, and 56 ± 3 as below: (6M/6F for radiographs, ex-vivo CT and histology of femurs 6M/6F for torsional testing of femurs)
2	24/M 24/F		Bupivacaine HCl	4 mg	0.53 ml	
3	24/M 24/F		Placebo collagen-matrix implant	NA	1x2 cm	
4	24/M 24/F		Bupivacaine collagen-matrix implant (low dose)	4 mg	1x1 cm	
5	24/M 24/F		Bupivacaine collagen-matrix implant (high dose)	8 mg	1x2 cm	

Frequency of dosing: Single dose

Number/Sex/Group: 24

Dose volume: See table

Formulation/Vehicle: See table

Route of administration: UNASSIGNED

Species: RAT

Strain: SPRAGUE-DAWLEY

Age / Sexual Maturity: Males 10 weeks old at shipping, females 15 weeks old at shipping

Comment on Study Design and Conduct: The objective of this study was to evaluate the potential effect of the test material, Bupivacaine collagen-matrix implant, on bone healing in a rat **osteotomy bone** model based on microCT parameters, radiography, histopathology, and torsional testing. Male and female rats had a

PEEK (polyetheretherketone) plate secured to the lateral aspect of the left femur followed by a mid-shaft osteotomy. The in-life portion of this study was approximately 85 days in duration.

Dosing Solution Analysis: Test article use was documented by the Testing Facility.

Biomarker⁺

Biomarker Evaluated: Other
If "Other" was selected: Bone osteotomy with torsional testing, microCT and radiography to evaluate bone strength and healing
Purpose of Biomarker: Safety

Observations and Results**Mortality**

Observations were started at least 7 days prior to surgery. On the day of surgery, Day 0, rats were observed prior to surgery and during recovery. Rats were then observed twice daily for at least 3 days after surgery and then at least once daily for the duration of the study. The last clinical observation was in the morning prior to necropsy.

Clinical Signs

Clinical observations were started at least 7 days prior to surgery. On the day of surgery, Day 0, rats were observed prior to surgery and during recovery. Rats were then observed twice daily for at least 3 days after surgery and then at least once daily for the duration of the study. The last clinical observation was in the morning prior to necropsy. Assessments included but were not limited to activity, posture, respiration, hydration status, and overall body condition.

Each rat had a normal physical exam during acclimation.

Body Weights

Body weights were collected for randomization and then weekly for the duration of the study.

No statistically significant differences were observed in all males and females between treatment groups at either the Day 28 or Day 56 time points.

Food Consumption

Food consumption was monitored but not measured.

Ophthalmoscopy, ECG, Hematology, Clinical Chemistry, and Urinalysis – None.

Gross Pathology

Animals were euthanized at the designated study end points of Day 28 $\pm$ 4 days or Day 56 $\pm$ 4 days unless otherwise noted.

- After euthanasia, femurs from six pre-designated male and six pre-designated female rats from each group had their left femur (osteotomy side) harvested and stripped of soft tissue. The femurs were then wrapped in saline-soaked gauze, placed in a bag or suitable container, and stored at -20°C. These femurs were shipped frozen at the end of the necropsy for torsional testing.

Organ Weights – None.

Necropsy Results

As shown in the Table, the most frequent observations were hematomas associated with the processing and evaluation in the Day 28 groups across all treatment groups. Hematomas were observed in 16 of 56 animals in the females and 8 of 60 males. By the Day 56 time point, the numbers of hematomas had declined to 6 of 58 females. No hematomas were observed in the Day 56 male groups. Hematomas were observed across all treatment groups and were not considered related to treatment.

Table: Incidence of Hematomas*

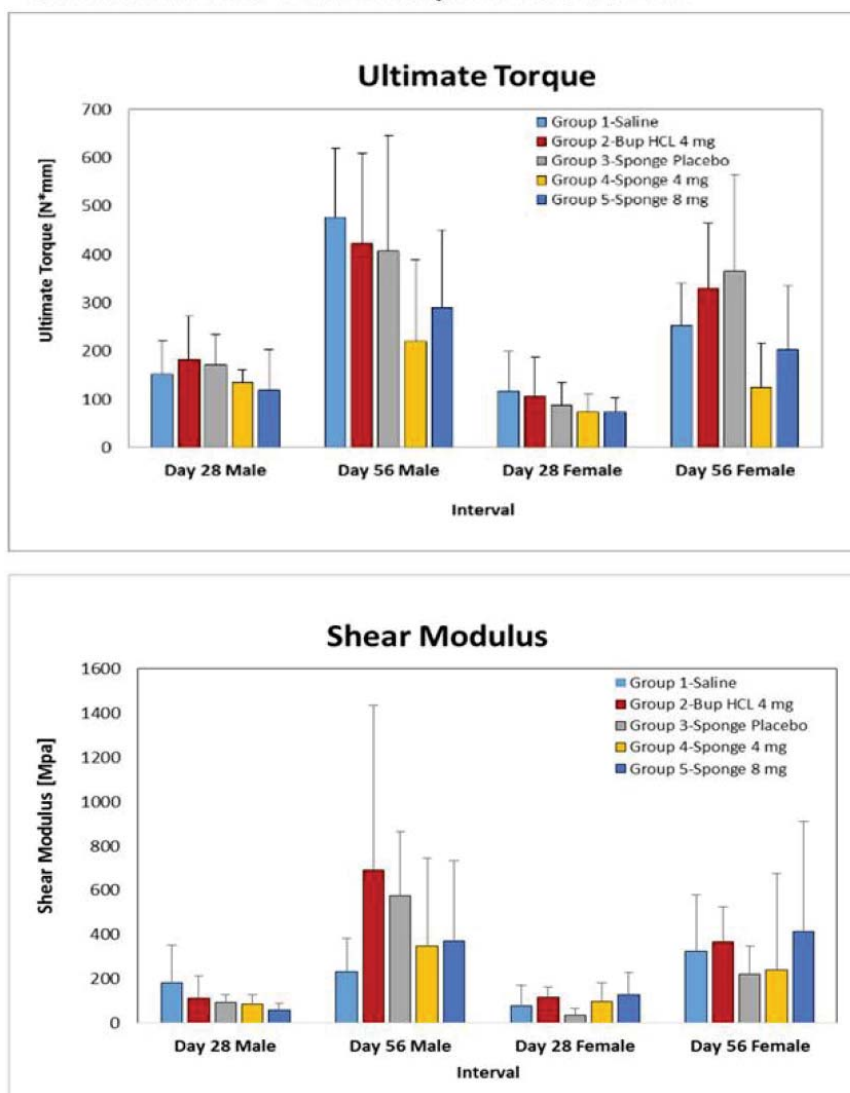
Sex	Day	Treatment				
		Saline	Bupivacaine HCl	Placebo collagen-matrix implant	Bupivacaine collagen-matrix implant 4 mg	Bupivacaine collagen-matrix implant 8 mg
Female	28	4	4	4	1	3
Male	28	1	1	2	2	2
Female	56	1	3	0	2	0
Male	56	0	0	0	0	0

*Number of animals with a hematoma at necropsy is recorded.

In addition to hematomas, infections at the surgical site, that had not been evident during the in-life portion of study, were observed. These infections were infrequent and only observed at the Day 56 time point.

Torsional Testing Results - Standard deviations were large; however, the 4 mg and 8 mg Bupivacaine collagen-matrix implant groups trended lower in torsional strength than the controls (Groups 1-3) in both the male and female groups at both study intervals. Statistical analysis of the ultimate torque and shear modulus results did not reveal any statistically significant differences between treatment groups in males or females at either Day 28 or 56. Graphical depiction of the results are presented below from the Appendix of the study report.

5. GRAPHICAL SUMMARY OF ULTIMATE TORQUE AND SHEAR MODULUS



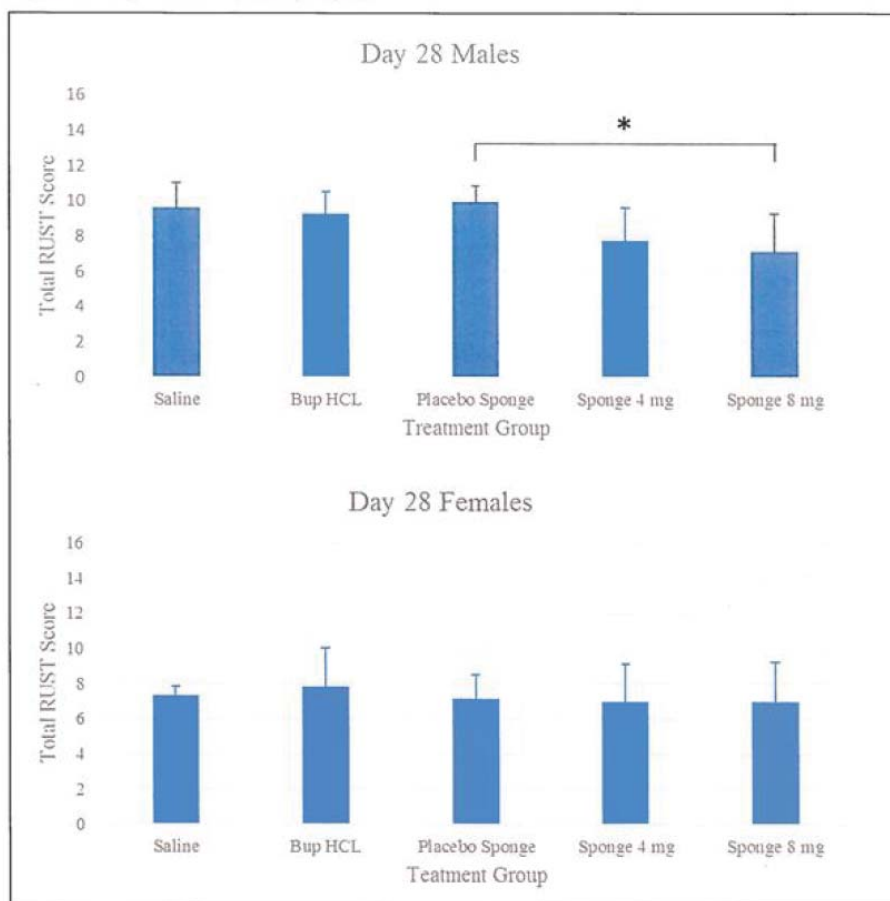
Radiography Results - The Bupivacaine collagen-matrix implant 8 mg in males and 4 and 8 mg in females inhibited bone healing in both male and female rats when assessed radiographically at Day 56 in this model. The images were score using the

Radiographic Union Scale for Tibial Fractures (RUST) as described in the table below, reproduced from the Appendix of the report:

Table 1. Radiographic Union Scale for RUST evaluation

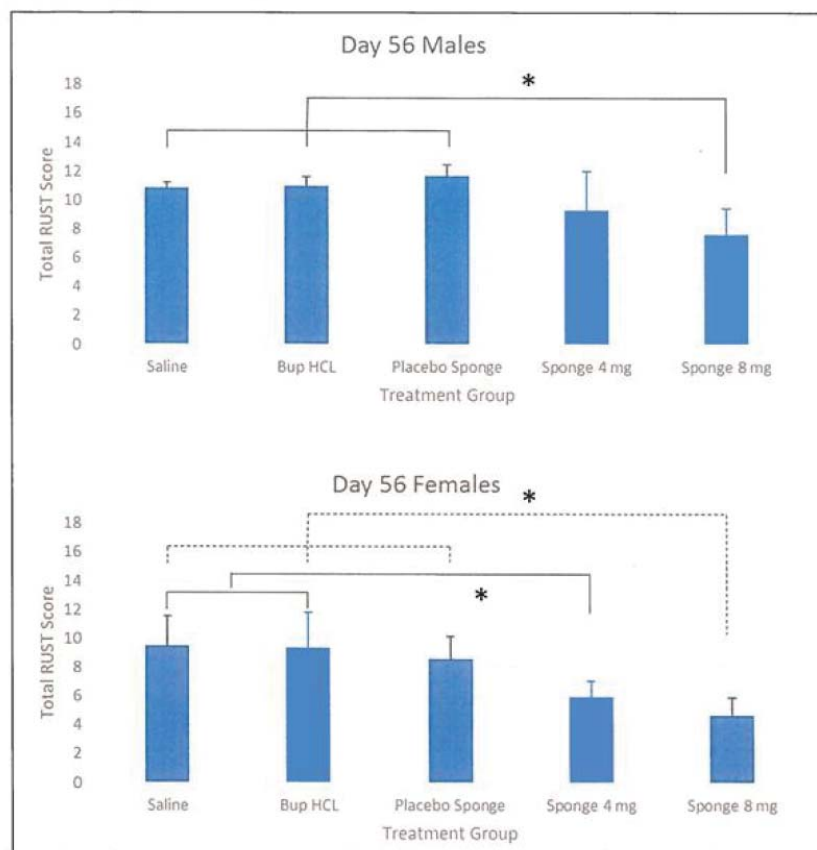
Score per cortex	Callus	Fracture Line
1	Absent	Visible
2	Present	Visible
3	Present	Invisible

Figure 6. Day 28 RUST Scoring Results



The Day 28 RUST scores. RUST scores are shown as mean with standard deviations. The 8 mg XARACOLL test sponge was lower than the Placebo Sponge in the Males at Day 28 (p value = 0.0394). No differences were observed between treatment groups in the Day 28 Females. * = statistical significance by Tukey-Kramer pairwise multiple comparison test of mean.

Figure 7. Day 56 Rust Scoring Results

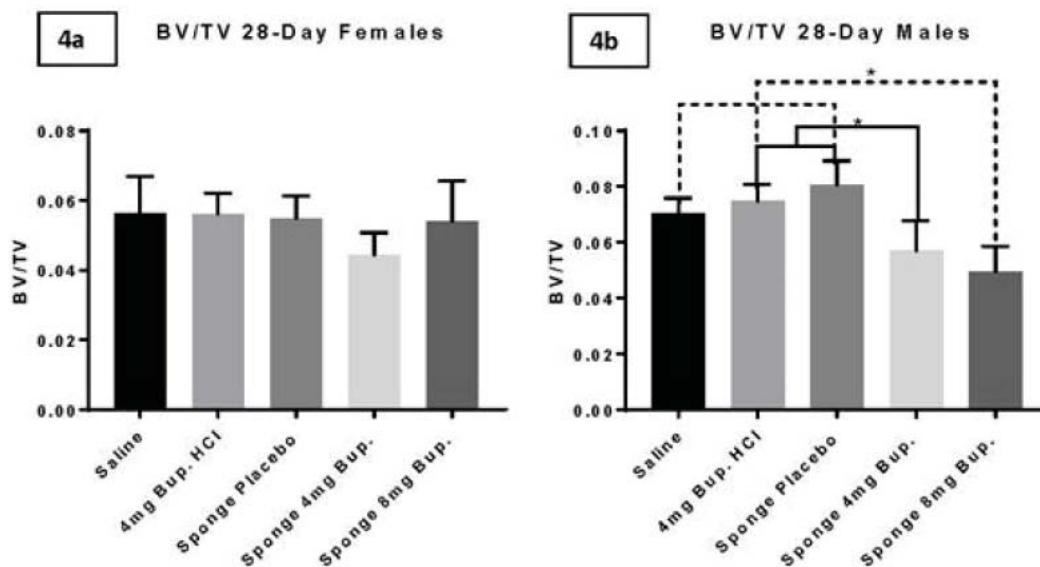


Day 56 RUST scores. RUST scores are shown as mean with standard deviations. In the Males at Day 56, the 8 mg XARACOLL test sponge was significantly lower than Saline ($p=0.0100$), BUP HCL ($p=0.0063$), and Placebo Sponge ($p=0.0009$) treatments. In the Females at Day 56, the 4 mg XARACOLL test sponge was significantly lower than Saline ($p=0.0135$) and BUP HCL ($p=0.0244$) treatments. In addition, the 8 mg XARACOLL test sponge was significantly lower than Saline ($p=0.0005$), BUP HCL ($p=0.0012$), and Placebo Sponge ($p=0.0074$). * = statistical significance by Tukey-Kramer pairwise multiple comparison test of mean.

MicroCT - The general trend that osteotomies in contact with the 4 mg and 8 mg Bupivacaine collagen-matrix implant had less bone volume and a lower polar moment of inertia (PMOI) than the saline, 4 mg bupivacaine HCl, or placebo collagen-matrix implant treated osteotomies. This was most consistent for the 8 mg Bupivacaine collagen-matrix implant groups for both males and females at 56 days. However, the 4 mg Bupivacaine collagen-matrix implant group also had significantly less BV/TV for males at 28 days and females at 56 days and significantly lower PMOI in females at 56 days. Based upon evidence that lower bone volume fraction is associated with diminished bone strength, significantly less robust healing was observed and therefore the fracture site was weaker as measured by microCT parameters in rats where the osteotomy was in contact with the 8 mg Bupivacaine collagen-matrix implant. The summary of the findings are depicted in the figure below, reproduced from the Appendix of the study report:

BV/TV Outcome Measure

Bone Volume over Total Volume evaluates the ratio of the bone measured within a given volume divided by the total volume. The scans were performed to ensure that the total volume was consistent between samples. The three-dimensional renderings are organized by treatment group, gender, and time point of euthanasia and can be found in Appendix 3. The mean BV/TV was compared between the 5 treatment groups (saline, 4mg Bupivacaine HCl, placebo sponge, 4mg Bupivacaine sponge, and 8mg Bupivacaine sponge) at 28 and 56 days post-surgery and subdivided by gender. There was no statistical difference between treatment groups for the 28 day females (**Figure 4a**). For the 28-day males, the 8mg Bupivacaine sponge had less bone within the defect volume than saline, 4mg Bupivacaine HCl and the placebo sponge groups (**Figure 4b**). The 4mg Bupivacaine sponge group had less bone than the 4mg Bupivacaine HCl and placebo sponge groups (**Figure 4b**). At day 56, the females which received either the 4mg or 8mg Bupivacaine sponge had significantly less bone volume/total volume than the saline, 4mg Bupivacaine HCl, and the placebo sponge groups (**Figure 4c**). At day 56, the males that received an 8mg Bupivacaine sponge had significantly less bone volume/total volume than the saline, 4mg Bupivacaine HCl, and the placebo sponge groups (**Figure 4d**).



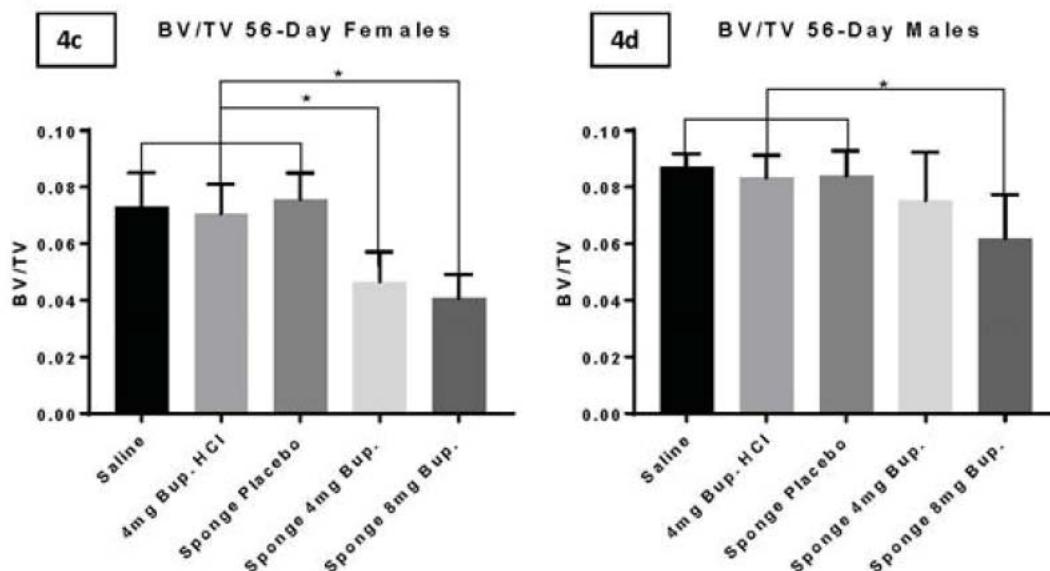


Figure 4 (a-d): Mean BV/TV. 4a: 28-Day Females BV/TV. There were no statistical differences between treatment groups ($p > 0.05$). 4b: 28-Day Males BV/TV. The 8mg Bupivacaine sponge treatment had significantly less bone formation than the saline, 4mg Bupivacaine HCl and placebo sponge groups ($* = p < 0.05$). The 4mg Bupivacaine sponge treatment had significantly less bone formation than the 4mg Bupivacaine HCl and the placebo sponge ($* = p < 0.05$). 4c: 56-Day Females BV/TV: Both Bupivacaine sponge treatment groups had significantly less bone formation than the saline, 4mg Bupivacaine HCl and the placebo sponge treatment groups ($* = p < 0.05$). 4d: 56-Day Males BV/TV: The 8mg Bupivacaine sponge treatment group had significantly less bone formation than the saline, 4mg Bupivacaine HCl and the placebo sponge groups ($* = p < 0.05$).

Histopathology

Adequate Battery: Not for a full toxicology study. The examination was limited to bone.

Peer Review: No.

- Femurs from the remaining six male and six female rats in each treatment group were subjected to digital radiography after euthanasia and prior to disarticulation of the left femur. This was performed under GLP compliance. After radiographs were taken, the left femurs were collected leaving tissues intact near the osteotomy site. Muscle and other tissues at both ends of the femur were removed, and the proximal end of the femur was removed to allow for fixative to penetrate the medullary cavity. The femurs were then immersion fixed in 10% neutral buffered formalin (NBF).
- After digital radiography, the formalin-fixed femurs were sent for MicroCT imaging. Bone volume, total volume, the ratio of bone volume to total volume, polar moment of inertia, and cortical thickness were measured.
- Femurs were radiographed, trimmed, processed, and embedded with the PEEK plate intact and the tissues overlying the osteotomy site intact. Radiography of all samples was performed to optimize sectioning.

Results were evaluated semi-quantitatively using the following scoring system:

Parameter		Score				
		0	1	2	3	4
Fracture Repair Response	Callus Formation	None	Mild (<50%)	Moderate (>50%)	Full bony callus across defect	Not Applicable (NA)
	Bone Union	No new bone in fracture line – nonunion	Mild bridge (<50%)	Moderate bridge (>50%)	Full bone bridge union	NA
	Cortex Remodeling	No remodeling	Mild remodeling (<50%)	Moderate remodeling (>50%)	Full remodeling cortex	NA
Tissue Response Around Osteotomy and Lateral to PEEK	Fibrous Connective Tissue (CT) Formation (i.e., Fibrosis)	No new fibrous tissue	Presence of minimal or mild new fibrous tissue	Presence of moderate new fibrous tissue	Disruption of normal tissue architecture and presence of moderately dense fibrous tissue	Severe deposition of moderately dense to dense fibrous tissue
	Inflammation: Overall, PMNs, mononuclear inflammatory cells (lymphocytes, histiocytes, macrophages), giant cells	None	Rare 1-5/hpf ^a	Mild, 5-10/hpf	Moderate, heavy infiltrate	Marked, tissue filled with inflammatory cells
	Necrosis	None	Minimal	Mild	Moderate	Marked

^ahpf = high powered field (400x).

The pathology report summarized the findings for the various groups as follows (Group 1 = saline control, Group 2 = bupivacaine control, Group 3 = placebo sponge control, Group 4 = Bupivacaine collagen-matrix sponge 4 mg, and Group 5 = Bupivacaine collagen-matrix sponge 8 mg).

Time Point	Parameter	Control						Test			
		G1		G2		G3		G4		G5	
		n	M ± SD	n	M ± SD	n	M ± SD	n	M ± SD	n	M ± SD
Day 28	Callus Formation	12	2.2 ± 0.7	12	2.1 ± 0.8	12	2.2 ± 0.8	10	1.4 ± 0.8	11	1.2 ± 1.0
	Bone Union	12	1.6 ± 0.8	12	1.5 ± 0.8	12	1.4 ± 0.9	10	1.3 ± 0.7	11	0.5 ± 0.7
	Cortex Remodeling	12	0.9 ± 0.5	12	0.6 ± 0.5	12	0.3 ± 0.5	10	0.6 ± 0.5	11	0.3 ± 0.5
Day 56	Callus Formation	12	2.8 ± 0.6	11	2.8 ± 0.4	11	2.6 ± 0.5	12	1.8 ± 1.2	12	1.3 ± 1.1
	Bone Union	12	2.7 ± 0.5	11	2.5 ± 0.7	11	2.1 ± 0.3	12	1.7 ± 0.9	12	1.4 ± 0.9
	Cortex Remodeling	12	1.5 ± 0.7	11	1.6 ± 0.5	11	1.4 ± 0.5	12	0.8 ± 0.6	12	0.7 ± 0.8

G = Group; n = Sample Size; M = Mean; SD = Standard Deviation.

Text Table 1. Healing Response at Osteotomy (Males and Females Combined). The healing response to the osteotomy site, in the region of Test and Control Article placement, was graded semi-quantitatively for callus formation, bone union, and cortex remodeling.

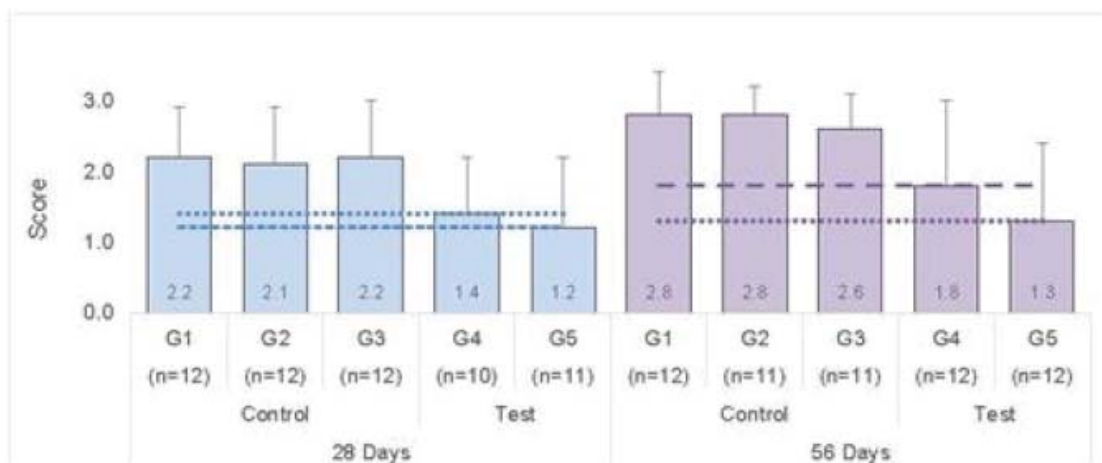


Figure 1. Callus Formation (Males and Females Combined). The callus formation over to the osteotomy site, in the region of Test and Control Article placement, was graded semi-quantitatively. Data represents semimean $\pm$ standard deviation (SD) and graphically demonstrates the data presented in Text Table 1.

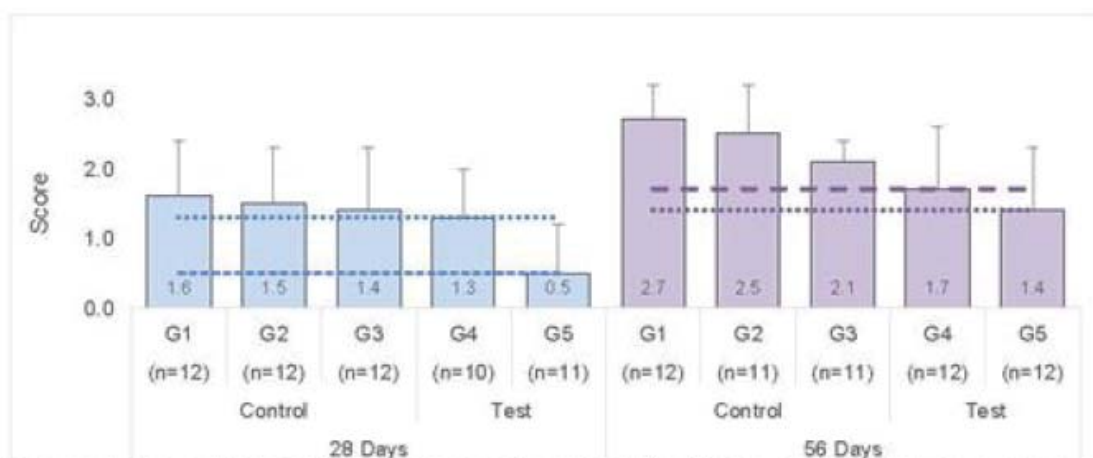


Figure 2. Bone Union (Males and Females Combined). The degree of bone union over to the osteotomy site, in the region of Test and Control Article placement, was graded semi-quantitatively. Data represents mean $\pm$ SD and graphically demonstrates the data presented in Text Table 1.

Histopathology Results – Treatment of a mid-shaft osteotomy with the Bupivacaine collagen-matrix implant demonstrated a dose dependent inhibition of bone healing in males on Day 28, and in both males and females on Day 56, and an increase in fibrosis around the osteotomy site in the males at Day 28, compared to a placebo collagen-matrix implant, saline, or bupivacaine HCl alone.

Special Evaluation – none other than reported.

Toxicokinetics – None.

10.2 Extractables and Leachables Studies

During the previous review cycle, the Applicant was informed that the extractable/leachable (E/L) evaluation submitted with the resubmission (SDN 9) dated 2/2/2018 was not considered adequate as a clear E/L correlation was not shown. Also notable was that the leachables study had only tested drug product lots that were 22 months or older to support the proposed 2-year expiry. We advised the Applicant to perform a rigorous extractables study of the primary and secondary packaging. From this robust extractables study, identify and determine target leachables, and then develop a method to detect those leachables in the drug product. The Applicant initiated new extractables and leachables studies during the review cycle and submitted a newly conducted extractables study (Study 10713; SDN 33; 9/20/2018) prior to the PDUFA date. The CMC review team informed us that the newly conducted extractables study was considered adequate in terms of extraction conditions and analytical methods used. However, the new leachables studies could not be submitted prior the PDUFA date. Consequently, the complete response letter dated 11/30/2018 included the following deficiency from the nonclinical perspective.

