

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

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

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Indication: Post-operative pain management
Applicant: Heron Therapeutics, Inc.
Clinical Review Division: Division of Anesthesiology, Addiction Medicine,
and Pain Medicine (DAAP)
Reviewer: Jaime D'Agostino, PhD
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Template Version: September 1, 2010

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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY.....	11
1.1	INTRODUCTION	11
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	11
1.3	RECOMMENDATIONS	16
2	DRUG INFORMATION.....	26
2.1	DRUG	26
2.2	RELEVANT INDS, NDAs, BLAs AND DMFs.....	27
2.3	DRUG FORMULATION	28
2.4	COMMENTS ON NOVEL EXCIPIENTS	29
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	55
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN.....	144
2.7	REGULATORY BACKGROUND	144
3	STUDIES SUBMITTED.....	151
3.3	PREVIOUS REVIEWS REFERENCED.....	162
4	PHARMACOLOGY	162
4.1	PRIMARY PHARMACOLOGY	162
4.2	SECONDARY PHARMACOLOGY	163
4.3	SAFETY PHARMACOLOGY	163
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	163
5.1	PK/ADME	163
6	GENERAL TOXICOLOGY.....	165
6.1	SINGLE-DOSE TOXICITY	166
6.2	REPEAT-DOSE TOXICITY	175
7	GENETIC TOXICOLOGY.....	228
7.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES)	228
8	CARCINOGENICITY.....	235
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	235
9.2	EMBRYONIC AND FETAL DEVELOPMENT+	257
10	SPECIAL TOXICOLOGY STUDIES	261
10.1	WOUND HEALING AND BONE STUDIES.....	261
10.2	JUVENILE ANIMAL STUDIES.....	289
11	INTEGRATED SUMMARY AND SAFETY EVALUATION	290
12	APPENDIX/ATTACHMENTS.....	307
12.1	CONSULTS FROM DARS	307

12.2 LITERATURE CITED 313

Table of Tables

Table 1: Relevant INDs, NDAs, and DMFs.....27

Table 2: Composition of HTX-011 28

Table 3: Amount of AP135 in Different Formulations 29

Table 4: Reproductive Toxicology Studies with APF18A 30

Table 5: Summary of Local Effects Following AP135 Treatment 32

Table 6: Summary of Systemic Effects Following AP135 Treatment 35

Table 7: DMSO FDA-approved Products as Per IID 36

Table 8: DMSO Literature Cited by Applicant.....36

Table 9 Pharmacokinetic Parameters in Rats Orally Exposed to 1000 mg/kg DMSO (Study 35-30).....39

Table 10 Pharmacokinetic Parameters in Rabbits Orally Exposed to 1000 mg/kg DMSO (Study 35-31).....39

Table 11 Pharmacokinetic Parameters in Humans Undergoing TKA Surgery and Receiving Zynrelef 40

Table 12: Triacetin in FDA-approved Products as per CDER IID 41

Table 13 Triacetin Levels in Plasma of Female Rats 43

Table 14 Individual Triacetin Concentrations from Clinical Study 306 44

Table 15 Triacetin Exposure Margins Between Rat and Human as Calculated by the Applicant.....45

Table 16 Estimated Steady State Concentrations for Glycerol and Acetic Acid Following Dosing with 400 mg/12 mg HTX-011 48

Table 17: Maleic Acid FDA-approved Products as Per CDER IID 49

Table 18 Toxicokinetic Parameters of Maleic Acid in Rabbits 51

Table 19: Summary of Local Effects Potentially Associated with Excipients.....54

Table 20: Summary of Systemic Effects Potentially Associated with Excipients.....54

Table 21: Bupivacaine Drug Substance Specifications 57

Table 22: Meloxicam Drug Substance Specifications 59

Table 23: HTX-011 Drug Product Specifications (Part 1).....63

Table 24: HTX-011 Drug Product Specifications (Part 2).....64

Table 25: HTX-011 Drug Product Specifications (Part 3).....65

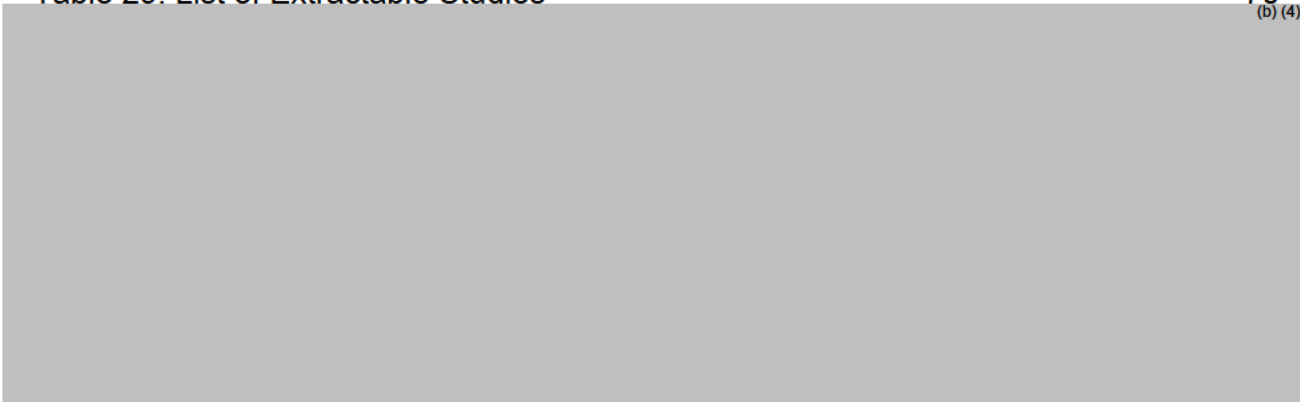
Table 26: Specifications for AP135 67

Table 27: Primary Container Closure 68

Table 28: Preparation and Administration Components 69

Table 29: List of Extractable Studies 70

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Table 85: Pharmacology Studies Submitted	152
Table 86: Pharmacodynamic Studies Submitted-Part 1	152
Table 87: Pharmacodynamic Studies Submitted-Part 2	153
Table 88: Pharmacodynamic Studies Submitted-Part 3	154
Table 89: Safety Pharmacology Studies Submitted	154
Table 90: Pharmacokinetics Studies Submitted	155
Table 91: Pharmacokinetic Studies Submitted (Continued)	155
Table 92: Toxicology Studies Submitted-Part 1	156
Table 93: Toxicology Studies Submitted-Part 2	157
Table 94: Toxicology Studies Submitted-Part 3	158
Table 95: Toxicology Studies Submitted-Part 4	159
Table 96: Toxicology Studies Submitted-Part 5	160
Table 97: Toxicology Studies Submitted-Part 6	161
Table 98: Highest Concentrations of Bupivacaine and Meloxicam in Plasma, Milk, and/or Urine of Minipig Sows and Neonate Piglets Following a Single SC Administration of HTX-011 to Sows.....	164
Table 99: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Rats	164
Table 100: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Dogs	165
Table 101: Composition of Test Articles Used in Nonclinical Studies Supporting Development of HTX-011-56	166
Table 102: Overview of Single-dose Toxicity Studies Conducted by Applicant	167
Table 103 Formulations Tested in Study Number 33-48	170
Table 104 Experimental Design of Study Number 33-48	170
Table 105 Histopathological Findings on Day 3 at the Primary Injection Site, Males...	172
Table 106 Histopathological Findings on Day 3 at the Secondary Injection Site, Males	172
Table 107 Histopathological Findings on Day 14 (Recovery) at the Primary Injection Site, Males.....	173
Table 108 Histopathological Findings on Day 14 (Recovery) at the Secondary Injection Site, Males.....	173

Table 109 Histopathological Findings on Day 3 at the Primary Injection Site, Females	174
Table 110 Histopathological Findings on Day 3 at the Secondary Injection Site, Females	174
Table 111 Histopathological Findings on Day 14 (Recovery) at the Primary Injection Site, Females.....	175
Table 112 Histopathological Findings on Day 14 (Recovery) at the Secondary Injection Site, Females.....	175
Table 113 Experimental Design of Study 33-85	178
Table 114: Incidence of Clinical Observations in Males	179
Table 115: Incidence of Clinical Observations in Females.....	180
Table 116: Notable Changes in Hematology Parameters in Male Rats	181
Table 117: Notable Changes in Clinical Chemistry Parameters in Rats	182
Table 118: Gross Observations in Male Rats	183
Table 119: Gross Observations in Female Rats.....	183
Table 120: Systemic Histopathological Observations	185
Table 121: Chronic Inflammation (Incidence).....	186
Table 122: Chronic Inflammation, Active (Incidence)	186
Table 123: Granulomatous Inflammation (Incidence).....	186
Table 124: Hemorrhage (Incidence)	187
Table 125: Presence of Test Material (Incidence)	187
Table 126: Chronic Inflammation, Recovery Animals.....	187
Table 127: Bupivacaine Toxicokinetic Parameters.....	188
Table 128: Meloxicam Toxicokinetic Parameters	189
Table 129: Levels of Polymer Breakdown Products in the Urine	190
Table 130 Experimental Design of Study 34-84	194
Table 131: Incidence of Clinical Observations in Males	195
Table 132: Incidence of Clinical Observations in Females.....	195
Table 133: Notable Changes in Hematology Parameters in Male Rats	197
Table 134: Notable Changes in Hematology Parameters in Female Rats	197
Table 135: Notable Changes in Clinical Chemistry Parameters in Male Rats.....	198
Table 136: Notable Changes in Clinical Chemistry Parameters in Female Rats	198
Table 137: Urine pH in Male and Female Rats	199
Table 138: Gross Observations in Male Rats (Incidence)	199
Table 139: Gross Observations in Female Rats (Incidence).....	199
Table 140: Organ Weights in Male Rats.....	200
Table 141: Organ Weights in Female Rats	200
Table 142: Infiltrate of Mononuclear Cells (Incidence)	202
Table 143: Fibrosis (Incidence)	202
Table 144: Edema (Incidence)	202
Table 145: Erythrocyte Accumulation (Incidence)	203
Table 146: Ulcer (Incidence).....	203
Table 147: Acute Inflammatory Exudate (Incidence).....	203
Table 148: Bupivacaine Toxicokinetic Parameters.....	204
Table 149 Experimental Design of Study 33-86	208
Table 150: Incidence of Edema at the Injection Site	210

Table 151: Average Severity of Edema in Vehicle Control Group.....	210
Table 152: Body Weights.....	211
Table 153: Hematology Parameters in Male Dogs.....	212
Table 154: Hematology Parameters in Female Dogs.....	213
Table 155: Clinical Chemistry Parameters in Male Dogs	214
Table 156: Clinical Chemistry Parameters in Female Dogs	215
Table 157: Gross Observations in Male Dogs.....	216
Table 158: Gross Observations in Female Dogs.....	216
Table 159: Organ Weights in Male Dogs.....	217
Table 160: Organ Weights for Female Dogs	217
Table 161: Percentage of Main Study Animals with Chronic Multifocal Inflammation ..	221
Table 162: Percentage of Recovery Animals with Chronic Multifocal Inflammation....	221
Table 163: Percentage of Main Study Animals with Active Chronic Multifocal Inflammation	221
Table 164: Percentage of Main Study Animals with Multifocal Granulomatous Inflammation	222
Table 165: Percentage of Recovery Animals with Multifocal Granulomatous Inflammation	222
Table 166: Percentage of Main Study Animals with Multifocal Fibrosis	222
Table 167: Percentage of Recovery Animals with Multifocal Fibrosis	223
Table 168: Percentage of Main Study Animals with Multifocal Hemorrhage	223
Table 169: Percentage of Main Study Animals with Multifocal Presence of Test Material	223
Table 170: Bupivacaine PK Parameters.....	225
Table 171: Meloxicam PK Parameters	227
Table 172: Plate Incorporation in Absence of S9	231
Table 173: Plate Incorporation in Presence of S9	232
Table 174: Positive Control Data.....	233
Table 175: Historical Control Data.....	234
Table 176: Litter Viability	260
Table 177: Sex and Sex Ratio of Litter.....	260
Table 178: Treatment Groups Hernia Repair Study	264
Table 179: Clinical Signs Rabbit Hernia Repair Study	265
Table 180: Dermal Observations of Day 3 Sacrifice Group.....	267
Table 181: Dermal Observations of Day 15 Sacrifice Group.....	267
Table 182: Dermal Observations on Day 15	267
Table 183: Wound Observations of Day 3 Sacrifice Group (Incidence)	268
Table 184: Wound Observations of Day 15 Sacrifice Group (Incidence)	268
Table 185: Last Day of Wound Hemorrhage	269
Table 186: Hematology Findings on Day 3, Percentage of Saline Control	270
Table 187: Hematology Findings on Day 15, Percentage of Saline Control	271
Table 188: Gross Findings of Day 3 Sacrifice Group	272
Table 189: Gross Findings of Day 15 Sacrifice Group	272
Table 190: Histological Findings of Day 15 Sacrifice Group	273
Table 191: Clinical Signs	277
Table 192: Bone Mineral Content of Left Femur (Percent of Saline Control)	279

Table 193: Bone Mineral Content of Right Femur (Percent of Saline Control).....	279
Table 194: Bone Mineral Density of Left Femur (Percent of Saline Control).....	279
Table 195: Bone Mineral Density of Right Femur (Percent of Saline Control)	279
Table 196: Healing Score of Femur.....	280
Table 197 Experimental Design of Study 33-54	282
Table 198: Dermal Observations in Day 4 Sacrifice Animals, Male	284
Table 199: Dermal Observations in Day 4 Sacrifice Animals, Female	284
Table 200: Dermal Observations in Day 15 Sacrifice Animals, Male	284
Table 201: Dermal Observations in Day 15 Sacrifice Animals, Female	284
Table 202: Wound Observations on Day 4.....	285
Table 203: Wound Observations on Day 8.....	286
Table 204: Gross Pathology of the Incision Site, Day 4	287
Table 205: Gross Pathology of the Incision Site, Day 15	287
Table 206: Summary of Histopathological Findings, Day 4	288
Table 207: Summary of Histopathological Findings, Day 15.....	289
Table 208 Timeline of the Pediatric Development Plan.....	290
Table 209 Comparison of Bupivacaine Exposure in Nonclinical Studies to Clinical Study 206.....	300

Table of Figures

Figure 1 DMSO Exposure in Humans Undergoing TKA Surgery Receiving Zynrelef 40
Figure 2 Maleic Acid Exposure in Humans Undergoing TKA Surgery Receiving Zynrelef 52
.....
Figure 3: Levels of (b) (4) Leachable Study 3.....122
Figure 4: Levels of (b) (4) Leachable Study 3 132
Figure 5: Levels of (b) (4) Leachable Study 4 137
.....
Figure 6: Levels of (b) (4) Leachable Study 4 139
.....
Figure 7: Body Weight in Rat Bone Defect Study 278

1 Executive Summary

1.1 Introduction

The Applicant, Heron Therapeutics Inc, has developed a fixed-combination drug containing bupivacaine and meloxicam, which they referred to as HTX-011 during development. HTX-011 is a viscous solution that is formulated in a proprietary tri(ethylene glycol)-based poly(orthoester) polymer. The Applicant developed HTX-011 for application into a surgical site to reduce postoperative pain for 72 hours and reduce the need for opioid analgesics. The Applicant has submitted a 505(b)(2) application relying on the Agency's previous finding of safety for MARCAINE® (bupivacaine HCl injection; NDA 16964) and MOBIC® (meloxicam; NDA 20938) with additional support from published literature. However, the latest 356h form indicates that the Applicant is also relying on two other bupivacaine NDAs (18053 and 18304). From a nonclinical perspective, these three FDA-approved bupivacaine labels contain the same data.