You have not provided an adequate extractables/leachables evaluation to support the safety of your proposed container closure system. Specifically, although your extraction study, submitted September 20, 2018 (SDN 33), was capable of detecting compounds at the requested safety concern threshold of (b) (4) mcg/day, you did not identify many of the compounds detected above this safety threshold. Further, you did not provide adequate leachables data from multiple batches at release where compounds identified in the extraction study were targeted using validated methods.

To address this deficiency:

Identify the compounds detected in the extraction studies completed to date and evaluate the drug product stability batches for the presence of any extractable detected at (b) (4) mcg/day or higher. Provide a toxicological risk assessment for any leachable compound present in the drug product at (b) (4) mcg/day or greater.

The CMC review team also included the following deficiency pertaining to the E/L evaluation:

The leachables assessment you have provided is incomplete. An analytical method for the detection of leachables has not been provided, and therefore, a correlation between the extractables and leachables cannot be made. Leachables testing should be performed on (b) (4) manufactured product due to (b) (4)

To address this deficiency:

- From the robust extractables profiles of both primary and secondary packaging, identify and determine the target leachables.
- Develop methods which can detect these leachables in the drug product.
- Test 3 batches of drug product at multiple time-points on stability with emphasis on (b) (4) manufactured product that includes ink labeling on individual blisters as planned for commercial product.

We also refer you to USP <1663> and <1664> for additional information.

To address these deficiencies, the Applicant submitted the following data:

- Robust extraction study (Study 10713) performed according to USP <1663> of the primary and secondary container closure systems after (b) (4). This study was actually submitted at the end of the previous review cycle (SDN 33; 9/20/2018) and is summarized below.
- Leachable studies on six Xaracoll lots according to USP <1664>.
 - o Primary leachables stability study ((b) (4) Study 11000) tested 3 (b) (4) manufactured XARACOLL lots. Data from samples under accelerated stability at 40°C/75% RH and at the intended storage condition (25°C/60% RH) from release to 6 months were provided with this resubmission. The Applicant noted testing through 36 months is ongoing.
 - o Supplemental leachables stability study ((b) (4) 10830) tested 3 XARACOLL lots. In this case, the lots were approximately 4 months beyond the end of manufacturing when placed on stability. Data from samples under accelerated stability at 40°C/75% RH and at the intended storage condition (25°C/60% RH) for up to 6 months were provided with this resubmission. The Applicant noted testing through 36 months is ongoing.
- The Applicant did not identify the unknown compounds detected above the safety concern threshold of (b) (4) mcg/day in the extraction study. However, they did provide data showing that all unknown compounds detected above (b) (4) mcg/day in the extraction study can also be detected above the AET in the newly conducted leachables studies. This was consistent with advice given by FDA in a Type C meeting held in 2019 (refer to meeting minutes dated 10/29/2019 in DARRTS).

Extractables

Study title: Controlled Extraction Study Associated with a Molded Blister and (b) (4) Lid (b) (4) (Primary Packaging) Supporting a Collagen-Based Surgical Implant

Note, this newly conducted extractables study was submitted at the end of the previous review cycle and is summarized here.

Study no.: 10713

Study location: SDN 33; 9/20/2018; [\\cdsesub1\evsprod\nda209511\0032\m3\32-body-data\32r-reg-info\extractables-report-18-3412.pdf](#)

Conducting lab: (b) (4)

Components tested:

Reviewer's comments: The CMC drug product reviewer informed that the newly conducted extraction study was considered adequate in terms of extraction conditions and analytical methods used. As noted above and in the nonclinical review from the previous cycle dated 11/2/2018, there were also several unknown compounds detected above (b) (4) mcg/day in the extractables study. However, the Applicant has employed comparable analytical methods for the extractable and leachable studies and has provided additional data to demonstrate that any compound, including the unknown compounds, detected above (b) (4) mcg/day in the extractables study can be detected above the AET in the leachables studies. The CMC reviewer confirmed that this is the case (see CMC review for the adequacy of this approach).

Leachables

The Applicant's table below provides a summary of the lots tested, stability storage conditions, and time points evaluated for both the newly conducted primary and

supplemental leachables studies submitted with this resubmission. Notably, only data up to 6 months were provided from both studies. However, the CMC review noted that the totality of the leachables data, including the new data and data submitted during the previous review cycle, are adequate to support the proposed expiry of 2 years (see CMC review).

Purpose	Study	Lots Used in Study	Age on initial test date (b) (4)	Storage conditions and testing time points
Primary Leachables Stability Study	11000	19001802 19001902 19011802	(b) (4) manufactured)	25°C/60 %RH: T= <u>0, 1.5, 3, 6</u> , 9, 12, 18, 24, 30, 36 months 40°C/75 %RH: T= <u>0, 1.5, 3, 6</u> months
Supplemental Leachables Stability Study	10830	18022611 18032611 18042611	4	25°C/60 %RH: T= <u>0, 3, 6</u> , 9, 12, 18, 24, 30, 36 months 40°C/75 %RH: T= <u>0, 3, 6</u> months

(a) Underlined, bolded timepoints have been completed to date.

The methods used for the new leachables studies are summarized in the Applicant's table below.

Compound Class	Analytical Method	(b) (4) SOP	Method Validation Report
Fatty Acids	Quantitative Test for Selected Fatty Acids as Potential Leachables in XARACOLL (Bupivacaine HCL-Collagen Matrix Implant) using HPLC/DAD Techniques	(b) (4)	10829B.3467
Non-Volatile Compounds	Quantitative Test for Selected Non-Volatiles as Potential Leachables in XARACOLL (Bupivacaine HCL-Collagen Matrix Implant) using HPLC/DAD Techniques	(b) (4)	10829A.3466
Semivolatile Compounds	Quantitative Test for Semivolatiles as Potential Leachables in XARACOLL (Bupivacaine HCL-Collagen Matrix Implant) using GC-MS Techniques	(b) (4)	10829C.3468

Summary Results

Stability data from the primary leachables study (Study 11000) through 6 months demonstrated that three leachables, including (b) (4) were detected; however, they were observed at concentrations below their respective quantitation limits in all lots at all conditions including both accelerated and normal storage conditions. The limits of quantitation employed were verified by the CMC review to be as low as the recommended AETs based on SCTs of (b) (4) mcg/day to ensure that the validated methods employed would be able to detect and quantify any compound at (b) (4) mcg/day.

Table 7: Stability Data for Observed Leachable Compounds for Stability Study 11000 at 25°C/ 60% RH

			Lot 19001802				Lot 19001902				Lot 19011802			
			(µg/dose)				(µg/dose)				(µg/dose)			
	LOD ^a	LOQ	0 m	1.5 m	3 m	6 m	0 m	1.5 m	3 m	6 m	0 m	1.5 m	3 m	6 m
(b) (4)	NA ^b	(b) (4)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
(b) (4)		(b) (4)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
			<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

(b) (4)

Table 8: Stability Data for Observed Leachable Compounds for Stability Study 11000 at 40°C/ 75% RH

			Lot 19001802				Lot 19001902				Lot 19011802			
			(µg/dose)				(µg/dose)				(µg/dose)			
	LOD ^a	LOQ	0 m	1.5 m	3 m	6 m	0 m	1.5 m	3 m	6 m	0 m	1.5 m	3 m	6 m
(b) (4)	NA ^b	(b) (4)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
(b) (4)		(b) (4)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
			<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

(b) (4)

Stability data from the supplemental leachables study (Study 10830) through 6 months demonstrated that two compounds, (b) (4) and (b) (4) were observed at concentrations below their respective quantitation limits in all lots at all conditions. One compound (b) (4) was observed at values that ranged (without a trend) from below the LOQ up to a maximum amount of (b) (4) mcg/day, which was observed in Lot 18022611 at T = 0. However, the CMC reviewer informed us that the peak identified as (b) (4) is actually a contaminant and should be disregarded as a leachable. Refer to the CMC review for more details.

Table 9: Stability Data for Observed Leachable Compounds for Stability Study 10830 at 25°C/ 60% RH

			Lot 18022611				Lot 18032611				Lot 18042611			
			(µg/dose)				(µg/dose)				(µg/dose)			
	LOD ^a	LOQ	0 m ^c	1.5 m	3 m	6 m	0 m ^c	1.5 m	3 m	6 m	0 m ^c	1.5 m	3 m	6 m
(b) (4)	NA ^b	(b) (4)	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ
(b) (4)		(b) (4)	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ
(b) (4)		(b) (4)	NT	<LOQ	(b) (4)	(b) (4)	NT	<LOQ	<LOQ	(b) (4)	NT	(b) (4)	<LOQ	(b) (4)

(b) (4)

Table 10: Stability Data for Observed Leachable Compounds for Stability Study 10830 at 40°C/ 75% RH

			Lot 18022611				Lot 18032611				Lot 18042611			
			(µg/dose)				(µg/dose)				(µg/dose)			
			0 m ^c	1.5 m	3 m	6 m	0 m ^c	1.5 m	3 m	6 m	0 m ^c	1.5 m	3 m	6 m
(b) (4)	LOD ^a	LOQ	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ
	NA ^b	(b) (4)	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ
	(b) (4)	(b) (4)	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ	<LOQ	NT	<LOQ	<LOQ
			(b) (4)	NT	<LOQ	<LOQ	(b) (4)	NT	(b) (4)	<LOQ	(b) (4)	NT	(b) (4)	<LOQ

Reviewer's notes and conclusions:

The Applicant addressed the deficiencies related to the extractable and leachable studies by conducting new extractable and leachable studies. The CMC review team has verified that:

- The extractable studies were considered adequate in terms of extraction conditions and analytical methods.
- The compounds selected for targeting in the leachables study were reasonable based on the extractable data. The Applicant did not identify all unknown extractable compounds above (b) (4) mcg/day. However, the validated methods employed in the leachables studies were capable of detecting any compound, including unknowns, above (b) (4) mcg/day.
- The leachable studies were considered adequately designed in terms of number of batches, time points, stability storage conditions, and analytical methods.
- Two leachable compounds were detected in the leachables study; however, they were not observed at or above (b) (4) mcg/day in any batch, any time point, and under any storage condition. Therefore, no toxicological risk assessments were warranted to justify the safety of leachables.
- There was good correlation between the extractable and leachables studies.
- The E/L data are adequate to qualify the safety of the container closure system and leachables that may arise from the packaging process of the drug product.

11 Integrated Summary and Safety Evaluation

See Executive Summary.

12 Appendix/Attachments

N/A

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

/s/

JAY H CHANG on behalf of GARY P BOND
08/21/2020 04:52:16 PM

JAY H CHANG
08/21/2020 04:53:26 PM

RICHARD D MELLON
08/21/2020 04:54:46 PM
I concur.

**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: NDA 209511

Supporting document/s: SDN 1, 9, 31, 33 - SDN 1 (original submission), 9
(Resubmission after RTF), 31 (DS specs), 33
(extractables study)

Applicant's letter date: October 31, 2016 (Original Submission), February 2,
2018 (Resubmission after RTF)

CDER stamp date: October 31, 2016 and February 2, 2018

Product: XARACOLL® (Bupivacaine Hydrochloride Collagen-
Matrix Implants)

Indication: Surgical site placement to produce postsurgical
analgesia following (b) (4)

Applicant: Innocoll Pharmaceuticals

Review Division: Division of Anesthesia, Analgesia, and Addiction
Products

Reviewer: Gary Bond, PhD

Team Leader: Jay H. Chang, PhD

Supervisor: R. Daniel Mellon, PhD

Division Director: Sharon Hertz, MD

Project Manager: Allison Meyer

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 209511 are owned by Innocoll Pharmaceuticals or are data for which [name of applicant] has obtained a written right of reference. Any information or data necessary for approval of Innocoll Pharmaceuticals that 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 209511.

TABLE OF CONTENTS

1	EXECUTIVE SUMMARY.....	3
1.1	INTRODUCTION AND REGULATORY HISTORY	3
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	3
1.3	RECOMMENDATIONS	5
2	DRUG INFORMATION.....	10
2.1	DRUG	10
2.2	RELEVANT INDs, NDAs, BLAs AND DMFs.....	11
2.3	DRUG FORMULATION	11
2.4	COMMENTS ON NOVEL EXCIPIENTS	12
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	12
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	20
2.7	REGULATORY BACKGROUND	20
3	STUDIES SUBMITTED	23
3.1	STUDIES REVIEWED	23
3.2	STUDIES NOT REVIEWED.....	24
3.3	PREVIOUS REVIEWS REFERENCED.....	25
4	PHARMACOLOGY	25
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	25
5.1	PK/ADME	25
5.2	TOXICOKINETICS.....	29
6	GENERAL TOXICOLOGY	37
6.1	SINGLE-DOSE TOXICITY	37
7	GENETIC TOXICOLOGY.....	51
7.1	IN VITRO REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES)	51
7.2	IN VITRO ASSAYS IN MAMMALIAN CELLS.....	59
7.3	IN VIVO CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY)	74
7.4	OTHER GENETIC TOXICITY STUDIES.....	79
8	CARCINOGENICITY.....	79
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	79
10	SPECIAL TOXICOLOGY STUDIES	83
10.1	EXTRACTABLE STUDIES	83
10.2	LEACHABLES STUDIES.....	98
11	INTEGRATED SUMMARY AND SAFETY EVALUATION	101
12	APPENDIX/ATTACHMENTS.....	104

1 Executive Summary

1.1 Introduction and Regulatory History

NDA 209511 was submitted by Innocoll Pharmaceuticals for marketing approval of Xaracoll, which is an implantable combination product containing bupivacaine and purified Type I bovine collagen, for the proposed indication of placement into a surgical site to produce postsurgical analgesia following (b) (4). Each Xaracoll implant contains 100 mg bupivacaine HCl. The maximum recommended human dose (MRHD) is 300 mg (3 x 100 mg implants), which is equivalent to 266.4 mg bupivacaine base. The NDA was submitted via the 505(b)(2) pathway relying on the Agency's previous findings of safety of the listed drug Marcaine (NDA 16964; approved 1972) and literature.

NDA 209511 was originally submitted on 10/31/2016. However, the Division issued a Refuse-to-File letter on 12/23/2016 with issues from almost all disciplines. Of note, the Applicant changed the collagen component of the product during development and did not test the to-be-marketed (TBM) product in pivotal nonclinical toxicology studies and the comparative human bioavailability (BA) study. Only studies with the older formulation were submitted to the NDA. In addition, an adequate extractables/leachables study was not included with the NDA. With this resubmission, the Applicant included a new GLP toxicology study and new human comparative BA study using the TBM product as well as a new extractables/leachables evaluation. Also of note, when the Applicant originally submitted the NDA, they referenced both listed drugs Marcaine and (b) (4) as part of the 505(b)(2) NDA submission; however, only Marcaine is referenced in this resubmission. This is significant because the clinical BA study demonstrated that the AUC exposure level to bupivacaine following XARACOLL treatment at the MRHD exceeded the listed product Marcaine by approximately 2X (e.g., AUC_{0-last} (h•ng/mL) of 19493 vs 9708, respectively), which is not surprising given the bupivacaine HCl dose of 300 mg in XARACOLL vs 175 mg in Marcaine. Therefore, the referenced drug product does not support the safety of the higher exposures obtained via this drug product formulation. As such, general toxicity studies in two species (at least one non-rodent) are needed to justify the systemic safety of the bupivacaine exposure through XARACOLL administration. However, the NDA included only one rat toxicity study, which did not provide adequate AUC coverage to justify the systemic safety of XARACOLL. The nonclinical data are discussed below.

1.2 Brief Discussion of Nonclinical Findings

There are no nonclinical concerns with the drug substance specifications. The proposed drug product specifications are not in compliance with the recommendations outlined in ICH M7 for mutagenic impurities. Specifically, the proposed specifications for (b) (4) at NMT (b) (4) ppm, which results in a potential total daily intake of NMT (b) (4) mcg per device, technically exceeds the ICH M7 threshold of toxicological concern of (b) (4) mcg/day for an acute use product if it released entirely on the first day of dosing. The Applicant's justification for safety was based on a standard established by

ISO for implantable devices of up to (b) (4) mg as per ISO 10993-07 (per the Applicant). It is not clear what the ISO standard was based on. Review of the existing data on this compound suggest that it does test positive in the Ames assay as a mutagen. However, there are two published rat and two published mouse dermal toxicity studies, which report negative findings for carcinogenicity. As the entire (b) (4) mcg/implant may not be released in the first day of administration and given the published carcinogenicity study results, the proposed specification is not likely to result in a significantly increased risk. Nonetheless, the actual drug product batch analyses suggest that the specification may be able to be tightened. This should be addressed in the second cycle. All other drug product specifications are acceptable. The Applicant also adequately addressed the safety of all (b) (4) and elemental impurities in accordance with ICH Q3C and Q3D.

The drug product technically contains a novel excipient, bovine collagen, because this compound is not currently listed in the FDA Inactive Ingredients Database (IID) for use in a subcutaneously implanted device. The Applicant conducted biocompatibility studies in accordance with the International Standards Organization (ISO) which were reviewed by CDRH to address their regulatory requirements. As a novel excipient, CDER requires adequate justification for the safety of this excipient as well in accordance with CDER guidance. Given the fact that collagen is an endogenous compound and the body is well equipped to metabolize and process this material, only local tissue toxicity studies with the formulation were deemed necessary to inform the safety of this novel excipient.

To characterize the systemic and local safety of the drug product formulation, the Applicant conducted an extensive literature review for bupivacaine and submitted a pivotal GLP 56-day rat toxicology study testing the local tissue effects of the to-be-marketed bupivacaine collagen sponge implant in a wound closure model. They also evaluated wound healing qualitatively in this study. The new GLP toxicology study results suggest the potential for a clear local tissue response which includes microscopic evidence of marked necrosis of tissue surrounding the implant and a chronic-active inflammatory state consistent with the implantation of a foreign material. Mild to moderate necrosis was noted in the saline group, the bupivacaine injection control group, and the collagen matrix alone implant group. Only the bupivacaine collagen implant groups demonstrated marked local tissue necrosis. There was no clear impact on wound healing in this study based on clinical and gross macroscopic observations alone suggesting that the enhanced local tissue response did not translate into significant clinical impact. The existing human local safety data should be leveraged to confirm that these local changes are not considered clinically adverse. The implanted material was no longer clearly present in tissue somewhere between 35 and 56 days post implantation.

During development, the Division noted that although not required for approval of the product, the standard battery of genetic toxicity studies for bupivacaine would ideally be completed to appropriately inform labeling. The Applicant completed the requested studies. Bupivacaine HCl was negative for genotoxicity in a standard test battery: 1) in

vitro reverse mutation assay in bacterial cells (Ames), 2) in vitro mammalian cell gene mutation test (L5178Y/TK+/- mouse lymphoma assay), and 3) in vivo clastogenicity assay in rodent (micronucleus assay). However, the high dose tested in the in vivo study did not result in frank toxicity, therefore the study is not considered valid based on current standards. The results should not be included in labeling without further justification for the high dose selection. Given the apparent greater exposure to bupivacaine via this product relative to the referenced product (2 times the AUC_{0-last}), this study can be required to be completed as per OND policy.

The Applicant submitted a literature review of the published studies to address the requirements outlined in the Pregnancy Labeling and Lactation Rule (PLLR). Review of the published studies submitted did not identify data that substantively changed the overall conclusions of the referenced reproductive and developmental toxicity studies in the Marcaine labeling. As such, no additional labeling recommendations are provided at this time.

1.3 Recommendations

1.3.1 Approvability

From a pharmacology toxicology perspective, there are inadequate nonclinical data to justify the safety of XARACOLL and therefore we cannot recommend approval at this time. Specifically, the Applicant has not submitted adequate extractables/leachables data to characterize the safety of the drug product container closures system. Second, the existing toxicology study does not provide adequate coverage for the proposed systemic toxicity of bupivacaine via this product, particularly because the AUC_{0-last} for this product exceeds that of the referenced drug product. As such, adequate toxicity data in two species are required. Third, the in vivo micronucleus assay did not test adequate doses to be considered valid and will be required to be repeated unless justified otherwise. Finally, the Applicant did not provide adequate safety justification for the levels of (b) (4) in the final drug product taking into considerations the recommendations outlined in ICH M7.

Deficiencies:

1. You have not provided an adequate characterization of the systemic safety of bupivacaine exposures via your drug product formulation. Specifically, based on the existing human pharmacokinetic data, your product results in an $AUC_{(0-last)}$ that is twice that of the referenced product. Your existing toxicology data in the rat model do not test exposures that provide coverage for the human exposures via your product.

To address this deficiency:

Conduct adequate toxicology studies in two species that provide adequate coverage for the proposed human exposures via your drug product (AUC and C_{max}).

2. You have not provided a valid in vivo micronucleus assay for bupivacaine. Specifically, the high dose selected for the assay did not result in frank toxicity.

To address this deficiency:

Repeat the in vivo micronucleus assay for bupivacaine testing doses that result in frank toxicity in accordance with the ICH guidance document: *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use*, available at <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074931.pdf>.

3. You have not provided an adequate extractables/leachables evaluation to support the safety of your proposed container closure system. Specifically, although your extraction study, submitted September 20, 2018 (SDN 33), was capable of detecting compounds at the requested safety concern threshold of (b) (4) mcg/day, you did not identify many of the compounds detected above this safety threshold. Further, you did not provide adequate leachables data from multiple batches at release where compounds identified in the extraction study were targeted using validated methods.

To address this deficiency:

Identify the compounds detected in the extraction studies completed to date and evaluate the drug product stability batches for the presence of any extractable detected at (b) (4) mcg/day or higher. Provide a toxicological risk assessment for any leachable compound present in the drug product at (b) (4) mcg/day or greater.

4. You have not provided adequate justification for the proposed specification for (b) (4) in the drug product formulation.

To address this deficiency:

Either reduce the specification for (b) (4) to NMT (b) (4) mcg/day or provide an adequate toxicological risk assessment for this compound to justify the proposed specification in accordance with the ICH guidance document: *M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*, available at <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM347725.pdf>.

1.3.2 Additional Non-Clinical Recommendations

None at this time.

1.3.3 Labeling

Formal labeling negotiations have not been completed to date given the complete response recommendation. The labeling suggestions below have not been fully

discussed with the review team or the Applicant. The reader is referred to the second cycle review and ultimately an approval letter for final labeling content.

Marcaine label (2012)	Applicant's Proposed Labeling (April 2018, Version 1.2)	Recommended Labeling	Rationale/Comment
	HIGHLIGHTS OF PRESCRIBING INFORMATION INDICATIONS AND USAGE XARACOL (b) (4) [REDACTED] for placement into the surgical site to produce postsurgical analgesia following (b) (4) (1). USE IN SPECIFIC POPULATIONS • Pregnancy: based on animal data, may cause fetal harm (8.1).	HIGHLIGHTS OF PRESCRIBING INFORMATION INDICATIONS AND USAGE XARACOLL is an implantable combination product containing bupivacaine, an amide local anesthetic, and purified Type I bovine collagen, that is indicated for placement into the surgical site to produce postsurgical analgesia following (b) (4) (1). USE IN SPECIFIC POPULATIONS • Pregnancy: May cause fetal harm (8.1).	Established pharmacological class needs to be included here in the Highlights. See clinical review for final indication. This has been deleted in recent labels for other drugs and is appropriate here – Maternal Health call.
Pregnancy Category C: 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	8.1 Pregnancy Risk Summary There are no studies conducted with XARACOLL in pregnant women. Bupivacaine hydrochloride produced developmental toxicity when administered subcutaneously to pregnant rats and rabbits at clinically relevant doses. Based on animal data, advise pregnant women of the potential risks to a fetus. The background risk of major birth defects and miscarriage for the indicated population is unknown. However, the background risk in the	8.1 Pregnancy Risk Summary There are no studies conducted with XARACOLL in pregnant women. Bupivacaine hydrochloride produced developmental toxicity when administered subcutaneously to pregnant rats during implantation through weaning and to rabbits during the period of organogenesis at clinically relevant doses. Based on animal data, advise pregnant women of the potential risks to a fetus. The background risk of major birth defects and	Information from approved Marcaine label (2012) formatted per the Pregnancy and Lactation Labeling Rule (PLLR), amended by adding appropriate dose ratios and dosing periods to comply with PLLR requirements. The literature was reviewed and there was no information that was deemed warranted for the label at this time. Standard PLLR label language. We defer to

anesthesia or analgesia. (See Labor and Delivery)	U.S. general population of major birth defects is 2-4% and of miscarriage is 15-20% of clinically recognized pregnancies.	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.	the Maternal Health and clinical review teams on clinical labeling language.
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/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 1/5th the MRHD on a BSA basis.	<i>Data</i> <i>Animal Data</i> Bupivacaine hydrochloride was administered subcutaneously to rats at doses of 4.4, 13.3 and 40 mg/kg and to rabbits at doses of 1.3, 5.8 and 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). No embryo-fetal effects were observed in rats at up to 40 mg/kg, a dose that caused increased maternal lethality. This dose is approximately 1.3 times the daily maximum recommended human dose (MRHD) of 300 mg when calculated on a mg/m ² body surface area (BSA) for a 60 kg woman. An increase in embryo-fetal deaths was observed in rabbits at the high dose (1.4 times the MHRD based on BSA) in the absence of maternal toxicity. (b) (4) No Observed Adverse Effect Level (b) (4) approximately 0.4 times the MRHD on a BSA basis.	<i>Data</i> <i>Animal Data</i> Bupivacaine hydrochloride was administered subcutaneously to rats at doses of 4.4, 13.3 and 40 mg/kg and to rabbits at doses of 1.3, 5.8 and 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). No embryo-fetal effects were observed in rats at up to 40 mg/kg, a dose that caused increased maternal lethality. This dose is approximately 1.3 times the daily maximum recommended human dose (MRHD) of 300 mg when calculated on a mg/m ² body surface area (BSA) for a 60 kg woman. An increase in embryo-fetal deaths was observed in rabbits at the high dose (approximately 1.4 times the MHRD on a BSA basis) in the absence of maternal toxicity. The developmental No Observed Adverse Effect Level in rabbits is approximately 0.4 times the MRHD on a BSA basis.	The Applicant included appropriate exposure margins.
In a rat pre- and post-natal development study (dosing from implantation through weaning) conducted at	In a rat pre- and post-natal development study (dosing from implantation through weaning) conducted at	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 mg/kg/day, 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.	subcutaneous doses of 4.4, 13.3, and 40 mg/kg/day, decreased pup survival was observed at the high dose (approximately 1.3 times the daily MRHD on a BSA basis).	subcutaneous doses of 4.4, 13.3, and 40 mg/kg/day, decreased pup survival was observed at the high dose (approximately 1.3 times the daily MRHD on a BSA basis).	
Nursing Mothers: Bupivacaine has been reported to be excreted in human milk suggesting that the nursing infant could be theoretically exposed to a dose of the drug. Because of the potential for serious adverse reactions in nursing infants from bupivacaine, a decision should be made whether to discontinue nursing or not administer bupivacaine, taking into account the importance of the drug to the mother.	8.2 Lactation Risk Summary Bupivacaine has been reported to be excreted in human milk suggesting that the nursing infant could be theoretically exposed to a dose of the drug. There is no available information on effects of the drug in the breastfed infant or effects of the drug on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for XARACOLL and any potential adverse effects on the breastfed infant from XARACOLL or from the underlying maternal condition.	8.2 Lactation Risk Summary Bupivacaine has been reported to be excreted in human milk suggesting that the nursing infant could be theoretically exposed to a dose of the drug. There is no available information on effects of the drug in the breastfed infant or effects of the drug on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for XARACOLL and any potential adverse effects on the breastfed infant from XARACOLL or from the underlying maternal condition.	We defer to the Maternal Health and clinical review teams on clinical labeling language.
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. In general, the progression of anesthesia is related to the diameter, myelination, and conduction velocity of	12.1 Mechanism of Action (b) (4) bupivacaine 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. Clinically, the order of loss of nerve function is (1) pain, (2) temperature, (3) touch, (4) proprioception, and	12.1 Mechanism of Action Local anesthetics such as bupivacaine 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. In general, the progression of anesthesia is related to the diameter, myelination, and conduction velocity of	Applicant has deleted the bolded sentence from the Marcaine label without an explanation. It should be retained.

affected nerve fibers. Clinically, the order of loss of nerve function is as follows: (1) pain, (2) temperature, (3) touch, (4) proprioception, and (5) skeletal muscle tone.	(5) skeletal muscle tone.	affected nerve fibers. Clinically, the order of loss of nerve function is (1) pain, (2) temperature, (3) touch, (4) proprioception, and (5) skeletal muscle tone.	
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.	13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenesis</u> Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine have not been conducted. <u>Mutagenesis</u> Bupivacaine was not mutagenic or clastogenic in a bacterial reverse mutation assay, an in vitro mammalian cell gene mutation test, and an in vivo mammalian erythrocyte micronucleus assay. <u>Impairment of Fertility</u> The effect of bupivacaine on fertility has not been determined.	13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenesis</u> Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine have not been conducted. <u>Mutagenesis</u> Bupivacaine was not mutagenic or clastogenic in a bacterial reverse mutation assay and an in vitro mammalian cell gene mutation test. <u>Impairment of Fertility</u> The effect of bupivacaine on fertility has not been determined.	From Marcaine label. The Applicant conducted new genotox studies with bupivacaine (all three) and XARACOLL extracts (first two) for this NDA. The in vivo study is omitted until justified as valid or repeated. Consistent with Marcaine. No reliable PLLR-related literature search data was identified.

2 Drug Information

2.1 Drug

CAS Registry Number - 18010-40-7 (anhydrous), 14252-80-3 (monohydrate)

Generic Name - Bupivacaine hydrochloride

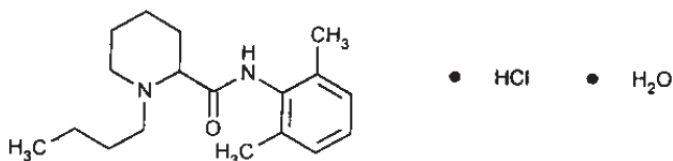
Code Name - NR

Chemical Name - 1-Butyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide hydrochloride monohydrate

Molecular Formula/Molecular Weight - C₁₈H₂₈N₂O·HCl·H₂OMolecular Weights

342.90 monohydrate
 324.89 anhydrous
 288.43 free base anhydrous

Structure or Biochemical Description



Pharmacologic Class - amide local anesthetic

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
16964	Marcaine	DAAAP	0.25% injection	Approved	10/3/1972	Local anesthesia	Hospira

IND#	Drug	Status	Division	Indication	Stamp Date	Sponsor
77127	Bupivacaine Collagen Matrix	Active	DAAAP	Post-surgical pain	3-1-2007	Innocoll Pharmaceuticals

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
(b) (4)				

2.3 Drug Formulation

Composition - Xaracoll is a white to off-white drug/device combination product composed of a (b) (4) purified collagen matrix as the device component containing the amide local anesthetic bupivacaine HCl as the drug substance component. The product configuration is a 5 × 5 × 0.5 cm matrix implant consisting of purified type I bovine collagen prepared as (b) (4). The final collagen/bupivacaine (b) (4).

(b) (4)

(b) (4) Each final (b) (4) drug

product matrix contains 75 mg of collagen and 100 mg of bupivacaine HCl. The composition of each product matrix is shown in the table below.

Xaracoll Composition

Component	Quality standard	Quantity per matrix	Function
Bovine type I collagen, purified	(b) (4)	75 mg	Excipient
Bupivacaine HCl	USP	100 mg	Drug substance

(b) (4)

2.4 Comments on Novel Excipients

Technically, bovine Type I collagen is a novel excipient because it has not been previously used in an FDA-approved drug product for subcutaneous administration or in a wound closure environment as per the FDA Inactive Ingredients Database (IID). As such, the existing toxicology studies, in conjunction with the ISO studies reviewed by CDRH were submitted to justify the safety of this excipient. Because bovine collagen is comparable to human collagen (almost identical amino acid sequences and antigenic determinants) and exposure to this excipient is expected to be comparable to collagen levels found in the body, genetic toxicology studies and reproductive and developmental toxicology studies were not required to support this NDA.

The collagen used in this product is derived from bovine Achilles tendons obtained from (b) (4) closed herds and certified bovine spongiform encephalopathy free. The reader is referred to the CMC quality review for details and the CDRH review for biocompatibility assessments, which included genetic toxicology studies and an evaluation of extractables and leachables from the collagen matrix.

Because bovine collagen is comparable to human collagen (almost identical amino acid sequences and antigenic determinants) and exposure to this excipient and its degradation products is expected to be comparable to human collagen found ubiquitously in the body, genetic toxicology studies and reproductive and developmental toxicology studies were not required to support this NDA. Although collagen will likely take up to 56 days to degrade and be removed from the implantation site, the lack of genetic toxicity concern and relatively short duration of exposure for this indication preclude the need for carcinogenicity studies.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance

The drug substance (bupivacaine HCl) specifications are listed in the two tables that follow, one for the substance manufacturer and an (b) (4) one for the drug manufacturer. At the maximum daily dose of 300 mg bupivacaine, according to ICH Q3A(R2), the appropriate qualification threshold is (b) (4) % or (b) (4) mg per day intake (whichever is lower).

1. 300 mg at (b) (4) % is (b) (4) mg, making this the threshold level

While the manufacturer specification is (b) (4) % for (b) (4) the Applicant will control it to NMT (b) (4) %, which is acceptable.

All impurities and degradants are within ICH Q3A(R2) thresholds.

Bupivacaine HCl Drug Substance Specifications from Manufacturer

Test	Acceptance criteria	Method
Appearance	White crystalline powder.	Visual
Identification A: IR	Must comply with reference.	Infrared spectroscopy
Identification B: UV	Spectrum: $\lambda_{\max}$ = (b) (4) nm	Ultraviolet spectroscopy
Identification (b) (4) (b) (4)	Must comply.	Chemical test
pH	(b) (4)	USP
Clarity of solution	Solution must be clear and colorless.	Ph. Eur.
Acidity	pH > (b) (4)	Ph. Eur.
Alkalinity	pH < (b) (4)	Ph. Eur.
(b) (4)	(b) (4) %	(b) (4)
Water	(b) (4) %	
Heavy metals	Max. (b) (4) ppm	USP
(b) (4)	Max. (b) (4) %	USP/Ph. Eur.
(b) (4)	Max. (b) (4) ppm	HPLC
(b) (4)	Max. (b) (4) %	GC
Any other impurity	Max. (b) (4) %	GC
Sum of impurities	Max. (b) (4) %	GC
Assay titration	(b) (4)	Ph. Eur.
Bioburden ²	Typically < (b) (4) CFU/g	Microbiological test
Endotoxin ²	Typically < (b) (4) EU/g	Microbiological test

² Bioburden and endotoxin are not regulatory specifications but are performed for information only

Bupivacaine HCl Drug Substance Specifications from Applicant

Test procedure	Acceptance criteria	Method reference
Appearance ¹	White crystalline powder or colorless crystals	Visual inspection according to Ph. Eur.
Identification A: infrared absorption	Complies with USP reference IR spectrum	USP <197S>
Identification B: UV absorption	Absorption at (b) (4) nm complies	USP <197U>
Identification (b) (4) presence of (b) (4)	Positive for (b) (4)	USP <191>
pH	(b) (4)	USP <791>
Water content ¹	(b) (4)	USP <921>
Residue on ignition	NMT (b) (4) %	USP <281>
Heavy metals	NMT (b) (4) % ((b) (4) ppm)	USP <231>
Limit of residual solvents	NMT (b) (4) %	USP monograph
Chromatographic purity TLC ¹	Any single impurity NMT (b) (4) % Sum of impurities NMT (b) (4) %	USP monograph
Assay titration ¹	(b) (4) % (b) (4)	USP monograph
Bioburden ¹	NMT (b) (4) CFU/g combined TAMC & TYMC	USP <61>
(b) (4)	Max. (b) (4) %	COA

¹ Included in annual testing program.

(b) (4)

CFU = colony forming units; TAMC = total aerobic microbial count; TYMC = total yeast & molds count.

The Applicant also conducted an assessment aligned with ICH M7 ((Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk)) on actual and potential (b) (4) related impurities listed in the table below using the following Quantitative Structure-Activity Relationship (QSAR) methodologies and systems:

Expert rule-based methodology and parameters:	Leadscope genetox expert alerts v4 (System: Leadscope Model Applier v2.2.2.3); the domain assessment was turned on
Statistical-based methodology and parameters:	Leadscope Salmonella statistical-based QSAR model v3, Leadscope E.coli/TA102 statistical-based QSAR model v1 (System: Leadscope Model Applier v2.2.2.3); domain assessment was turned on
Genetic toxicity database used for searching:	Leadscope SAR genetox 2017
Rodent carcinogenicity database used for searching:	Leadscope SAR carcinogenicity 2017

(b) (4)

(b) (4)

Results – Chemical 1 has an M7 classification of (b) (4)
(b) (4) Chemical 2 has an M7 classification of (b) (4)

(b) (4) This actual/potential impurity, as with the first one, is not listed in the specifications and is assumed to be NMT (b) (4) % (b) (4) ppm or (b) (4) mcg at a maximum dose of 300 mg bupivacaine) the same as (b) (4) For this actual/potential impurity considered positive in silico, the Toxicological Threshold of Concern (TTC) for a potential genotoxin is (b) (4) mcg for the single dose Xaracoll, above which M7 lists to test the actual/potential impurity separately in a bacterial mutagenicity assay. The (b) (4) impurity, being related to (b) (4), then has an upper limit of (b) (4) mcg in 300 mg of Bupivacaine and has been reported to be at (b) (4) mcg per dose. Based on these rationalizations, there are no safety concerns under current conditions.

Drug Product

Specifications for the drug product are listed in the following table:

XARACOLL Drug Product Specifications

Final testing	Acceptance criteria	SOP
Characteristics/identity		
Appearance	White to off white porous sponge	QC6-008
Identity bupivacaine (HPLC)	Sample RT $\pm$ (b) (4) % of reference standard RT	QC6-009
DSC	Report Results	TBD
SDS-PAGE	Conforms to Type I Collagen Standard	TBD
Purity testing		
(b) (4)	$\leq$ (b) (4) ppm	QC6-057
(b) (4)	$\leq$ (b) (4) ppm	QC6-012
(b) (4)	$\leq$ (b) (4) ppm	QC6-012
Related substances by GC	Any single impurity $\leq$ (b) (4) % Total impurities $\leq$ (b) (4) %	QC6-010
Related substance (b) (4) (HPLC)	$\leq$ (b) (4) %	QC6-009
Assay testing		
(b) (4)	(b) (4)	QC6-008
(b) (4)	(b) (4)	QC6-008 USP (b) (4)
Assay bupivacaine HCl (HPLC)	(b) (4)	QC6-009
Content uniformity (HPLC)	AV NMT (b) (4) Complies to USP <905>	QC6-009 USP <905>
Dissolution (HPLC)	30 min: (b) (4) 120 min: (b) (4) 360 min: NLT (b) (4)	QC6-011 QC6-009 USP <711>
Microbiological testing		
Bacterial endotoxins	$\leq$ (b) (4) EU/implant	QC8-020 USP <85>
Sterility	Sterile	QC8-014 USP <71>

NMT = not more than; NLT = not less than; AV = acceptance value; EU = endotoxin units

SOP = analytical method

Issues of interest are the three highlighted (b) (4) chemicals above (b) (4)

(b) (4)

2. All lots at release have been reported to meet the specification of NMT (b) (4) ppm.

Reviewer's comment: The proposed specification of (b) (4) is acceptable since the potential total daily intake (TDI) based on the MRHD of XARACOLL is within ICH M7 limits for an acute indication.

(b) (4)

(b) (4)

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Reviewer's comment: We acknowledge that CDER and CDRH may have different acceptable daily limits for genotoxic impurities; however, Applicants must meet ICH limits for marketing application of drug products evaluated by CDER. Therefore, the specification for (b) (4) must be within the acceptable daily intake of (b) (4) mcg/day in accordance with ICH M7 for an acute indication. That being said, the drug product batch data showed that the highest level observed in stability studies is (b) (4) ppm, which would result in a maximum TDI of (b) (4) mcg/day. Therefore, the Applicant must tighten the specification so that the TDI does not exceed ICH M7 limits.

(b) (4) at <= (b) (4) ppm – According to ICH Q3C, the acceptable level is (b) (4) ppm

(b) (4)

(b) (4)

In summary, as all lots at release have been reported to meet the specifications for (b) (4), the test is not performed on stability, (b) (4). In addition, the proposed specification for (b) (4) is acceptable per ICH M7. Therefore, none of the chemicals and possible (b) (4)-related impurities are in excess of safety concern levels.

2.6 Proposed Clinical Population and Dosing Regimen

Xaracoll is proposed for preemptive treatment of postsurgical pain following (b) (4). Each bupivacaine HCl collagen-matrix contains 100 mg bupivacaine HCl (equivalent to 88.8 mg of bupivacaine) (b) (4) in 75 mg of collagen in a matrix measuring approximately 5 cm x 5 cm x 0.5 cm. The proposed dose of Xaracoll is 300 mg (equivalent to a total of 266.4 mg of bupivacaine), achieved by implanting 3 x 100 mg collagen matrices during soft tissue surgery at multiple layers in the soft tissue (e.g., between the fascia/muscle closure and in the layers below the skin closure). Xaracoll was studied primarily in hernioplasty and hysterectomy, but these two surgeries are representative models of different types of soft tissue surgeries, given their unique anatomic considerations, surgical approaches and placement of Xaracoll in the surgical wound.

2.7 Regulatory Background

Bupivacaine has been marketed in the United States for over 20 years as Marcaine. This New Drug Application is being submitted using a 505(b)(2) regulatory pathway with Marcaine (NDA 016964 – October 3, 1972) as the listed drug.

NDA 209511 was originally submitted on October 31, 2016. However, the Division issued a Refuse-to-File (RTF) letter on October 31, 2016 with issues from almost all disciplines.

2.8 Container Closure System

The container closure system for XARACOLL consists of primary container closure packaging components (see table below), an implant carrier frame (triplet carrier for single blisters), and a secondary packaging pouch.

Primary Container Closure System Components

Component	Description	Regulatory reference	Supplier
Primary container - blister	<u>Dimensions:</u> 63.2 mm × 63.2 mm × 15 mm (outer size) 48–54 mm × 48–54 mm conical (inner size) <u>Material:</u>	21 CFR 177.1315 178.3297 178.3770	(b) (4)
Primary closure - (b) (4)	<u>Dimensions:</u> 289 mm × 289 mm 64.5 mm × 74.0 mm <u>Material:</u>	21 CFR 177.1520	

The following figures show pictures of the primary container closure components.



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To justify the safety of the container closure system, the Applicant submitted extractables and leachables studies, which are reviewed in Section 10 of this review.

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3 Studies Submitted

- includes submissions of 10/31/2016 and 2/2/2018

3.1 Studies Reviewed

Study Number	Study Title	GLP/nonGLP	Review Center ^a
4.2.2.2 Absorption			
(b) (4) 134508	A Study to Investigate the Rate of Drug Release from a Bupivacaine-Collagen Implant in Male Beagle Dogs	nonGLP	CDER
00134510	An Acute Single Dose Toxicokinetic Study of Bupivacaine Collagen Implant in Sprague Dawley Rats	GLP	CDER
4.2.3 Toxicology			
4.2.3.1 Single Dose Toxicity			
Rat Subcutaneous			
00134509	A 56-Day Bupivacaine Collagen Implant Toxicity Study in Sprague Dawley Rats	GLP	CDER
4.2.3.3 Genotoxicity			
4.2.3.3.1 In vitro			
AE26TM.502ICH (b) (4)	Bacterial Reverse Mutation Assay	GLP	CDER
V0023_311	Bacterial Reverse Mutation Study with a Dose Range Finding Study	GLP	CDRH
AE26TM.704ICH (b) (4)	In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay)	GLP	CDER
V0573_200	Genotoxicity: Dose Range Finding Study with a Definitive Mouse Lymphoma Assay	GLP	CDRH
4.2.3.3.2 In vivo			
AE32HH.125M012ICH (b) (4)	In Vivo Mammalian Erythrocyte Micronucleus Assay in Rats	GLP	CDER
4.2.3.6 Local Tolerance			
TI 251_800	ISO Intracutaneous Study in Rabbits	GLP	CDRH
TI 261_300	ISO Guinea Pig Maximization Sensitization Test	GLP	CDRH
4.2.3.7 Other Toxicity Studies			
4.2.3.7.6 Impurities			
Referred to as: ICH M7 Assess	ICH M7 Compliant Assessment of Two Bupivacaine Impurities	NA	CDER
4.2.3.7.7 Other			
GLP-06-2964-GI	L929 MEM Elution Test - ISO	GLP	CDRH
GLP-2018-0182	Elution Cytotoxicity Test - ISO	GLP	CDRH

TU010_807	USP Rabbit Pyrogen Study, Material Mediated	GLP	CDRH
TU010_807/S	USP Rabbit Pyrogen Study, Material Mediated (Sponge only)	GLP	CDRH

- Submitted 10/31/2016 NA – Not Applicable

- Submitted 2/2/2018

a – Center for Devices and Radiological Health (CDRH), Center for Drug Evaluation and Research (CDER)

3.2 Studies Not Reviewed

Study Number	Study Title	GLP/nonGLP	Review Center ^a
4.2.2 Pharmacokinetics			
4.2.2.1 Analytical Methods and Validation Reports			
(b) (4)-134503	Validation of an UHPLC/MS/MS Assay for the Determination of Bupivacaine Concentrations in Rat Plasma	nonGLP	CDER
(b) (4)-134505	Validation of an UHPLC/MS/MS Assay for the Determination of Bupivacaine Concentrations in a Collagen Implant	nonGLP	CDER
C0022_000	Infrared Spectroscopy	nonGLP	CDER
4.2.2.2 Absorption			
(b) (4)-134506	A Toxicokinetic Study of a Bupivacaine Collagen Implant in Sprague Dawley Rats	GLP	CDER
(b) (4) 009/04-001	Comparison of Local and Systemic Drug Release and Kinetics from Three Collagen-Based Drug Delivery Systems in Laboratory Dogs	GLP	CDER
(b) (4) 009/05-001	Comparison of Local and Systemic Drug Release and Kinetics from Collagen-Based Drug Delivery Systems Containing Gentamicin, Bupivacaine and Ciprofloxacin in Laboratory Dogs	GLP	CDER
4.2.2.7 Other Pharmacokinetic Studies			
(b) (4) 36	Content Uniformity of 100 mg Bupivacaine HCl within a 75 mg Collagen Matrix 5 cm x 5 cm	nonGLP	CDER
4.2.3 Toxicology			
4.2.3.1 Single Dose Toxicity			
Rat Subcutaneous			
(b) (4)-134501	An Ascending-Dose Study of Bupivacaine Collagen Implant with a 14-Day Observation Period in Sprague Dawley Rats	nonGLP	CDER
(b) (4)-134502	A 56-Day Bupivacaine Collagen Implant Study in Sprague Dawley Rats	GLP	CDER
4.2.3.7 Other Toxicity Studies			
4.2.3.7.7 Other			
January 26, 2018 (b) (4)	Safety Assessment of Leachables for INL-001 and its Container Closure System	NA	CDER

June 21, 2018 (b) (4)	Risk Assessment of Leachables from Xaracoll	NA	CDER
September 18, 2018 (b) (4)	Controlled Extraction Study Associated with (b) (4) Blister (b) (4) (Primary Packaging) Supporting a Collagen-Based Surgical Implant	NA	CDER

3.3 Previous Reviews Referenced

No previous reviews were referenced.

4 Pharmacology

No new pharmacology studies were submitted with this NDA. The pharmacology of bupivacaine is well characterized. According to the reference product Marcaine label:

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. In general, the progression of anesthesia is related to the diameter, myelination, and conduction velocity of affected nerve fibers. Clinically, the order of loss of nerve function is as follows: (1) pain, (2) temperature, (3) touch, (4) proprioception, and (5) skeletal muscle tone.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study title: A Study to Investigate the Rate of Drug Release from a Bupivacaine-Collagen Implant in Male Beagle Dogs

Study no.: 134508
Study report location: <\\cdsesub1\evsprod\nda209511\0000\m4\42-stud-rep\422-pk\4222-absorp\134508-134508.pdf> (b) (4)
Conducting laboratory and location: (b) (4)
Date of study initiation: January 20, 2016
GLP compliance: No
QA statement: No
Drug lot #, and % purity: Bupivacaine-collagen implant, Lot **14021606**, not the "to be marketed" drug product (exp. date: 30-Jun-2017)

Key Study Findings

The objective of the study was to evaluate the drug release profile of a bupivacaine collagen implant following surgical placement into the subcutaneous tissue of dogs via a midline abdominal incision.

- Residual implant material was retrieved after surgical implantation and evaluated for bupivacaine levels. Bupivacaine-collagen implants (i.e., bupivacaine sponges) were administered once surgically to 1 group of male Beagle dogs. Group 1 consisted of 8 males, and 2 males were implanted with a sponge each week over a total of 4 weeks. The implant was placed on top of the abdominal incision and underneath the skin. Each animal was surgically implanted with one-half of a sponge, each 2.5 cm x 5 cm (nominal bupivacaine dose level of 50 mg). Animals were euthanized at 0.25, 0.5, 1, 2, 4, 8, 12, or 24 hours after surgery on Study Day 0 for collection of the sponges and subsequent evaluation of residual bupivacaine.

Residual Bupivacaine HCl Content in Collagen Sponge Implant Samples

Animal Number	Collection Time on Study Day 0 (Hours Post-Dose)	Bupivacaine Concentration (ng/mL)	Residual Bupivacaine Content (mg)	Residual Bupivacaine HCl Content (mg)
6883	0.25	1,150,000	23.0	25.9
5446	0.5	1,020,000	20.4	23.0
5496	1	946,000	18.9	21.3
7427	2	638,000	12.8	14.4
6559	4	595,000	11.9	13.4
8069	8	427,000	8.5	9.6
8457	12	213,000	4.3	4.8
5316	24	21,200	0.4	0.5

- Following surgical implantation of bupivacaine sponges into male Beagle dogs via a midline abdominal incision, residual implant material was retrieved at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after implantation and subsequently evaluated for bupivacaine HCl content. Bupivacaine HCl was released from the sponges over time with approximately 48.2% released after 15 minutes (0.25 hour), 57.4% released after 1 hour, 73.2% released after 4 hours, 80.8% released after 8 hours, and 99.0% after 24 hours following implantation.

Methods

Doses: Bupivacaine HCl dose ranged from 4.1-6.0 mg/kg.

Frequency of dosing: Single dose

Route of administration: Subcutaneous surgical implant

Dose volume: Each animal was surgically implanted with one-half of a sponge, each 2.5 cm x 5 cm (nominal bupivacaine dose level of 50 mg).

Formulation/Vehicle: The test article was a white to off-white porous 5 cm x 5 cm (5 mm thick) sponge with a nominal concentration of 100 mg of bupivacaine HCl (b) (4)

Species/Strain: Male Beagle dogs

Number/Sex/Group: The number of animals used on study was the minimum number required to yield scientifically meaningful data to accomplish the goals of this study.

Age: 6-10 months old

Weight: 8.3-12.2 kg at the initiation of dose administration

Satellite groups: None

Unique study design: Assessment of the residual implants was conducted by using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC/MS/MS) analysis.

Deviation from study protocol: Actual ages ranged from 9 to 27 months

Observations and Results

Mortality

All animals were observed twice daily for mortality.

No mortality was reported.

Clinical Signs

All animals were observed twice daily for moribundity. Clinical examinations were performed once daily for at least 6 consecutive days during acclimation, prior to dosing, and 1-2 hours post-dosing. Detailed physical examinations were performed on Study Days -5 and 0.

There were no clinical observations associated with implantation of the bupivacaine sponges.

Body Weights

Individual body weights were recorded on Study Day 0 (prior to dosing). Body weight data were collected for administration of anesthesia only and all dogs received the same dose (50 mg bupivacaine HCl in one-half of a sponge).

No Food Consumption, Ophthalmoscopy, ECG, Hematology, Clinical Chemistry, Urinalysis, Gross Pathology, Organ Weights, Histopathology, and Toxicokinetic evaluations were conducted.

Special Evaluation

A single animal per time point was euthanized at 0.25, 0.5, 1, 2, 4, 8, 12, or 24 hours after surgery for bupivacaine-collagen implant collection and subsequent evaluation of residual bupivacaine.

Residual Bupivacaine HCl Content in Collagen Sponge Implant Samples

Animal Number	Collection Time on Study Day 0 (Hours Post-Dose)	Bupivacaine Concentration (ng/mL)	Residual Bupivacaine Content (mg)	Residual Bupivacaine HCl Content (mg)
6883	0.25	1,150,000	23.0	25.9
5446	0.5	1,020,000	20.4	23.0
5496	1	946,000	18.9	21.3
7427	2	638,000	12.8	14.4
6559	4	595,000	11.9	13.4
8069	8	427,000	8.5	9.6
8457	12	213,000	4.3	4.8
5316	24	21,200	0.4	0.5

Following surgical implantation of bupivacaine sponges into male Beagle dogs via a midline abdominal incision, residual implant material was retrieved at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after implantation and subsequently evaluated for bupivacaine HCl content. Bupivacaine HCl was released from the sponges over time with approximately 48.2% released after 15 minutes, 57.4% released after 1 hour, 73.2% released after 4 hours, 80.8% released after 8 hours, and 99.0% after 24 hours following implantation.

Toxicokinetics

Blood samples for possible toxicokinetic evaluation were collected from all surviving animals at 0.5, 1, 2, 4, 8, 12, 18, and 24 hours after dose administration on StudyDay 0. **Blood samples were not evaluated.**

Dosing Solution Analysis

No assessments of formulation homogeneity, stability, or concentrations were conducted due to the test article being a finished product, which was labeled with storage information and expiration date.

5.2 Toxicokinetics

Study title: An Acute Single Dose Toxicokinetic Study of Bupivacaine Collagen Implant in Sprague Dawley Rats

Study no.: 00134510
 Study report location: <\\cdsesub1\evsprod\nda209511\0008\m4\42-stud-rep\422-pk\4222-absorp\134510\134510.pdf>
 Conducting laboratory and location: (b) (4)
 Date of study initiation: June 1, 2017
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Xaracoll, Lot 15041002, the “to be marketed” drug product, Exp. date: Feb 2020 [ID No. 170118], purity not reported
 Marcaine 0.5%, Lot 73265DD, Exp. date: 01 Jan 2019 [ID No. 170162], purity not reported

Key Study Findings

- The objective of this study was to evaluate the toxicokinetic profile of a bupivacaine collagen implant over a 6-day post-surgical period following surgical placement into both the peritoneal cavity and subcutaneous tissue of Sprague Dawley rats via a midline abdominal incision. In addition, residual implant material was retrieved at 24, 48, 72, 96, and 144 hours after dosing and evaluated for bupivacaine levels. The test article is intended to be used as a single-dose implant in surgical incisions for relief of post-surgical pain.

Study Group Assignment

Group No.	Treatment	Dose (mg)* Males/Females	Dose (mg/kg)** Males/Females	No. of Animals	
				Males	Females
1	Bupivacaine Injection	6/4	15.3/16.1	6	6
2	Bupivacaine Collagen Sponge	12/8	31.0/33.0	15	15
3	Bupivacaine Collagen Sponge	24/12	61.9/49.1	15	15

* = As the hydrochloride (HCl) salt

** = Calculated based on mean body weight of animals on Study Day 0

- Following surgical infiltration or implantation of bupivacaine, systemic exposure of bupivacaine was observed at all dose levels. Mortality was noted for males (3) and females (1) in the high-dose bupivacaine sponge group. These deaths were likely due to bupivacaine toxicity. Exposure based on $AUC_{(0-t)}$, increased in a roughly dose-proportional fashion across all doses for the bupivacaine injectable and bupivacaine sponges, and there were no notable differences in exposure between male and female rats. The increase in C_{max} for bupivacaine injectable compared to either dose of bupivacaine sponge was less than dose-proportional. In general,

overall exposure in males and females was similar (within approximately 2-fold) in terms of $AUC_{(0-t)}/D$ for the bupivacaine sponge and bupivacaine injectable. The C_{max}/D values for males and females were consistently lower for both dose levels of bupivacaine sponge compared to bupivacaine injectable even though the bupivacaine sponge doses were higher.

- Of the 24 sponges collected at 24 hours after implantation, residual bupivacaine was measured in 5 animals (6 sponges) across the 2 bupivacaine sponge groups. Residual bupivacaine was not observed in any implant after the 24-hour time point. In most cases, these sponge concentrations were > 2-fold lower than the observed C_{max} in the respective groups with an exception of 1 of the internal sponges recovered from 1 male in the 12 mg dose group, which had a residual concentration that was 4.7-fold higher than the mean C_{max} for this group. However, this animal's individual bupivacaine plasma concentrations were in agreement with concentrations from other animals at the same time points within the group. The T_{max} was observed at 0.5 hours postdose and $T_{1/2}$ ranged from 1.42 to 1.61 hours in the injectable bupivacaine group and T_{max} was observed at 1 hour postdose and $T_{1/2}$ ranged from 2.06 to 5.30 hours in the bupivacaine sponge groups.

Summary of Toxicokinetic Parameters

Analyte	Treatment ID	Dose (mg/kg)	Gender	AUC(0-t) (h*ng/mL)	AUC (0-t)/D	C _{max} (ng/mL)	C _{max} /D	T _{max} (h)	T _{1/2} (h)
Bupivacaine	1	6	Male	2710	452	935	156	0.5	1.42
		4	Female	2410	603	1100	275	0.5	1.61
	2	12	Male	5840	487	773	64.4	1	4.65
		8	Female	6080	760	1250	156	1	NR
	3	24	Male	12200	509	1450	60.5	1	5.30
		12	Female	8900	742	1730	144	1	2.06

NR = Not reportable

Doses:

Study Group Assignment

Group No.	Treatment	Dose (mg)*	Dose (mg/kg)**	No. of Animals	
		Males/Females	Males/Females	Males	Females
1	Bupivacaine Injection	6/4	15.3/16.1	6	6
2	Bupivacaine Collagen Sponge	12/8	31.0/33.0	15	15
3	Bupivacaine Collagen Sponge	24/12	61.9/49.1	15	15

* = As the hydrochloride (HCl) salt

** = Calculated based on mean body weight of animals on Study Day 0

Frequency of dosing: Once
 Route of administration: Group 1 animals were dosed by injection into the muscle and subcutaneous tissue around the incision. Group 2–3 animals had 1 sponge placed within the peritoneal cavity and 1 in the subcutaneous area of the surgical incision.
 Dose volume: Not applicable (see dose area in table).

Formulation/Vehicle: Group 1 – not reported (likely 0.9% saline). Groups 2 & 3 – bupivacaine collagen sponge. Details of composition not reported in study report.