NDA 211988 was originally submitted on September 21st, 2018 and was not approved in the first review cycle due to deficiencies from numerous disciplines. The complete response letter dated April 30th, 2019 included nonclinical deficiencies related to excipient safety qualification, drug product specifications, and extractable leachables evaluations. The Applicant resubmitted on September 26th, 2019, but the NDA was not approved due to nonclinical deficiencies. The second complete response letter dated June 26th, 2020 indicated that the nonclinical deficiencies related to excipient safety qualification and drug product specifications were not adequately addressed. Refer to the Regulatory History section of this review for the specific language conveyed in the complete response letters. The Applicant resubmitted on November 12th, 2020.

1.2 Brief Discussion of Nonclinical Findings

The drug product formulation contains several "new" excipients as defined by the FDA guidance document as excipients used at higher doses, concentrations, or routes of administration than in previously approved drug products. Specifically, the proposed doses of AP135, dimethyl sulfoxide (DMSO), triacetin, and maleic acid exceeded levels present in FDA-approved drug products as per the CDER Inactive Ingredients Database (IID). The Applicant was informed of the need to adequately qualify the safety of these new excipients in their NDA early in development. The Applicant relied on the nonclinical safety studies with the drug product formulation to characterize the local and systemic safety of the excipients and are described in detail below. These studies did not address the potential for reproductive or developmental toxicity from the excipients. In the original submission, adequate data were provided to characterize the reproductive and developmental safety profile of AP135 at the maximum recommended human dose (MRHD). However, the Applicant did not adequately justify the safety of DMSO, maleic acid, and triacetin with respect to the standard reproductive and developmental toxicology battery endpoints in the original submission or their resubmission following the first complete response (e.g., second review cycle). In their resubmission to the second Complete Response, (e.g., third review cycle), the Applicant

provided adequate PK bridging data, along with additional literature data, to support that the levels of the excipients in the drug product are of minimal risk at the MRHD.

General toxicology studies were submitted to characterize the toxicological potential of the drug product formulation. These studies were also specifically designed to address the local safety of meloxicam as this is a new route of administration and the systemic safety of bupivacaine since exposure of bupivacaine from the drug product exceeded the reference product both in terms of AUC_{0-24h} and overall duration of exposure.

The pivotal studies were two 28-day repeat-dose studies (rat and dog by subcutaneous injection, Studies 33-85 and 33-86) conducted with the final clinical formulation, HTX-011. These two studies provided the only clear product-specific information characterizing the local tissue effects of the to-be-marketed formulation. Local effects observed in nonclinical studies throughout development consisted of a prolonged inflammatory response, including areas of tissue necrosis. The findings appear to be related primarily to the vehicle (similar levels of excipients but no active drug products). The local tissue findings were discussed with the clinical review team during development and the clinical team concluded that clinical studies could proceed with adequate monitoring and informed consent to determine if the nonclinical adverse effects translated into adverse clinical findings.

In terms of local toxicity, the primary finding following treatment with HTX-011 and the HTX-011 vehicle in both rats and dogs in the pivotal studies was an inflammatory response that is consistent with a foreign body reaction. This was primarily characterized by persistent chronic inflammation that was still present up to 28 days following injection. The clinical significance of prolonged chronic inflammation is unclear. Based on a minipig study (Study 33-54) by wound instillation, HTX-011 appears to only have a very minor impact on soft tissue wound healing consistent with herniorrhaphy procedures. However, it must be kept in mind that when the dose volume was normalized to the size of the incision site (estimated incision sizes for the surgeries proposed in the clinical studies were provided by the clinical team) the minipig study (1 mL/cm) was lower than the proposed clinical surgeries (herniorrhaphy, 3-4 cm, 2.5-3.4 mL/cm; (b) (4)

(b) (4) The local effects appear to be primarily driven by the presence of the tri(ethylene glycol)-based poly(orthoester) polymer (AP135) since similar local effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer (b) (4). Since the clinical formulation was used in the repeat-dose studies, the studies adequately characterize the local toxicity of the drug product, including meloxicam and the excipients, at a margin of 1x. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in determining if the chronic inflammation noted in the animals translates to clinically meaningful adverse effects.

Given the prolonged and rather robust local tissue response, the potential impact of the drug product on a variety of surgical meshes was tested in nonclinical models. The results suggest that the drug product did not appear to impact the integrity of the

surgical meshes tested nor translate into meaningful changes in wound healing. In addition, to support the proposed bunionectomy study and indication, the impact of the drug product on bone healing and integrity was studied in an animal model. The study did not suggest a clear meaningful delay in bone healing, a finding that was further and carefully monitored in clinical studies.

While the 28-day studies were conducted under exaggerated conditions (each injection site received 2-3 injections) similar dermal effects were observed after single injections with the HTX-011 vehicle in rats (Studies 33-48 and 33-55) suggesting the findings in the repeat-dose studies are relevant for a single administration. The findings in the single-dose studies with the HTX-011 vehicle were more severe (e.g., necrosis) than in the repeat-dose studies. This is likely due to the increased volume of injection used in these studies. Increased volume of injection was also linked to increased severity in the 28-day dog study where the HTX-011 vehicle (5.2-8.9 mL) showed increased severity and incidence of local findings compared to the HTX-011 clinical formulation (2.3-3.9 mL). It is difficult to translate the increased volumes into a clinically meaningful dose except to say that increased amounts of locally applied HTX-011 increases the risk for local toxicity.

In terms of systemic safety, the primary finding following treatment with HTX-011 and the HTX-011 vehicle in the pivotal studies was a change in hematology parameters, primarily an increase in reticulocytes, although increases in white blood cells, platelets, leukocytes, eosinophils, neutrophils, monocytes, and lymphocytes were also observed. The effects seem to be derived from the AP135 polymer since similar effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4)). This is likely due to breakdown of the polymer into smaller fragments that are then systemically distributed.

More severe effects were seen in the 28-day dog study where a larger volume of injection was used for the HTX-011 vehicle which resulted in higher exposure to the excipients (2.4-2.5x at the MRHD based on BSA scaling). These effects included decreased activity, decreased body weight, and increased organ weights (liver, thymus, adrenal, and kidney). It is likely that these effects are not relevant for a single-dose exposure since no effects were seen in single-dose studies using the HTX-011 vehicle in rats at exposure levels of the excipients of ~7.2x those of the MRHD based on BSA scaling. Increased hematology parameters were seen in one single-dose rat study using the HTX-011 vehicle at excipient exposures of 0.9-1.5x those of the MRHD based on BSA scaling. Based on all of the studies, increased hematology parameters occur at excipient exposures of ~0.5-1.5x those of the MRHD based on BSA scaling. We acknowledge that there is clinical experience with the drug product that included monitoring for changes in hematology parameters and defer to the clinical review team in determining if these increases, consistent with an inflammatory reaction, also occur in the clinical setting and are clinically relevant.

(b) (4)

In clinical bioavailability studies, the exposure (AUC) to bupivacaine via the drug product formulation at the proposed maximum daily dose of 400 mg bupivacaine exceeded that of the referenced drug products (e.g., Marcaine), and therefore additional data were needed to qualify the systemic safety of bupivacaine for the proposed clinical use. The pivotal 28-day nonclinical studies in rats and dogs included exposure data at 24 and 48 hours but not AUC_{inf} . Compared to human exposures in Clinical Study 209 (total knee arthroplasty), the rat exposures were ~ 0.5x and 0.3x for AUC_{0-24} and AUC_{0-48} , respectively, and the dog exposures were ~ 1.0x and 0.8x for AUC_{0-24} and AUC_{0-48} , respectively for bupivacaine. Because the bupivacaine AUC exposure levels in rats were lower than in humans, the Applicant conducted a 14-day toxicity study in rats by subcutaneous injection (dosing every 48 hours) of HTX formulations containing only bupivacaine (not the to-be-marketed formulation) and used a higher dose than in the previous rat study. The toxicological findings in the 14-day rat study were similar to the results of the 28-day studies with HTX-011, which included an inflammatory response that is consistent with a foreign body reaction and changes in hematology parameters. It is difficult to assess if the magnitudes of the changes are more severe in this study compared to previous studies which used lower doses of bupivacaine due to differences in dosing volumes, formulations, and durations of treatment. The combined male and female systemic exposure (AUC_{inf}) of bupivacaine achieved in the study (52,900 ng*h/mL) was higher than the highest reported bupivacaine exposure reported in the Applicant's clinical trials (Clinical Study 209, total knee arthroplasty, 31,173 h*ng/mL) by a factor of ~1.7x. Of note, while the data from the 28-day dog study is not ideal (AUC_{inf} was not calculated) it does provide data in a second species that had similar exposure to bupivacaine over 24 hours as humans exposed to 400 mg bupivacaine (~1x). The only systemic finding in dogs at this exposure level was an increase in reticulocytes. Taken together with the results from the 14-day rat study which achieved a higher exposure multiple, the safety of the 400 mg dose of bupivacaine is supported by the

nonclinical data as long as the clinical team does not believe that changes in hematology parameters are of a clinical concern considering the human experience.

To justify the safety of the container closure system, the Applicant conducted multiple extraction and leachable studies to identify potential leachables from the (b) (4) and the container closure system. The extractable and leachable testing in the original submission did not provide adequate leachable data to permit substantive toxicological evaluation of the safety of the container closure system (b) (4) of the drug product. Specifically, the Applicant did not test at least three drug product stability batches over the entire course of shelf life to the proposed expiry, did not perform leachable studies for all compounds identified above the safety concern threshold (b) (4) (mcg/day), did not adequately identify the compounds they detected, and did not provide adequate justification for the use of a surrogate approach for (b) (4) found in the drug product. The Applicant addressed these deficiencies in their resubmission and based on discussions with the chemistry, manufacturing, and controls (CMC) team, the extractable and leachable testing was considered adequate.

In total, eight individual leachables were identified above the AET across the five leachable studies: (b) (4)

(b) (4). The Applicant provided a detailed risk assessment for the leachables and PDEs were derived for the compounds based on the data available. The maximum detected levels of the leachables were all below the calculated PDEs and are not expected to be of a safety concern.

The drug substance impurity and drug product degradant specifications are acceptable. The deficiency related to the drug product degradant (b) (4) was addressed in the first resubmission by reducing the specification to NMT (b) (4)%, which is acceptable based on the qualification thresholds in ICH Q3B(R2). The deficiency related to the drug product degradant (b) (4) was addressed in the second resubmission by reducing the specification to NMT (b) (4)% based on the amount of (b) (4) in the nonclinical batches. The Applicant also reduced the upper limit of the specification for DMSO to (b) (4)% based on the DMSO concentrations in clinical lots tested in Phase 2b and Phase 3 studies.

The Applicant has adequately addressed all of the nonclinical deficiencies identified in the previous submissions and from the nonclinical perspective there are no outstanding concerns that would preclude approval. In terms of the safety of the drug product, the nonclinical data indicated that a local inflammatory response and changes in hematology parameters occurs in animals at doses that are predicted to be comparable to those in humans. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in determining if these effects also occur in the clinical setting.

1.3 Recommendations

1.3.1 Approvability

The Applicant adequately addressed all of the nonclinical deficiencies identified in the first and second cycle. The nonclinical studies did identify a potential risk for alterations in hematology parameters and a robust locally chronic inflammation reaction. However, we recognize that clinical data were obtained to determine if these nonclinical findings translate into meaningful clinical adverse effects or not. As such, the NDA may be approved from the nonclinical perspective with the proposed labeling changes and post marketing requirements. The reader is referred to the clinical review for justification of the clinical effects observed.

1.3.2 Additional Nonclinical Recommendations

Once approved, the following PREA PMRs will be required:

1. Conduct a juvenile animal study in the rodent model to characterize the impact of DMSO on the developing brain to support clinical studies in pediatric patients under three years of age.
2. Conduct a juvenile animal study in an appropriate model to characterize the impact of meloxicam on the developing kidney, liver, lung, and testes to support clinical studies in pediatric patients under two years of age.

1.3.3 Labeling

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

Applicant's Proposed Labeling	Reviewer's Recommended Changes	Rationale for Changes/Comments
(b) (4)		

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2 Drug Information

2.1 Drug

CAS Registry Number: 38396-39-3 (bupivacaine); 71125-38-7 (meloxicam)

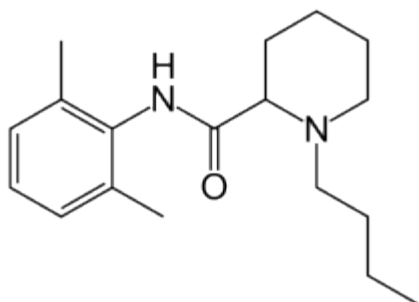
Generic Name: bupivacaine (base) and meloxicam (base)

Code Names: Zynrelef, HTX-011, and HTX-011-056

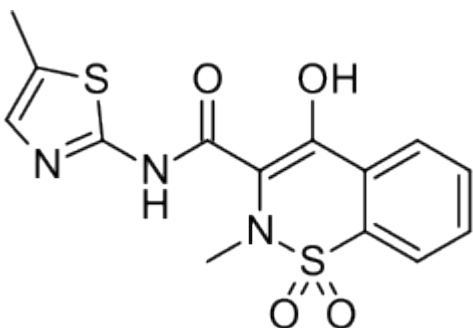
Chemical Name: (RS)-1-butyl-N-(2,6-dimethylphenyl)piperidine-2-carboxamide (bupivacaine) and 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide (meloxicam)

Molecular Formula/Molecular Weight: $C_{18}H_{28}N_2O$ / 288.43 g/mol (bupivacaine); $C_{14}H_{13}N_3O_4S_2$ / 351.40 g/mol (meloxicam)

Structure or Biochemical Description:



Bupivacaine



Meloxicam

Pharmacologic Class: Amide local anesthetic (bupivacaine); nonsteroidal anti-inflammatory drug (meloxicam)

2.2 Relevant INDs, NDAs, BLAs and DMFs

The development program for this NDA was completed under IND 125927, originally submitted to the Agency on 5/7/2015.

The Applicant is submitting the current New Drug Application under section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act. As such, they are referencing MARCAINE™ (bupivacaine, NDA 016964), Bupivacaine hydrochloride (NDA 18053), Sensorcaine (NDA 18304), and MOBIC® (meloxicam, NDA 020938) as the listed drugs.

Table 1: Relevant INDs, NDAs, and DMFs

<i>IND/NDA/DMF</i>	<i>Drug/Compound</i>	<i>Sponsor</i>	<i>Division</i>	<i>Status</i>
IND 125927	HTX-011 (bupivacaine and meloxicam)	Heron Therapeutics Inc	DAAP	Active
NDA 016964	Marcaine (bupivacaine hydrochloride)	Hospira Inc	DAAP	Approved (10/03/1972)
NDA 020938	Mobic (Meloxicam)	Boehringer Ingelheim Pharmaceuticals Inc	DAAP	Approved (04/13/2000)
NDA 18053	Bupivacaine HCl	Hospira Inc	DAAP	Approved (4/17/1978)
NDA 18304	Sensorcaine	Fresenius Kabi USA LLC	DAAP	Approved (12/30/1981)
DMF	(b) (4)			
DMF				
DMF				
DMF				
DMF				
DMF				

DMF	(b) (4)
DMF	(b) (4)

The Applicant also notes that the vented vial spike has been cleared by the Center for Devices and Radiological Health (CDRH) via the 510(k) process ((b) (4)). The syringes are also cleared by CDRH via the 510(k) process ((b) (4)).

2.3 Drug Formulation

HTX-011 is an extended-release solution containing a fixed-dose combination of bupivacaine and meloxicam. HTX-011 uses AP135, a highly viscous polymer to provide extended release of the active ingredients. Dimethyl sulfoxide (DMSO) and triacetin are included as (b) (4), respectively.

Table 2: Composition of HTX-011

Component	Concentration (% w/w)	Concentration (mg/g)	Concentration (mg/mL)	Function	Reference to Quality Standard
Bupivacaine base	2.50	25.0	29.25	Active ingredient	Manufacturer
Meloxicam	0.075	0.75	0.88	Active ingredient	USP, Ph Eur
AP135	62.375	623.75	729.788	Release-controlling polymeric excipient	Manufacturer
Dimethyl sulfoxide	10.00	100	117	(b) (4)	USP, Ph Eur
Triacetin	25.00	250	292.5		USP, Ph Eur
Maleic acid	0.05	0.5	0.585		NF, Ph Eur
Totals	100.00	1000.0	1170		

Abbreviations: AP135, tri(ethylene glycol) poly(orthoester); USP, United States Pharmacopeia; Ph Eur, European Pharmacopoeia; NF, National Formulary

Table from page 6 of the Applicant's Drug Product Summary for HTX-011 (2.3.P)

The Applicant proposed a maximum recommended human dose (MRHD) of 400 mg bupivacaine and 12 mg of meloxicam. This dose would require administration of 13.68 mL of the drug product solution. Based on the total daily dose, AP135, DMSO, triacetin, and maleic acid are all considered new excipients as defined by the FDA guidance document because the dose and/or route of administration exceed that of any other FDA-approved drug product to date. During development the Agency advised the Applicant that the levels of the excipients in the drug product appear to exceed those listed in the CDER Inactive Ingredients Database (IID) and must be adequately qualified for systemic safety.

2.4 Comments on Novel Excipients

Based on the MRHD and route of administration, there are multiple new excipients in this drug product that require adequate justification for safety. Each of these will be discussed below.

AP135

AP135 is a polymeric excipient that provides slow diffusional release of the active pharmaceutical ingredients over approximately three days. It is part of the tri(ethylene glycol) poly(orthoester) (TEG-POE) class of polymers (b) (4)

AP135 is not found in the CDER Inactive Ingredients Database (IID). A similar polymeric excipient, APF18A, is used in the Applicant's FDA-approved and marketed product SUSTOL (granisetron, NDA 022445) for subcutaneous injection. (b) (4)

Data from the development of this polymer was submitted as part of the current NDA.

Table 3: Amount of AP135 in Different Formulations

<i>Formulation</i>	<i>Percentage of excipient (w/w)</i>	<i>AP135 concentration</i>	<i>Amount at MRHD¹</i>
HTX-011			(b) (4)
HTX-011 vehicle (HTX-024-002)			
Sustol			
APF18A			

¹ Maximum recommended human dose for HTX-011 is 400 mg/12 mg bupivacaine/meloxicam or 13.68 mL. Maximum recommended human dose for Sustol is 10 mg (0.42 mL) based on product labeling.

The amount of AP135 that a patient would be exposed to by use of SUSTOL at the MRHD from SUSTOL's labeling is about (b) (4) times less than the amount of AP135 at the MRHD of HTX-011. Therefore, the presence of AP135 in SUSTOL does not qualify the levels of this excipient in HTX-011.

The Applicant conducted nonclinical studies with APF18A as part of the development of SUSTOL, including repeat-dose toxicity studies, a genetic toxicity assessment (bacterial forward mutation, mouse lymphoma, and forward mutation assays) and reproductive toxicity studies (fertility and early embryonic development in rat, embryo-fetal development in rat and rabbit, and pre- and post-natal development in rat, see table below) and submitted these to this NDA. The genetic toxicity studies were negative for

mutation or clastogenicity. The only treatment-related effects observed in the reproductive studies were local tissue effects at the injection site. Local effects were characterized in more detail in the repeat-dose studies, including histopathological analysis of the injection sites. The repeat-dose studies also characterized the systemic effects of APF18A and the relevance of the local and systemic findings are discussed below. In addition to nonclinical studies with APF18A, the Applicant also characterized the local and systemic effects of AP135 as part of the HTX-011 vehicle used in multiple studies for the current NDA.

Table 4: Reproductive Toxicology Studies with APF18A

Study #	Type	AP135 (mg/mL)	AP135 (mg/kg)	HED (mg)	Systemic Safety margin¹
27-02	Fertility and early embryonic development (rat)	(b) (4)			
25-09	Embryo-fetal development (rat)				
25-10	Embryo-fetal development (rabbit)				
27-03	Pre- and post-natal development				

¹ Calculated as a ratio of the non-clinical human equivalent dose divided by the amount of AP135 at the maximum recommended human dose (b) (4) mg AP135).

Although the rabbit EFD study noted in the table above does not provide complete coverage for the proposed clinical exposure and the high dose tested did not result in clear maternal toxicity, the Applicant concluded that the high dose tested was the maximum feasible dose (MFD) based on the material oozing from the injection sites at larger volumes. Given this feasibility limitation and because the study was able to dose close to the predicted exposure to APF18A via this formulation and the existing adverse effects noted in the labeling for both meloxicam and bupivacaine, no further studies will be required to address the potential embryo-fetal effects of APF135 for the intended clinical use.

Local effects:

The 90-day studies with the APF18A polymer were conducted in rat and dog (Studies 25-07 and 25-08). Dosage was by subcutaneous administration (0.25 mL in rats and 1 mL in dogs) every 24 hours across six dosage sites (16 injections total per site). Local injection site reactions observed consistently in both studies included dermal findings of erythema and edema, macroscopic observations of thickening of the skin, and multiple microscopic findings (chronic inflammation of multiple regions, necrosis/degeneration, fibrosis, and edema). Other findings seen primarily in one species included erosion, ulceration, hyperplasia, and hemorrhage. In general, findings were mostly no longer present following a 6-week recovery period, except for edema and erythema in rats, which was still present, but the severity and incidence was greatly reduced.

Similar injection site findings were seen with the HTX-011 vehicle (HTX-024-002) in 28-day repeat-dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous

injection (0.13-0.19 mL in rats and 5.2-8.9 mL in dogs) every 48 hours across six dosage sites (2-3 injections total per site). In rats, scabbing was seen at the injection sites and microscopic observations included chronic and granulomatous inflammation. In dogs, edema, swelling, and scabs were observed at the injection site and microscopic observations included chronic inflammation, granulomatous inflammation, fibrosis, and hemorrhage. Of note, in both studies chronic inflammation was still present at the end of the study, suggesting that it persists for up to 26-28 days.

When interpreting results from these studies, it must be kept in mind that the studies were conducted under exaggerated conditions; the 90-day study had a total of 16 injections per injection site and the 28-day studies had a total of 2-3 injections per injection site. This exceeds the single dose of HTX-011 that would be administered clinically. However, similar local site reactions were seen following a single subcutaneous dose of the HTX-011 vehicle in rats (Studies 33-48 and 33-55) suggesting that local effects of the polymer seen in repeat-dose studies are likely relevant for the clinical dosing scheme.

The results from both studies together suggest that AP135 contributes to a local tissue reaction. This is not unexpected as the response appears to be consistent with a foreign body reaction. However, some of the findings suggest that the effects may be more adverse than a typical inflammatory reaction, such as the observations of necrosis, erosion, and ulceration. These effects were seen in the APF18A studies, which were of a longer duration and used a higher concentration of AP135 as compared to repeat-dose studies with the HTX-011 vehicle ((b) (4)). It is unlikely that the longer duration plays a role since necrosis was also observed in single-dose rat studies using the HTX-011 vehicle (Studies 33-48 and 33-55). Therefore, it is more likely that the higher dosage of AP135 contributed to the increased severity of findings.

In addition to a higher concentration of AP135, it appears increasing the injection volume may also play a role. The single-dose rat studies (Studies 33-48 and 33-55) where potentially adverse findings were observed used increased injection volumes (0.5 mL and 2 mL) compared to the rat 28-day repeat-dose study with the HTX-011 vehicle (Study 33-85, 0.13-0.19 mL) where only inflammation was observed microscopically. A similar trend of dose volume and severity/incidence of local reactions was seen in the 28-day dog study with HTX-011 (Study 33-86). Dogs treated with the HTX-011 vehicle (5.2-8.9 mL) had increased incidence and severity of injection site findings compared to animals receiving the HTX-011 drug product (2.3-3.9 mL), despite the two formulations having similar levels of AP135. While increased dosage volume appears to result in a more severe local reaction, it is difficult to meaningfully translate the dose volumes to the clinical dose.

The majority of the findings observed in studies with both APF18A and the HTX-011 vehicle either completely recovered, or severity and incidence was greatly reduced by the end of the recovery periods. Based on the 28-day studies with the HTX-011 vehicle, chronic inflammation was the longest lasting effect at the injection site, which was still present to some extent around 28 days later. Chronic inflammation was not observed in

recovery animals in the APF18A studies, likely due to the extended recovery period in these studies (6 weeks).

In summary, the local toxicity of AP135 was adequately characterized. Treatment with APF18A and the HTX-011 vehicle, resulted in multiple injections site findings. The injection site findings were, for the most part, consistent with a foreign body reaction to injected material. However, in some cases more severe findings were observed, likely related to the higher concentration of AP135 in APF18A or the use of a large injection volume. In general, the findings recovered with time; the most persistent finding was chronic inflammation, which lasted about 28 days following treatment with the HTX-011 vehicle. It is difficult to put the local findings into context since both adverse (necrosis) and likely tolerable (chronic inflammation) findings were seen with clinically relevant concentrations of AP135. In addition, it is unclear what the clinical significance of chronic inflammation would be as it appears to be a normal, though robust, reaction to foreign material. In that regard, only a minor effect on wound healing was observed in a GLP mini-pig study (Study 33-54). Therefore, we acknowledge that there is clinical experience with HTX-011 from the Phase 2 and 3 studies and defer to the clinical review team in regard to if the local findings in nonclinical studies are acceptable from a risk-benefit perspective considering the clinical data. However, it appears that more severe findings were seen when either the concentration of AP135 exceeded the clinical formulation or an increased volume of injection was used in the animal studies. Therefore, we would recommend caution regarding increased dosing in humans.

Table 5: Summary of Local Effects Following AP135 Treatment

Study	AP135 (W/W)	Injection site	Injection volume
Rat, 90-day, APF18A, 25-07	(b) (4)	Dermal observations: erythema and edema Macroscopic changes: abrasion and thickening Microscopic observations: hyperplasia, chronic inflammation, necrosis, edema, erosion, ulceration, fibrosis	0.25 mL
Dog, 90-day, APF18A, 25-08	(b) (4)	Dermal observations: edema Macroscopic changes: thickening Microscopic observations: chronic inflammation, granulomatous inflammation, necrosis, edema, hemorrhage, fibrosis	1 mL
Rat, 28-day, HTX-011 vehicle, 33-85	(b) (4)	Dermal observations: scab Macroscopic changes: scab Microscopic observations: chronic inflammation, granulomatous inflammation	0.13-0.19 mL
Dog, 28-day, HTX-011 vehicle, 33-86	(b) (4)	Dermal observations: edema, scab Macroscopic changes: thickening	5.2-8.9 mL

		Microscopic observations: chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage	
Rat, single, HTX-011 vehicle, 33-48	(b) (4)	Dermal observations: edema Macroscopic changes: scab, dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, crust	2 mL
Rat, single, HTX-011 vehicle, 33-55		Dermal observations: not conducted Macroscopic changes: dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, fibrosis	0.5 mL

Systemic effects:

The 90-day studies with the APF18A polymer were conducted in rat and dog (Studies 25-07 and 25-08). Dosage was by subcutaneous administration (0.25 mL in rats and 1 mL in dogs) every 24 hours across six injection sites (16 injections total per site). Based on body weights at the beginning of the study, rats were exposed to 531.8-1008.6 mg/kg of AP135 ((b) (4)) and dogs were exposed to 93.6-170.2 mg/kg ((b) (4)). While these studies address the direct exposure to the excipient, a few things must be kept in mind. The first is that the studies were multiple-dose studies, which is exaggerated compared to single-dose exposure from the clinical product. The second is that the route of administration was subcutaneous injection as opposed to direct instillation into the surgical area as intended clinically. It is unclear how the different route of administration would affect systemic exposure to the excipient.

In rats, systemic effects observed following APF18A treatment included increases in hematology parameters (platelets, white blood cells, neutrophils, monocytes, reticulocytes (f), and lymphocytes (f)), decreased clinical chemistry parameters (total protein, albumin, albumin/globulin ratio, and calcium (f)), increased organ weights (spleen, adrenal (f), and ovary (f)), and increased incidence of microscopic findings (spleen hematopoiesis, lung alveolus histiocytes, kidney nephropathy (m), kidney corticomedullary junction mineralization (f), myeloid hyperplasia of the sternum and femur marrow, and lymphoid hyperplasia in multiple lymph nodes). A majority of these findings are consistent with a nonspecific immune system response to a foreign material, likely the result of breakdown products of the polymer entering systemic circulation. Findings in dogs were limited to changes in clinical chemistry parameters (decreased albumin (m), increased globulin (m), and decreased albumin/globulin ratio). The more limited number of systemic findings in dogs compared to rats is likely related to the dog receiving a much lower mg/kg dose of APF18A.

Similar systemic findings were seen with the HTX-011 vehicle in 28-day repeat-dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection (0.13-0.19 mL in

rats and 5.2-8.9 mL in dogs) every 48 hours across six injection sites (2-3 injections total per site). This resulted in rats being exposed to 646.4 mg/kg of AP135 (b) (4) and dogs being exposed to 760.5 mg/kg (b) (4). When interpreting the results of these studies it is important to keep in mind that the HTX-011 vehicle contained multiple excipients beyond just the AP135 polymer. In addition, the study was a repeat-dose study by a slightly different route of exposure as was discussed for the APF18A studies.

Findings in the rat study were limited to altered hematology parameters in male rats (increased platelets, neutrophils, eosinophils, and reticulocytes). In dogs, multiple systemic effects were observed including: hypoactivity, decreased body weight (f), altered hematology parameters (increased white blood cells, platelets, leukocytes, eosinophils, neutrophils, monocytes, and reticulocytes; decreased red blood cells, HGB, HCT, and lymphocytes), altered clinical chemistry parameters (increased globulin, decreased albumin (f), decreased albumin/globulin ratio, increased LDH, and decreased creatinine clearance), and increased organ weights (liver, thymus (m), adrenal (f), and kidney (f)). The more robust systemic reaction to the HTX-011 vehicle in dogs is likely related to the large dose of vehicle in this study. This is supported by the observation of very limited systemic findings with the HTX-011 drug product in the same study (limited to increased reticulocytes in males and increased kidney weight in females) which delivered an AP135 dose of 321.1 mg/kg (b) (4).

Taken together, the potential systemic toxicity of AP135 was adequately characterized across multiple studies. Potentially adverse effects (histopathological findings and/or decreased body weight) were seen in the rat study with APF18A at approximately 0.52-0.98x the MRHD (400 mg/12 mg) and in the dog study with the HTX-011 vehicle at a dose of 2.5x the MRHD (400 mg/12 mg). The adverse effects observed in these studies may not be clinically relevant since they are likely the result of repeat dosing and the drug product is indicated for a single treatment. In that regard, these adverse effects were not observed in single-dose studies with the HTX-011 vehicle by subcutaneous injection at approximately 0.93-7.2x the MRHD (400 mg/12 mg). The most consistent systemic effect was an increase in hematology parameters that were seen in dogs with APF18A at approximately 0.31-0.57x, in rats with the HTX-011 vehicle at approximately 0.63x, and in dogs with HTX-011 at approximately 1.1x the MRHD (400 mg/12 mg). Altered hematology parameters were also seen in one of the two single dose rat studies with the HXT-011 vehicle at approximately 0.93-1.5x the MRHD, suggesting they may be clinically relevant even after a single exposure. Changes in hematology parameters were measured in the clinical studies and we defer to the clinical team in terms of interpreting if the observed effects in nonclinical studies are acceptable from a risk-benefit perspective considering the clinical data.