Species/Strain: Crl:CD(SD) male and female rats

Number/Sex/Group: 6/sex in the comparator group and 15/sex in the test article-treated groups

Age: Approximately 67 days old at receipt

Weight: Not reported

Satellite groups: None

Unique study design: No

Deviation from study protocol: Nothing significant

Study Design

Group Number	Treatment ^a	Dose Area/Sponge (cm ²) M/F	Total Dose Area (cm ²) M/F	Nominal Bupivacaine HCl Dose (mg) M/F	Number of Animals ^b	
					Males	Females
1	Bupivacaine HCl injectable	NA	NA	6/4 ^c	6	6
2	Bupivacaine sponge	1.5/1	3/2	12/8	15	15
3	Bupivacaine sponge	3/1.5	6/3	24/12	15	15

^a Group 1 animals were dosed by infiltration into the muscle and subcutaneous tissue around the incision while Group 2 and 3 animals had 1 bupivacaine sponge placed within the peritoneal cavity and 1 in the subcutaneous area of the surgical incision.

^b Group 1 animals were euthanized and discarded following the final blood collection (Study Day 1). Group 2 and 3 animals (3/sex/group) were assigned to necropsy at 24, 48, 72, 96, and 144 hours (± 2 hours) after dosing for implant retrieval and subsequent analysis for residual bupivacaine.

^c Group 1 males received 1.2 mL and females received 0.8 mL of bupivacaine HCl (5 mg/mL).

Study Group Assignment

Group No.	Treatment	Dose (mg)* Males/Females	Dose (mg/kg)** Males/Females	No. of Animals	
				Males	Females
1	Bupivacaine Injection	6/4	15.3/16.1	6	6
2	Bupivacaine Collagen Sponge	12/8	31.0/33.0	15	15
3	Bupivacaine Collagen Sponge	24/12	61.9/49.1	15	15

* = As the hydrochloride (HCl) salt

** = Calculated based on mean body weight of animals on Study Day 0

The dose levels on this study were selected based on another study where the NOAEL was 24 mg in males and 8 mg in females. Clinical signs were limited to those associated with systemic bupivacaine toxicity.

Surgery

Group 1: Group 1 had bupivacaine injectable infiltrated into the abdominal wall

(musculature) and subcutaneous tissues (see Appendix 1 - Study Protocol and Deviations). This was done after the abdominal cavity was closed but before the skin was closed. The bupivacaine was injected into both sides of the incision parallel to the tissue plane using approximately 3 injections/side in each layer (musculature and subcutaneous) as needed until the total volume was delivered. Group 1 males and females received 1.2 and 0.8 mL of injectable bupivacaine, respectively.

Group 2: Group 2 males received 2 bupivacaine sponges, each 1 cm x 1.5 cm, while the Group 2 females received 2 bupivacaine sponges, each 1 cm x 1 cm each.

Group 3: Group 3 males received 2 bupivacaine sponges, each 2 cm x 1.5 cm, while Group 3 females received 2 bupivacaine sponges, 1 cm x 1.5 cm each.

Group 2 and 3 surgery: Total doses were the same for all animals per sex within a group and were determined by the size of the sponge implanted. The first bupivacaine sponge was implanted within the abdominal cavity and held in place by incorporating the implant into the first layer of sutures so that it laid against the peritoneum under the incision. The abdominal cavity was closed using 4-0 vicryl in a simple interrupted pattern. A second sponge of the same size was placed on top of the abdominal incision and beneath the skin. This sponge was tacked at the corners with 4-0 vicryl to keep it in place before the skin was closed with 9 mm wound clips. The surgeries were staggered over 4 days. The day of dosing/surgery was Study Day 0.

Observations and Results

Food consumption, ophthalmoscopy, ECG, hematology, clinical chemistry, urinalysis, organ weights, and histopathology evaluations were not conducted in this toxicokinetic study.

Mortality

All animals were observed twice daily, once in the morning and once in the afternoon, for mortality. Animals found dead were examined macroscopically as soon as possible to remove the residual sponges.

Three males and 1 female in the high-dose bupivacaine sponge group were found dead during the surgical recovery period, within approximately 1.5 hours of implantation. Clinical findings for these animals included tremors, pale body and extremities. Clear material around the mouth in one male. The only remarkable macroscopic finding for these animals was foamy contents in the trachea for one male. These deaths were reported to likely be due to bupivacaine toxicity. All other animals survived to the scheduled euthanasia.

Clinical Signs

All animals were observed twice daily, once in the morning and once in the afternoon, for moribundity. Clinical examinations were performed at the time of dosing/surgery, 2–3 hours following dosing/surgery, and daily during the study period. The absence or presence of findings was recorded for individual animals at the scheduled intervals. Detailed physical examinations were conducted on all animals within 4 days of receipt, on the day of randomization, on Study Day 0 (prior to dosing), and on the day of the scheduled necropsy.

Increased incidences of dilated pupils were noted for males and females in the high-dose bupivacaine sponge group at 2–3 hours postdosing. Tremors were noted during the surgical recovery period for 1 male and 1 female in the high-dose bupivacaine sponge group, including one male that was found dead. Hunched posture was noted for 1 female in thigh-dose sponge group. From the detailed physical examinations and daily observations, it was noted that findings of yellow material on the urogenital and anogenital areas, ventral trunk, and/or hindlimbs were noted for males and females in the low- and high-dose bupivacaine sponge groups. These findings were generally noted on the day after surgery/dosing. These findings were attributed to the test article. No other test article-related clinical findings were noted. All other clinical findings in the test article-treated groups were noted with similar incidence in the comparator group, were limited to single animals, were not noted in a dose-related manner, and/or were common findings for laboratory rats of this age and strain.

Body Weights

Body weights were only taken for dosing purposes (1 dose).

Mean body weights (standard deviation) were 391 (14.3), 387 (9.8), & 388 g (10.8) for males and 249(6.8), 242 (10.9) & 244 (7.2) for females for Groups 1, 2, & 3, respectively, indicating comparable dosing weights.

Gross Pathology (animals found dead only)

A complete necropsy was conducted on all animals found dead. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. Group 1 animals were euthanized after the final blood collection (Study Day 1) and discarded without examination. Group 2 and 3 animals were euthanized at 24, 48, 72, 96, and 144 hours after dosing (± 2 hours), and the remnants of both implants were retained and weighed. Carcasses were then discarded without further examination.

At the time of the scheduled euthanasia, the internal sponges of 19/56 animals were displaced within the abdominal cavity. Sponge degradation and bupivacaine absorption in the peritoneal cavity would not be affected since the sponge would still have been in contact with the peritoneum and abdominal organs. Review of the bioanalytical sponge data revealed that only 2 of these displaced sponges (from a 24-hour necropsy animal)

had any residual bupivacaine. There were no apparent differences in bupivacaine sponge concentrations between the internal sponges that remained attached to the incision and those that were displaced. There were no apparent differences in plasma bupivacaine levels as a result of the internal sponge becoming displaced.

Toxicokinetics

For Group 1, blood samples for toxicokinetics (approximately 500 mcL/time point) were collected prior to dosing and at 0.083, 0.25, 0.5, 1, 4, 8, 12, and 24 hours after dose administration on Study Day 0 (day of dosing). For Groups 2 and 3, blood samples for toxicokinetics were collected prior to dosing/surgery and at 0.083, 0.25, 0.5, 1, 4, 8, 12, 24, 36, 48, 72, and 96 hours after dose administration on Study Day 0. For Group 1, blood was collected from 3 animals/sex at each time point. For Groups 2 and 3, blood was collected from ≤ 6 animals/sex/group at each time point.

Toxicokinetic values were as follows:

Toxicokinetic Parameters for Bupivacaine in Rats

Analyte	Group	Dose (mg)	Gender	AUC(0-t) (h*ng/mL)	AUC(0-t)/D	C _{max} (ng/mL)	C _{max} /D	T _{max} (h)	T _{1/2} (h)
Bupivacaine	1	6	Male	2710	452	935	156	0.5	1.42
		4	Female	2410	603	1100	275	0.5	1.61
	2	12	Male	5840	487	773	64.4	1	4.65
		8	Female	6080	760	1250	156	1	NR
	3	24	Male	12,200	509	1450	60.5	1	5.30
		12	Female	8900	742	1730	144	1	2.06

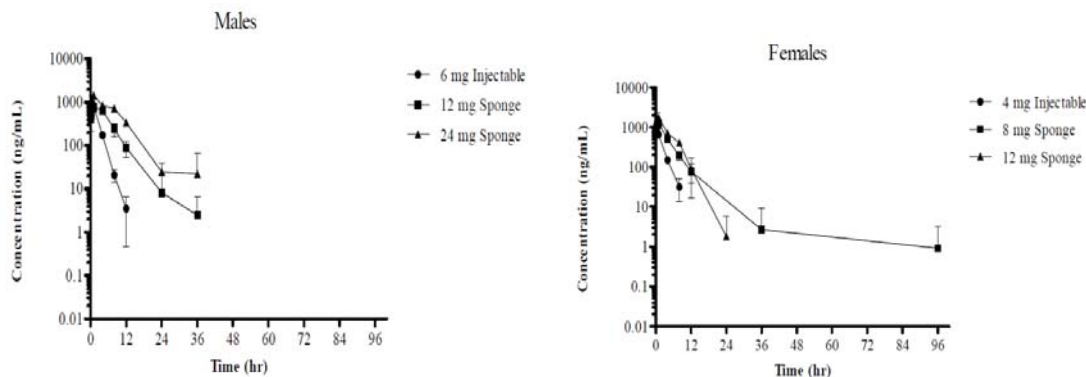
NR = Not reportable

Pre-dose concentrations of bupivacaine were not measurable in any group. Following intramuscular/subcutaneous injection and surgical implantation of bupivacaine, systemic exposure for bupivacaine was observed at all dose levels. Exposure for bupivacaine increased as bupivacaine collagen sponge dose increased from 12 to 24 mg in males and 8 to 12 mg in females in a dose-proportional manner, and there were no notable differences in exposure between male and female rats. Exposure based on AUC_(0-t), increased in a roughly dose-proportional fashion across all doses for the bupivacaine injectable and bupivacaine sponges. The increase in C_{max} for bupivacaine injectable compared to either dose of bupivacaine sponge was less than dose-proportional.

In general, overall exposure in males and females was similar (within approximately 2-fold) in terms of AUC_(0-t)/D for the bupivacaine sponge and bupivacaine injectable. The C_{max}/D values for males and females were consistently lower for both dose levels of bupivacaine sponge compared to bupivacaine injectable even though the bupivacaine sponge doses were higher.

The T_{max} was observed at 0.5 hours postdose and T_{1/2} ranged from 1.42 to 1.61 hours in the injectable bupivacaine group and T_{max} was observed at 1-hour postdose and T_{1/2} ranged from 2.06 to 5.30 hours in the bupivacaine sponge groups. The majority of the

bupivacaine was eliminated from the plasma by 24 hours (see figures) as it was from the sponge.



Evaluation of Residual Bupivacaine

Group 2 and 3 animals (≤ 3 /sex/timepoint) were euthanized at 24, 48, 72, 96, and 144 hours (± 2 hours) after dosing/surgery. The remnants of both implants were collected.

Of the 24 sponges collected at 24 hours after implantation, residual bupivacaine was measured in 5 animals (6 sponges) across the 2 bupivacaine sponge groups; internal sponge for 1 Group 2 female, internal sponge for 2 Group 2 males, and external sponge for 1 Group 2 and 2 Group 3 males. Residual bupivacaine was not observed in any implant after the 24-hour time point. In most cases, these sponge concentrations were > 2 -fold lower than the observed C_{max} in the respective groups with an exception of 1 of the internal sponges recovered from 1 male in the 12 mg dose group, which had a residual concentration that was 4.7-fold higher than the mean C_{max} for this group. However, this animal's individual bupivacaine plasma concentrations were in agreement with concentrations from other animals at the same time points within the group.

Residual Bupivacaine HCl Content in Collagen Sponge Implant Samples

Animal Number	Collection Time on Study Day 0 (Hours Post-Dose)	Bupivacaine Concentration (ng/mL)	Residual Bupivacaine Content (mg)	Residual Bupivacaine HCl Content (mg)
6883	0.25	1,150,000	23.0	25.9
5446	0.5	1,020,000	20.4	23.0
5496	1	946,000	18.9	21.3
7427	2	638,000	12.8	14.4
6559	4	595,000	11.9	13.4
8069	8	427,000	8.5	9.6
8457	12	213,000	4.3	4.8
5316	24	21,200	0.4	0.5

Dosing Solution Analysis

No assessments of stability, concentration, or sample homogeneity were conducted because these are finished products and concentration and stability were provided by the Sponsor.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: A 56-Day Bupivacaine Collagen Implant Toxicity Study in Sprague Dawley Rats

Study no.:	00134509
Study report location:	\\cdsesub1\evsprod\nda209511\0008\m4\42-stud-rep\423-tox\4231-single-dose-tox\134509\134509.pdf
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	May 18, 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- Xaracoll, Lot 15041002, the “to be marketed” drug product, Exp. date: Feb 2020 [ID No. 170118], purity not reported - Marcaine 0.5%, Lot 73265DD, Exp. date: 01 Jan 2019 [ID No. 170162], purity not reported - Xaracoll Placebo Sponge, Lot 15020502, Exp. date: 31 Mar 2018, [ID No. 170117], purity not reported - 0.9% sodium chloride for injection, USP (Lot 66-351-DK), purity not reported

Key Study Findings

This study was intended to evaluate the local and systemic tolerability of a bupivacaine collagen implant over a 56-day post-surgical period following surgical placement into both the peritoneal cavity and subcutaneous tissue of Sprague Dawley rats via a midline abdominal incision. The test article is proposed to be used as a single-dose implant in surgical incisions for relief of post-surgical pain. The “to-be-marketed” drug product was tested. Key findings are summarized below:

- Surgical implantation of bupivacaine sponges to Crl:CD(SD) rats at nominal dose levels of 12 and 24 mg bupivacaine HCl (mean dose of 30.8 and 61.4 mg/kg) for males and 8 and 12 mg bupivacaine HCl (mean dose of 32.3 and 48.4 mg/kg) for females was generally well tolerated.
- Mortality, food consumption, and body weight were unaffected by treatment. Blood samples for clinical pathology evaluations (hematology, coagulation, and

serum chemistry) were collected at each scheduled necropsy from all animals assigned to the scheduled necropsy (Study Days 3, 14, 28, 35 [placebo collagen sponge group], and 56).

- Based on gross and microscopic findings, there was good local and systemic tolerability of bupivacaine collagen implants over a 56-day post-surgical period, with no notable impact observed on wound healing. Note that wound healing endpoints were limited and no tensile strength assessments were included. No systemic toxicity was noted. Local clinical observations of increased incidence of edema (very slight to severe) were noted for males and females in the high-dose bupivacaine sponge group. No gross findings at the incision were reported at necropsy.
- There was an increased severity, but not incidence, of coagulative necrosis of the abdominal wall muscle in both males and females in the low- and high-dose bupivacaine sponge groups on Study Day 3 compared to each control group, as well as a slightly increased severity, but not incidence, of fibroplasia and mononuclear cell infiltration at the incision site in the collagen sponge and low- and high-dose bupivacaine sponge groups on Study Day 14. At subsequent time points, there was no difference in coagulative necrosis, fibroplasia and/or fibrosis, or mononuclear cell infiltration between groups, reflecting a healing process with recovery and thus no difference in outcome at later time points (i.e., no adverse impact on wound healing).
- The quantity of collagen sponge material progressively decreased with increasing time as part of the healing process but attrition rate was greater at the external implant site when compared to the internal implant site. No collagen sponge material could be microscopically discerned via evaluation of routine H&E-stained tissues at Day 56 at any external or internal implantation site in Groups 3-5.
- The lowest-observed-adverse-effect-level (LOAEL) is the low dose of 12 mg bupivacaine (30.8 mg/kg - 6 cm² total bupivacaine collagen sponge dose) for males and 8 mg bupivacaine (32.3 mg/kg - 3 cm² total bupivacaine collagen sponge dose) for females.

Methods

Doses:

Dose Concentration

Group Number	Treatment	Dose Area/Sponge (cm ²) M/F	Total Dose Area (cm ²) M/F	Nominal Bupivacaine HCl Dose (mg) M/F
1	0.9% Saline	NA	NA	0
2	Bupivacaine HCl injectable ^a	NA	NA	6/4
3	Collagen sponge ^b	3/1.5	6/3	0
4	Bupivacaine sponge ^b	1.5/1	3/2	12/8
5	Bupivacaine sponge ^b	3/1.5	6/3	24/12

NA = Not applicable; M = Male; F = Female

^a Group 1 and 2 animals were dosed by injection into the muscle and subcutaneous tissue around the incision.^b Group 3–5 animals had 1 sponge placed within the peritoneal cavity and 1 in the subcutaneous area of the surgical incision.

Mean Bupivacaine HCl Dose (mg/kg) M/F
0/0
15.5/16.3
0/0
30.8/32.3
61.4/48.4

Frequency of dosing:	Single dose
Route of administration:	Injection or implant in surgical incisions
Dose volume:	Groups 1 & 2 injections (1.2 and 0.8 mL for males and females, respectively). Groups 3, 4, & 5, not applicable (NA) as received sponges.
Formulation/Vehicle:	Groups 1 & 2 saline. Groups 3, 4, & 5, NA.
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	12/sex except Group 3 (placebo collagen sponge) at 15/sex
Age:	Approximately 66 days old at receipt followed by a minimum 13-day acclimation period
Weight:	364-420 g (male), 277-318 g (female)
Satellite groups:	No
Unique study design:	Group 1 and 2 animals were dosed by injection into the muscle and subcutaneous tissue around the incision. Group 3–5 animals had 1 sponge placed within the peritoneal cavity and 1 in the subcutaneous area of the surgical incision.
Deviation from study protocol:	Nothing remarkable

Study Method

Each group consisted of 12 rats/sex, with the exception of Group 3, which consisted of 15 rats/sex. Animals (≤ 3 /sex/time point) were euthanized at 3, 14, 28, 35 (Group 3 only), and 56 days after dosing.

Bupivacaine collagen implants (also referred to as bupivacaine sponges) were administered once surgically to 2 groups (Groups 4 and 5) of rats. Each animal was surgically implanted with 2 bupivacaine sponges of equal size via an abdominal incision, 1 placed and secured within the abdominal cavity (incorporated into the abdominal closure) and 1 placed and secured within the subcutaneous tissues. The nominal bupivacaine HCl dose levels were 12 and 24 mg (mean dose of 30.8 and 61.4 mg/kg) for Group 4 and 5 males, respectively, and 8 and 12 mg (mean dose of 32.3 and 48.4 mg/kg) for Group 4 and 5 females, respectively.

The comparator article, bupivacaine HCl injectable, was administered via infiltration once into the abdominal wall (musculature) and subcutaneous tissues to Group 2. Placebo collagen sponge implants were administered once surgically to Group 3 animals in a manner similar to Groups 4 and 5. The 0.9% saline control, was administered via infiltration, once into the abdominal wall (musculature) and subcutaneous tissues to Group 1.

Group 4 males received 2 bupivacaine sponges, each 1 cm x 1.5 cm, while the Group 4 females received 2 bupivacaine sponges, each 1 cm x 1 cm. Group 3 and 5 males received 2 bupivacaine sponges, each 2 cm x 1.5 cm, while Group 3 and 5 females received 2 bupivacaine sponges, 1 cm x 1.5 cm each.

Group 1 and 2 animals received 1.2 mL (males) or 0.8 mL (females) of 0.9% saline (Group 1) or injectable bupivacaine HCl (Group 2; mean dose of 15.5 and 16.3 mg/kg), respectively.

Observations and Results

Mortality

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

There were no treatment related fatalities.

One unscheduled euthanasia occurred in one female rat in Group 3 was found in extremis on Study Day 0 with a portion of jejunum protruding outside of the abdominal cavity due to self-evisceration at the surgery site. Histologic findings were unremarkable. The cause of death is unscheduled euthanasia subsequent to self-trauma of the surgical site resulting in evisceration and is not related to the collagen sponge.

One animal was found dead during the course of the toxicity study. One female rat in Group 2 was found dead on Study Day 24 following an accidental fatal crushing injury after escape from her cage and entrapment behind a bank of cages. The cause of death is trauma and is not related to bupivacaine injection.

All other animals survived to the scheduled necropsies.

Clinical Signs

Detailed physical examinations were conducted on all animals within 4 days of receipt, on the day of randomization, Study Day 0, weekly (± 2 days) during the study period, and on the days of the scheduled necropsies. All animals were observed twice daily, once in the morning and once in the afternoon, for moribundity. Clinical examinations were performed at the time of dosing/surgery, 2–3 hours following dosing/surgery, and daily during the study period.

There were no test article-related clinical observations. Observations of swollen facial area were related to the use and fit of Elizabethan collars to prevent the rats from reaching their incisions. All other clinical observations in the test article-treated groups were noted with similar incidence across groups, were limited to single animals, were not noted in a dose-related manner, and/or were common findings for laboratory rats of this age and strain.

Surgical Site Observations

Surgical sites were examined on Study Days 1–10 and occasionally longer for erythema, edema, and other dermal findings. Erythema and edema were evaluated in accordance with the method of Draize based on a 4-step grading system of very slight, slight, moderate, and severe. No formal wound healing observation techniques were employed (e.g., tensile strength assessment).

In the following two tables (males and females), total overall incidences of erythema and edema and total number of animals involved were reported. No other wound healing evaluations were conducted. Observations were apparently not always adequate to identify reversibility (see examples later).

(SURGICAL SITE OBSERVATIONS - MALES)
 PROJECT NO.: 00134509M A BUPIVACAINE COLLAGEN IMPLANT STUDY IN RATS
 SPONSOR: INNOCOLL TECHNOLOGIES SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS

----- M A L E -----

TABLE RANGE: GROUP:	1	06-20-17 TO 07-02-17 2	3	4	5
DERMAL OBS					
-SCORED, NOT REMARKABLE	94/12	88/12	124/15	90/12	77/12
-NO ERYTHEMA	5/ 2	11/ 5	5/ 2	9/ 3	19/ 7
-ERYTHEMA - VERY SLIGHT	0/ 0	0/ 0	0/ 0	0/ 0	3/ 3
-NO EDEMA	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
-EDEMA - VERY SLIGHT	2/ 2	8/ 4	2/ 1	6/ 2	12/ 5
-EDEMA - SLIGHT	2/ 1	3/ 3	2/ 2	2/ 2	4/ 4
-EDEMA - MODERATE	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
-EDEMA - SEVERE	1/ 1	0/ 0	1/ 1	1/ 1	4/ 4
1- SALINE	2- BUP INJECT/6	3-COLLAGEN SPONGE	4- BUP SPONGE/12	5- BUP SPONGE/24	

(SURGICAL SITE OBSERVATIONS - FEMALES)
 PROJECT NO.: 00134509F A BUPIVACAINE COLLAGEN IMPLANT STUDY IN RATS
 SPONSOR: INNOCOLL TECHNOLOGIES SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS

----- F E M A L E -----

TABLE RANGE: GROUP:	1	06-20-17 TO 07-02-17 2	3	4	5
DERMAL OBS					
-SCORED, NOT REMARKABLE	91/12	74/12	122/15	90/12	75/12
-NO ERYTHEMA	10/ 2	24/ 5	5/ 2	8/ 4	21/ 7
-ERYTHEMA - VERY SLIGHT	0/ 0	1/ 1	0/ 0	1/ 1	3/ 2
-ERYTHEMA - SLIGHT	0/ 0	0/ 0	2/ 2	0/ 0	0/ 0
-NO EDEMA	0/ 0	1/ 1	0/ 0	1/ 1	0/ 0
-EDEMA - VERY SLIGHT	6/ 2	8/ 4	4/ 2	4/ 2	17/ 7
-EDEMA - SLIGHT	2/ 1	10/ 5	2/ 2	3/ 3	2/ 2
-EDEMA - MODERATE	0/ 0	3/ 3	1/ 1	0/ 0	1/ 1
-EDEMA - SEVERE	2/ 2	3/ 3	0/ 0	1/ 1	4/ 4
1- SALINE	2- BUP INJECT/4	3-COLLAGEN SPONGE	4- BUP SPONGE/8	5- BUP SPONGE/12	

Very slight erythema was observed in high dose bupivacaine sponge males thru 10 days. Very slight erythema was observed in bupivacaine, and low- and high-dose bupivacaine sponge females thru 10 days.

Generally increased incidences of edema (very slight to severe) at the incision site were noted for males and females in the high-dose bupivacaine sponge group when compared to the saline control and placebo collagen implant groups, but all groups exhibited edema, even the saline and sponge only suggesting that the response was a combination of surgery and treatment. Increased incidence and severity was considered test article-related and likely associated with the bupivacaine.

With a set ~10⁺ day observation period after surgery, reversibility of effects was not

always determined. For example, high-dose bupivacaine sponge Male 2092's 12th day and Female 2184's 13th day, was 6-29-17 and 6-30-17, respectively, had effects still observable.

2092	M	BUP SPONGE/24	DERMAL OBS	06-27-17	8:22	P	SCORED, NOT REMARKABLE
				06-28-17	9:51	P	SCORED, NOT REMARKABLE
				06-29-17	15:14	P	ERYTHEMA - VERY SLIGHT
				06-29-17	15:14	P	EDEMA - VERY SLIGHT
2184	F	BUP SPONGE/12	DERMAL OBS	06-26-17	13:35	P	NO ERYTHEMA
				06-26-17	13:35	P	EDEMA - VERY SLIGHT
				06-27-17	8:28	P	SCORED, NOT REMARKABLE
				06-28-17	9:57	P	EDEMA - VERY SLIGHT
				06-28-17	9:57	P	NO ERYTHEMA
				06-29-17	15:21	P	SCORED, NOT REMARKABLE
				06-30-17	8:59	P	NO ERYTHEMA
				06-30-17	8:59	P	EDEMA - SEVERE

In addition, a high-dose bupivacaine sponge female last two (2) days scores (Days 13 & 14 showed remarkable but possibly questionable recovery from severe edema to not remarkable in one day.

2173	F	BUP SPONGE/12	DERMAL OBS	06-22-17	9:41	P	SCORED, NOT REMARKABLE
				06-23-17	9:44	P	SCORED, NOT REMARKABLE
				06-24-17	8:12	P	SCORED, NOT REMARKABLE
				06-25-17	7:18	P	SCORED, NOT REMARKABLE
				06-26-17	13:35	P	SCORED, NOT REMARKABLE
				06-27-17	8:29	P	NO ERYTHEMA
				06-27-17	8:29	P	EDEMA - VERY SLIGHT
				06-28-17	9:58	P	EDEMA - VERY SLIGHT
				06-28-17	9:58	P	NO ERYTHEMA
				06-29-17	15:21	P	NO ERYTHEMA
				06-29-17	15:21	P	EDEMA - VERY SLIGHT
				06-30-17	9:00	P	NO ERYTHEMA
				06-30-17	9:00	P	EDEMA - SEVERE
				07-01-17	7:18	P	SCORED, NOT REMARKABLE

Swelling and/or discoloration of the incision site (noted as swollen abdominal area and abdomen blue, respectively) were noted in all groups, but the incidence was higher in the high-dose bupivacaine sponge group animals. These observations were noted shortly after surgery and were attributed to seromas (clear-yellowish serum fluid), which resolved over a period of 1 to 6 days. Seromas are sometimes observed after abdominal surgery and are of uncertain relationship to the test article since they were not observed in a previous toxicokinetic study.

There were no other potential bupivacaine sponge-related clinical observations. Observations of swollen facial area were related to the use and fit of Elizabethan collars to prevent the rats from reaching their incisions. All other clinical observations in the test article-treated groups were noted with similar incidence across groups, were limited to single animals, were not noted in a dose-related manner, and/or were common findings for laboratory rats of this age and strain.

Body Weights

Individual body weights were recorded within 4 days of receipt, on the day of randomization, Study Day 0, weekly ($\pm$ 2 days) during the study period, and on the

day prior to the first day of the scheduled necropsies (nonfasted). Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights (fasted) were recorded on the days of the scheduled necropsies.

Body weights and body weight changes were unaffected by bupivacaine sponge administration and there were no differences between all five (5) test groups.

Food Consumption

Cage food weights were recorded once weekly ($\pm$ 2 days) beginning following randomization and throughout the study period and also on Study Day 2. Food consumption was calculated as g/animal/day for the corresponding body weight intervals.

Food consumption was unaffected by bupivacaine sponge administration and there were no differences between all five (5) test groups.

Ophthalmoscopy - none

ECG - none

Clinical Pathology

Blood samples for clinical pathology evaluations (hematology, coagulation, and serum chemistry) were collected at each scheduled necropsy from all animals assigned to the scheduled necropsy (Study Days 3, 14, 28, 35 [placebo collagen sponge group], and 56). The animals were fasted overnight prior to blood collection.

Hematology and Coagulation

The following parameters were evaluated:

Total leukocyte count (WBC)	Mean platelet volume (MPV)
Erythrocyte count (RBC)	Differential leukocyte count -
Hemoglobin (HGB)	Percent and absolute
Hematocrit (HCT)	-Neutrophil (NEU)
Mean corpuscular volume (MCV)	-Lymphocyte (LYMPH)
Mean corpuscular hemoglobin (MCH)	-Monocyte (MONO)
Mean corpuscular hemoglobin concentration (MCHC)	-Eosinophil (EOS)
Platelet count (Platelet)	-Basophil (BASO)
Prothrombin time (PT)	-Large unstained cell (LUC)
Activated partial thromboplastin time (APTT)	Fibrinogen
Reticulocyte count	Red cell distribution width (RDW)
Percent (RETIC)	Platelet estimate ^a
Absolute (RETIC Absolute)	Red cell morphology (RBC Morphology) ^a

() Designates tabular abbreviation

^a Presented on individual tables if a manual differential was performed, and the manual data were accepted and reported instead of the automated differential data

There were no bupivacaine sponge-related alterations in hematology and coagulation parameters and there were no differences between all five (5) test

groups.

While there were some differences between test groups (bupivacaine, sponge, & bupivacaine sponge) and saline control, nothing was notable as changes were not observed at other time points, were only noted in a single sex, did not occur in a dose-related manner, and/or mean levels were reported to be within the (b) (4) historical control data (not provided).