Table 6: Summary of Systemic Effects Following AP135 Treatment

Study	Dose of AP135 in mg/kg (HED in mg⁴)	Effects	Safety Margin³
Rat, 90-day, APF18A, 25-07	531.8-1008.6 (b) (4)	↑ hematology parameters, altered clinical chemistry, ↑ organ weights, microscopic findings	0.52-0.98x ¹
Dog, 90-day, APF18A, 25-08	93.6-170.2 (b) (4)	altered clinical chemistry	0.31-0.57x ¹
Rat, 28-day, HTX-011 vehicle, 33-85	646.4 (b) (4)	↑ hematology parameters	0.63x ²
Dog, 28-day, HTX-011 vehicle, 33-86	760.5 (b) (4)	↓ activity, ↓ BW, altered hematology parameters, altered clinical chemistry, ↑ organ weights	2.5x ²
Dog, 28-day, HTX-011, 33-86	321.1 (b) (4)	↑ reticulocytes	1.1x ²
Rat, single dose, HTX-011 vehicle, 33-55	957.8-1584.4 (b) (4)	↑ hematology parameters	0.93-1.5x ¹
Rat, single dose, HTX-011 vehicle, 33-48	3755.6-7455.6 (b) (4)	none	3.6-7.2x ¹

¹ Safety margins calculated based on initial body weights. The mg/kg dose would decrease over the course of the study as animals weight increased.

² These studies used a constant volume/kg throughout the study.

³ Safety margins are compared to the MRHD of 400 mg/12 mg of bupivacaine/meloxicam.

⁴ Human equivalent dose calculated based on body surface area and a 60 kg individual

Overall Conclusion for AP135:

The local and systemic toxicity of AP135 was adequately characterized across multiple animal studies using different formulations. Locally, the primary finding at clinically relevant concentrations was a local inflammatory reaction at the injection site which could be considered a foreign body reaction. Chronic inflammation persisted for around 28 days. In addition, increased amounts of the excipient (either due to an increased concentration of AP135 or increased dosage volumes) appeared to cause severe local reactions, although it is difficult to translate the increased volumes in a meaningful way to compare to the clinical dose. The only systemic finding after a single exposure was changes in hematology parameters at 0.93-1.5x the level of AP135 at the MRHD (400 mg/12 mg), although no systemic effects were seen in a second single-dose study at 3.6-7.2x the level of AP135 at the MRHD (400 mg/12 mg). In repeat-dose studies, hematology parameters were affected at 0.31-0.57x (dog) and 0.63x (rats) the level of AP135 at the MRHD (400 mg/12 mg). Adverse systemic effects were observed following repeat exposure at 0.52-0.98x the level of AP135 at the MRHD (400 mg/12 mg) in rats and in the dog study with the HTX-011 vehicle at 2.5x the MRHD (400 mg/12 mg); however, these effects are likely the result of exaggerated exposure conditions and not entirely clinically relevant. Taken together, the data suggest the potential for both local and systemic effects at clinically relevant doses and therefore AP135 is not

completely qualified for safety in the nonclinical studies. However, we acknowledge that there is clinical experience from the Phase 2 and 3 studies with the formulation and defer to the clinical review team in terms of interpreting if the observed effects in nonclinical studies translate into adverse effects in the clinical setting.

Dimethyl Sulfoxide (DMSO)

Dimethyl sulfoxide (DMSO) was included in the drug product as a (b) (4) (b) (4)%. The amount of DMSO in the drug product is (b) (4) % w/w or (b) (4) mg/g. This results in a total of (b) (4) mg of DMSO administered at the MRHD ((b) (4)). However, the drug product has a specification of (b) (4) % DMSO which would result in the maximal potential amount of DMSO being (b) (4) mg/g or (b) (4) mg at the MRHD. DMSO has been used in multiple approved products based on the CDER Inactive Ingredients Database (IID), primarily dosed by the topical route. Based on the levels of DMSO in this drug product, the use of DMSO in this drug product is novel based on total dose via the intended clinical route of administration. The IID provides the following information on FDA-approved drug products that contain DMSO.

Table 7: DMSO FDA-approved Products as Per IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency Per Unit Dose	Comment on Adequacy
Dimethyl Sulfoxide	IV (Infusion)	Powder, For Injection Suspension, Lyophilized	NA	Inadequate information and route
Dimethyl Sulfoxide	Subcutaneous	Implant	104 mg	Inadequate dose
Dimethyl Sulfoxide	Topical	Dressing	NA	Inadequate information and route
Dimethyl Sulfoxide	Topical	Lotion	45.5% W/W	Inadequate information on exposure via this route
Dimethyl Sulfoxide	Topical	Solution	45.5% W/W	Inadequate information on exposure via this route

Based on the information provided above, the proposed dose of DMSO via the SC route of injection clearly exceeds that of any other FDA-approved drug product. In the absence of adequate exposure information, the systemic coverage via the topical drug products is not clear. As such, adequate safety justification is required. The Applicant noted that DMSO is present in the IID as noted in the table above. To support the safety, they reference three publications, summarized below:

Table 8: DMSO Literature Cited by Applicant

Reference	Applicant Conclusion	Doses Tested	Comment on Adequacy
Noel 1975	Based on studies in the dog, pig, rabbit, and rat, the lenticular nucleus of	Various doses in multiple studies summarized.	Inadequate but supportive. Review of studies with a focus on

	the eye is a target organ of toxicity.		lenticular changes. Lacks adequate detail for independent substantive review.
Vogin 1970	Oral NOEL in an 18-month rhesus monkey study was 3 mg/kg/day	HED = 58 mg/60 kg person.	Inadequate dose to inform risk assessment
De la Torre 1981	No significant or long-lasting effects noted following IV administration of 3 g/kg DMSO in a 40% solution for 9 days.	HED = 58064 mg/60 kg person (47 times the MRHD of DMSO).	Inadequate. Supportive at best given study limitations. The study only tested 6 male animals. Battery of tissues examined via histopathology not fully described. May be limited to muscle, lymph nodes, salivary gland and peripheral nerve.

The above published studies were reviewed to determine if these data could be adequate to address the general toxicity of DMSO. Collectively, the data are supportive at best but suggest that DMSO has been tested in numerous species, including rodent and non-rodent, to inform the potential target organs of toxicity. However, the general toxicity of DMSO was characterized in part via the pivotal toxicology studies testing the drug product formulation.

DMSO was included in HTX-011 and the HTX-011 vehicle that were tested in the 28-day repeat dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection and the rat single-dose studies (Studies 33-55 and 33-48) by subcutaneous injection. These studies can be used to help characterize the presence of DMSO in the drug product from a local and general toxicology perspective. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section.

To address the potential reproductive and developmental toxicity of DMSO, the Applicant cited the Organization for Economic Cooperation and Development's (OECD) Screening Information Dataset (SIDS) dossier for DMSO (OECD 2000). The OECD evaluated unpublished studies in this document and a summary of their evaluation is provided here. This included a GLP oral reproduction/developmental toxicity screening study in rats, a GLP oral developmental toxicity study in rats, and a GLP oral developmental toxicity study in rabbits. No reproductive or developmental toxicity was seen in the reproduction/developmental toxicity screening study or the rabbit developmental study (highest dose tested was 1000 mg/kg). Developmental/maternal toxicity (decreased body weight gain in dams manifesting as decreased fetal weight and reduced/delayed ossification of the ribs) was observed in the rat developmental study at 5000 mg/kg but no effects were seen at 1000 mg/kg. Thus, the overall NOAEL for reproductive/developmental toxicity is 1000 mg/kg, which is a human equivalent dose of 9,677.4 mg based on body weight scaling and a 60 kg individual. Thus, the levels of

DMSO in the drug product (b) (4) mg) are covered by the available nonclinical reproductive/developmental toxicity data based on body surface area.

The Applicant also performed PK studies in animals and humans to bridge exposure to DMSO following oral exposure in animals to exposure at the MRHD in patients undergoing TKA. Two animal studies were conducted; Study 35-30 in rats and Study 35-31 in rabbits. In Study 35-30, pregnant rats were orally dosed with DMSO from GD 6 through 17 and nonpregnant female and male rats were orally dosed with DMSO for 5 consecutive days. In Study 35-31, pregnant rabbits were orally dosed with DMSO from GD 6 through 19. In both studies, AUC_{0-inf} and C_{max} were similar on the first and last days of dosing in all animals (see tables below), suggesting that DMSO exposure does not increase with time. However, it must be pointed out that the duration of dosing in the Applicant's PK studies was shorter than some of the durations of exposure in the developmental/reproductive studies they are attempting to bridge from. For example, rats in the Applicant's PK study were only exposed for 11 days, while exposure in the reproductive study they referenced was much longer (~10 weeks). This is not a concern for the purpose the Applicant is using the PK data for since even if exposure was higher following prolonged exposure using the shorter-term values would represent a more conservative approach to deriving safety margins.

The Applicant also measured PK in patients undergoing TKA during clinical Study 306 and the PK data was reported in Study 35-42. DMSO was released quickly with a T_{max} of 4-6 hours. DMSO was still quantifiable in about half the subjects at 48 hours post dose but the level detected was very low. This was the last time point measured in the cohort that received the maximum recommended human dose. A second cohort which received a slightly lower dose (300 mg/ 9 mg vs. the MRHD of 400 mg/ 12 mg) had measurements out to 72 hours. In this cohort, only 1/14 subjects had detectable levels of DMSO at 60 and 72 hours post dose.

Both AUC_{0-inf} and C_{max} were higher in animals receiving oral doses of 1000 mg/kg than in TKA patients receiving the MRHD. AUC_{0-inf} was 47 (nonpregnant) to 55 (pregnant) times higher in rats receiving oral doses of 1000 mg/kg compared to TKA patients receiving the MRHD and 72 times higher in pregnant rabbits receiving oral doses of 1000 mg/kg compared to TKA patients receiving the MRHD. C_{max} was ~125 times higher in rats and ~146 times higher in rabbits as compared to TKA patients receiving the MRHD. Since the nonclinical reproductive and developmental toxicity studies cited by the Applicant did not show any evidence of reproductive or developmental effects with oral doses of DMSO up to 1,000 mg/kg and the systemic exposure to DMSO in these studies would be higher than exposure from the drug product at the MRHD, the nonclinical data support that there is minimal risk for developmental or reproductive toxicity from DMSO in the drug product.

Table 9 Pharmacokinetic Parameters in Rats Orally Exposed to 1000 mg/kg DMSO (Study 35-30)

DMSO Dose (mg/kg/day)	Day	Sex	C _{max} (µg/mL)	T _{max} (h)	t _{1/2} (h)	AUC ₀₋₂₄ ^a (µg•h/mL)	AUC _{last} ^b (µg•h/mL)	AUC _{inf} ^c (µg•h/mL)
1000	GD 6	Pregnant Females	1110	1	1.62	7210	7200	7200
		GD 17	Pregnant Females	973	0.5	3.50	7130	6990
	1	Male	1000	0.5	2.63	6300	6300	6310
		Female	979	1	1.28	6000	5970	5990
		Combined	989	0.5	2.34	6150	6150	6160
	5	Male	999	0.5	1.16	6710	6680	6690
		Female	953	0.5	1.33	5650	5610	5640
		Combined	976	0.5	1.24	6180	6150	6170

DMSO = dimethyl sulfoxide; GD = Gestation Day.

^a AUC₀₋₂₄ is area under the plasma concentration-time curve from time zero to 24 h post-dose.

^b AUC_{last} is area under the plasma concentration-time curve from time zero to the last quantifiable time-point post-dose.

^c AUC_{inf} is area under the plasma concentration-time curve from time zero to infinity.

Table from page 719 of the Applicant's study report for Study 35-30

Table 10 Pharmacokinetic Parameters in Rabbits Orally Exposed to 1000 mg/kg DMSO (Study 35-31)

DMSO Dose (mg/kg/day)	Day	C _{max} (µg/mL)	T _{max} (h)	t _{1/2} (h)	AUC ₀₋₂₄ ^a (µg•h/mL)	AUC _{last} ^b (µg•h/mL)	AUC _{inf} ^c (µg•h/mL)
1000	GD 6	1170	4	4.70	11900	11900	12200
	GD 19	1180	1	3.79	9290	9080	9470
	R _c ^d	1.01	NA	NA	0.781	0.763	0.776

DMSO = dimethyl sulfoxide; GD = Gestation Day; NA = not applicable.

^a AUC₀₋₂₄ is area under the plasma concentration-time curve from time zero to 24 h post-dose.

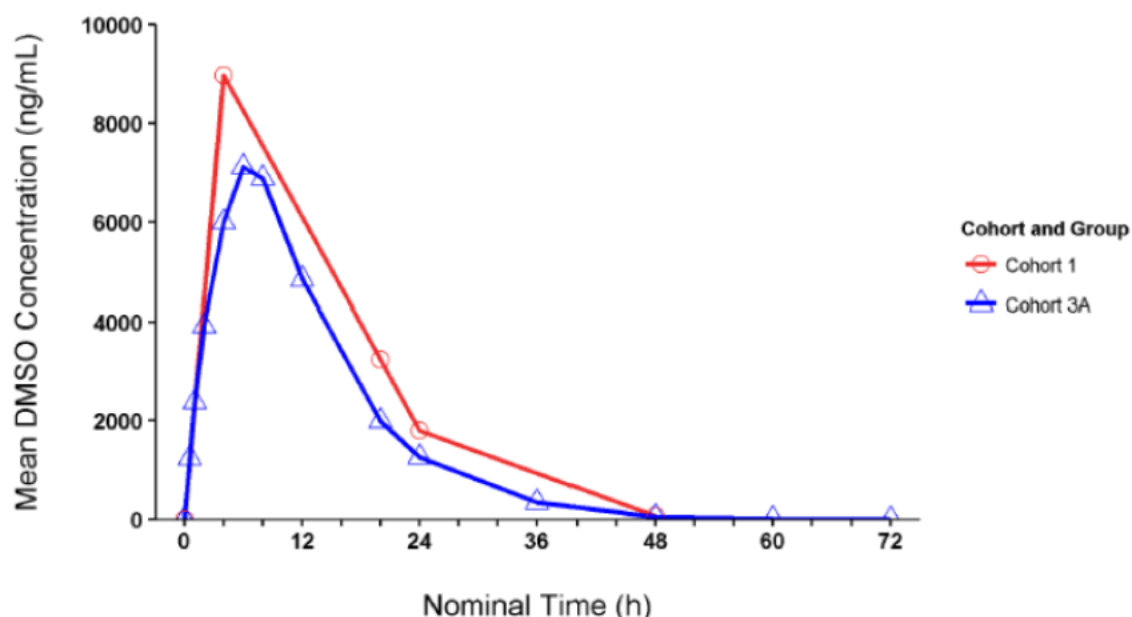
^b AUC_{last} is area under the plasma concentration-time curve from time zero to the last quantifiable time-point post-dose.

^c AUC_{inf} is area under the plasma concentration-time curve from time zero to infinity.

^d R_c is accumulation ratio of parameter values on GD 19/ GD 6

Table from page 706 of the Applicant's study report for Study 35-31

Figure 1 DMSO Exposure in Humans Undergoing TKA Surgery Receiving Zynrelef



Subjects in Cohort 1 had a planned treatment of HTX-011 400 mg/12 mg (14 mL containing 1600 mg DMSO); Subjects in Cohort 3A had a planned treatment of HTX-011 300 mg/9 mg (10.5 mL containing 1200 mg DMSO)

Figure from page 1667 of the Applicant's study report for Study 35-42

Table 11 Pharmacokinetic Parameters in Humans Undergoing TKA Surgery and Receiving Zynrelef

Cohort	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	T _{last} (h)	AUC _{inf} (h*ng/mL)	AUC ₀₋₂₄ (h*ng/mL)	AUC ₂₄₋₄₈ (h*ng/mL)	AUC ₄₈₋₇₂ (h*ng/mL)	t _{1/2} (h)
Cohort 1 (N=49)	8040 (56.3)	4.08 (1.07 - 24.68)	127000 ^c (70.0)	47.95 ^c (23.53 - 49.38)	132000 ^a (56.8)	120000 ^b (55.4)	14200 ^a (60.7)	762 ^a (68.1)	5.77 ^a (19.5)
Cohort 3A (N=14)	7620 (33.3)	6.08 (3.72 - 8.10)	96700 (48.1)	37.12 (23.68 - 73.28)	97900 (47.9)	88900 (43.1)	7030 (122)	296 (347)	5.52 (28.3)

N = Sample size

Values presented as Geometric Mean (Geometric CV%), except T_{max} and T_{last}, which are presented as Median (Minimum, Maximum).

Subjects in Cohort 1 had a planned treatment of HTX-011 400 mg/12 mg; Subjects in Cohort 3A had a planned treatment of HTX-011 300 mg/9 mg

^aN=23; ^bN=26; ^cN=27

Table from page 1673 of the Applicant's study report for Study 35-42

Triacetin

(b) (4). The amount of triacetin in the drug product is (b) (4) mg/g. This results in a total of (b) (4) mg of triacetin administered at the MRHD for HTX-011 ((b) (4) mg/g).

Triacetin has been used in multiple approved products, primarily dosed by the oral route, as identified by the IID. The information from the IID is reproduced in the table below:

Table 12: Triacetin in FDA-approved Products as per CDER IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency Per Unit Dose
Triacetin	Endocervical	Gel	NA
Triacetin	Oral	Capsule	6.42 mg
Triacetin	Oral	Capsule, Coated Pellets	1.21 mg
Triacetin	Oral	Capsule, Enteric Coated Pellets	5.1 mg
Triacetin	Oral	Capsule, Extended Release	46.7 mg
Triacetin	Oral	Capsule, Sustained Action	2.76 mg
Triacetin	Oral	Concentrate	50 mg/1 mL
Triacetin	Oral	Powder, For Oral Suspension	1.02 mg/5 mL
Triacetin	Oral	Suspension	0.32 mg/1 mL
Triacetin	Oral	Suspension, Extended Release	1.18 mg/5 mL
Triacetin	Oral	Tablet	6 mg
Triacetin	Oral	Tablet (Immed./Comp. Release), Film Coated	0.72 mg
Triacetin	Oral	Tablet, Chewable	1.36 mg
Triacetin	Oral	Tablet, Coated	1 mg
Triacetin	Oral	Tablet, Delayed Action, Enteric Coated	6 mg
Triacetin	Oral	Tablet, Delayed Release	1.82 mg
Triacetin	Oral	Tablet, Extended Release	8 mg
Triacetin	Oral	Tablet, Film Coated	15.12 mg
Triacetin	Oral	Tablet, Sustained Action	1.96 mg
Triacetin	Transdermal	Patch	22.1 mg
Triacetin	Transdermal	Patch, Controlled Release	22.1 mg

Based on the MRHD and route of administration, none of the previously approved drug products provide adequate coverage for the proposed dose of triacetin. The Applicant justifies the safety by stating that triacetin is considered generally recognized as safe (GRAS). However, GRAS determinations for foods are in the context of how the material is used in food and oral toxicity data. No data regarding food consumption or metabolism was provided. The Applicant cited a summary of toxicity findings but provided no references other than an LD₅₀ study in rats and mice. From a general toxicology perspective, the NDA lacks reference to adequate data to support the systemic safety of the proposed levels of triacetin.

Triacetin was included in HTX-011 and the HTX-011 vehicle that were tested in the 28-day repeat-dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection and the rat single-dose studies (Studies 33-55 and 33-48) by subcutaneous injection. These studies can be used to help characterize the presence of triacetin in the

drug product. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section. These studies only address the potential local and general systemic toxicity of triacetin leaving a need for additional data to address potential reproductive and developmental.

To address the potential reproductive and developmental toxicity of triacetin, the Applicant cited an unpublished GLP combined oral repeat-dose and reproduction/developmental toxicity study in rats submitted to the Japanese Ministry of Health and Welfare. A translated copy of the study was provided by the Applicant for review and the study was also reviewed by the Organization for Economic Cooperation and Development (OECD 2002a). No findings on reproductive or developmental factors were observed in this study up to a dose of 1000 mg/kg, the highest dose tested. PK data were not collected in this study and no data were available in the OECD document in terms of rat oral absorption to bridge exposure in animals to humans.

In order to demonstrate that the oral study would adequately address human exposure to triacetin by the clinical route of administration, the Applicant conducted an oral PK study in rats (Study 35-34) to estimate triacetin exposure under the same conditions as the rat oral developmental/reproductive toxicity study. The Applicant treated nonpregnant female rats by oral gavage with triacetin for 5 days and measured triacetin in the blood on the 5th day. Triacetin was rapidly metabolized and primarily only detectable above the limit of quantitation (LOQ) at the first two time points (5 and 15 minutes). Triacetin was seen at later time points in a few individual animals but it was not consistent across the whole study. Overall, the data suggested that orally administered triacetin is rapidly metabolized in animals and that systemic exposure is primarily to metabolites of triacetin.

Table 13 Triacetin Levels in Plasma of Female Rats

	Time (hr)										
	0.0	0.0833	0.25	0.50	0.75	1.0	2.0	4.0	8.0	12	24
Animal	Triacetin Concentration (ng/mL)										
1	(b) (4)										
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
Mean	0.00	29.4	3230	3.60	0.00	7.35	0.00	0.00	0.923	0.00	0.00
SD	0.00	37.7	5550	6.24	0.00	12.7	0.00	0.00	1.60	0.00	0.00

Blank space = not a sampling time point. Concentration values of 0.00 ng/mL were below the lower limit of assay quantitation (2.04 ng/mL)

SD: standard deviation

Table from page 95 of the Applicant's study report for Study 35-34

The Applicant also measured exposure to triacetin in humans following clinical administration of the drug product during total knee arthroplasty (Study 306; PK data in Study 35-42) to see how triacetin exposure compared to animals. Due to the rapid metabolism of triacetin by esterases, an esterase inhibitor needed to be added at the time of blood collection so that further metabolism would not occur before measurement. Due to this limitation, the Applicant could only collect data from the ongoing part of the study. Only data from nine patients receiving a 400 mg/ 12 mg dose of HTX-011 were available at the time of the second resubmission. Of the nine patients, only two had detectable levels above the LOQ (2 ng/mL). The first patient had a detectable level at the first time point (0.5 hour) which was barely above the LOQ and the second patient had detectable levels out to the 6-hour time point.

Table 14 Individual Triacetin Concentrations from Clinical Study 306

Patient	Triacetin Concentration (ng/mL)											
	Time (h)											
	0	0.5	1	2	4	6	8	12	20	24	48	72
(b) (6)												(b) (4)
n	9	9	9	9	8	8	7	8	9	9	6	5
Mean	0.00	15.8	0.878	0.00	0.333	1.09	0.00	0.00	0.00	0.00	0.00	0.00
SD	0.00	46.3	2.63	0.00	0.940	3.08	0.00	0.00	0.00	0.00	0.00	0.00
Median	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Samples reported as 0.00 ng/mL were below the limit of assay quantitation (< 2.00 ng/mL)
 NR = not reported; NS = no sample; SD = standard deviation

Table from page 10 of the Applicant’s risk assessment for triacetin (Study 35-43)

Based on the animal and human data, the Applicant concluded that there was low to no exposure in humans and that the exposure in rats was substantially higher. This was based on a comparison of the estimated C_{max} between rats and humans since non compartmental analysis for a determination of AUC could not be performed on the human data. C_{max} in rats was determined to be 3,230 ng/mL as compared to 15.8 ng/mL in humans. However, the animal C_{max} was driven by a single time point in a single animal that was substantially higher (>200 fold) than any other measurement and above the upper LOQ. Removing this value results in an estimated C_{max} of 29.4 ng/mL in rats, which is very close to the human estimate of 15.8 ng/mL. However, the Applicant’s report noted that despite being an out-of-trend value, there was no evidence to conclude that the value was not valid. Further, the human C_{max} is also driven by a single relatively high time point.

Table 15 Triacetin Exposure Margins Between Rat and Human as Calculated by the Applicant

Analyte	Study Species/ Sex	Test Article	Dose ^a	Route	Day	n	C _{max} (ng/mL)	AUC ₀₋₂₄ (h·ng/mL)	Animal:Human Safety Margin (fold) ^b	
									C _{max}	AUC
Triacetin	35-34 Rat/F	Triacetin in 3% acacia in distilled water	1,000 mg/kg/day	Oral gavage	5	12	3,230	713	204	NC
Triacetin	306 Human/ M, F	HTX- 011	400 mg/ 12 mg (4 g triacetin)	Instillation into surgical site	1	9	15.8	NC ^c		

^a For clinical Study 306, bupivacaine dose/meloxicam dose (excipient dose) is presented.

^b Safety margins are calculated as the ratio of the mean animal exposure: mean human exposure.

^c No patient had 3 consecutive timepoints with quantifiable triacetin levels; therefore, mean AUC values could not be calculated.

Abbreviations: AUC = area under the concentration-time curve; AUC₀₋₂₄= area under the concentration-time curve from Time 0 to 24 hours; C_{max} =maximum observed concentration; F= female(s); M= male(s); NC=could not be calculated.

Table from page 13 of the Applicant's risk assessment for triacetin (Study 35-43)

Another potential issue to consider when comparing animal and human exposure is that the earliest collection time point in humans was 30 minutes post dosing as compared to 5 mins in animals. Based on preliminary in vitro experiments with rat and human blood (Study 35-40), the half-life of triacetin in whole blood is very short (rat: 20 seconds; human: 25-88 seconds) suggesting that the timing of sample collection could be a critical factor. To illustrate the importance of the choice of sampling times, if 30 mins was chosen as the first time point in animals, the results would be almost identical to humans; that is there would only be detectable levels in one of three animals and the estimated C_{max} (7.35 ng/mL) would be less than in humans. Based on in vitro release data of triacetin from the drug product, the majority of triacetin is released over the first 12 hours and about half of the total amount is released between 6 and 12 hours, which suggests that triacetin could be released into systemic circulation 30 minutes after dosing. However, the available PK data from the one TKA patient with multiple values suggests that triacetin may be released relatively early from the drug product. Based on the data it cannot be ruled out that higher levels of triacetin may occur at early time points with the drug product.

In regard to the PK data, it is the FDA reviewer's opinion that the data are not robust enough to make a direct comparison of systemic exposure of triacetin between animals and humans. Since noncompartmental analysis could not be conducted on the human data, the only comparison was based on C_{max}, which was driven by a single large datapoint for both humans and rats. Overall, the systemic exposure to triacetin appears to be low in both animals and humans and suggests that the primary systemic exposure is to metabolites of triacetin.

While there was evidence for some systemic exposure to triacetin, the Applicant claims that the fetus would not be exposed to any triacetin as triacetin would not be expected to be able to cross the placenta. While no direct data for triacetin were presented, triacetin is a triglyceride and the Applicant provided literature references describing triglyceride metabolism and placental transfer during pregnancy (see Study 35-43 for a full list of references). The general consensus across the review articles was that triglycerides do not cross the placenta intact and are first metabolized by lipoprotein lipases to produce fatty acids, glycerol, and other glycerides, which can then enter the placental cells. Thus, it is expected that what little triacetin is available in systemic circulation would be converted to glycerol and acetic acid before crossing the placenta.

Based on the low exposure to triacetin demonstrated in humans following administration of the drug product and the data supporting a lack of transfer across the placenta, it is reasonable to evaluate the risk of triacetin in terms of its major metabolites, glycerol and acetic acid. Reproductive and developmental toxicity data were available for both glycerol and acetic acid and the information provided by the Applicant are summarized below.

For glycerol, the Applicant cited an OECD SIDS document (OECD 2002b). Glycerol was tested in a two-generation reproduction study administered orally as a 20% solution in water each day for 8-12 weeks (males and females were dosed for 8 weeks prior to mating with continued dosing in females until weaning). No effects were found on the reproductive efficiency of the parents, nor the growth, fertility, and reproductive performance of the F1 generation. In addition, no histological changes were observed in the tissues of both the F1 and F2 generation. A NOAEL of 2000 mg/kg was identified. While the study data were considered limited by the OECD, it was considered the most reliable study available and valid with restrictions. A developmental toxicity study with glycerol was also reviewed in the OECD SIDS document and a copy of the study report was provided by the Applicant. No developmental toxicity was seen in the rat (up to 1310 mg/kg), mouse (1280 mg/kg), or rabbit (up to 1180 mg/kg) following dosing by oral gavage. As with the reproductive toxicity study, the study data was considered limited by the OECD but was considered the most reliable study available and valid with restrictions.

The NOAEL for reproductive effects was 2000 mg/kg (HED of 19.3 g by BSA) and the developmental toxicity NOAELs were >1000 mg/kg (HED of 12.7 g for rats and 22.8 g for rabbits by BSA). The OECD document indicated that glycerol is readily absorbed by the intestine with slower absorption in the stomach; however, a quantitative estimate was not available. Thus, there is limited information in order to bridge the oral animal data with glycerol to the potential exposure to glycerol following administration of the drug product by the clinical route of administration. Assuming the entire amount of triacetin in the drug product is converted into glycerol and acetic acid and systemically available, exposure would be to a maximum of 1.7 g of glycerol from the drug product at the MRHD (4 g of triacetin = 18.33 mmol triacetin based on a molar mass of 218.2 g/mol; each mol of triacetin = 1 mol of glycerol; molar mass of glycerol is 92.094 g/mol). However, even assuming a poor absorption of 10% for glycerol in the animal studies

and 100% absorption of triacetin following wound instillation, the estimated amount of glycerol tested in the animal studies would still be similar to the amount in the drug product (0.7x for rat developmental, 1.3x for rabbit developmental, and 1.1x for rat reproduction).

For acetic acid, the Applicant cited an OECD SIDS document for ethyltriacetoxysilane (OECD 2005) which evaluated animal reproductive and developmental studies of exposure to acetic acid or similar chemicals. Fertility was evaluated in an oral study with sodium acetate in mice to assess fertility up to a dose of 60 mg/kg with treatment for a week before breeding, during the 9-day breeding period (females), and throughout pregnancy, lactation, and weaning of the offspring. No effects on fertility were observed. The OECD concluded that the study was valid with restrictions. Presumably, higher doses were not tested in the study due to the corrosivity of acetic acid and potential for stomach lesions. For example, 7-day range-finding studies with ethyltriacetoxysilane (which rapidly hydrolyses into acetic acid) indicated that a maximum dose level of less than 20 mg/kg/day would be needed to avoid stomach lesions that resembled acetic acid toxicity.

An oral developmental study with acetic acid (5% apple cider vinegar) was conducted with doses of up to 1600 mg/kg by gavage in mice, rats, and rabbits. The study is described as 1600 mg/kg acetic acid in the OECD document, but this amount of acetic acid would be expected to cause severe stomach irritation. The Applicant submitted a copy of the original study report and it appears that the dose is 1600 mg/kg of 5% acetic acid which would correspond to a dose of 80 mg/kg acetic acid and is more in line with the observations in the range-finding study described above. There were no maternal or developmental effects observed in any of the animals tested of all three species.