Clinical Chemistry

The following parameters were evaluated:

Albumin	Total cholesterol (Cholesterol)
Total protein	Calcium
Globulin [by calculation]	Chloride
Albumin/globulin ratio (A/G Ratio) [by calculation]	Phosphorus
Total bilirubin (Total BILI)	Potassium
Urea nitrogen	Sodium
Creatinine	Sorbitol dehydrogenase (SDH)
Alkaline phosphatase (ALP)	Triglycerides (Triglyceride)
Alanine aminotransferase (ALT)	Creatine kinase (CK)
Aspartate aminotransferase (AST)	Lactate dehydrogenase (LDH)
Gamma glutamyltransferase (GGT)	Bicarbonate (HCO ₃)
Glucose	Appearance ^a

() Designates tabular abbreviation

^a Includes degree of hemolysis, lipemia, and icterus; presented on individual other chemistry data tables

There were no bupivacaine sponge-related alterations in clinical chemistry parameters and there were no differences between all five (5) test groups.

While there were some differences between test groups (bupivacaine, sponge, & bupivacaine sponge) and saline control, nothing was notable as changes were not observed at other time points, were only noted in a single sex, did not occur in a dose-related manner, and/or mean levels were within the (b) (4) historical control data.

Urinalysis - none

Gross Pathology

Animals (≤ 3/sex/time point) were euthanized at 3, 14, 28, 35 (Group 3 only), and 56 days after dosing. A complete necropsy was conducted on all animals. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted).

Adrenal glands Aorta Bone with marrow Femur Sternum Bone marrow smear (from femur) ^a Brain Cervix Diaphragm Epididymides ^b Eyes with optic nerve ^c Gastrointestinal tract Esophagus Stomach Duodenum Jejunum Ileum Cecum Colon Rectum Heart Incision site (included the abdominal wall and skin) Kidneys Larynx Liver (sections of 2 lobes) Lungs (including bronchi, fixed by inflation with fixative)	Lymph nodes Axillary Mandibular Mesenteric Ovaries with oviducts ^d Pancreas Peripheral nerve (sciatic) Peyer's patches Pharynx Pituitary Prostate Salivary glands (mandibular) Seminal vesicles Skeletal muscle (rectus femoris) Skin with mammary gland ^e Spinal cord (cervical, thoracic, lumbar) Spleen Testes ^b Thymus Thyroid (with parathyroids) ^d Tongue Trachea Urinary bladder Uterus Vagina Gross lesions (when possible)
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^a Bone marrow smears were obtained at scheduled necropsies and from the animal euthanized in extremis, but not placed in formalin; slides were not examined.

^b Fixed in modified Davidson's solution

^c Fixed in Davidson's solution

^d Oviducts and parathyroids were examined if in plane of section and in all cases where a gross lesion of the organ was present.

^e For females; a corresponding section of skin was taken from the same anatomic area in males.

Days 3, 14, 28, 35 (sponge only), & 56) - No test article-related gross findings were noted at necropsy. Any gross findings observed were considered incidental, commonly observed in this strain and age of rats, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to implantation of the bupivacaine-impregnated collagen sponge.

Organ Weights – No organ weights were measured.

Histopathology

Microscopic examination was performed on the bolded tissues listed in the previous Table from all animals.

Adequate Battery – Adequate tissues sampled but not all evaluated. Not an issue.

Peer Review – No.

Histological Findings - There were no microscopic findings indicating systemic toxicity at any time point in this study for any of the test groups. Collagen sponge material was observed within the internal surgical implant site at Days 3, 14, 28 (Groups 3-5), and 35 (Group 3 only). Collagen sponge material was observed within the external

surgical implant site at Days 3, 14, and 28 (Groups 3-5) but was not observed at the external implant site at Day 35 (Group 3 only). Following are tables and text summarizing surgical site observations on the different days for each group.

Group Number	Treatment
1	0.9% Saline
2	Bupivacaine HCl injectable ^a
3	Collagen sponge ^b
4	Bupivacaine sponge ^b
5	Bupivacaine sponge ^b

^a Group 1 and 2 animals were dosed by injection into the muscle and subcutaneous tissue around the incision.

^b Group 3–5 animals had 1 sponge placed within the peritoneal cavity and 1 in the subcutaneous area of the surgical incision.

Microscopic findings observed but not listed in the following observations, were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of test articles.

Day 3

Summary of Microscopic Findings – Terminal Euthanasia (Day 3)

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg)	0	6	0	12	24	0	4	0	8	12
No. Animals Examined	3	3	3	3	3	3	3	3	3	3
Site, Surgical, 1 (external sponge)										
Necrosis	(3) ^a	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Minimal	0	0	1	0	0	1	0	1	0	0
Mild	2	2	2	0	0	2	2	0	0	0
Moderate	1	1	0	0	0	0	1	2	0	0
Marked	0	0	0	3	3	0	0	0	3	3
Foreign material	(0)	(0)	(3)	(3)	(3)	(0)	(0)	(3)	(3)	(3)
Moderate	0	0	3	3	3	0	0	3	3	3
Site, Surgical, 2 (internal sponge)										
Foreign material	(0) ^a	(0)	(3)	(3)	(3)	(0)	(0)	(3)	(3)	(3)
Minimal	0	0	0	1	0	0	0	0	0	1
Mild	0	0	3	2	3	0	0	3	3	2

^a Numbers in parentheses represent the number of animals with the finding.

Necrosis comprised regions of acute coagulative necrosis affecting the abdominal skeletal musculature and associated connective tissues, typically in proximity to the apparent incision margins and was observed on Day 3 only demonstrating resolution by Day 14. Incidence of necrosis was uniform across all Groups as all animals were affected. Minimal-to-moderate severity of necrosis was observed in all control groups (Groups 1-3). However, both sexes in Groups 4 & 5 (low- and high-dose bupivacaine

sponges) demonstrated greater severity characterized by transmural and generally more widespread regions of coagulative necrosis when compared with control Groups 1-3. As there were no apparent differences in severity of necrosis between Groups 4 and 5, no dose-dependent trend was observed. On that basis, it appears that severity of necrosis was an effect of the bupivacaine-impregnated collagen sponge regardless of dose. There was no apparent trend or histologic finding in subsequent time points to indicate that greater severity of necrosis at Day 3 corresponded to a given outcome at later time points (such as increased fibrosis or atrophy).

A diagnosis of foreign material was used exclusively for histologic identification of collagen sponge material. It is not a toxicity but an observation with severity related to the amount of sponge in a given section with quantification being variable due to sectioning for histology.

Day 14

Summary of Microscopic Findings – Terminal Euthanasia (Day 14)

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg)	0	6	0	12	24	0	4	0	8	12
No. Animals Examined	3	3	3	3	3	3	3	3	3	3
Site, Surgical, 1 (external sponge)										
Fibroplasia	(3) ^a	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Mild	2	2	0	0	1	0	0	0	0	0
Moderate	1	1	3	2	0	3	2	3	3	2
Marked	0	0	0	1	2	0	1	0	0	1
Infiltration, mononuclear cell	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Mild	3	2	0	0	0	2	0	0	0	0
Moderate	0	1	3	3	2	1	3	2	3	2
Marked	0	0	0	0	1	0	0	1	0	1
Foreign material	(0)	(0)	(3)	(3)	(3)	(0)	(0)	(3)	(3)	(3)
Minimal	0	0	3	3	3	0	0	3	3	3
Site, Surgical, 2 (internal sponge)										
Fibroplasia	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Minimal	3	3	0	1	0	3	3	2	2	2
Mild	0	0	3	2	1	0	0	1	1	0
Moderate	0	0	0	0	2	0	0	0	0	1
Foreign material	(0)	(0)	(3)	(3)	(3)	(0)	(0)	(3)	(2)	(3)
Minimal	0	0	0	1	0	0	0	0	1	2
Mild	0	0	3	1	3	0	0	3	1	1
Moderate	0	0	0	1	0	0	0	0	0	0

^a Numbers in parentheses represent the number of animals with the finding.

Consistent with the healing process, fibroplasia occurred in all animals of all groups at the external and internal implant sites at Day 14. Slightly increased severity was observed in groups with any type of collagen sponge and also increased with bupivacaine sponge.

Fibroplasia was characterized by numerous plump spindle cells (fibroblasts)

accompanied by small to moderate amounts of fibrillar eosinophilic extracellular material (intrinsic/native collagen) as well as neovascularization (not graded separately). At subsequent time points with all groups (Sponge only on Day 35) (Days 28 and 56), there was no difference in fibroplasia and/or fibrosis across groups, reflecting a healing process with recovery.

For the external sponge, infiltration of mononuclear cells occurred with similar incidence in both sexes across all groups, with slightly increased severity occurring with bupivacaine HCL (Group 2) and any type of collagen sponge implant. Mononuclear cell infiltration was characterized by infiltration of numerous macrophages, with fewer lymphocytes; neutrophils were intermittently observed but were not graded separately. At subsequent time points (Days 28 and 56), there was no difference in fibroplasia and/or fibrosis between groups, reflecting a healing process with recovery with no difference in outcome.

Collagen sponge material was identified at external and internal implantation sites in all animals in Groups 3-5 except for one low dose bupivacaine sponge female and is likely a sectioning variation effect. The external implant site at Day 14 had uniformly minimal quantities in all animals. The internal implant site generally contained somewhat larger portions (mild to moderate) of collagen sponge material with histologic evidence of degradation that will be discussed later.

Day 28

Summary of Microscopic Findings – Terminal Euthanasia (Day 28)

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg)	0	6	0	12	24	0	4	0	8	12
No. Animals Examined	3	3	3	3	3	3	2	3	3	3
Site, Surgical, 1 (external sponge)										
Foreign material	(0) ^a	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Minimal	0	0	1	0	0	0	0	0	0	0
Site, Surgical, 2 (internal sponge)										
Foreign material	(0)	(0)	(1)	(1)	(3)	(0)	(0)	(1)	(1)	(0)
Minimal	0	0	1	1	3	0	0	1	1	0

^a Numbers in parentheses represent the number of animals with the finding.

At the Day 28 time point, only one (1) Group 3 male had collagen sponge detectable at the external implant site. This animal also had a small remnant of collagen sponge present at the internal implantation site. Presence of collagen sponge material, while minimal in quantity, occurred with greater incidence at the internal implant site at Day 28 (affecting a total of 7 animals as noted in the table). The greater incidence in Group 5 males is likely due to sectioning variability.

The incidence of collagen implant material detection was lower for the external site at Day 28. When considered in the context of Day 14 microscopic findings (with

uniformly and consistently greater attrition of collagen sponge material at the external implantation site regardless of Group), this sustained pattern suggests a greater degree of collagen sponge attrition at the external implantation site.

Day 35 (sponge only group)

Summary of Microscopic Findings – Terminal Euthanasia (Day 35)

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg)	0	6	0	12	24	0	4	0	8	12
No. Animals Examined	0	0	3	0	0	0	0	3	0	0
Site, Surgical, 2 (internal sponge)										
Foreign material	(-)	(-)	(3) ^a	(-)	(-)	(-)	(-)	(1)	(-)	(-)
Minimal	-	-	3	-	-	-	-	1	-	-

^a Numbers in parentheses represent the number of animals with the finding.

At Day 35, collagen sponge material was not detected at any external implant site. However, 4 animals in the sponge only Group 3 (3 males and 1 female), contained small fragments of collagen sponge material associated with the peritoneal aspect of the internal implant site.

Microscopic changes observed at the surgery site were a continued, appropriate, and typical healing response to include fibrosis and minimal to mild atrophy of the abdominal wall skeletal musculature that was similar between all Groups.

Day 56

Summary of Microscopic Findings – Terminal Euthanasia (Day 56)

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg)	0	6	0	12	24	0	4	0	8	12
No. Animals Examined	3	3	3	3	3	3	3	3	3	3
Site, Surgical, 2 (internal sponge)										
Atrophy: abdominal wall; myofiber	(3) ^a	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Minimal	3	2	0	2	2	3	2	1	1	2
Mild	0	1	3	1	1	0	1	2	2	1

^a Numbers in parentheses represent the number of animals with the finding.

On Day 56, abdominal wall skeletal muscle atrophy was observed with similar incidence in all Groups. The presence of muscle wall atrophy in all animals was reported as a typical result of surgical intervention at this site. Slightly increased severity (mild) of abdominal wall muscle atrophy was observed in all groups other than the saline group suggesting that severity is associated with the presence of any collagen sponge or bupivacaine treatment and is therefore not exclusively a bupivacaine sponge effect.

Special Evaluation - none

Toxicokinetics – none

Separate toxicokinetics studies conducted in other studies.

Dosing Solution Analysis

Documentation regarding the purity and stability of the test article is on file with the Sponsor (b) (4). No assessments of stability, concentration, or sample homogeneity were conducted because these are finished products, and concentration and stability were provided by the Sponsor.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay (Bupivacaine HCl)

Study no.:	AE26TM.502ICH. (b) (4)
Study report location:	\\cdsesub1\evsprod\nda209511\0000\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\ae26tm-502\ae26tm-502ich-(b) (4).pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 14, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- Bupivacaine hydrochloride (HCl) - 100% (provided by Sponsor in the study protocol), 99.3% (per Certificate of Analysis from Syntacoll), 99.5% (per Certificate of Analysis (b) (4))
	- Dimethylsulfoxide vehicle – (b) (4)
	(b) (4) 99.99% purity
	- positive controls

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535	Rat	2-aminoanthracene	1.0
TA100, TA1537		(b) (4)	2.0
WP2 <i>uvrA</i>		Purity 97.5%	15
TA98	None	2-nitrofluorene	1.0
		(b) (4)	
		Purity 99.4%	
		sodium azide	
TA100, TA1535	None	(b) (4)	1.0
		Purity 99.6%	
		9-aminoacridine	
		(b) (4)	
TA1537	None	Purity 99.4%	75
		methyl methanesulfonate	
		(b) (4)	
		Purity 100.0%	
WP2 <i>uvrA</i>	None		1,000

Key Study Findings

The purpose of this study was to evaluate the mutagenic potential of bupivacaine hydrochloride by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* strain WP2 *uvrA* in the presence and absence of an exogenous metabolic activation system. Dimethyl sulfoxide (DMSO) was used as the vehicle.

- In the preliminary toxicity assay, the dose levels tested were 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 mcg per plate. Neither precipitate nor background lawn toxicity were observed. Reductions in revertants were observed with some conditions beginning at 3333 or at 5000 mcg per plate. Based upon these results, the maximum dose tested in the mutagenicity assay was 5000 mcg per plate.
- In the mutagenicity assay, the dose levels tested were 50, 150, 500, 1500 and 5000 mcg per plate. Neither precipitate nor background lawn toxicity were observed. Reductions in revertants were observed with tester strain WP2 *uvrA* at 5000 mcg per plate in the presence and absence of S9 activation. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.
- All criteria for a valid study were met as described in the protocol. These results indicate bupivacaine hydrochloride was negative for the ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* strain.

Methods

Strains:	<i>Salmonella typhimurium</i> histidine auxotrophs TA98, TA100, TA1535, and TA1537, and <i>Escherichia coli</i> WP2 <i>uvrA</i>
Concentrations in definitive study:	50, 150, 500, 1500 and 5000 mcg per plate.
Basis of concentration selection:	Preliminary toxicity assays at doses of 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 mcg per plate
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	See table above
Formulation/Vehicle:	DMSO for bupivacaine HCl
Incubation & sampling time:	Overnight cultures were prepared by inoculating from the appropriate frozen permanent stock into a vessel, containing 30 to 50 mL of culture medium. To assure that cultures were harvested in late log phase, the length of incubation was controlled and monitored. Following inoculation, each flask was placed in a shaker/incubator programmed to begin shaking at 125 to 175 rpm and incubating at 37±2°C for approximately 12 hours before the anticipated time of harvest. Each culture was monitored spectrophotometrically for turbidity and was harvested at a percent transmittance yielding a titer of greater than or equal to 0.3x10 ⁹ cells per milliliter.

Test Method

The test system was exposed to the test article using the regulatory accepted plate incorporation methodology originally described by Ames *et al.* (Mutation Research, 31:347-364, 1975) and updated by Maron and Ames (Mutation Research, 113:173-215, 1983). On the day of use in each assay, all tester strain cultures were checked for the appropriate genetic markers.

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats that were injected intraperitoneally with Aroclor™ 1254 (200 mg/mL in corn oil) at a dose of 500 mg/kg, five days before sacrifice.

Study Validity

The following criteria were met and the mutagenicity assay was considered valid:

- All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvrB* gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvrA* cultures must demonstrate the deletion in the *uvrA* gene.
- All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2 *uvrA*, 10-60.
- To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to 0.3×10^9 cells/mL.
- The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of three non-toxic dose levels is required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met:
 - (1) A >50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count.
 - (2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

Evaluation of Test Results

For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated and are reported.

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article as specified below:

Strains TA1535 and TA1537

Data sets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean vehicle control value.

Strains TA98, TA100 and WP2 *uvrA*

Data sets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2.0-times the mean vehicle control value.

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response was evaluated as negative if it was neither positive nor equivocal.

Dose Formulation Analysis

Dose formulations were sent to the analytical chemistry laboratory at (b) (4) for analysis. Concentration analysis indicated that the actual mean concentrations of the analyzed low dose (0.500 mg/mL) and high dose (50.0 mg/mL) formulations were (b) (4) % and (b) (4) % of their respective target concentrations with < (b) (4) % relative standard deviation (RSD). These results indicate that the formulations were accurately prepared, and the test guideline recommended limit dose was achieved. No test article was detected in the vehicle control sample.

Results

- No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions or the S9 and sham mixes.
- Based upon the results of the preliminary toxicity assay, the dose levels selected for the mutagenicity assay were 50.0, 150, 500, 1500 and 5000 mcg per plate. Neither precipitate nor background lawn toxicity were observed. Reductions in revertants were observed with tester strain WP2 *uvrA* at 5000 mcg per plate in the presence and absence of S9 activation
- No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation (see Tables).

Without S-9 activation:

Mutagenicity Assay without S9 activation

Study Number: AE26TM.502ICH. (b) (4)

Study Code: AE26TM

Experiment: B1

Date Plated: 7/24/2015

Exposure Method: Plate incorporation assay

Evaluation Period: 7/30/2015

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	Bupivacaine hydrochloride	5000 µg	18	6	0.9	17 ^A , 13 ^A , 25 ^A
		1500 µg	14	5	0.7	10 ^A , 19 ^A , 13 ^A
		500 µg	23	7	1.2	30 ^A , 17 ^A , 21 ^A
		150 µg	26	9	1.4	33 ^A , 30 ^A , 16 ^A
		50.0 µg	18	5	0.9	22 ^A , 18 ^A , 13 ^A
	DMSO	100 µL	19	4		21 ^A , 22 ^A , 15 ^A
TA100	Bupivacaine hydrochloride	5000 µg	49	10	0.5	46 ^A , 60 ^A , 40 ^A
		1500 µg	73	9	0.8	67 ^A , 83 ^A , 68 ^A
		500 µg	77	10	0.9	75 ^A , 88 ^A , 68 ^A
		150 µg	77	10	0.9	84 ^A , 82 ^A , 66 ^A
		50.0 µg	81	8	0.9	74 ^A , 80 ^A , 89 ^A
	DMSO	100 µL	90	6		96 ^A , 84 ^A , 89 ^A
TA1535	Bupivacaine hydrochloride	5000 µg	8	3	0.6	10 ^A , 5 ^A , 10 ^A
		1500 µg	15	4	1.2	10 ^A , 17 ^A , 17 ^A
		500 µg	13	2	1.0	13 ^A , 11 ^A , 15 ^A
		150 µg	17	9	1.3	16 ^A , 26 ^A , 8 ^A
		50.0 µg	11	2	0.8	11 ^A , 9 ^A , 13 ^A
	DMSO	100 µL	13	4		15 ^A , 16 ^A , 9 ^A
TA1537	Bupivacaine hydrochloride	5000 µg	4	3	0.8	5 ^A , 1 ^A , 6 ^A
		1500 µg	9	2	1.8	10 ^A , 9 ^A , 7 ^A
		500 µg	7	2	1.4	7 ^A , 9 ^A , 5 ^A
		150 µg	6	3	1.2	3 ^A , 6 ^A , 8 ^A
		50.0 µg	4	3	0.8	2 ^A , 8 ^A , 3 ^A
	DMSO	100 µL	5	3		7 ^A , 2 ^A , 7 ^A

Key to Automatic Count Flags

^A: Automatic count

Mutagenicity Assay without S9 activation

Study Number: AE26TM.502ICH (b) (4)
 Experiment: B1
 Exposure Method: Plate incorporation assay

Study Code: AE26TM
 Date Plated: 7/24/2015
 Evaluation Period: 7/30/2015

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
WP2uvrA	Bupivacaine hydrochloride	5000 µg	6	4	0.3	10 ^A , 6 ^A , 3 ^A
		1500 µg	12	6	0.6	7 ^A , 11 ^A , 18 ^A
		500 µg	16	6	0.8	10 ^A , 18 ^A , 21 ^A
		150 µg	21	4	1.1	25 ^A , 21 ^A , 18 ^A
		50.0 µg	22	1	1.1	22 ^A , 23 ^A , 21 ^A
	DMSO	100 µL	20	2		18 ^A , 19 ^A , 22 ^A
TA98	2NF	1.0 µg	461	208	24.3	355 ^A , 327 ^A , 701 ^A
TA100	SA	1.0 µg	850	58	9.4	783 ^A , 881 ^A , 887 ^A
TA1535	SA	1.0 µg	674	4	51.8	677 ^A , 670 ^A , 676 ^A
TA1537	9AAD	75 µg	299	68	59.8	306 ^A , 363 ^A , 228 ^A
WP2uvrA	MMS	1000 µg	452	40	22.6	422 ^A , 497 ^A , 437 ^A

Key to Positive Controls

2NF 2-nitrofluorene
 SA sodium azide
 9AAD 9-Aminoacridine
 MMS methyl methanesulfonate

Key to Automatic Count Flags

^A: Automatic count

With S-9 activation

Mutagenicity Assay with S9 activation

Study Number: AE26TM.502ICH (b) (4)
 Experiment: B1
 Exposure Method: Plate incorporation assay

Study Code: AE26TM
 Date Plated: 7/24/2015
 Evaluation Period: 7/30/2015

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	Bupivacaine hydrochloride	5000 µg	19	5	0.9	25 ^A , 18 ^A , 15 ^A
		1500 µg	22	3	1.0	25 ^A , 21 ^A , 19 ^A
		500 µg	24	7	1.1	17 ^A , 25 ^A , 30 ^A
		150 µg	23	2	1.1	22 ^A , 22 ^A , 26 ^A
		50.0 µg	24	9	1.1	34 ^A , 22 ^A , 16 ^A
	DMSO	100 µL	21	4		21 ^A , 17 ^A , 25 ^A
TA100	Bupivacaine hydrochloride	5000 µg	62	11	0.7	72 ^A , 65 ^A , 50 ^A
		1500 µg	105	12	1.2	93 ^A , 116 ^A , 105 ^A
		500 µg	118	10	1.4	123 ^A , 125 ^A , 107 ^A
		150 µg	112	19	1.3	130 ^A , 114 ^A , 93 ^A
		50.0 µg	103	5	1.2	109 ^A , 101 ^A , 100 ^A
	DMSO	100 µL	85	9		79 ^A , 95 ^A , 82 ^A
TA1535	Bupivacaine hydrochloride	5000 µg	9	4	0.8	10 ^A , 13 ^A , 5 ^A
		1500 µg	13	3	1.2	15 ^A , 15 ^A , 10 ^A
		500 µg	18	4	1.6	18 ^A , 22 ^A , 14 ^A
		150 µg	11	3	1.0	9 ^A , 9 ^A , 15 ^A
		50.0 µg	14	1	1.3	15 ^A , 13 ^A , 13 ^A
	DMSO	100 µL	11	2		9 ^A , 13 ^A , 11 ^A
TA1537	Bupivacaine hydrochloride	5000 µg	11	4	1.0	14 ^A , 13 ^A , 6 ^A
		1500 µg	12	4	1.1	10 ^A , 9 ^A , 16 ^A
		500 µg	11	2	1.0	11 ^A , 10 ^A , 13 ^A
		150 µg	12	4	1.1	11 ^A , 16 ^A , 9 ^A
		50.0 µg	10	4	0.9	13 ^A , 11 ^A , 5 ^A
	DMSO	100 µL	11	3		15 ^A , 9 ^A , 9 ^A

Key to Automatic Count Flags

^A: Automatic count

Mutagenicity Assay with S9 activation

Study Number: AE26TM.502ICH, (b) (4)
 Experiment: B1
 Exposure Method: Plate incorporation assay

Study Code: AE26TM
 Date Plated: 7/24/2015
 Evaluation Period: 7/30/2015

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
WP2uvrA	Bupivacaine hydrochloride	5000 µg	13	2	0.4	15 ^A , 13 ^A , 11 ^A
		1500 µg	17	3	0.6	16 ^A , 21 ^A , 15 ^A
		500 µg	26	12	0.9	40 ^A , 18 ^A , 21 ^A
		150 µg	24	5	0.8	22 ^A , 30 ^A , 21 ^A
		50.0 µg	28	3	1.0	31 ^A , 27 ^A , 25 ^A
	DMSO	100 µL	29	9		26 ^A , 39 ^A , 23 ^A
TA98	2AA	1.0 µg	629	80	30.0	539 ^A , 659 ^A , 690 ^A
TA100	2AA	2.0 µg	768	121	9.0	742 ^A , 899 ^A , 662 ^A
TA1535	2AA	1.0 µg	104	10	9.5	101 ^A , 115 ^A , 96 ^A
TA1537	2AA	2.0 µg	68	4	6.2	72 ^A , 64 ^A , 67 ^A
WP2uvrA	2AA	15 µg	283	71	9.8	202 ^A , 312 ^A , 334 ^A

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic Count Flags

^A: Automatic count

Conclusion

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that, under the conditions of this study, bupivacaine hydrochloride did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9. The study was concluded to be negative and no further testing was warranted.

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7.2 In Vitro Assays in Mammalian Cells

Study title: *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK^{+/-} Mouse Lymphoma Assay) (Bupivacaine HCl)

Study no.: AE26TM.704ICH (b) (4)
Study report location: [\\cdsesub1\evsprod\nda209511\0000\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\ae26tm-704\ae26tm-704ich-\(b\)\(4\).pdf](#)
Conducting laboratory and location (b) (4)
Date of study initiation: June 29, 2015
GLP compliance: Yes (ICH 2011 and OECD 1997 testing guidelines)
QA statement: Yes
Drug, lot #, and % purity: - Bupivacaine hydrochloride (HCl), Lot 0005235568, 100% (provided by Sponsor in the study protocol), 99.3% (per Certificate of Analysis from Syntacoll), 99.5% (b) (4)
- Dimethylsulfoxide (DMSO), Lot SHBF1863V. 99.97% purity
- Water, Lot AZK195967, purity cell culture grade
Positive controls:
- Methyl methanesulfonate (MMS), Lot A0346325, 99% purity
- 7,12-Dimethyl-benz(a)anthracene (DMBA), Lot SLBH2894V, 98% purity

Key Study Findings

The purpose of this study was to evaluate the genotoxic potential of bupivacaine hydrochloride (HCL) by quantitating forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells (increased number of cells) and the size of the resulting colonies (smaller). L5178Y/TK^{+/-} cells, normally diploid at the thymidine kinase (TK) locus, were exposed to appropriate concentrations of test article, as well as the concurrent positive and vehicle controls in the presence and absence of an exogenous metabolic activation system (Aroclor-induced rat liver S9) in order to assess mutagenic activity (L5178Y/TK^{+/-} → L5178Y/TK^{-/-}).

Based on the results of the preliminary toxicity assay, cultures were treated in the mutagenicity assay at appropriate concentrations in a 4-hour treatment with S9, a 4-hour treatment without S9 and a 24-hour treatment without S9. No visible precipitate was observed at the beginning or end of treatment, and the test article did not have an adverse impact on the pH of the cultures.

All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met. These results indicate bupivacaine HCl was negative in the L5178Y/TK^{+/-} Mouse Lymphoma Assay, under the conditions of the assay, and according to the criteria of the test protocol.

Methods

- Cell line: L5178Y/TK^{+/-} mouse lymphoma cells
- heterozygous at the normally diploid thymidine kinase (TK) locus.
- Concentrations in definitive study: 2.84, 10.0, 41.0, 62.0, 122, 163, and 325 mcg/mL (4-hour treatment with S9), 2.84, 10.0, 22.0, 41.0, 81.0, 163, and 325 mcg/mL (4-hour treatment without S9) and 10.0, 22.0, 41.0, 81.0, 163, and 325 mcg/mL (24-hour treatment without S9)
- Basis of concentration selection: Results of the preliminary toxicity assay where bupivacaine hydrochloride monohydrate was prepared in DMSO at concentrations from 1.34 to 343 mcg/mL (active ingredient) using 4-hour treatments with and without S9, and a 24-hour treatment without S9 (the maximum concentration of active ingredient evaluated was > than the 1 mM limit dose for this assay).
- Negative control: Dimethylsulfoxide (DMSO)
- Positive control: Methyl methanesulfonate (MMS) when no metabolic activation and 7,12-dimethylbenz(a)anthracene (DMBA) with metabolic activation
- Formulation/Vehicle: DMSO - DMSO was selected as the solvent of choice based on the solubility of the test article and compatibility with the target cells.
- Incubation & sampling time: Target cells were treated for 4 hours in the presence and absence of S9, and for 24 hours in the absence of S9, by incorporation of the test article vehicle mixture in the treatment medium.