The NOAEL for fertility was 60 mg/kg (HED of 0.6 g by BSA) and the developmental toxicity NOAELs were 80 mg/kg (HED of 0.8 g for rats and 1.5 g for rabbits by BSA). There was no oral absorption data included in the OECD document but the European Chemicals Agency (ECHA) registration dossier for acetic acid contained a reference that indicated oral absorption in rats is nearly 100% at doses similar to those used in the reproductive and developmental studies (20 mg/rat; 40-80 mg/kg assuming the rats weighed between 0.25 and 0.5 kg). Assuming the entire amount of triacetin in the drug product is converted into glycerol and acetic acid and systemically available, exposure would be to a maximum of 3.3 g of acetic acid from the drug product at the MRHD (4 g of triacetin = 18.33 mmol triacetin based on a molar mass of 218.2 g/mol; each mole of triacetin = 3 mol of acetic acid; molar mass of acetic acid is 60.052 g/mol). Even if acetic acid was completely absorbed in the animal studies, the doses used in the animal studies are still below the amount of acetic acid anticipated from the drug product at the MRHD.

While the studies with acetic acid may not cover the anticipated level from the drug product, there was presumably some exposure to acetic acid in the rat study with triacetin as well. Assuming all of the triacetin dosed in the study was absorbed and converted into acetic acid, the animals would have been exposed to 826 mg/kg acetic

acid, which is a HED of ~8 g by BSA scaling. This value is about 2.4x the amount of estimated acetic acid at the human MRHD. However, as pointed out earlier, no data on oral absorption of triacetin in rat were available to adjust for potential differences in the route of administration used in animals and the method of clinical dosing.

It must be kept in mind that the metabolites of triacetin are endogenous molecules that are involved in a number of important physiological processes. As such, the body would be expected to be able to utilize the metabolites and they would be unlikely to disrupt normal biological processes. For example, acetate plays a key role in nutrition and metabolic processes including the formation of acetyl-CoA as part of the tricarboxylic acid cycle. Glycerol also plays key roles in metabolism, such as gluconeogenesis, and its utilization is increased during pregnancy. Due to their important roles in biological processes, both metabolites can be found at relatively high endogenous levels in human circulation.

The Applicant estimated steady state concentrations of the metabolites that would be expected from treatment of HTX-011 at the MRHD to compare to endogenous levels of these metabolites in humans. The Applicant assumed a 12-hour duration of release based on their in vitro release data and that the entire 4 g of triacetin would be converted into glycerol and acetic acid over this time period. They then used published clearance values for glycerol and acetic acid to calculate the steady state concentrations of the metabolites. The estimated steady state concentrations of glycerol and acetic acid from the drug product were 1.7-8.2 times lower than reported endogenous levels.

Table 16 Estimated Steady State Concentrations for Glycerol and Acetic Acid Following Dosing with 400 mg/12 mg HTX-011

Triacetin metabolite (mmol) ¹	Estimated metabolic rate (mmol/h) ²	Endogenous clearance ³	Estimated Steady State Concentration, C _{ss} (mM) ⁴	Mean Endogenous level (mM) ⁵	Ratio of endogenous to HTX-011
Glycerol 18.33	1.54	37.5	0.011	Normal: 0.06 Pregnant: 0.09	Normal: 5.5x Pregnant: 8.2x
Acetic Acid 54.99	4.58	32.4	0.033	Fasted: 0.055 Postprandial: 0.14	Fasted: 1.7x Postprandial: 4.2x

¹ 4 g of triacetin = 18.33 mmol triacetin based on a molar mass of 218.2 g/mol; each mole of triacetin = 1 mol of glycerol (molar mass of 92.094 g/mol) and 3 mol of acetic acid (molar mass of 60.052 g/mol)

² mmol of metabolite /12 hours; 12-hour release based on in vitro release data

³ Johnston 1982 for glycerol and Richards, 1982 for acetic acid

⁴ C_{ss} = production rate/clearance; 60 kg for glycerol (body weights not reported) and 71 kg (reported body weight in Richards, 1982)

⁵ glycerol normal: Johnston 1982, Tibbling, 1969, and Pelkonen, 1967; glycerol pregnant: Persson, 1975; acetic acid fasted: Kveim, 1979, Tollinger, 1979, and Pomare, 1985; acetic acid postprandial: Tollinger, 1979 and Pomare, 1985

Table was modified by the reviewer based on the table presented on page 11 of the Applicant's risk assessment for triacetin (Study 35-43)

When taken together, the data supports a low risk for reproductive or developmental toxicity from the level of triacetin in the drug product. Triacetin has a short half-life in rat and human blood and the animal/human PK data suggest that systemic exposure to triacetin is low and transient. Further, any systemic triacetin is not expected to cross the placenta. Thus, it appears that exposure will primarily be to the metabolites of triacetin, which are expected to be glycerol and acetic acid. Neither triacetin, nor its metabolites, showed any evidence of reproductive or developmental toxicity in animal studies following oral exposure. For triacetin and glycerol, it appears that exposure in the animal studies was sufficient to support the safety of the levels of triacetin in the drug product. This was not the case for acetic acid since dosing in animals by the oral route is limiting by stomach toxicity due to the corrosiveness of acetic acid. However, it is likely that sufficient levels of acetic acid were generated from triacetin in the rat combined reproductive/developmental study based on the Applicants rat PK experiment to support the levels expected from triacetin in the drug product dosed at the MRHD. Further, the estimated human exposure to acetic acid and glycerol from the drug at the MRHD was below endogenous values reported in the literature. Since both molecules are important for multiple physiological process, it is expected that the body will be able to utilize the metabolites without consequence. Based on a weight of the evidence approach, the levels of triacetin in the drug product are not expected to result in reproductive or developmental toxicity.

Maleic Acid

(b) (4)
 The amount of maleic acid in the drug product is 0.05% w/w or 0.5 mg/g. This results in a total of 8.0 mg of maleic administered at the MRHD for HTX-011 (13.68 mL * 1.17 mg/mL * 0.5 mg/g). Maleic acid has been used in multiple approved drug products by different routes according to the IID, including intramuscular, intravenous, and oral, as noted in the table below.

Table 17: Maleic Acid FDA-approved Products as Per CDER IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency per unit dose
MALEIC ACID	INTRAMUSCULAR	INJECTION	0.01%W/V
MALEIC ACID	INTRAMUSCULAR	POWDER, FOR INJECTION SOLUTION	NA
MALEIC ACID	INTRAVENOUS	INJECTION	0.01%
MALEIC ACID	IV(INFUSION)	INJECTABLE	0.01%
MALEIC ACID	IV(INFUSION)	INJECTION	ADJ PH
MALEIC ACID	ORAL	CAPSULE	2MG
MALEIC ACID	ORAL	SYRUP	1.73MG/5ML
MALEIC ACID	ORAL	TABLET	4MG

ADJPH = adjust PH as necessary

Of the products listed above, the oral syrup product can be dosed up to 12 teaspoons per day (~59 mL), which would correlate with a maximum daily dose (MDD) of 20.41 mg maleic acid. This exceeds the amount of maleic acid that a patient would be exposed to at the MRHD (6.1 mg). However, the oral route is not ideal for justifying the clinical route of exposure due to potential differences in absorption and metabolism and therefore the approved products do not qualify this excipient for general systemic exposure.

Approved products listed in the IID had a lower concentration of maleic acid than HTX-011 (0.1 mg/mL vs. 0.585 mg/mL in HTX-011). Thus, maleic acid in HTX-011 cannot be qualified for local safety based on the approved products.

Maleic acid was included in HTX-011, but not the HTX-011 vehicle, that was tested in the 28-day repeat-dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection. The studies using HTX-011 can be used to help characterize the presence of maleic acid in the drug product. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section.

The Applicant cited a publicly available Environmental Protection Agency (EPA) action memorandum to address the systemic safety of the proposed dose of maleic acid (EPA, 2006). The EPA reference cited studies with maleic anhydride but that the results could be applied to maleic acid due to rapid hydrolysis of maleic anhydride to maleic acid. The EPA reference contained results of a multi-generation reproduction study and a teratogenic study in rats. The study reports were not provided by the Applicant. According to the action memorandum, in a multigenerational study in rats, maleic anhydride was administered by oral gavage at 0, 20, 55, or 150 mg/kg/day. Renal lesions were noted at all doses. A LOAEL for the study was described as 20 mg/kg/day. The EPA memo states that there were no effects noted on fertility at doses up to 55 mg/kg/day. Further, no adverse effects on litter size and pup survival were noted at doses up to 150 mg/kg/day. In a separate dedicated embryo-fetal development study, the EPA memo noted that there were no external abnormalities, soft tissue abnormalities or skeletal abnormalities when female rats were treated with 0, 30, 90, or 140 mg/kg/day maleic anhydride from Gestation Day 6 to 15. The memo reported that the maternal NOAEL was the high dose of 140 mg/kg.

No reproductive or developmental effects were seen at human equivalent doses of 532 mg (reproductive) and 1354.8 mg (teratogenic), which is 67x and 169x times the levels a patient would be exposed to at the MRHD, respectively. Normally, the Agency does not accept summary information to support the safety of novel excipient use. However, because the EPA is a U.S. regulatory agency, we will accept these summary data based on their conclusions. The Applicant identified oral bioavailability data in the rat which suggests that maleic acid is about 30% orally bioavailable in the Sprague Dawley rat (Wu et al., 2016). The safety margins would still be sufficiently high even if it is assumed that instillation into the wound would result in 100% bioavailability of maleic acid with adjusted safety margins of 20x and 51x.

The Applicant submitted a study testing the developmental toxicity of oral maleic acid in rabbits (Study 34-74). The full review of the study can be found in Section 9 of this review. No effect on developmental toxicity was observed in the study. The highest dose tested was 20 mg/kg, which is a human equivalent dose of 387.1 mg based on body surface area and a 60 kg person, which is 48x the levels a patient would be exposed to at the MRHD. This dose produced no evidence of maternal toxicity and therefore was not ideally designed as a toxicology study but could be justified as a sufficiently high enough dose as it was expected to provide ~50-fold exposure compared to the MRHD by BSA scaling. When comparing animal to human exposure, it appears that the C_{max} is about 50-fold or greater in animals at 20 mg/kg compared to the MRHD in humans (see below for more information regarding human PK).

The Applicant performed PK studies in pregnant rabbits and humans to bridge exposure to maleic acid following oral exposure in animals to exposure at the MRHD in patients undergoing TKA. In Study 35-32, pregnant rabbits were orally dosed with 20 mg/kg/day maleic acid from GD 6-18. This was the same as the high dose in the developmental study. AUC_{0-24h} and C_{max} were similar following the first (GD 6) and last (GD 18) dose.

Table 18 Toxicokinetic Parameters of Maleic Acid in Rabbits

Maleic Acid Dose (mg/kg/day)	Day	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-24}^a (ng•h/mL)	AUC_{last}^b (ng•h/mL)	AUC_{inf}^c (ng•h/mL)
20	GD 6	3080	2	ND	6620	6220	ND
	GD 18	2810	1	0.707	6680	6410	6690
	R_c^d	0.912	NA	NA	1.01	1.03	NA

GD = Gestation Day; NA = not applicable; ND = not determined.

^a AUC_{0-24} is area under the plasma concentration-time curve from time zero to 24 h post-dose.

^b AUC_{last} is area under the plasma concentration-time curve from time zero to the last quantifiable time-point post-dose.

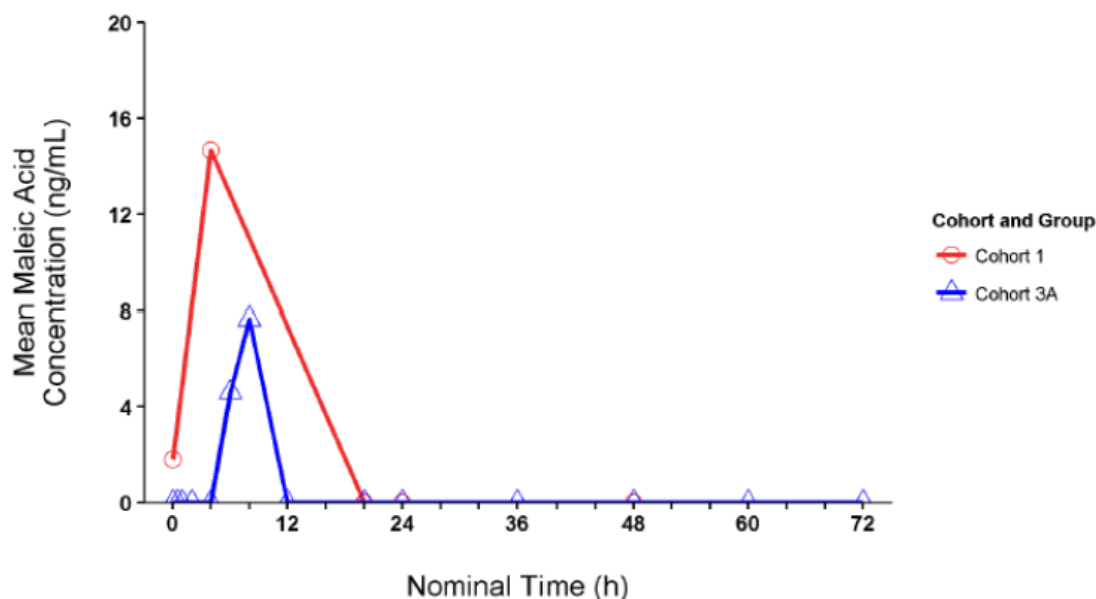
^c AUC_{inf} is area under the plasma concentration-time curve from time zero to infinity.

^d R_c is accumulation ratio of parameter values on GD 18/ GD 6

Table from page 118 of the Applicant's study report for Study 35-32

The Applicant also measured PK in patients undergoing TKA during Clinical Study 306 and the PK data was reported in Study 35-42. Maleic acid was measured in two cohorts; one receiving 400 mg/12 mg (Cohort 1) and one receiving 300 mg/9 mg (cohort 3A). In both cohorts, only a small percentage of patients (Cohort 1: 12/50; Cohort 3A: 2/14) had quantifiable concentrations of maleic acid at any time point. Due to the small number of quantifiable time points, noncompartmental analysis could not be completed to calculate an AUC. Based on the measured values, maleic acid increases to a maximal plasma level around 4-8 hours and then drops to unquantifiable levels. For values below the LLOQ, the Applicant extrapolated the concentrations of maleic acid and very low levels of maleic acid were seen up to 72 hours in half of the samples.

Figure 2 Maleic Acid Exposure in Humans Undergoing TKA Surgery Receiving Zynrelef



Subjects in Cohort 1 had a planned treatment of HTX-011 400 mg/12 mg (14 mL containing 8 mg maleic acid); Subjects in Cohort 3A had a planned treatment of HTX-011 300 mg/9 mg (10.5 mL containing 6 mg maleic acid)

Figure from page 1669 of the Applicant's study report for Study 35-42

Both AUC_{0-24h} and C_{max} were higher in animals receiving oral doses of 20 mg/kg than in TKA patients receiving the MRHD. C_{max} and AUC_{0-24h} in TKA patients could not be quantified but were determined to be <0.05 mcg/mL (the LLOQ; 0.038 mcg/mL based on extrapolated values) and <1.2 h*mcg/mL (LLOQ for 24 hrs), respectively. Using these values as a conservative estimate, C_{max} in animals was at least 56-times higher and AUC_{0-24h} was at least 5.6 times higher in animals compared to humans. The estimate for AUC_{0-24h} of 1.2 h*mcg/mL to derive the safety margin is a large overestimate since it assumes the plasma concentration was 0.05 mcg/mL at all time points over 24 hours.

Since maleic acid exposure was low in humans, this could indicate that maleic acid is being rapidly metabolized and that exposure is predominantly to metabolites of maleic acid. The Applicant provided data to support that maleic acid is eliminated by conversion to malic acid and excreted as CO_2 . The Applicant reasons that malic acid does not possess a concern for reproductive or developmental safety since it is an intermediary of the citric acid cycle.

Since the nonclinical rabbit developmental toxicity study conducted by the Applicant did not show any evidence of developmental effects with oral doses of maleic acid up to 20 mg/kg and the systemic exposure to maleic acid in these studies would be higher than exposure from the drug product at the MRHD, the oral developmental study is adequate to support that the levels of maleic acid in the drug product at the MRHD are not expected to result in developmental toxicity.

Characterization of the excipients by nonclinical data

As noted above, DMSO, triacetin, and maleic acid in the drug product were not characterized for either local or systemic safety based on use in other approved products. However, the Applicant did test these excipients as part of the drug product and vehicle in the nonclinical studies. An important distinction is that the HTX-011 vehicle that was tested did not contain maleic acid, but the HTX-011 drug product itself did.

In terms of local toxicity, the HTX-011 vehicle (containing DMSO and triacetin) resulted in an inflammatory reaction that is consistent with a foreign body material. This was characterized as chronic inflammation that lasted for up to 28 days. A similar reaction was seen with the HTX-011 drug product (containing DMSO, triacetin, and maleic acid). Based on studies with APF18A, the inflammatory reaction is very likely driven by the presence of AP135, although a contribution of the other excipients cannot be completely ruled out. More severe findings, such as necrosis, were seen in the single-dose rat studies but this is possibly due to the increased injection volume used.

In the worst-case scenario, the excipients could have contributed to the observed local inflammatory reaction. It is unclear what the clinical significance of chronic inflammation would be as it appears to be a normal reaction to a foreign material. In that regard, there did not appear to be a significant effect on wound healing in a GLP mini-pig study (Study 33-54). We also acknowledge that there is clinical experience with the excipients from the Phase 2 and 3 studies and defer to the clinical review team regarding if the local findings in non-clinical studies would be of a safety concern. If the clinical team finds the chronic inflammation as tolerable, then the excipients are characterized for local safety at a margin of 1x, as the concentrations of DMSO, triacetin, and maleic acid in the nonclinical studies are the same as those used in the clinical formulation.

In terms of systemic effects, the primary findings with the HTX-011 vehicle (containing DMSO and triacetin) were changes in hematology parameters with similar effects observed with HTX-011 (containing DMSO, triacetin, and maleic acid). Again, it is likely that these changes are the result of AP135 as similar findings were seen with APF18A, although a contribution of the other excipients cannot be completely ruled out. More severe effects (decreased body weights and decreased activity) were seen in the repeat-dose dog study with the HTX-011 vehicle (Study 33-86) but these were not seen in rat studies or when HTX-011 was given at a lower dose. In the worst-case scenario, the excipients contributed to the hematology findings, which could be considered an adverse effect. We acknowledge that there is clinical experience with the excipients from the Phase 2 and 3 studies, which included some monitoring for hematology changes, and defer to the clinical review team regarding if the changes in non-clinical studies would be of a safety concern. If the clinical team finds the changes in hematology parameters tolerable, then the excipients are characterized for general systemic safety at a margin of up to 1.4x (see table below).

While the above studies help characterize the general systemic effects of the excipients, no studies were conducted with any of the individual excipients, HTX-011, or the HTX-011 vehicle to address the potential reproductive toxicology of the excipients. The Applicant did provide data to address the reproductive/developmental toxicity of all of the excipients as described in the previous sections.

Table 19: Summary of Local Effects Potentially Associated with Excipients

Study	Excipients	Injection site	Injection volume
Rat, 28-day, HTX-011 vehicle, 33-85	DMSO: 10%* Triacetin: 25%	Dermal observations: scab Macroscopic changes: scab Microscopic observations: chronic inflammation, granulomatous inflammation	0.13-0.19 mL
Dog, 28-day, HTX-011 vehicle, 33-86	DMSO: 10%* Triacetin: 25%	Dermal observations: edema, scab Macroscopic changes: thickening Microscopic observations: chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage	5.2-8.9 mL
Rat, single, HTX-011 vehicle, 33-48	DMSO: 10%* Triacetin: 25%	Dermal observations: edema Macroscopic changes: scab, dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, crust	2 mL
Rat, single, HTX-011 vehicle, 33-55	DMSO: 10%* Triacetin: 25%	Dermal observations: not conducted Macroscopic changes: dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, fibrosis	0.5 mL

(b) (4)

Table 20: Summary of Systemic Effects Potentially Associated with Excipients

<i>Study</i>	<i>Excipients in mg/kg (HED in mg)</i>	<i>Effects</i>	<i>Safety Margin³</i>
Rat, 28-day, HTX-011 vehicle, 33-85	DMSO: 99.5 (963) Triacetin: 248.6 (2406)	Increased hematology parameters	0.60x ²
Rat, 28-day, HTX-011, 33-85	DMSO: 99.5 (963) Triacetin: 248.6 (2406) Maleic acid: 0.50 (4.8)	Increased hematology parameters	0.60x ²
Dog, 28-day, HTX-011 vehicle, 33-86	DMSO: 117 (3900) Triacetin: 292.5 (9750)	Decreased activity, decreased BW, altered hematology parameters, altered clinical chemistry, increased organ weights	2.4x ²
Dog, 28-day, HTX-011, 33-86	DMSO: 51.5 (1717) Triacetin: 128.7 (4290) Maleic acid: 0.26 (8.7)	Increased reticulocytes	1.1x ²
Rat, single dose, HTX-011 vehicle, 33-55	DMSO: 147-244 (1423-2361) Triacetin: 368-609 (3561-5894)	Increased hematology parameters	0.89-1.4x ¹
Rat, single dose, HTX-011 vehicle, 33-48	DMSO: 578-1147 (5594-11100) Triacetin: 1444-2868 (13974-27755)	None	3.5-6.9x ¹

¹ Safety margins calculated based on initial body weights. The mg/kg dose would decrease over the course of the study as animals weight increased.

² These studies used a constant volume/kg throughout the study.

³ Safety margins are compared to the MRHD of 400 mg/12 mg of bupivacaine/meloxicam. The safety margin for DMSO was calculated based on 10% DMSO in the drug product. (b) (4)

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Specifications

Bupivacaine

The drug substance specifications for bupivacaine base are shown in the Applicant's table below. At the MRHD of 400 mg bupivacaine, according to ICH Q3A(R2), the appropriate qualification threshold is 0.15% or 1 mg per day intake (whichever is lower). Based on this the Applicant's specifications for (b) (4) and any unspecified impurity are acceptable.

The drug substance contains one residual solvent, (b) (4) is listed in ICH Q3C as a (b) (4) with a concentration limit of (b) (4) ppm so the Applicant's proposed specification of (b) (4) ppm is acceptable.

The adequacy of the remaining specifications is discussed below.

(b) (4)

(b) (4)

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Table 21: Bupivacaine Drug Substance Specifications

Attribute		Acceptance Criteria	Test Method Reference
Appearance		White to off-white crystalline powder or crystals or granules	Visual
Identification	IR	Complies with reference spectrum	USP <197A>, Ph Eur 2.2.24
	Chromatographic	Complies with reference	TLC
Water content (% w/w)		(b) (4)	USP <921>, Ph Eur 2.5.12
Residue on ignition/sulfated ash (% w/w)			USP <281>, Ph Eur 2.4.14
Loss on drying (% w/w)			USP <731>, Ph Eur 2.2.32
Assay (% w/w)			Titration
Chromatographic purity (% w/w)	(b) (4)		HPLC
	Any unspecified impurity		
	Total impurities		
(b) (4)		GC	
(b) (4)		GC	
(b) (4)		GC	
Bacterial endotoxins (EU/g)		USP <85>, Ph Eur 2.6.14	
Microbiological limits (CFU/g)	Total aerobic microbial count ^a	USP <61>, Ph Eur 2.6.12	
	Total combined yeasts/molds count ^a		

Abbreviations: IR, infrared; USP, United States Pharmacopeia; Ph Eur, European Pharmacopoeia; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; GC, gas chromatography; % w/w, percent weight/weight; ppm, parts per million; EU, endotoxin units; CFU, colony forming units; NMT, not more than

^a Per USP <1111> and Ph Eur 5.1.4; 10² is 200 CFU/g.

Table from page 1 of the Applicant's specification document (3.2.S.4.1)

Meloxicam

The drug substance specifications for meloxicam are shown below. At the MRHD of 12 mg meloxicam, according to ICH Q3A(R2), the appropriate qualification threshold is 0.15% or 1 mg per day intake (whichever is lower). Based on this, the Applicant's specifications for all impurities are acceptable.

All of the specifications for the residual solvents are within ICH Q3C. ICH Q3C does not specifically delineate (b) (4), but refers to (b) (4) which, as per the guidance, (b) (4) ICH Q3C notes that the PDE for (b) (4) ppm concentration. The proposed specification of NMT (b) (4) ppm (b) (4) would result in (b) (4) mcg of (b) (4) at the MRHD of 12 mg meloxicam ((b) (4)) via this product which is below the amount of (b) (4) present at the PDE of (b) (4) mg ((b) (4)). The specification is acceptable.

Table 22: Meloxicam Drug Substance Specifications

Attribute		Acceptance Criteria	Test Method Reference
Appearance		Pale yellow powder	Visual
Identification	IR	Complies with reference spectrum	USP <197K>, Ph Eur 2.2.24
	HPLC	The retention time of the major peak of the sample corresponds to that of the reference standard	USP monograph
Loss on drying (% w/w)		NMT 0.5	USP <731>, Ph Eur 2.2.32
Residue on ignition/sulfated ash (% w/w)		NMT 0.1	USP <281>, Ph Eur 2.4.14
Assay, HPLC (% w/w on dried basis)		98.0 – 102.0	USP monograph
Assay, titration (% w/w on dried basis)		99.0 – 101.0	Ph Eur monograph
Related substances (HPLC) (% w/w)	USP related compound A and Ph Eur impurity A ^a	NMT 0.1	HPLC, USP monograph Procedure 1, Ph Eur monograph
	USP related compound B and Ph Eur impurity B ^b	NMT 0.1	
	USP methyl meloxicam and Ph Eur impurity C ^c	NMT 0.05	
	USP ethyl meloxicam and Ph Eur impurity D ^d	NMT 0.05	
	Any unspecified impurity	NMT 0.10	
	Total impurities	NMT 0.3	
Residual solvents (ppm)	(b) (4)		GC
Bacterial endotoxins (EU/g)	(b) (4)		USP <85>, Ph Eur 2.6.14

Table from page 1 the Applicant's specification document (3.2.S.4.1).

Drug Product

The specifications for the drug product are located in the tables below. At the MRHD of 400 mg bupivacaine, according to ICH Q3B(R2), the appropriate qualification threshold is 0.2% or 3 mg per day intake (whichever is lower). Based on this, the Applicant's specifications for any unspecified impurity are acceptable. The specification for

(b) (4) was NMT (b) (4) in the original submission, which was higher than ICH Q3B(R2). This was identified as a deficiency. In the resubmission, the Applicant has reduced the specification to (b) (4) which is acceptable.

At the MRHD of 12 mg meloxicam, according to ICH Q3B(R2), the appropriate qualification threshold is 0.5% or 200 mcg per day intake (whichever is lower). Based on this, the Applicant's specifications for all meloxicam-related substances are acceptable.

The adequacy of the specifications for other impurities, (b) (4) are discussed below.

(b) (4)

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DMSO

DMSO is an excipient used (b) (4) in HTX-011 (b) (4). The Applicant set a specification for DMSO as the content in the final drug product can vary. The Applicant originally set a specification (b) (4) in the original submission, although it did not appear that (b) (4) % DMSO was ever tested in nonclinical or clinical studies and the Applicant did not provide any safety justification for (b) (4) % DMSO. This was not listed as a deficiency in the first cycle but was raised as a non-hold comment in the second cycle.

The Applicant proposed to reduce the upper limit of the specification to (b) (4) % based on the DMSO concentrations in clinical lots tested in Phase 2b and Phase 3 studies. Thus, we defer to the clinical team in regard to whether the (b) (4) % is acceptable based on the clinical data.

Table 23: HTX-011 Drug Product Specifications (Part 1)

Attribute	Acceptance Criteria	Method Reference
Appearance (Visual)	(b) (4)	
Dynamic Viscosity at 25°C		
Molecular Mass Distribution (GPC)		
Particulate Matter by Light Obscuration		
Identification of Bupivacaine and Meloxicam by HILIC ^a		
Identification of Bupivacaine and Meloxicam by HPLC ^a		
Bupivacaine Assay (HPLC)		
Bupivacaine Related Substances (Area % by HPLC)		

Table from page 1 of the Applicant's Specifications document (3.2.P.5.6)

Table 24: HTX-011 Drug Product Specifications (Part 2)

Attribute	Acceptance Criteria	Method Reference
Bupivacaine Dose Uniformity ^a	(b) (4)	
Meloxicam Assay (HPLC)		
Meloxicam Related Substances (Area % by HPLC)		
Meloxicam Dose Uniformity ^a		
In Vitro Release (see Table 2 for additional Level 2 and Level 3 acceptance criteria)		
(b) (4)		
Container Closure Integrity (Dye Ingress) ^b		
Bacterial Endotoxins		

Table from page 2 of the Applicant's specifications document (3.2.P.5.6)

Table 25: HTX-011 Drug Product Specifications (Part 3)

Attribute	Acceptance Criteria	Method Reference
Sterility ^a	No growth	(b) (4)
Extractable Volume ^a	60 mg bupivacaine / 1.8 mg meloxicam	(b) (4)
	200 mg bupivacaine / 6 mg meloxicam	
	300 mg bupivacaine / 9 mg meloxicam	
	400 mg bupivacaine / 12 mg meloxicam	

Abbreviations: GPC, gel permeation chromatography; GC, gas chromatography; HPLC, high performance liquid chromatography; M_w, weight average molecular weight; M_n, number average molecular weight; LMW, low molecular weight; LC, label claim; NMT, not more than; NA, not applicable; AV, acceptance value; HILIC, hydrophilic interaction liquid chromatography; NLT, not less than; ppm, parts per million; USP, United States Pharmacopeia; Ph Eur, European Pharmacopoeia

^a Tested on release only

^b Tested on stability only

Table from page 3 of the Applicant's specifications document (3.2.P.5.6)

(b) (4)

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(b) (4)

Container Closure System

HTX-011 is a solution filled into a 10 or 20 mL (b) (4) clear glass vial with a 20 mm opening (b) (4). The vials are closed with 20 mm (b) (4) stoppers coated with (b) (4) and capped with 20 mm aluminum overseals (b) (4).

(b) (4)
The preparation and administration components include

syringes, a vented vial spike, Luer lock applicators, tip caps, and instructions for use. The sealed glass vial is considered the primary container closure.

Table 27: Primary Container Closure

Description	Manufacturer	DMF	Specification Number
10 mL (b) (4) clear glass vial with a 20 mm opening			
20 mL (b) (4) clear glass vial with a 20 mm opening			
20 mm (b) (4) gray (b) (4) stopper with (b) (4)			
20 mm Aluminum overseal with a matte flip-off cap ^a			

Abbreviations: DMF, Drug Master File; NA, not applicable

^a Different flip-off cap colors are used for each of the product configurations (Table 3).

^b Aluminum overseal with matte flip-off cap is not in direct contact with the product.