Principle of the Test Method

Cells deficient in thymidine kinase (TK) due to the mutation TK^{+/-} → TK^{-/-} are resistant to the cytotoxic effects of the pyrimidine analogue trifluorothymidine (TFT). Thymidine kinase proficient cells are sensitive to TFT, which causes the inhibition of cellular

metabolism and halts further cell division. Thus, mutant cells are able to proliferate in the presence of TFT, whereas normal cells, which contain thymidine kinase, are not.

Treatment of Target Cells

Prior to use in the assay, L5178Y/TK^{+/-} cells were cleansed to reduce the frequency of spontaneously occurring TK^{-/-} cells. The S9 was prepared from male Sprague-Dawley rats that were injected intraperitoneally with Aroclor™ 1254 (200 mg/mL in corn oil) at a dose of 500 mg/kg, five days before sacrifice.

Six or seven concentrations were tested using duplicate cultures at appropriate dose intervals based on the results of the preliminary toxicity test. Treatment was carried out by combining 100 mcL of test article dose formulation, vehicle or positive control dose formulation, F₀P medium (Fischer's medium plus antibiotics and sodium pyruvate), and S9 mix (as appropriate) with 6×10^6 L5178Y/TK^{+/-} cells in a total volume of 10 mL. Each S9-activated 10-mL culture contained 4 mL S9 mix (final S9 concentration of 1.0%). Cultures were capped tightly and incubated with mechanical mixing at $37 \pm 1^\circ\text{C}$ for 4 or 24 hours.

For the definitive assay only, at the end of the exposure period, the cells were washed with culture medium and collected by centrifugation. The cells were resuspended in 20 mL F₁₀P (F₀ plus 10% horse serum plus sodium pyruvate) on Day 1 and in 10 mL F₁₀P on Day 2, and incubated at $37 \pm 1^\circ\text{C}$ for two days following treatment. Cell population adjustments to 3×10^5 cells/mL were made as follows:

- 4-hour treatment – 1 and 2 days after treatment.
- 24-hour treatment – immediately after test article removal, and 2 and 3 days after treatment.

Selection of the Mutant Phenotype

Cells from selected dose levels were cultured in triplicate with 2-4 mcg TFT/mL (trifluorothymidine/mL) at a density of 1×10^6 cells/100 mm plate in cloning medium containing 0.22 to 0.24% agar. For estimation of cloning efficiency at the time of selection of those same cultures, 200 cells/100 mm plate were cultured in triplicate in cloning medium without TFT (viable cell (VC) plate). Cultures were incubated under standard conditions ($37 \pm 1^\circ\text{C}$ in a humidified atmosphere of $5 \pm 1\%$ CO₂ in air) for 11 or 12 days. The total number of colonies per culture was determined for the VC plates and the total relative growth calculated. The total number of colonies per TFT plate was then determined for those cultures with $\geq 10\%$ total growth (including at least one concentration between 10 and 20% total growth, if possible). Colonies were counted and the diameter of the TFT colonies from the positive control and vehicle control cultures were determined over a range from 0.2 to 1.1 mm.

Data Endpoint Calculation Formula

Data Calculations:

$$\text{Cloning Efficiency (CE, \%)} = \frac{\text{number of VC colonies}}{\text{number of cells plated}} \times 100$$

$$\text{Mutant Frequency (MF)} = \frac{\text{number of TFT colonies}}{(\text{number of cells plated})(\text{CE})}$$

$$\text{Induced MF (IMF)} = \text{MF}_{\text{treated}} - \text{MF}_{\text{controls (average)}}$$

$$\text{Suspension Growth (SG; 4-hour)} = \frac{\text{Day 1 cells/mL}}{0.3 \times 10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cells/mL}}{\text{Day 1 cells/mL}_{(\text{adjusted})}}$$

$$\text{Suspension Growth (SG; 24-hour)} = \frac{\text{Day 0 cells/mL}}{0.3 \times 10^6 \text{ cells/mL}} \times \frac{\text{Day 1 cells/mL}}{\text{Day 0 cells/mL}_{(\text{adjusted})}} \times \frac{\text{Day 2 cells/mL}}{\text{Day 1 cells/mL}_{(\text{adjusted})}}$$

$$\text{Relative SG (\%)} = \frac{\text{SG}_{\text{treated}}}{\text{SG}_{\text{controls (average)}}} \times 100$$

$$\text{Relative Total Growth (RTG, \%)} = \frac{\text{CE}_{\text{treated}}}{\text{CE}_{\text{control (average)}}} \times \text{RSG}$$

Study Validity

Vehicle Controls

The average spontaneous mutant frequency of the vehicle control cultures must be within 35 to 140 TFT-resistant mutants/ 10^6 surviving cells. Low spontaneous mutant frequencies, i.e., 20 to 34 mutants/ 10^6 surviving cells, were considered acceptable if small colony recovery was demonstrated (Mitchell et al., Mutation Research 394:177-303, 1997). The average cloning efficiency of the vehicle controls must be between 65 and 120% and the total suspension growth between 8 to 32 for the 4-hour exposure and 20 to 180 for the 24-hour exposure (Moore et al., EMM 40:292-299, 2002; EMM 47:1-5, 2006; and Mutation Research 627: 36-40, 2007).

Positive Controls

The mutant frequency for at least one dose of each positive control must meet the criteria for a positive response. The mutant frequency for at least one dose of one of the positive controls must induce an increase in small colony mutants according to the following criteria: Induced Mutant Frequency (IMF) positive control $\geq 300 \times 10^{-6}$ mutants with 40% small colonies or small colony IMF for positive control $\geq 150 \times 10^{-6}$ (Moore et al., EMM 40:292-299, 2002 and EMM 47:1-5, 2006).

Test Article-Treated Cultures

Cultures treated with a minimum of four concentrations of test article must be evaluated and their mutant frequencies reported. Results may be accepted, with justification,

when only three concentrations of test article are evaluated and otherwise meet the other criteria for a valid test. The highest test article concentration must produce 80 to 90% toxicity unless limited by solubility or the maximum required concentration. In the case of a test article with a steep toxicity curve (no concentrations with 10 to 20% survival), the results may be considered acceptable if a concentration spacing of ≤ 2 -fold is used and the highest concentration tested showed $< 20\%$ survival or total kill (Sofuni et al., Environ. Molec. Mutagen. 29:434-438, 1997).

Evaluation of Test Results

The cytotoxic effects of each treatment condition were expressed relative to the vehicle-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency for each treatment condition was calculated by dividing the mean number of colonies on the TFT-plates by the mean number of colonies on the VC-plates and multiplying by the dilution factor (2×10^{-4}), and was expressed as TFT-resistant mutants/ 10^6 surviving cells. The induced mutant frequency (IMF) was defined as the mutant frequency of the treated culture minus the mutant frequency of the vehicle control cultures. The International Workshop on Genotoxicity established a Global Evaluation Factor (GEF) for a positive response at an IMF of ≥ 90 mutants/ 10^6 clonable cells at the Aberdeen meeting in 2003 (Moore et al., EMM 47:1-5, 2006).

In evaluation of the data, increases in induced mutant frequency which occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. All conclusions were based on scientific judgment; however, the following criteria are presented as a guide to interpretation of the data (Moore et al., EMM 47:1-5, 2006).

- A result was considered positive if a concentration-related increase in mutant frequency was observed in the treated cultures and one or more treatment conditions with 10% or greater total growth exhibit induced mutant frequencies of ≥ 90 mutants/ 10^6 clonable cells (based on the average mutant frequency of duplicate cultures). If the average vehicle control mutant frequency was > 90 mutants/ 10^6 clonable cells, a doubling of mutant frequency over the vehicle would also be required (Mitchell et al., Mutation Research 394:177-303, 1997).
- A result was considered negative if the treated cultures exhibit induced mutant frequencies of less than 90 mutants/ 10^6 clonable cells (based on the average mutant frequency of duplicate cultures) and there was no concentration-related increase in mutant frequency.

There are some situations in which a chemical would be considered negative when there was no culture showing between 10 to 20% survival (Office of Food Additive Safety, 2001).

- There was no evidence of mutagenicity (e.g., no dose response or increase in induced mutant frequencies between 45 and 89 mutants/10⁶) in a series of data points within 100 to 20% survival and there was at least one negative data point between 20 and 25% survival.
- There was no evidence of mutagenicity (e.g., no dose response or increase in induced mutant frequencies between 45 and 89 mutants/10⁶) in a series of data points between 100 to 25% survival and there was also a negative data point between 10 and 1% survival. In this case, it would be acceptable to count the TFT colonies of cultures exhibiting <10% total growth.

Dose Formulation Analysis

All samples were submitted to the analytical chemistry laboratory at (b) (4) for analysis. All dosing formulations analyzed met the acceptance criteria of (b) (4) % of target concentration and met $\leq$ (b) (4) % relative standard deviation (RSD). Actual concentration analysis indicated that the actual mean concentrations of the analyzed low dose (0.3 mg/mL) and high dose (34.3 mg/mL) formulations were (b) (4) and (b) (4) % of their respective target concentrations. No test article was detected in the vehicle control samples.

Stability analysis indicated that the test article in DMSO, at a concentration of 0.288 mg/mL, was stable at room temperature for at least three hours. Stability of the dose formulations was also determined as part of Ames Assay for bupivacaine hydrochloride ((b) (4) Study No. AE26TM.5021CH. (b) (4)). The results of the additional stability analysis indicated that the test article in DMSO, at concentrations of 0.504 and 52.0 mg/mL, was stable at room temperature for at least three hours.

Results

Preliminary Toxicity Assay

Results of the preliminary toxicity assay are presented in Table 1. Bupivacaine hydrochloride monohydrate was prepared in DMSO and evaluated in a preliminary toxicity assay at concentrations from 1.34 to 343 mcg/mL (active ingredient) using 4-hour treatments with and without S9, and a 24-hour treatment without S9 (the maximum concentration of active ingredient evaluated was > than the 1 mM limit dose for this assay; see Deviations). No visible precipitate was observed at the beginning or end of treatment, and the test article did not have an adverse impact on the pH or osmolality of the cultures [626 and 616 mmol/kg for the vehicle control and the highest treatment concentration (343 µg/mL active ingredient), respectively].

Relative suspension growth (RSG) was 11, 74, and 13% at concentrations of 172 mcg/mL (4-hour treatment with S9) and 343 mcg/mL (4- and 24-hour treatments without S9), respectively. RSG was 0% at a concentration of 343 mcg/mL using a 4-hour treatment with S9.

TABLE 1

**PRELIMINARY TOXICITY ASSAY USING BUPIVACAINE HYDROCHLORIDE (AS MONOHYDRATE)
4- and 24-Hour Exposures**

DOSE LEVEL (µg/mL)	PRECIP	CELL CONCENTRATION (cells/mL x 10 ⁶)			SUSPENSION GROWTH	
		DAY 0	DAY 1	DAY 2	TOTAL	% OF CONTROL

4HR NON-ACTIVATED CULTURES

SOLVENT A	✓	Not applicable for 4-hour exposure	1.800	1.555	31.1	100
SOLVENT B	✓		1.951	1.437	31.1	
1.34			1.854	1.377	28.4	91
2.68			1.854	1.395	28.7	92
5.36			1.817	1.393	28.1	90
10.7			1.769	1.348	26.5	85
21.4			1.744	1.479	28.7	92
42.9			1.658	1.380	25.4	82
85.8			1.576	1.455	25.5	82
172			1.499	1.363	22.7	73
343			1.358	1.528	23.0	74

4HR S9-ACTIVATED CULTURES

Induced Rat Liver

SOLVENT A	✓	Not applicable for 4-hour exposure	1.762	1.438	28.2	100
SOLVENT B	✓		1.702	1.459	27.6	
1.34			1.679	1.510	28.2	101
2.68			1.598	1.562	27.7	100
5.36			1.467	1.443	23.5	84
10.7			1.229	1.438	19.6	70
21.4			1.015	1.468	16.6	59
42.9			0.845	1.542	14.5	52
85.8			0.606	1.336	9.0	32
172			0.243	0.958	3.2	11
343			0.076	0.149	0.0	0

24HR NON-ACTIVATED CULTURES

SOLVENT A	✓	1.014	1.131	1.724	73.3	100
SOLVENT B	✓	1.011	1.017	1.834	69.9	
1.34		1.014	1.141	1.803	77.3	108
2.68		1.109	1.167	1.741	83.5	116
5.36		0.999	1.093	1.749	70.7	99
10.7		1.021	1.156	1.682	73.5	103
21.4		0.965	1.180	1.712	72.2	101
42.9		1.011	1.026	1.752	67.3	94
85.8		0.972	0.910	1.826	59.8	83
172		0.660	0.955	1.809	42.2	59
343		0.262	0.466	1.842	9.5	13

Solvent = DMSO

A and B are duplicate cultures

Mutagenesis Assay

The results of the mutagenicity assay are presented in Tables 2 through 4. Colony size distributions for the positive and vehicle control cultures are presented in Figures 1 through 3.

Based on the results of the preliminary toxicity assay, cultures were treated in the mutagenicity assay at concentrations of 2.84, 10.0, 41.0, 62.0, 122, 163 and 325 mcg/mL (4-hour treatment with S9), 2.84, 10.0, 22.0, 41.0, 81.0, 163 and 325 mcg/mL (4-hour treatment without S9) and 10.0, 22.0, 41.0, 81.0, 163 and 325 mcg/mL (24-hour

treatment without S9). No visible precipitate was observed at the beginning or end of treatment, and the test article again did not have an adverse impact on the pH of the cultures. Cultures treated at concentrations of 2.84, 10.0, 41.0, 122 and 163 mcg/mL (4-hour treatment with S9), 2.84, 10.0, 22.0, 81.0 and 325 mcg/mL (4-hour treatment without S9), and 10.0, 22.0, 41.0 and 81.0 mcg/mL (24-hour treatment without S9) exhibited 14 to 91%, 45 to 103% and 18 to 93% RSG, respectively, and were cloned (cultures treated at other concentrations were discarded prior to cloning because a sufficient number of higher concentrations was available, or due to excessive toxicity). Relative total growth of the cloned cultures ranged from 15 to 96% (4-hour treatment with S9), 46 to 107% (4-hour treatment without S9) and 17 to 105% (24-hour treatment without S9). No increases in induced mutant frequency ≥ 90 mutants/ 10^6 clonable cells were observed under any treatment condition.

Trifluorothymidine-resistant colonies for the positive and vehicle control cultures were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS and DMBA positive controls yielded the expected increase in small colonies (verifying the adequacy of the methods used to detect small colony mutants) and large colonies.

All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met.

TABLE 2

**DATA SUMMARY FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS TREATED WITH BUPIVACAINE
HYDROCHLORIDE (AS MONOHYDRATE)
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Mutagenesis Assay (4-hour exposure)**

DOSE LEVEL (µg/mL)	PRECIP.	% SUSP. GROWTH	VC COLONIES				TFT COLONIES				TOTAL MUTANT FREQUENCY (PER 10 ⁶ CELLS)	INDUCED MUTANT FREQUENCY (PER 10 ⁶ CELLS)	% RELATIVE TOTAL GROWTH
			PLATE COUNTS				PLATE COUNTS						
			1	2	3	MEAN	1	2	3	MEAN			
SOLVENT A		100	214	180	182	192	52	33	50	45	47	N/A	100
SOLVENT B			169	178	166	171	39	46	59	48	56		
2.84 A		103	142	164	146	151	31	44	25	33	44	-7	86
2.84 B		98	148	138	134	140	55	30	44	43	61	10	76
10.0 A		94	141	144	159	148	62	44	61	56	75	24	77
10.0 B		99	198	196	196	197	81	56	48	62	63	11	107
22.0 A		88	182	148	157	162	47	46	74	56	69	17	79
22.0 B		93	188	165	149	167	50	39	36	42	50	-2	86
41.0 A		88	****	****	****		****	****	****				
41.0 B		86	****	****	****		****	****	****				
81.0 A		73	166	142	145	151	39	45	58	47	63	11	61
81.0 B		78	133	130	148	137	65	52	50	56	81	30	59
163 A		57	****	****	****		****	****	****				
163 B		54	****	****	****		****	****	****				
325 A		45	196	171	201	189	39	59	56	51	54	3	47
325 B		56	145	135	163	148	43	42	40	42	56	5	46
POSITIVE CONTROL: Methyl methanesulfonate (MMS) (µg/mL)													
20		38	35	38	30	34	134	144	134	137	800	748	7
15		50	80	58	77	72	184	163	176	174	487	435	20
MEAN SOLVENT TOTAL SUSPENSION GROWTH: 18.5													
MEAN SOLVENT CLONING EFFICIENCY: 91%													
MEAN SOLVENT MUTANT FREQUENCY: 52 (PER 10 ⁶ CELLS)													

Solvent = DMSO

A and B are duplicate cultures

**** - Discarded prior to cloning because a sufficient number of higher concentrations was available

TABLE 3

**DATA SUMMARY FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS TREATED WITH BUPIVACAINE
HYDROCHLORIDE (AS MONOHYDRATE)
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Mutagenesis Assay (4-hour exposure)**

DOSE LEVEL (µg/mL)	PRECIP.	% SUSP. GROWTH	VC COLONIES				TFT COLONIES				TOTAL MUTANT FREQUENCY (PER 10 ⁶ CELLS)	INDUCED MUTANT FREQUENCY (PER 10 ⁶ CELLS)	% RELATIVE TOTAL GROWTH
			PLATE COUNTS				PLATE COUNTS						
			1	2	3	MEAN	1	2	3	MEAN			
SOLVENT A		100	187	150	133	157	63	45	47	52	66	N/A	100
SOLVENT B			138	122	150	137	51	42	42	45	66		
2.84 A		91	141	135	142	139	48	38	86	57	82	16	87
2.84 B		89	151	146	175	157	58	58	66	61	77	11	96
10.0 A		70	194	165	159	173	53	66	53	57	66	1	83
10.0 B		72	161	144	168	158	48	42	44	45	57	-9	77
41.0 A		38	166	176	187	176	82	68	74	75	85	19	46
41.0 B		36	160	148	144	151	68	*	81	75	99	33	37
62.0 A		30	****	****	****		****	****	****				
62.0 B		29	****	****	****		****	****	****				
122 A		19	168	131	166	155	104	91	81	92	119	53	20
122 B		25	133	158	165	152	92	65	88	82	107	42	26
163 A		15	159	134	137	143	78	89	69	79	110	44	15
163 B		14	164	164	172	167	95	90	90	92	110	44	16
325 A		0	+	+	+		+	+	+				
325 B		0	+	+	+		+	+	+				
POSITIVE CONTROL: 7,12-dimethylbenz(a)anthracene (DMBA) (µg/mL)													
1.5		21	107	81	90	93	235	246	280	254	547	482	14
1		41	130	101	121	117	224	252	227	234	399	334	32
MEAN SOLVENT TOTAL SUSPENSION GROWTH: 13.7													
MEAN SOLVENT CLONING EFFICIENCY: 73%													
MEAN SOLVENT MUTANT FREQUENCY: 66 (PER 10 ⁶ CELLS)													

Solvent = DMSO

A and B are duplicate cultures

* - Plate lost due to contamination

**** - Discarded prior to cloning because a sufficient number of higher concentrations was available

+ - Discarded prior to cloning due to excessive toxicity

**DATA SUMMARY FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS TREATED WITH BUPIVACAINE
HYDROCHLORIDE (AS MONOHYDRATE)
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Mutagenesis Assay (24-hour exposure)**

Solvent = DMSO A and B are duplicate cultures
 * - Plate lost due to contamination
 + - Discarded prior to cloning due to excessive toxicity

Figure 1

Mutagenesis Assay with Bupivacaine Hydrochloride (as Monohydrate)
without Activation, 4-Hour Exposure
Colony Size Distribution in the Absence of Metabolic Activation
(Non-Activated Positive Control Compared with Solvent Control)

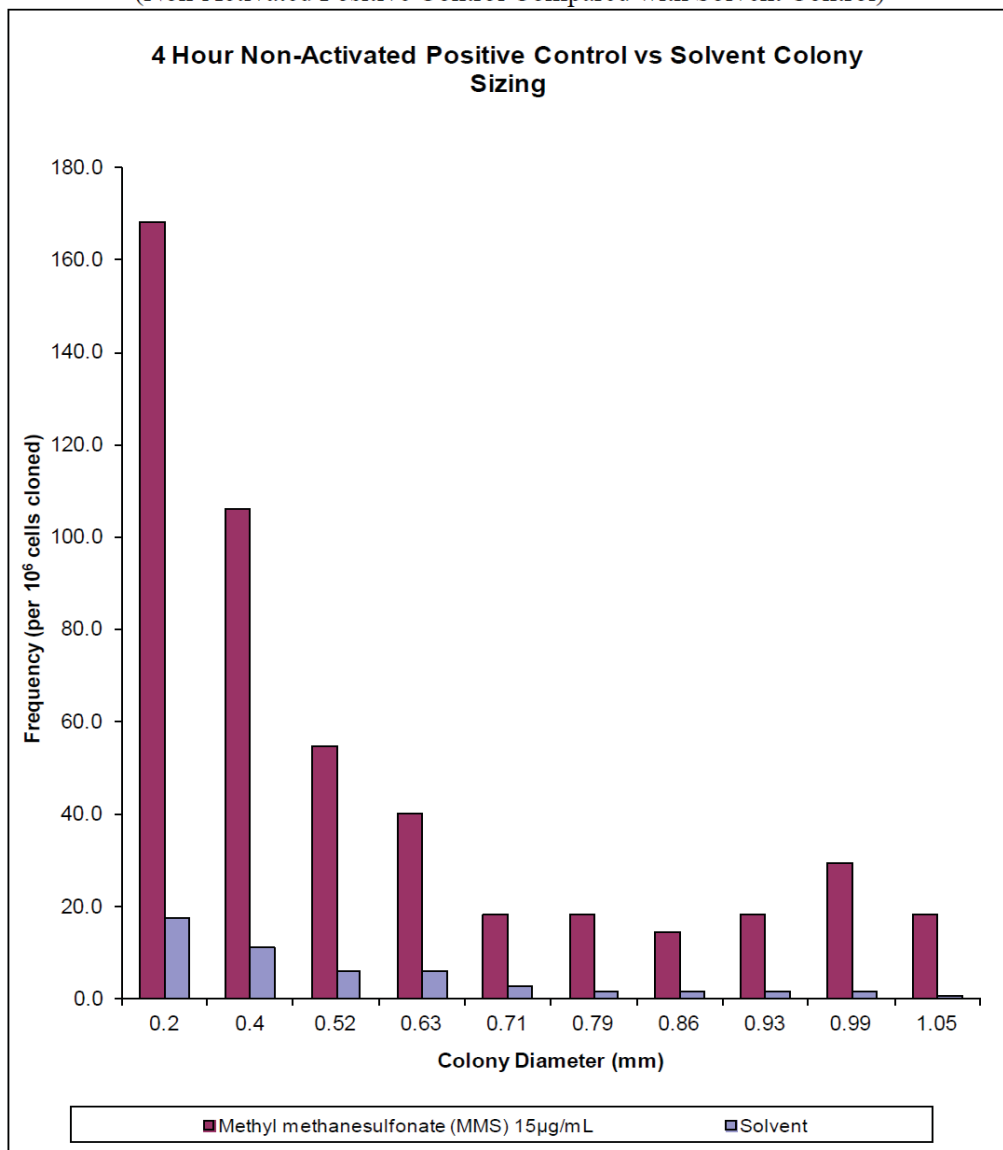


Figure 2

Mutagenesis Assay with Bupivacaine Hydrochloride (as Monohydrate)
with Activation, 4-Hour Exposure
Colony Size Distribution in the Presence of Metabolic Activation
(S9-Activated Positive Control Compared with Solvent Control)

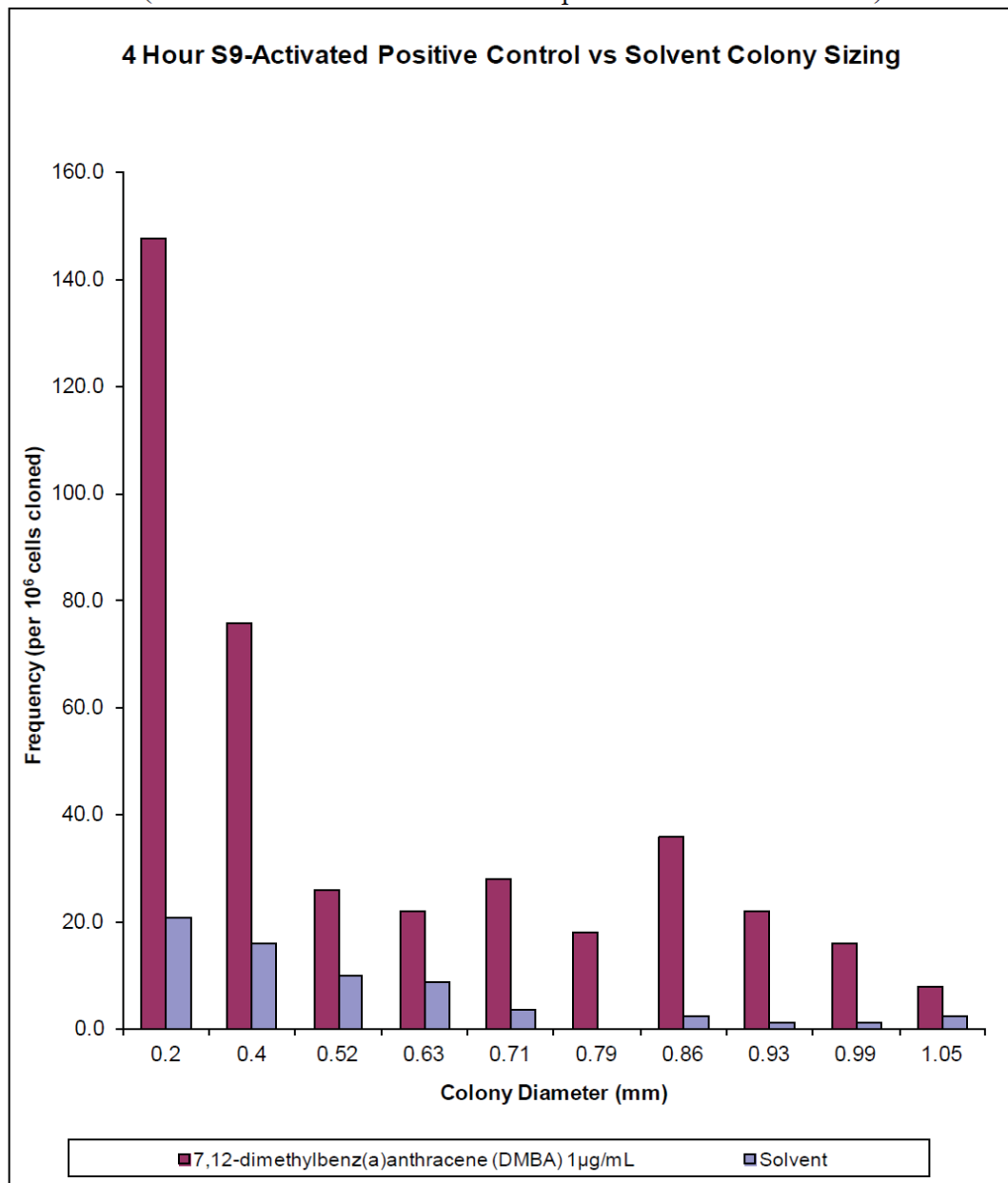
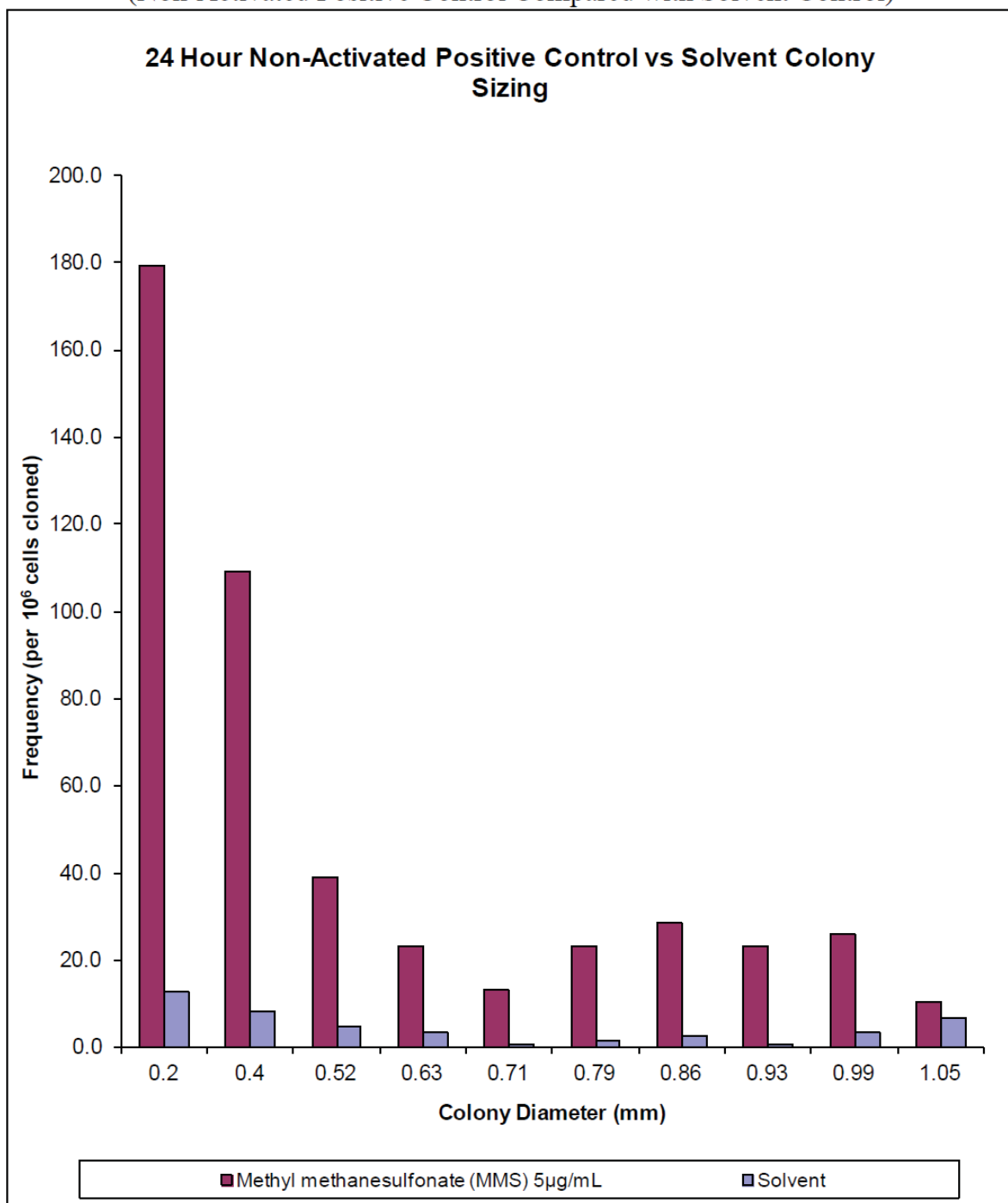


Figure 3

Mutagenesis Assay with Bupivacaine Hydrochloride (as Monohydrate)
without Activation, 24-Hour Exposure
Colony Size Distribution in the Absence of Metabolic Activation
(Non-Activated Positive Control Compared with Solvent Control)



Conclusion

These results indicate bupivacaine hydrochloride was not genotoxic in the L5178Y/TK⁺ Mouse Lymphoma Assay, under the conditions, and according to the criteria, of the test protocol.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: In Vivo Mammalian Erythrocyte Micronucleus Assay in Rats (Bupivacaine HCl)

Study no: AE32HH.125M012ICH. (b) (4)
Study report location: [\\cdsesub1\evsprod\nda209511\0000\m4\42-stud-rep\423-tox\4233-genotox\42332-in-vivo\ae32hh-125\ae32hh-125m012ich-\(b\)\(4\).pdf](\\cdsesub1\evsprod\nda209511\0000\m4\42-stud-rep\423-tox\4233-genotox\42332-in-vivo\ae32hh-125\ae32hh-125m012ich-(b)(4).pdf) (b) (4)
Conducting laboratory and location: (b) (4)
Date of study initiation: July 17, 2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: - Bupivacaine hydrochloride (HCl), USP 0.5%, Lot 46-328-DK, assumed 100% purity
- 0.9% Saline, Lot 311114

Key Study Findings

The test article, bupivacaine hydrochloride injection, USP 0.5%, was evaluated for its clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in rat bone marrow.

- Saline solution (0.9%) was used as the vehicle. Test and/or control article formulations were administered at various dose volumes by subcutaneous injection. Positive control [cyclophosphamide (CP)] data was from (b) (4) historical data.
- The definitive assay dose levels tested were 5, 10, and 20 mg/kg (Maximum Tolerated Dose from toxicity studies with lethality at 30 mg/kg). No clinical signs of toxicity other than piloerection were observed in any bupivacaine HCl test group including no treatment-related body weight changes.
- No appreciable reductions in the PCEs/EC ratio in the test article groups compared to the vehicle control group was observed indicating the test did not induce cytotoxicity. No statistically significant increase in the incidence of MnPCEs in the test article treated groups was observed relative to the vehicle control groups. The historical positive control induced a statistically significant increase in the incidence

of MnPCEs. The number of MnPCEs in the vehicle control groups did not exceed the historical control range.

- Under the conditions of this study, the administration of bupivacaine hydrochloride injection, USP 0.5% at doses up to and including a dose of 20 mg/kg was concluded to be negative in the In Vivo Mammalian Erythrocyte Micronucleus Assay in Rats. However, the highest dose tested did not produce frank toxicity (maximum tolerated dose) and therefore the study is not considered valid as per ICH S2(R1).

Methods

Doses in definitive study:

Group	Treatment	Dose Level (mg/kg)	Dose Volume ^A (mL/kg)	Euthanasia Time (hrs after treatment)	
				24	48
				Number of Male Rats	
1	Vehicle	0	4	5	5
2	Test Article	5	1	5	0
3	Test Article	10	2	5	0
4	Test Article	20	4	5	5

^ABased upon individual body weight

Frequency of dosing: Single dose on Day 0
 Route of administration: All dose formulations were administered by subcutaneous injection.
 Dose volume: See table above
 Formulation/Vehicle: 0.9% saline
 Species/Strain: Sprague-Dawley rats (169.1 – 191.2 g, 6 weeks old)
 Number/Sex/Group: 5
 Satellite groups: No
 Basis of dose selection: Doses based on previous toxicity study/data with 20 mg/kg as the maximum tolerated dose (MTD)
 Negative control: 0.9% saline
 Positive control: None – Historical control report

Methods

- Femoral bone marrow was collected at approximately 24 or 48 hours after the single dose
- At least four slides were prepared from each animal (2 as back-up)
- The standard staining procedure employed permits the differentiation by color of polychromatic and normochromatic erythrocytes (bright orange PCEs and ghost-like, dark green NCEs, respectively).
- The criteria for the identification of micronuclei are those of Schmid (Mutation Res. 31:9-15,1975). Micronuclei are brightly stained bodies that generally are round and that generally are between 1/20 and 1/5 the size of the PCE. The frequency of micronucleated cells was recorded with cells containing one or more micronuclei counted as one micronucleated PCE (MnPCE). At least 4000 PCEs/animal were

scored for the presence of micronuclei (MnPCEs) whenever possible. In addition, at least 500 total erythrocytes (PCEs + NCEs) were scored per animal to determine the proportion of PCEs as an index of bone marrow cytotoxicity.

Study Validity

The assay was conducted according to established procedures of Heddle, Mutation Res. 18:187-190, 1973, Mavournin et al., Mutation Res. 239:29-80, 1990, and Hayashi et al., Mutation Res. 312: 293-304, 1994). No deviations from the protocol or assay-method SOPs occurred during the conduct of this study.

Criteria for Determination of a Valid Test

The following criteria were met, making this a valid test.

Vehicle Controls

The group mean frequency of cells with MnPCEs must be within the 95% control limits of the distribution of the historical negative control database.

Positive Controls

The frequency of cells with MnPCEs must be significantly greater than the concurrent vehicle control ($p \leq 0.05$). In this study, historical controls were used for positive control on data for Sprague-Dawley rats dating from 2012-2014 (tables only were provided, not full reports).

Parameter	Positive Control					
	%PCE (in Total Erythrocytes)		%MnPCE (Individual animal)		%MnPCE (Studies)	
	Males	Females	Males	Females	Males	Females
Mean ³	48.8	46.4	2.26	2.10	2.26	2.10
Standard Deviation	8.0	8.0	0.82	0.86	0.71	0.79
95% Control Limits ⁴	32.8-64.9	30.3-62.4	0.62-3.89	0.38-3.83	0.84-3.67	0.52-3.69
Range ⁵	12.0-72.7	26.6-58.4	0.50-5.70	0.60-4.75	0.64-4.34	0.90-4.19

- 0 Positive control article: Cyclophosphamide monohydrate (CP); Doses: 10 to 50 mg/kg; Routes of administration: IV, IP, PO. Bone marrow collection time: 24 hours post-dose.

Test Conditions

Five animals/group were available for analysis.

Analyzable Concentrations

At least 4000 PCEs/animal were scored for the presence of micronuclei (MnPCEs) whenever possible. In addition, at least 500 total erythrocytes (PCEs + NCEs) were scored per animal to determine the proportion of PCEs as an index of bone marrow cytotoxicity. If PCE proportions <20% of vehicle control value are considered excessively cytotoxic and the animal data may be excluded from evaluation.

Maximum Dose Evaluated

The maximum dose evaluated for micronucleus induction must, in the absence of cytotoxicity or MFD, a dose of 2000 mg/kg/ body weight/day (limit dose) is used.

- In the absence of cytotoxicity or MFD, a dose of 2000 mg/kg/ body weight/day (limit dose) is used.

Evaluation of Test Results

All criteria for a valid test were met and determination of a positive response did not occur. The following criteria were used to determine if a positive occurred.

- At least one of the doses exhibits a statistically significant increase when compared with the concurrent negative control ($p \leq 0.05$), and
- When multiple doses are examined at a particular sampling time, the increase is dose-related ($p \leq 0.05$), and
- Results of the group mean or of the individual animals are outside the 95% control limit of the historical negative control data.
- The test article was considered to have induced a clear negative response if none of the criteria for a positive response were met and there is evidence that the bone marrow was exposed to the test article (unless intravenous administration was used).

Results

Clinical Symptoms

No mortality occurred at any dose level during the course of the definitive assay. The only clinical sign observed was piloerection in the 20 mg/kg group. All other animals appeared normal throughout the observation period. While piloerection is not a serious toxicological symptom, since the dose selection was based on previous toxicity studies, the dose is considered adequate by this reviewer.

Bone Marrow Analysis

The incidence of mnPCEs per 20,000 PCEs scored (4000 PCEs/animal) and the proportion of polychromatic erythrocytes per total erythrocytes are summarized in the following table.

Micronucleus Assay - Summary of Bone Marrow Analysis

Treatment mg/kg	Gender	Time (Hrs)	Animals	%PCE (Mean +/- SD)	Change from Control (%)	% MnPCE (Mean +/- SD)	Number of MnPCE/PCE Scored
0.9% Saline Solution	M	24	5	50.7 ± 6.7	---	0.06 ± 0.02	11 /20000
Bupivacaine hydrochloride injection, USP 0.5%							
5	M	24	5	52.6 ± 7.7	4	0.08 ± 0.03	16 /20000
10	M	24	5	47.9 ± 4.9	-6	0.06 ± 0.02	12 /20000
20	M	24	5	54.2 ± 1.7	7	0.06 ± 0.03	12 /20000
CP							
40	M	24	5	41.9 ± 7.8	-17	5.41 ± 0.30**	1081 /20000
0.9% Saline Solution	M	48	5	56.6 ± 6.7	---	0.04 ± 0.03	8 /20000
Bupivacaine hydrochloride injection, USP 0.5%							
20	M	48	5	56.0 ± 3.8	-1	0.05 ± 0.03	10 /20000

*p < 0.05 or **p < 0.01, One-Way ANOVA with Post-Hoc analysis or T-Test

24 Hrs MnPCE Male GLM P-value = 0.425, R-sqr = 15.57%

Note: the cyclophosphamide (CP) positive control data is from historical control

- No appreciable reductions in the PCEs/EC ratio in the test article groups compared to the vehicle control group was observed indicating the test did not induce cytotoxicity.
- No statistically significant increase in the incidence of MnPCEs in the test article treated groups was observed relative to the negative control group.
- The number of MnPCEs in the vehicle control groups did not exceed the historical control range listed in the following table.

Negative Control ¹						
Parameter	%PCE (Individual Animals)		%MnPCE (Individual Animal)		%MnPCE (Studies)	
	Males	Females	Males	Females	Males	Females
Mean ³	51.6	50.9	0.05	0.04	0.05	0.04
Standard Deviation	5.7	4.7	0.06	0.05	0.04	0.04
95% Control Limits ⁴	40.3-62.9	41.5-60.3	0.00-0.17	0.00-0.15	0.00-0.14	0.00-0.12
Range ⁵	33.1-71.0	40.6-64.8	0.0-0.30	0.00-0.35	0.00-0.18	0.00-0.21

¹ Since no appreciable differences in the induction of MnPCEs by different vehicles and solvents (test article carriers) and different routes of administration were observed, this table contains data from carriers and routes of administration widely used during the conduct of contract studies. Vehicles: water, water soluble vehicles (methylcellulose, carboxymethylcellulose, dextrose), saline, corn oil and other vehicles. Routes of administration: intraperitoneal (IP), intravenous (IV), oral gavage (PO), subcutaneous (SC). Bone marrow collection time: 24 and 48 hours post-dose.

Under the conditions of this study, the administration of bupivacaine hydrochloride injection, USP 0.5% at doses up to and including a dose of 20 mg/kg was concluded to be negative in the In Vivo Mammalian Erythrocyte Micronucleus Assay in Rats. However, the highest dose tested does not appear to be a maximum tolerated dose.

Dose Formulation Analysis

The different dose levels necessary for this study were achieved based on application of differing volumes of the undiluted test article. Consequently, since no study specific formulations were prepared, the assessment of achieved concentration and homogeneity (formulations analysis) was not required in this study.

7.4 Other Genetic Toxicity Studies

Genotoxicity studies were conducted with extracts of the “to be marketed drug product” using ISO/USP procedures. These studies were reviewed as a consult by the FDA CDRH (Center for Devices and Radiological Health) reviewer, biologist Lixin Liu, PhD.

Testing results were negative for genotoxicity.

8 Carcinogenicity

No carcinogenicity studies were conducted or required for this single dose drug product.

9 Reproductive and Developmental Toxicology

9.1 Pregnancy Lactation and Labeling Rule (PLLR) Label Review

No new reproductive and developmental nonclinical toxicology studies were submitted with the NDA. Section 8 Pregnancy nonclinical data was as listed in the referenced approved drug Marcaine (Bupivacaine HCl – NDAs 18692/16964) but modified as part of the requirement under the Pregnancy Lactation and Labeling Rule (PLLR) to include such things as nonclinical to clinical dose ratios. In addition, a review and summary of available published literature regarding the potential effects of bupivacaine use on pregnancy, lactation, and effects on female and male fertility was conducted. This is also required for all NDA applications filed after June 30, 2015 re PLLR. Based on the original submission date of this NDA (pre-2015 on October 13, 2016), the Applicant was required to conduct a literature search, reviewed relevant articles, and included the information in Section 8 Pregnancy of the label if appropriate regarding nonclinical-based information. Information was reviewed, but no additional nonclinical information was added to the Xaracoll label in Section 8. Two separate searches were conducted. The first search from May 19, 2016 was initiated to support the original NDA submission that occurred October 13, 2016. The second search was conducted on September 29, 2017 in support of the NDA resubmission of February 2, 2018.

As part of this requirement under the Pregnancy Lactation and Labeling Rule (PLLR), a review and summary of available published literature regarding the potential effects of bupivacaine use on pregnancy, lactation and effects on female and male fertility was conducted by the Applicant using the following strategy, noting that this search is not limited to nonclinical data but all inclusive, including human references:

Search Strategy

Date of Search	Database	Search Terms ^a	Limiters ^b	Number of Results	Number of References Obtained
19 May 2016	Embase, BIOSIS, MEDLINE, PubMed Central	("bupivacaine") AND (pregnant OR pregnancy OR gestation OR gestational OR gestating OR lactating OR lactation OR breastfeeding OR "breast milk" OR "breast feeding") AND (safety OR effect*)	English only ^c	223 ^d	93
29 Sep 2017 ^e	MEDLINE, PubMed Central	("bupivacaine") AND (pregnant OR pregnancy OR gestation OR gestational OR gestating OR lactating OR lactation OR breastfeeding OR "breast milk" OR "breast feeding") AND (safety OR effect* OR toxic*)	English only	9	4

a - Limited to search terms mentioned in title or abstract.

b - Except as noted, there were no limitations with respect to time, open access, or geography.

c - Also excluded the following document types: Conference abstract, meeting abstract, English abstract, meeting poster, conference paper, literature review, editorial, note, letter, short survey, meta-analysis, practice guideline, news, consensus development conference, meeting summary, lectures, conference review, congress, correction, historical article, meeting paper.

d - After excluding duplicate results from in-house and consultant sources.

e - Limited to period of time between 19 May 2016 and 29 Sep 2017.

Twenty references were noted as being related to nonclinical studies. Of those twenty, only one study was considered potentially relevant for labeling related to toxicity and reviewed in more detail below (Danielsson et al. 1997). See all literature search nonclinical studies in Appendix 1 with reviewer comment about applicability for label.

Danielsson BR, Danielson MK, Boo EL, Arvidsson T, Halldin MM. Toxicity of bupivacaine and ropivacaine in relation to free plasma concentrations in pregnant rats: a comparative study. Pharmacol Toxicol. 1997;81(2):90-96.

Bupivacaine was administered subcutaneously to groups of 15 mated female Sprague-Dawley rats per group starting in late pregnancy (Gestation Day 15 to Lactation Day 15) at doses of 5.5, 15, and 18 mg/kg in a semi-GLP study (followed guidelines but no auditing reported). Maternal systemic toxicity, as evidenced by behaviors such as chewing, spasm, dyspnea, drowsiness, salivation, convulsions, and death occurred in a dose dependent manner at doses > 5.5 mg/kg. No differences between bupivacaine treated animals and controls were observed in litter size, offspring loss or weight of offspring at birth or on Days 7 and 21 after parturition nor were there any differences in the time to reach physical developmental milestones.

Methods: Clinical signs, mortality, body weights and food consumption were recorded regularly during the main study. The duration of gestation was also recorded and special attention was paid to labor and delivery. Efforts were also made to determine any adverse effects on lactation and nursing instincts of the mothers. The effects on the offspring were studied by recording litter size, pup and litter weight and pup loss at birth and on days 1 and 3 in the pilot studies and the main study, and also on days 7 and 21 after parturition in the main study. Physical development milestones, such as pinna unfolding, teeth eruption and eye opening, were studied in the offspring until termination of the main study on Day 21 after parturition.

Results: No completely trackable data (i.e., raw data) were provided. The only semi-trackable toxicity data was for clinical signs and mortality as a table was provided in which clinical symptoms were listed:

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

() – number of incidences of the event observed

Other reported study data (pharmacokinetic data not included in this review) was only supported by text and included:

- In some of the groups treated with bupivacaine, an increased incidence of postnatal death of the offspring was observed during the first few days after delivery. The increased incidence of postnatal death was highest in the 24 mg/kg group (around 50%). The increased postnatal loss of the offspring was most probably caused by impaired maternal care, since the majority of the dead neonates belonged to a few mothers who showed severe side effects and poor care of the newborn.
- The length of the gestation period varied between 21 and 23 days in all dose groups and there was no tendency towards shortening or prolongation of the gestation period in any group. The mean body weight gain and food consumption were essentially the same in all groups and no disturbance of parturition, nursing and lactation related to administration with bupivacaine was observed.
- No obvious intergroup differences in litter size, offspring loss or weight of the offspring at birth or on Days 7 and 21 after parturition were observed, nor was there any intergroup changes (including the control group) in the time of occurrence of the physical developmental milestones studied, which were expressed as incidences of litters with completed development of pinna unfolding on Day 4, teeth eruption on Day 12 and completed eye opening on Day 17.
- The absence of direct fetotoxicity of bupivacaine after maternal administration in pregnant rats was observed at the NOAEL. The increased incidence of postnatal death of the offspring was only observed at maternal plasma concentrations of bupivacaine which produced convulsions and maternal death. These deaths of offspring were considered secondary to impaired maternal care of the neonates, and surviving neonates showed the same physical development as animals in the control group, in body weight gain and occurrence of physical milestones.
- Notable but not confirmable – According to author, maternal adverse effects in pregnant rats are obtained at free concentrations 3-4 times higher than the free concentrations of bupivacaine in pregnant women after a standard therapeutic dose in Caesarean section.
 - o Proposed dose of 300 mg or 5 mg/kg
 - o NOAEL at 5.5 mg/kg (HED 0.9 mg/kg)

- o Dose ratio 0.2

Recommendation on PLLR-required literature search: No nonclinical studies identified by the PLLR-required literature search were considered necessary to further inform Section 8 Pregnancy part of the label by the Applicant (Owner) and this reviewer. The study noted above reported similar results as the pre- and postnatal toxicology study already described in the referenced product labeling which noted decreased pup survival after a SC doses of 40 mg/kg bupivacaine with an apparent NOAEL of 13.3 mg/kg.

10 Special Toxicology Studies

The Applicant submitted ISO/USP method studies, including cytotoxicity, local irritation, sensitivity, pyrogenicity, genotoxicity and short-term toxicity studies, to fulfill device-related requirements of the product. These studies were reviewed by a consultant CDRH reviewer. Refer to the CDRH review.

In addition, the Applicant performed the following extractables/leachables studies and risk assessments to justify the safety of the container closure system. Although the final XARACOLL drug product is a (b) (4), implantable product, (b) (4)

(b) (4) which make up part of the primary container closure system. (b) (4)

Therefore, there is potential for (b) (4)

1. **Analytical Report: Extractable Study for the Primary Packaging of Xaracoll**
(Study no.: (b) (4) 1702A-AREP01-V01)
2. **Analytical Report: Screening for Potential Leachables in Xaracoll Stability Study samples** (Study no.: (b) (4) 1704A-AREP01-V01)
3. **Safety Assessment of Leachables for INL-001 and its Container Closure System** (Study no.: NA – (b) (4))
4. **Risk Assessment of Leachables from Xaracoll** (Study no.: NA – (b) (4))
5. **Controlled Extraction Study Associated with a Molded Blister and (b) (4) Lid (b) (4) (Primary Packaging) Supporting a Collagen-Based Surgical Implant**
(study no.: NA – (b) (4))

10.1 Extractable studies

Study title: Extractable Study for the Primary Packaging of Xaracoll

Study no.: (b) (4) 1702A-AREP01-V01

Study location: SDN 9; 2/2/2018; [\\cdsesub1\evsprod\nda209511\0008\m3\32-body-data\32r-reg-info\extractable-report.pdf](#)

Conducting lab: (b) (4)

Components tested: The components tested are shown in the table below. The primary container system components, (b) (4),

(b) (4)

(b) (4) Secondary packaging components,

(b) (4)

Then, these

components were tested individually.

(b) (4)

Table 4: Sample weights for extraction

Component	Component Quantity	Sample Weight [g]	Extraction volume [mL]
Blister	3 (6.3 g)	6.3	20
(b) (4)	3 (1.2 g)	1.2	20
Triplet Carrier	1 (15.0 g)	15.0	30
Outer pouch	1 (11.0 g)	11.0 g	40
Label	1 (0.75 g)	0.75	5

Analytical Methods: The determination of semi-volatile components was done by GC-MS liquid injection, volatile components by Headspace-GC-MS, non-volatile components by HPLC-QToF-MS, and elemental impurities by ICP-MS according to USP <233>.

Analytical evaluation threshold (AET): The AET was calculated using the following equation, with an appropriate safety concern threshold (SCT) of (b) (4) mcg/day and employed an additional 50% safety factor.

(b) (4)

(b) (4)

Reviewer's comments:

Discussions with our OPQ drug product reviewer informed that the initial extractables and leachables studies submitted with the resubmission dated 2/2/2018 were not considered adequate from the CMC perspective as a clear extractable leachable correlation was not shown. Because this initial extraction study was not deemed adequate from the CMC perspective, a toxicological risk assessment on compounds identified in this study was not performed by this reviewer. See overall summary at the end of the leachables section for a discussion of the adequacy of the extractables/leachables evaluation from a nonclinical perspective.

Study title: Controlled Extraction Study Associated with a Molded Blister and
(b) (4) Lid (b) (4) (Primary Packaging) Supporting a Collagen-Based Surgical
Implant

Study no.: 10713

Study location: SDN 33; 9/20/2018; <\\cdsesub1\evsprod\nda209511\0032\m3\32-body-data\32r-reg-info\extractables-report-18-3412.pdf>

Conducting lab: (b) (4)

Components tested:

(b) (4) ID	Description	Sample Description for Report	Lot No.	Date Received
TST052918-08		(b) (4)	170811001	May 29, 2018
TST052918-09			161021001	May 29, 2018
TST052918-10			170614001	May 29, 2018
TST052918-11			16002311	May 29, 2018
TST061218-01			122053	June 12, 2018

(b) (4)

Reviewer's comments: The CMC drug product reviewer informed that the newly conducted extraction study was considered adequate in terms of extraction conditions

and analytical methods used. The primary goal of the extraction study is to identify what may leach from the container closure system components at above the AET and then monitor for those compounds in an appropriately designed leachables study. However, an adequate leachables assessment was not provided. Therefore, one potential approach to evaluate the safety of the container closure system, though not ideal, is to perform a toxicological risk assessment on compounds that were detected with TDIs above (b) (4) mcg/day from the extraction study as a worst-case scenario. However, in this case, there were numerous unidentified compounds detected in the pH (b) (4) extracts alone, which represent the most closely related to the clinical product, using HPLC-DAD/MS that exceeded a TDI of (b) (4) mcg/day. Therefore, an adequate risk assessment cannot be performed on the potential leachables detected in the extraction studies.

10.2 Leachables studies

Study title: Screening for Potential Leachables in Xaracoll Stability Study samples

Study no.: (b) (4) 1704A-AREP01-V01

Study location: SDN 9; 2/2/2018;

Conducting lab: (b) (4)

Materials tested: One matrix from each XARACOLL batch was analyzed per the table below. Note, that only aged products at least 22 months or older were evaluated for leachables.

Batch Number	25°C	40°C
10001706 (Drug 60month) SP-QC0015, Nov 2010, (78 months)	x	
10011706 (Drug 60month) SP-QC0015, Nov 2010, (78 months)	x	
10021706 (Drug 60 month) SP-QC0015, Nov 2010, (78 months)	x	
14021606 SP-QC0039, Dec 2014, (30 months)	x	
15012801 SP-QC0043, Jul 2015, (22 months)		x
15041002 SP-QC0043, Jul 2015, (22 months)	x	
15020502, July 2015 (Placebo 22 months)	x	

Analytical methods: The determination of semi-volatile components was done by GC-MS liquid injection, volatile components were determined by Headspace-GC-MS, and non-volatile components were determined by HPLC-QToF-MS.

Sample preparation methods: For each XARACOLL batch, one matrix was cut into separate Erlenmeyer flasks. All flasks were rinsed with acetone and dried before commencing the analyses. The volume of 10 mL was minimal dose to wet the whole sample for dissolution. The flasks were filled with the corresponding dissolution solvent, either IPA or collagenase, closed and stored at 37 °C for two hours. A dynamic temperature-controlled plate-shaker was used to aid the sample dissolution. For blank analysis, each dissolution solvent was filled in individual Erlenmeyer flasks. These samples were stored under the same conditions as the samples. For FET-HS-GC-MS analysis a Xaracoll matrix was cut into pieces and filled in a headspace vial without further preparation.

For evaluation purposes, peaks above a level of (b) (4) mcg/dose (dose equivalent to 3 implants) were reported, as well as any above the TTC of 60 mcg/dose.

Reviewer's comments:

Discussions with our CMC drug product reviewer informed that the initial extractables and leachables studies submitted with the resubmission dated 2/2/2018 were not considered adequate from the CMC perspective as a clear extractable leachable correlation was not shown.

An Information Request from CMC sent to the Applicant on 6/27/2018 stated:



To show an extractable leachable correlation:

- a. Perform a rigorous extractables study of the primary and secondary packaging. Consider refluxing the primary and secondary packaging in appropriate solvents at their boiling point for 24-48 hours.
- b. From the robust extractables profiles, identify and determine the target leachables.
- c. Develop a method which can detect these leachables in the drug product.

- d. Test drug product at multiple timepoints on stability.
- e. We refer you to USP <1663> and <1664>.

The Applicant responded on 7/10/2018:

New studies have been initiated at (b) (4) a US based lab recognized as a leader in E&L studies. From the results of the Controlled Extraction Study, target leachables will be identified and methods developed and validated to detect those leachables with predicted concentrations above the Safety Concern Threshold in the finished Xaracoll combination product. These methods would then be used to test lots at release and throughout stability. Innocoll will submit the reports for these new studies when available. In addition, in response to the FDA observations on the existing studies, Innocoll would like to provide clarification. Leachable study (b) (4) 1704A-AREP01-V01 was not intended to be a study using validated methods to quantify specific target leachables but rather an initial screening study to determine whether any of the extractables which were found in the packaging materials were ultimately identified as leachables in Xaracoll. This was necessary as the design of the extractables study ((b) (4) -1702A-AREP01-V01) did not allow for the identification and grouping of all of the extractables and thus a simple determination of specific target leachables was not possible.

As noted above, it has been recognized that these studies may not completely fulfill FDA expectations for E&L studies. Accordingly, new studies have been initiated to confirm that all E&L regulatory requirements are met. The results from these studies will be provided when available.

It is notable that the Applicant clarified that the submitted leachables study was an initial screening study and that it did not employ validated methods to quantify specific target leachables. An IR was sent to the Applicant to inquire about timeline for a new adequately designed leachables study, and the Applicant subsequently conveyed the following information on 8/16/2018:

The extractables study for the primary packaging was prioritized as these data are the most relevant due to being in direct contact with the product. The studies on secondary packaging are being performed to ensure a complete E&L package will be available from a single source but were initiated at a later date. The remaining studies are proposed as postmarketing commitments. This will include completing, in sequential order, the extractables study on secondary packaging, method development of up to four methods for target leachables, and validation of the methods. At this point, methods will be used on lots of various ages (e.g., clinical lots beyond proposed 2-year expiry, lots using commercial process from initial up to approximately 12 months age) to determine a worst-case leachable profile, including toxicological evaluation of these results. The

entire comprehensive package is expected to be submitted to the NDA by 31 January 2019.

After method validation is complete, the final stage will be a shelf-life study of commercial Xaracoll lots using the validated leachables methods from release through the proposed 24-month shelf life. Initial release data will be submitted by 28 February 2019, with 6 month updates available in August and February through February 2021 to cover the proposed 24-month shelf life.

In summary, adequate leachables data were not available during this review cycle to justify the safety of the container closure system. Therefore, a deficiency will be included in this regard as the NDA will not be approved during this review cycle. The reader is referred to the CMC review for further details regarding the adequacy of the studies completed.

11 Integrated Summary and Safety Evaluation

The Center for Devices and Radiological Health (CDRH) and the Center for Drug Evaluation and Research (CDER) conducted individual reviews of nonclinical studies that were conducted, consistent with relevant methodologies (e.g., drug device for CDRH and drug product for CDER).

Center for Devices and Radiological Health (CDRH) Review

Testing of drug product and collagen vehicle extracts as part of biocompatibility testing using International Organization for Standardization (ISO) and United States Pharmacopeia (USP) methods support that the collagen component of the Xaracoll combination product and the complete Xaracoll drug product are non-cytotoxic, non-irritant, non-sensitive, non-pyrogenic, non-genotoxic and non-toxic in short-term condition. The tested Xaracoll was the “to be marketed” drug product.

Center for Drug Evaluation and Research (CDER) Review

General Toxicity:

In a rat study with a 56-day observation period, after a single dose, the quantity of collagen sponge material progressively decreased with increasing time as part of the healing process but attrition rate was greater at an external implant site when compared to an internal implant site. No collagen sponge material could be microscopically discerned via evaluation of routine H&E-stained tissues at Day 56 at any external or internal implantation site in Groups 3-5. The lowest-observed-adverse-effect-level (LOAEL) is the low dose of 12 mg bupivacaine (30.8 mg/kg; 6 cm² total bupivacaine collagen sponge dose) for males and 8 mg bupivacaine (32.3 mg/kg; 3 cm² total bupivacaine collagen sponge dose) for females.

Listed in the table are human clinical study and rat nonclinical study dose levels and blood levels of bupivacaine at Maximum Recommended Human Dose (MRHD) dose levels Bupivacaine HCl as Marcaine (175 mg – 2.9 mg/kg for 60 kg body weight) and as Xaracoll (300 mg - 5 mg/kg for 60 kg body weight). On a Human Equivalent Dose Level (HED) basis, the nonclinical doses support the human doses of Marcaine at 175 mg and of Xaracoll of 300 mg.

While these values support human safety for this single dose drug product from a C_{max} standpoint, they do not support human safety based on AUC. On this basis, human safety is not supported by nonclinical data. This nonclinical study was not very robust in doses tested.

Human Equivalent Doses (HED), Blood Toxicokinetics, and Nonclinical:Clinical Dose Safety Ratios of Bupivacaine after a Single Dose of Marcaine (175 mg – 2.9 mg/kg) or Xaracoll (300 mg – 5 mg/kg) Subcutaneously ^a										
species	sex	Dose (mg/kg)	HED ^b	Tmax (hour)	T1/2 (hour)	Cmax (ng/mL)	AUC _{0-t} ng*hour/mL	Nonclinical:Clinical Dose Ratio at MRHD ^c		
								HED	Cmax	AUC
Marcaine										
Rat	Female	16.1	2.6	0.5	1.6	1,100	2,410	0.9	1.7	0.3
	Male	15.3	2.4	0.5	1.4	935	2,710	0.8	1.5	0.3
Human	Male & Female	2.9	NA ^d	1	9.1	641	8,837	NA	NA	NA
Xaracoll										
Rat	Female	32.3	5.2	1	NR ^e	1,250	6,080	1.1	1.9	0.3
		48.4	7.7	1	2.1	1,730	8,900	1.6	2.6	0.5
	Male	31	4.9	1	4.7	773	5,840	1.0	1.2	0.3
		61	9.8	1	5.3	1,450	12,200	1.9	2.2	0.7
Human	Male & Female	5^f	NA	3	18.9	663	18,187	NA	NA	NA

a - Clinical Study CNN-CB-022 and Nonclinical Study 00134510
– Xaracoll lot 15041002

b - Human Equivalent Dose (HED) based on cm² body surface area (BSA)

c - Maximum Recommended Human Dose

d - Not Applicable

e - Not Reported

f - t = 96 hours in rats and humans

Clearance:

In separate pharmacokinetics studies, the amount of bupivacaine in the sponge (dogs) and in the blood (rats) were evaluated after a single dose of Xaracoll. Essentially, all of the bupivacaine was cleared from the sponge (dogs) and from the blood (rats) by 24 hours.

Dogs:

The dose was approximately 50 mg/dog or 5 mg/kg. For this non-safety study, for reference purposes only, the HED is 2.7 mg/kg with a proposed MRHD of 5 mg/kg.

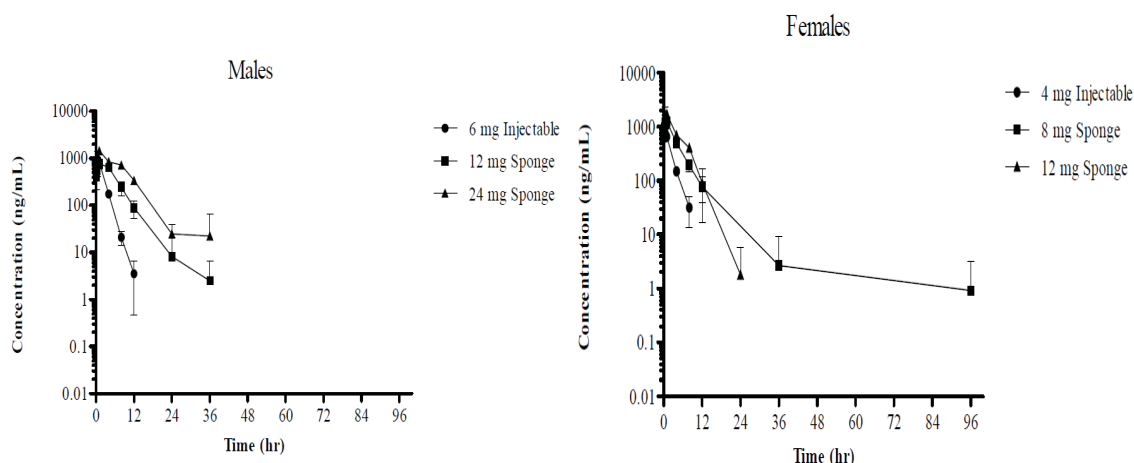
Residual Bupivacaine HCl Content in Collagen Sponge Implant Samples

Animal Number	Collection Time on Study Day 0 (Hours Post-Dose)	Bupivacaine Concentration (ng/mL)	Residual Bupivacaine Content (mg)	Residual Bupivacaine HCl Content (mg)
6883	0.25	1,150,000	23.0	25.9
5446	0.5	1,020,000	20.4	23.0
5496	1	946,000	18.9	21.3
7427	2	638,000	12.8	14.4
6559	4	595,000	11.9	13.4
8069	8	427,000	8.5	9.6
8457	12	213,000	4.3	4.8
5316	24	21,200	0.4	0.5

Rats:

The dose was approximately 61.9 mg/kg for males and 49.1 mg/kg for females. For this non-safety study, for reference purposes only, the HED is 9.9 mg/kg 7.9 mg/kg for females with a proposed MRHD of 5 mg/kg.

The mean concentrations of Bupivacaine in plasma of male and female rats following injection or surgical implantation of bupivacaine (Xaracoll)



Genotoxicity:

Bupivacaine was evaluated for potential genotoxicity. Bupivacaine HCl was negative for genotoxicity in a test battery: 1) In Vitro Reverse Mutation Assay in Bacterial Cells (Ames), 2) In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay), and 3) In Vivo Clastogenicity Assay in

Rodent (Micronucleus Assay). No carcinogenicity data was identified or generated for this single dose drug product.

In summary, the proposed maximum recommended human dose (MRHD) of 300 mg (5 mg/kg for a 60 kg human body weight), is not considered supported by nonclinical safety data with the duration of the exposure approximately 24 hours.

12 Appendix/Attachments

Appendix 1

Literature search-based nonclinical studies as part of Pregnancy Lactation and Labeling Rule (PLLR) Label Review

The following tables contain Applicant reviews of the 20 nonclinical studies identified in the PLLR-related literature search with review comments. One study (Danielsson et al. 1997) was considered potentially label worthy with more detailed review by this reviewer as contained in Section 9.

Animal Studies of Bupivacaine in Pregnancy

Hisayo O. Morishima, M.D. et al. Disposition of Bupivacaine and Its Metabolites in the Maternal, Placental, and Fetal Compartments in Rats. American Society of Anesthesiologists, 2000; 93:1069-74.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Morishima et al 2000 ⁵²	Pregnant Sprague-Dawley rats of 20-22 day gestation (term, 30 days)	- Total = 30 8 (PK group) 22 (transfer group)	- PK group: 1 mg/kg bolus BUP followed by IV infusion of 0.33 mg/kg/min over 15 min - Transfer group: IV infusion of 0.33 mg/kg/min BUP over 15 min	Distribution $t_{1/2}$	PK group: 1.2 ± 0.1 min	Fetal peak plasma conc	Transfer group: 320 ± 38 ng/mL
				Elimination $t_{1/2}$	PK group: 37.7 ± 2.4 min	Fetal:maternal plasma conc ratio at 0 h	Transfer group: 0.29
				V_d at steady-state	PK group: 3.86 ± 0.29 l/kg	Fetal:maternal placental conc ratio at 0 h	Transfer group: 0.63
				Clearance	PK group: 93.3 ± 8.6 mL/min/kg	HR	Transient, nonsignificant decrease from baseline in both groups
				Peak maternal plasma conc (after 15 min infusion)	PK group: $3,123 \pm 370$ ng/mL Transfer group: 1217 ± 130 ng/mL		

Reviewer Summary: This study was designed to determine the disposition of bupivacaine and its metabolites in the maternal, placental, and fetal compartments after a 1 mg/kg intravenous bolus followed by a 15-minute intravenous dosing at 0.33 mg/kg min in of 20-22 day pregnant rats with delivery either at the end of infusion or at 2 or 4 hours after dosing. Blood was sample at 0, 5, 15, 30, 60, 120, 180, and 240 minutes after infusion.

The elimination half-life of bupivacaine was 37.7 min. The major metabolite was 3'-hydroxybupivacaine. Bupivacaine and 3'-hydroxybupivacaine were present in all samples at the end of administration. The fetal to maternal concentration ratio of bupivacaine in plasma was 0.29, and in the placenta was 0.63. The amnion contained the highest bupivacaine concentration at 3-fold higher in the maternal and 11-fold higher than in the fetal plasma. At 4 hours after dosing, bupivacaine was no longer detectable in any maternal and fetal samples, whereas 3'-hydroxybupivacaine was still present in all tissues except the fetal plasma and heart.

The authors conclude that the data support the conclusion that bupivacaine does cross the placenta and levels of the drug and the 3'-hydroxybupivacaine metabolite can be detected in the fetus. The longer duration of exposure to the 3'-hydroxy metabolite is interesting and may be useful in the interpretation of any adverse findings in this species. However, as the existing rat embryo-fetal development and pre- and postnatal development study would have addressed the implications of this distribution, these data need not be included in labeling at this time.

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Danielsson BR, Danielson MK, Boo EL, Arvidsson T, Halldin MM. Toxicity of bupivacaine and ropivacaine in relation to free plasma concentrations in pregnant rats: a comparative study. *Pharmacol Toxicol.* 1997;81(2):90-96.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Danielsson et al 1997 ⁵³	Pregnant Sprague-Dawley rats at 15 days gestation	15	5.5, 15, or 18 mg/kg BUP SC injection	Occurrence of adverse effects	Chewing, spasm, dyspnea, drowsiness, salivation, and convulsions occurred in a dose-dependent manner at doses ≥ 18 mg/kg (plasma = 0.6 mg/L)	Litter data	No differences between BUP-treated animals and control were observed in litter size, offspring loss, or weight of the offspring at birth or on days 7 and 21 after parturition, nor were there any changes in the time of occurrence of the physical developmental milestones studied
				C _{max} : • 5.5 mg/kg • 15 mg/kg • 18 mg/kg	• 0.19 $\pm$ 0.05 mg/L • 0.63 $\pm$ 0.12 mg/L • 1.14 $\pm$ 0.41 mg/L C _{max} was reached in most cases within the first sampling interval (30 min) indicating that BUP is		
					rapidly absorbed from the site of injection.		
				T _{max} : • 5.5 mg/kg • 15 mg/kg • 18 mg/kg	• 0.6 $\pm$ 0.2 hr • 0.8 $\pm$ 0.3 hr • 0.5 $\pm$ 0 hr		
				t _{1/2} : • 5.5 mg/kg • 15 mg/kg • 18 mg/kg	• 15.0 $\pm$ 12.3 hr • 5.2 $\pm$ 2.2 hr • 2.7 $\pm$ 2.2 hr		
				Protein binding 0.5 hr after dosing: • 5.5 mg/kg • 15 mg/kg • 18 mg/kg	• 70.5 $\pm$ 6.8% • 67.4 $\pm$ 5.9% • 68.8 $\pm$ 6.9%		

Reviewer Comments: See Section 9.

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Fauza, DO et al., Prolonged Local Myometrial Blockade Prevents Preterm Labor After Fetal Surgery in a Leoprine Model. Journal of Pediatric Surgery, 1999, 34:4(April), 540-542.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Fauza et al 1999 ⁸⁴	Rabbits at 23 days gestation (term, 31 to 33 days)	5	0.5% BUP (5 mL) injected before hysterectomy; before closure myometrium incision injected with 1.5 mL suspension of polylactic co-glycolic microspheres with 75% (drug/polymer weight ratio) of BUP + 0.05% dex			Rate of abortion after fetal surgery	0 of 5 fetuses were aborted following surgery

Reviewer Comments: This study was completed as an animal model of post-operative premature labor. Although interesting the results are not appropriate for labeling.

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Morishima HO et al. Bupivacaine Toxicity in Pregnant and Nonpregnant Ewes. Anesthesiology, 1985, 63:134-139.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Morishima et al 1985 ⁸⁵	Pregnant sheep (ewes) at 130±2 days gestation (term, 148 days)	8	0.5 mg/kg/min, continuous infusion into jugular vein	Convulsions	- Dose: 1.9± 0.6 mg/kg (~6 min) - Serum conc: 4.2±0.4		
					µg/mL		
				Hypotension	• Dose: 3.8±0.6 mg/kg (8 min)*		
				Respiratory arrest	• Serum conc: 5.2 ±0.7 µg/mL (>8 and <11 min)*		
				Circulatory collapse	- Dose: 5.1±0.1 mg/kg (11.4 min)* - Serum conc: 5.5±0.8 µg/mL		
				*Significantly higher than non-pregnant controls			

Reviewer Comments: Nonclinical intravenous infusion not relevant for this indication.

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Santos AC, et al. Effect of Ropivacaine and Bupivacaine on Uterine Blood Flow in Pregnant Ewes, Anesth Analg, 1992; 74:62-67.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result

Santos et al 1992 ⁸⁶	Pregnant sheep (ewes) at 125-130 days gestation (term, 148 days)	15 total: 7 low-dose, 8 high-dose	- Continuous infusion for 60 minutes - Low dose: 0.077 mg/kg/min infusion for 15 min followed by 0.039 mg/kg/min for 45 min - High dose: 0.1 mg/kg/min for 15 min followed by 0.058 mg/kg/min for 45 min	Mean maternal plasma conc at 60 min (µg/mL)	- Low dose: 1.55±0.15 - High dose: 1.83±0.19	Mean fetal plasma conc at 60 min (µg/mL)	- Low dose: 0.28±0.09 - High dose: 0.32±0.02
				Maternal HR (bpm)	- Low dose: 96±7 (significant decrease from pre-infusion HR of 113±9) - High dose: 100±7	Fetal HR (bpm)	- Low dose: 163±2 - High dose: 152±10 - No significant differences from baseline
				Maternal MAP (mmHg)	- Low dose: 103±6 - High dose: 111±2 - No significant differences from baseline	Fetal MAP (mmHg)	- Low dose: 52±2 - High dose: 53±5 - No significant differences from baseline
				Maternal arterial pH	- Low dose: 7.50±0.02 - High dose: 7.52±0.02 - No sig differences from baseline	Fetal arterial pH	- Low dose: 7.41±0.02 - High dose: 7.41±0.01 - No sig differences from baseline
				Uterine blood flow (mL/min)	- Low dose: 456±60 - High dose: 420±61	CVP & IA	No significant changes from baseline
					No significant differences from baseline		

Reviewer Comments: Not relevant for proposed bupivacaine sponge insertion.

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Santos AC, et al. Comparative Systemic Toxicity of Ropivacaine and Bupivacaine in Nonpregnant and Pregnant Ewes, American Society of Anesthesiologists, 1995; 82:734-740.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Santos et al 1995 ⁸⁷	Pregnant and non-pregnant sheep (ewes) at 130-140 days gestation (term 148 days)	12	0.5% BUP at a rate of 0.5 mg/kg/min (continuous infusion) until circulatory collapse	Manifestations of systemic toxicity – convulsions, hypotension, apnea, and circulatory collapse	No significant difference between pregnant and nonpregnant animals in the doses or serum concentrations at which toxic manifestations occurred		

Reviewer Comments: Seems not relevant to label as sheep not typical nonclinical example and study was a bupivacaine versus ropivacaine comparison when ropivacaine was a new drug.

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Santos AC, DeArmas PI. Systemic toxicity of levobupivacaine, bupivacaine, and ropivacaine during continuous intravenous infusion to nonpregnant and pregnant ewes. Anesthesiology. 2001;95(5):1256-1264.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Santos et al 2001 ⁸⁸	Pregnant and non-pregnant sheep (ewes) near term of gestation	12 (pregnant)	0.52% BUP at a constant rate of 0.1 mL/kg/min (continuous IV infusion)	• Convulsions	Dose: 0.013±0.002 mmol/kg for pregnant ewes vs 0.014 ± 0.003 mmol/kg for non-pregnant ewes, P = 0.001		Fetal plasma conc: 90 min, 180 min
				More advanced manifestations of toxicity: - Hypotension - Apnea - Circulatory collapse	No significant differences between pregnant and nonpregnant ewes in dose required		

Reviewer Comments: No treatment-related effects in nontraditional nonclinical model so no label inclusion considered warranted.

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Santos AC et al. The Effects of Bupivacaine, L-Nitro-L-Arginine-Methyl Ester, and Phenylephrine on Cardiovascular Adaptations to Asphyxia in the Preterm Fetal Lamb. *Anesth Analg.* 1997; 85:1299-1306.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Santos et al 1997 ⁸⁹	Pre-term fetal lambs (from ewes at 112-114 days gestation)	13	0.07 mg/kg/min BUP for 15 min followed by 0.035 mg/kg/min BUP for 165 min (2-step IV infusion)	Maternal plasma conc: 90 min, 180 min	• 2.12±0.28 µg/mL • 2.06±0.22 µg/mL	Fetal plasma conc: 90 min, 180 min	• 0.34±0.09 µg/mL • 0.38±0.09 µg/mL
				Maternal HR	95±5 bpm	Fetal HR	172±5 bpm
				Maternal MAP	118±6 mmHg	Fetal MAP	50±2* mmHg
				Maternal pH	7.49±0.01	Fetal pH	7.33±0.03*
					*Significantly different from control	Effect on adaptation to asphyxia	• No change in MAP and acid-base state after 90 min of cord asphyxiation • Abolished asphyxia-induced increase in blood flow to myocardium and cerebral cortex but not to adrenals

Reviewer Comments: Topic not considered relevant to label as toxicokinetic data not presented.

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Fleming JA et al. The Effects of Bupivacaine on the Umbilical Circulation and Placental Gas Exchange in the Fetal Lamb. *Anesth Analg.* 1987; 66:1121-1126.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Fleming et al 1987 ⁹⁰	Chronically instrumented fetal lambs at 125-140 days gestation	7	• ~0.1 mg/kg/min BUP • Continuous infusion for 60-120 min • Infusion rates were selected to avoid neurologic toxicity and approach plasma BUP conc measured in cord blood after epidural anesthesia			Peak plasma conc	1.3±0.3 µg/mL (range: 0.2-3.2)
						Fetal HR	158±2* bpm (89% of control)
						Umbilical flow	187±20* mL x kg/min (94% of control)
						Fetal BP (UA)	50±1 mmHg
						Umbilical vascular resistance	0.24±0.04 mmHg/mL x kg/min
						UA pH	7.35±0.01
						BE	3.1±0.7 mM/L
						Epoch length for low-voltage ECoG	14 ±2 min
						Epoch length for high-voltage ECoG	12 ±2 min
							*Significantly different from control

Reviewer Comments: Not apparently relevant to label for subcutaneously administered drug as tested chronically catharized sheep and intravenous fetal dosing.

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Karsli B et al. Effects of Local Anesthetics on Pregnant Uterine Muscles. *Pol. J. Pharmacol.* 2003; 55:51-56.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Karsli et al 2003 ⁵⁵	Myometrial strips from pregnant albino rats at gestation 18-21 days	30	10 ⁻⁵ M to 10 ⁻⁴ M BUP in 10 mL organ bath	• Contraction duration • Contraction frequency	BUP statistically decreased contraction frequency and duration at a conc of 3 x 10 ⁻⁴ M		

Reviewer Comments: Not considered relevant to label as study was of isolated myometrium of pregnant rats.

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Moller RA et al. Effects of Progesterone on the Cardiac Electrophysiologic Action of Bupivacaine and Lidocaine. *Anesthesiology*. 1992; 76:604-608.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Moller et al 1992 ⁹¹	Hearts (right septal wall) from oophorectomized female white New Zealand rabbits treated with 59.8±11.0 ng/mL progesterone	7	3.5, 10.4, and 17.4 µM BUP (1, 3, and 5 µg/mL)	PF action potential	Significant anesthetic-induced decrease in PF action potential amplitude at conc of 10.4 µM		
				Inexcitability	Inexcitability was induced in 5 progesterone-treated tissues (compared with 2 controls)		
				V _{max}	Significantly greater depression of V _{max} at 3.5 (52%) and 10.4 µM compared to control		
				APD ₅₀	Slight, non-significant decreases in APD ₅₀		
				PF-VM	Complete PF-VM conduction block at 10.4-17.4 µM BUP		

Reviewer Comments: Animal model of oophorectomized rabbits and Purkinje fiber electrophysiology. There is no clear impact on current labeling.

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Arici G et al. The effects of bupivacaine, ropivacaine and mepivacaine on the contractility of rat myometrium. *Int J Obstetric Anesthesia*. 2004; 13:95-98.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Arici et al 2004 ⁵⁴	Myometrial strips from pregnant Wistar rats at 18-21 days gestation	10	Cumulative doses from 10 ⁻⁸ to 10 ⁻⁴ mol/L of BUP in 10 mL organ bath for 30 min at each dose level.	Force of contractions	Significantly reduced from baseline at a 10 ⁻⁴ mol/L, when contractility was 79.6±12.6% of baseline (P=0.005)		
				Frequency of contractions	No difference from baseline at any conc		

Reviewer Comments: In vitro data not likely contributory to label.

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Dong Y and Gao L. Effect of epidural block on 24-hour urine protein in pregnant rat models with preeclampsia. *Arch Gynecol Obstet*. 2012; 286:609–611.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result

Dong and Gao 2011 ⁹²	Wistar rats on the 14th day of pregnancy given L-NAME to model pre-eclampsia	10	0.125% of BUP (25 µl) twice daily for 7 days epidural anesthesia + SC L-NAME	Systolic BP on 20th day after pregnancy	• 131.6±11.1 (increased from 110.4±10.2 at baseline) • Significantly lower than control group given saline or L-NAME alone		
				24-hour urine protein on 20th day after pregnancy	• 32.06±8.18 (increased from 7.65±1.81) • Significantly lower than control group given saline or L-NAME alone		
				Epidural block can decrease 24-hour urine protein and systolic blood pressure in pregnant rat models with pre-eclampsia			

Reviewer Comments: Label value limited from 3-page article and dose route (epidural) not clearly relevant to proposed subcutaneous indication.

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Fishburne JI et al. Responses of the gravid uterine vasculature to arterial levels of local anesthetic agents. Am J Obstet Gynecol. 1979; 133 (7):753-761.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Fishburne et al 1979 ⁹³	Pregnant sheep (ewes) of 77-97 days gestation	26	• Intravascular infusion conc of	Uterine blood flow	25% reduction from initial UBF with 7.0 µg/mL of initial UBF		
			BUP was varied to yield blood levels of ~1, 3, and 10 µg/mL • Mean conc: 1.4 µg/mL (1.0-2.0); 4.2 µg/mL (2.9-7.0); 10.9 µg/mL (7.7-11.2)	Myometrial tonus	Regular and highly significant increase in myometrial tonus		

Reviewer Comments: Responses of the uterine vasculature and myometrium to uterine arterial infusions of local anesthetic agents evaluated in chronically prepared conscious ewes between not considered to impact current label.

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Santos AC et al. The Placental Transfer and Fetal Effects of Levobupivacaine, Racemic Bupivacaine, and Ropivacaine. Anesthesiology. 1999; 90:1698-1703.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Santos et al 1999 ⁹⁴	Pregnant ewes in good condition near term of gestation (148 days)	10	0.014 mL/kg/min intravascular catheter infusion to achieve maternal serum conc at 60 min of 1-1.5 µg/mL (routine epidural anesthesia for Caesarian delivery)	60 min post-infusion		60 min post-infusion	
				• Maternal HR • Maternal MAP • Maternal UBF • Maternal IA • Maternal CVP *Statistically significant decrease.	• 82±15 bpm* • 89±10 mmHg • 469±27 mL/min • 7±3 mmHg • 7±4 mmHg	• Fetal HR • Fetal MAP • Fetal pH (n=9) • Fetal PCO₂ • Fetal PO₂	• 150±16 bpm • 49±4 mmHg • 7.41±0.02 • 44±3 • 21±4

Reviewer Comments: Intravenous infusion of pregnant ewes not considered to impact labeling given human experience.

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Wei J et al. Effects of local anesthetics on contractions of pregnant and non-pregnant rat myometrium in vitro. Acta Physiologica Hungarica. 2014; 101(2):228-235.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Wei et al 2014 ⁵⁶	Myometrial strips from pregnant Sprague-Dawley rats at 18-20 days of gestation	8	- BUP was added at a cumulative conc from 0.01-300 $\mu\text{mol/L}$ to a 10 mL bath - Each strip was exposed for 15 min	Contraction amplitude	BUP significantly decreased amplitude of contractions in pregnant myometrium at conc of $\geq 100 \mu\text{mol/L}$		
				Contraction frequency	BUP had no significant effect on frequency of contractions		

Reviewer Comments: In vitro study of myometrium not considered of value to label.

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Nacitarhan C et al. The Effects of Opioids, Local Anesthetics and Adjuvants on Isolated Pregnant Rat Uterine Muscles. *Methods Find Exp Clin Pharmacol*. 29(4);273-276.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Nacitarhan et al 2007 ⁵⁹	Myometrial strips from pregnant Wistar rats	10	Increasing conc of BUP from 10^{-8} to 10^{-4} in a 20-mL tissue bath	% decrease in oxytocin-induced contractions	20.4 $\pm$ 7.4		
				Sensitivity (-log EC ₅₀)	4.2 $\pm$ 0.2		
				Contraction frequency	BUP did not cause a significant change in the frequency of oxytocin-induced contractions		

Reviewer Comments: In vitro study of longitudinal uterine smooth muscle not considered of value to label.

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Vincent RD, Jr., Chestnut DH, McGrath JM, Chatterjee P, Poduska DJ, Atkins BL. The effects of epidural anesthesia on uterine vascular resistance, plasma arginine vasopressin concentrations, and plasma renin activity during hemorrhage in gravid ewes. *Anesth Analg*. 1994; 78(2):293-300.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Vincent et al 1994 ⁶⁵	Pregnant sheep (ewes) at 118 days gestation (term, 145 days) who were bled at a constant rate	12	6-20 mL of 0.5% (additional BUP injected as needed until sensory level was at T8)	Maternal outcomes: • HR (beats/min) • MAP (mm Hg) • Stroke volume (mL) • Cardiac output (L/min) • UBF (mL/min) • PCWP (mm Hg) • SVR (dynes $\times$ cm^{-5} $\times$ s^{-1}) • UVR (dynes $\times$ cm^{-5} $\times$ s^{-1}) • pH • PO ₂ (mm Hg) • PCO ₂ (mm Hg)	- The bleeding volume required to reduce maternal MAP 40% below baseline was smaller ($P=0.0001$) in the epidural group than in the control group. - However, the additional bleeding volume necessary to maintain maternal MAP at that level for 1 h was similar in the two groups. - Maternal HR, CO, PCWP, CVP, hematocrit, pH PO₂, and PCO₂ were not significantly different between groups at any time. - Maternal MAP was significantly lower in the epidural group ($P=0.0001$) before hemorrhage but not after	Fetal outcomes: • HR (beats/min) • MAP (mmHg) • pH • PO ₂ (mmHg) • PCO ₂ (mmHg)	- Fetal HR and MAP were not significantly different between groups at any time. - Epidural anesthesia attenuated the increase in uterine vascular resistance during hemorrhage. However, there were no significant differences in fetal PO₂ or pH between groups.

Reviewer Comments: Article not found in submission and only abstract obtained but topic considered not considered relevant for label.

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Fanning RA et al. A Comparison of the Inhibitory Effects of Bupivacaine and Levobupivacaine on Isolated Human Pregnant Myometrium Contractility. Anesthesia and Analgesia. 2008; 107(4): 1303-1307.

Author, Year	Model	n	Dose	Maternal Outcomes		Fetal Outcomes	
				Safety Endpoint	Result	Safety Endpoint	Result
Fanning et al 2008 ³⁷	In Human Myometrial Strips vitro study	8	1x10 ⁻⁸ M to 3x10 ⁻⁴ M BUP in 10-15 mL organic baths	Contraction amplitude EC ₅₀	-4.21 M (level is 33 times higher than maximal plasma conc obtained with 30 mL of 0.5% BUP)		
				Peak-to-peak contraction interval EC ₅₀	-3.68M		

Reviewer Comments: Human term in vitro myometrium contractility study not considered relevant for nonclinical section of labeling.

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

/s/

GARY P BOND
11/02/2018
Xaracoll NDA Nonclinical Review

RICHARD D MELLON on behalf of JAY H CHANG
11/02/2018

RICHARD D MELLON
11/02/2018
I concur.