Table from page 3 of the Applicant's Container Closure System document (3.2.P.7)

Table 28: Preparation and Administration Components

Description	Manufacturer	Manufacturer Part Number	510(k) Clearance Number	Shelf Life	Storage Condition
VVS	(b) (4)				
2 mL (3 mL) ^a Luer lock syringe					
10 mL (12 mL) ^a Luer lock syringe					
Syringe tip cap					
LLA					

Abbreviations: VVS, vented vial spike; LLA, Luer lock applicator; NDA, New Drug Application

^a The first number indicates the nominal volume of the syringe, and the number in parentheses indicates the maximum volume.

^b-Refer to [Section 3.2.P.8.1.6](#).

Table from page 4 of the Applicant's Container Closure System document (3.2.P.7)

Extractables/Leachables Evaluation

To justify the safety of leachables arising from the container closure system or from (b) (4) processes that are in direct contact with the drug product, the Applicant performed extractables and leachables studies, which are described in the following documents:

- Module 3.2.P.2 Container Closure System
- Module 3.2.P.2 Manufacturing Process Development

The Applicant conducted extractable and leachable studies with two different contract testing labs, (b) (4) and (b) (4). Each contract lab took a different approach to extractable and leachable testing. For example, (b) (4) conducted a single extraction study, while (b) (4) conducted a total of three extraction studies. Each approach is reviewed separately in this section.

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2.6 Proposed Clinical Population and Dosing Regimen

HTX-011 is indicated for application into the surgical site to reduce pain for 72 hours. The Applicant proposed a limitation of use since safety and efficacy have not been established in highly vascular surgeries. Beyond this limitation of use, the Applicant is proposing a broad indication. However, based on internal discussions with the clinical team, the following surgeries are supported: unilateral bunionectomy, unilateral open inguinal hernia, and unilateral total knee arthroplasty. We defer to the clinical team on which surgeries are adequately supported with the approval of the NDA.

The Applicant proposed a maximum dose of 400 mg/12 mg (13.68 mL).

2.7 Regulatory Background

HTX-011 was originally developed under IND 125927, which was opened on May 7th, 2015. NDA 211988 was submitted on September 21st, 2018 and the NDA was fileable from the nonclinical perspective with no comments for the 74-day letter. Following review of the Applicant's NDA, the Division issued a complete response on April 30th, 2019. The complete response included the following nonclinical deficiencies.

3. You have not provided adequate data to support the safety of the DMSO or triacetin, in your drug product and the levels in the product exceed the maximum potency listed in the CDER Inactive Ingredient Database (IID) and are therefore considered novel. Specifically, your NDA did not include any discussion of the impact of these two excipients on the standard reproductive and developmental battery of studies.

Information needed to address this deficiency:

Submit adequate data to fully characterize the impact of the proposed doses of DMSO and triacetin on all endpoints normally characterized via the standard battery of reproductive and developmental toxicology studies. Should you elect to address this via literature, justify the adequacy of the literature based on current standard study protocols and provide copies of all referenced literature. In the absence of adequate published data, GLP nonclinical toxicology studies should be completed.

4. Your toxicological risk assessment for the excipient maleic acid, which exceeds the maximum potency listing in the CDER Inactive Ingredients Database (IID), does not address the potential impact of maleic acid on embryo-fetal development in a second species (typically rabbit).

Information needed to address this deficiency:

Submit adequate justification for the safety of the proposed maximum daily dose of maleic acid, specifically with respect to the effects of this compound on rabbit embryo-fetal development.

5. You have not provided adequate data to qualify the proposed drug product degradant (b) (4) which exceeds the ICH Q3B(R2) qualification threshold.

Information needed to address this deficiency:

Either tighten the drug product specification for (b) (4) to NMT (b) (4) % or provide adequate qualification in accordance with ICH Q3B(R2) as follows:

- a. Complete a minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
- b. In addition, conduct a repeat-dose toxicology study of appropriate duration to support the proposed indication. In this case, a study of 14 days should be completed.

Alternatively, provide adequate data to support your position that (b) (4)

However, we note that if you elect to pursue this option, the data may not address the local safety concerns via this drug product's proposed dosing regimen.

6. You have not provided adequate data to support the safety of the drug product specification for (b) (4). The proposed specification of NMT (b) (4) %

exceeds the appropriate qualification threshold of NMT (b) (4) % for a drug product with a maximum recommended human dose of greater than 2 grams as outlined in the ICH guidance for industry: *Q3B(R2) Impurities in New Drug Products*. (b) (4)

We also acknowledge that (b) (4) was detected in the clinical HTX-011 lot that was tested in the pivotal 28-day toxicology studies in dogs and rats; however, the level detected in these stability batches do not support the proposed specification.

Information needed to address this deficiency:

Either tighten the specification of (b) (4) to be within the qualification threshold of NMT (b) (4) % or provide adequate qualification in accordance with ICH Q3B(R2) as follows:

- a. Complete a minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
 - b. In addition, conduct a repeat-dose toxicology study of appropriate duration to support the proposed indication. In this case, a study of 14 days should be completed.
7. You have not provided adequate leachable data to permit substantive toxicological evaluation of the safety of the container closure system and the (b) (4) for this drug product. Specifically:
- a) You have not tested at least three drug product stability batches over the entire course of your proposed expiry (early, middle, late) in order to identify trends for leachables over the course of stability.
 - b) You have not tested your drug product for extractable compounds detected above the safety concern threshold (SCT) of (b) (4) mcg/day from the (b) (4) in Extractable Study 3 (RPT-694).
 - c) You have not fully identified all of the compounds above the SCT in your extraction studies.
 - d) In your risk assessment for the (b) (4) you have not provided adequate justification for the use of the (b) (4) as a surrogate for the (b) (4)

- e) The variability of (b) (4) present in the existing leachable data do not identify any clear trends and suggest the potential for these compounds to be derived from (b) (4)

Information needed to address this deficiency:

Conduct new leachables studies based on all of the extractables identified in Extractable Study 3 identified above the safety concern threshold (SCT) of (b) (4) mcg/day from the (b) (4) according to USP <1663> and <1664>. In addition, since Extractable Study 3 did not address non-volatile compounds, conduct new leachable studies based on the non-volatile compounds identified in Extractable Studies 1 (RPT-701) and 2 (RPT-693).

Of note, you used a conservative SCT of (b) (4) mcg presumably based on genotoxic concerns. However, for acute products (duration of < 1 month) an SCT of (b) (4) mcg/day can be used for genetic toxicity assessment. However, for general toxicity concerns, employ a SCT of (b) (4) mcg/day in the leachable study.

Include at least three to-be-marketed drug product batches over the entire course of your proposed expiry (early, middle, late) to identify trends for leachables over the course of stability. The three drug batches should be stored in the planned commercial container closure.

The leachable study should use an AET that accurately reflects the potential total daily intake of potential leachables from the drug product and how the product will be dosed. For example, if multiple vials are to be used to deliver a dose, this could affect the AET calculation.

We noted that multiple compounds were not fully identified from your extraction/leachable studies. This included the leachable identified in the final drug product samples from Leachable Study 3 as (b) (4). Provide descriptions of the methods and procedures used to attempt to identify these compounds and justify why these compounds could not be identified or provide further data supporting identification of these chemicals.

Include further justification to support that use of (b) (4) is appropriate to represent the potential toxicity of (b) (4) either by providing literature references or performing adequate QSAR analysis.

Provide a root-cause analysis to identify the source of the (b) (4) or confirm that the (b) (4) present are derived from either (b) (4). If they are derived from (b) (4), justify how you intend to adequately control (b) (4) to ensure product consistency and quality.

In addition to the comments above, the following general recommendations regarding the design and requirements for adequate extractable leachable safety assessment are provided to guide these studies:

The NDA submission must contain adequate information on potential leachables and extractables from the drug container closure system and/or drug product formulation, unless specifically waived by the Division. The evaluation of extractables and leachables from the drug container closure system or device should include specific assessments (b) (4) etc. Provide justification for the choice of solvents and conditions for the extraction studies (e.g., time, temperature, agitation). The results of the extraction studies should be used to assure that you are adequately monitoring the drug product stability samples for potential leachables from the primary or secondary container closure systems and from your analysis of data from any (b) (4) processes that suggest the potential for additional leachable compounds in the final drug product formulation. Your analytical evaluation threshold (AET) must be established to be able to detect, identify, and quantitate levels of compounds based on these thresholds or you must provide adequate justification that these thresholds are not possible to be met by current analytical methodology. If you cannot meet these thresholds, safety evaluations will be based on the limits of quantitation (LOQ). Your submission must include a detailed discussion of how you established your AET as well as justification for the limits of detection (LOD) and LOQ for the analytical methods used.

Evaluate at least three batches of your to-be-marketed drug product for leachables and include assessments at multiple timepoints over the course of your stability studies in order to identify trends in leachable levels over time. The materials tested should include any secondary container closure systems, if present, and be subjected to the same sterilization methods, as appropriate. These data are essential to determine the appropriate shelf life of your product.

For all drug products, establish your AET to be able to detect potentially carcinogenic or genotoxic compounds as per ICH M7 qualification thresholds (e.g., not more than 1.5 mcg/day or up to 120 mcg/day depending on the duration of treatment). However, from a general toxicology perspective, for parenteral products, the AET must be able to detect and identify any leachable that is present in the product at 5 mcg/day and higher, unless justified otherwise, to permit an adequate toxicological risk assessment.

For additional guidance on extractables and leachables testing, refer to the following documents:

- USP <1663>: Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems

- USP <1664>: Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems
- FDA guidance for industry: *Container Closure Systems for Packaging Human Drugs and Biologics*, available at, <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070551.pdf>

In your assessment, include a table listing all compounds, including the concentration in ppm, the experimental conditions, and the maximum daily exposure to these compounds based on the maximum daily dose of the product. The extractable/leachable data must be accompanied by an adequate toxicological risk assessment. Although a toxicological risk assessment based on the results of the extraction studies may be adequate to support the safety assessment during development, for your NDA, the final safety assessment should be based on your evaluation of leachables from at least three batches of your drug product tested at multiple timepoints over the course of your stability studies, as discussed above. The approach for toxicological evaluation of the safety of leachables must be based on good scientific principles and take into account the specific container closure system or patch, drug product formulation, dosage form, route of administration, and dose regimen (chronic or short-term dosing). The safety assessment should be specifically discussed in Module 2.6.6.8 (Toxicology Written Summary/Other Toxicity) of the NDA submission. The risk assessment should be based on the maximum level of each leachable detected in long-term stability samples that include any intended secondary container closure system(s) unless otherwise justified. Include copies of all referenced studies upon which a safety assessment is based.

- If you employ a Permissible Daily Exposure (PDE) assessment as described in ICH Q3C, provide justification for all safety factors employed.
- Published literature to support the safety of any compound rarely provides adequate detail of the study design and study results to permit a thorough independent evaluation of the data. Summary reviews, (e.g., BIBRA, CIR, HERA), although potentially useful to identify original source material, are not acceptable as the source material is not provided and the conclusions cannot be independently verified. Submission of any published study reports must be accompanied by a detailed comparison to modern toxicology study endpoints and any shortcomings of the study must be discussed and justification must be provided to support your assertion that these data are adequate to support the safety of your container closure system.
- Safety justifications based on analogous compounds are also not acceptable unless you can provide adequate data to support your conclusions that a risk assessment based on one compound can be logically interpolated to represent an adequate safety evaluation for your leachable/extractable. This should include

a detailed understanding of the absorption, distribution, metabolism, and elimination of the compounds and an adequate scientific bridge to interpolate a NOAEL for the extractable/leachable compound.

A Type A post-action meeting was held with the Applicant on August 7, 2019. The NDA was resubmitted on September 26th, 2019. Following review of the Applicant's resubmission, the Division issued a complete response on June 26th, 2019. The complete response included the following nonclinical deficiencies.

1. You have not provided adequate data to support your request for a waiver of reproductive and developmental studies for triacetin. Although you have concluded that the primary metabolites of triacetin do not represent a risk for reproductive and developmental effects, you have provided no data to support the conclusion that triacetin instilled into a wound at the maximum daily dose will not result in systemic exposure to the triacetin molecule.

Information needed to address this deficiency:

Provide data to support the conclusion that triacetin levels are not present in the systemic circulation via your drug product or conduct reproductive and developmental toxicology studies with triacetin via a route that mimics the clinical exposure to this excipient. If you elect to continue to rely upon the cited oral rat reproductive and developmental study, provide justification that the animals in that study were exposed to triacetin at levels comparable to those that will occur following clinical use of your drug product at the maximum recommended human dose.

2. You have not provided an adequate scientific bridge to the referenced oral reproductive and developmental studies submitted to qualify the safety of DMSO exposure via your drug product. Instillation of the drug product may result in higher C_{max} and AUC compared to oral studies and therefore it is not clear if the referenced studies adequately address the safety of your product.

Information needed to address this deficiency:

Provide data to support your conclusion that these referenced oral studies resulted in exposures to DMSO that exceed the exposure to DMSO via your drug product or conduct studies using an appropriate route of administration.

3. You have not provided adequate data to support the proposed drug product specification for (b) (4) at NMT (b) (4)%. The nonclinical and clinical lots tested to date in your development program did not contain this high of a percentage of (b) (4) and therefore does not qualify this specification. Further, you did not provide data to support your conclusion that (b) (4) (b) (4) would not contribute to the local tissue effects of the drug product.

Information needed to address this deficiency:

Either conduct a local tissue toxicity study that tests drug product that contains at least the maximum specified level of (b) (4) reduce the specification for (b) (4) to that which was tested in the nonclinical toxicology studies, or provide data to support your hypothesis that the (b) (4) in the product does not contribute to local tissue effects of the drug product.

4. Your embryofetal development study in rabbits tested maleic acid via an oral route of administration rather than the proposed route of administration of your drug product and you did not provide an adequate scientific bridge to support your conclusion that the study provides adequate characterization of the developmental effects of maleic acid exposures via installation into a wound.

Information needed to address this deficiency:

Submit justification that the oral toxicology study resulted in exposures (C_{max} and AUC) that provide adequate coverage for the exposure via your drug product when the product is instilled into a wound. In addition, submit the final study report for the oral maleic acid embryofetal development study in the rabbit to the NDA.

A Type A post-action meeting was held with the Applicant on September 2, 2020. The NDA was resubmitted on November 12th, 2020.

3 Studies Submitted

The Applicant submitted multiple nonclinical studies to support HTX-011 and are summarized in the tables below.

Table 85: Pharmacology Studies Submitted

Study Number	Type of Study	Test System	Method of Administration	Testing Facility
<i>Primary Pharmacodynamics</i>				
33-12	POP ^a	Domestic Piglet	Local Administration into the incision site	(b) (4)
33-14	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-13	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-24	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-61	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-68	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-71	POP ^a	Domestic Piglet	Local Administration into the incision site ^b	
33-87	Incision Model	Domestic Piglet	Local Administration into the incision site	
33-23	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-25	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-29	POP ^a	Domestic Piglet	Local Administration into the incision site	
<i>Secondary Pharmacodynamics</i>				
NA				
<i>Safety Pharmacology</i>				
33-62	In vitro	CHO	In vitro	(b) (4)
<i>Pharmacodynamic Drug Interactions</i>				
NA				

Abbreviation: POP, postoperative pain.

^a Incisional POP employing Von Frey methods for assessment of analgesia.

^b Eloxiject™ (meloxicam) 5 mg/mL Solution for Injection.

Table from Page 2 of the Applicant's Pharmacology Tabulated Summary (2.6.3)

Table 86: Pharmacodynamic Studies Submitted-Part 1

Heron Study No. ^{a, b}	Type of Study	Test Article ^c	Composition (w/w%)	MLX:BPV Ratio	BPV Dose/MLX Dose (mg)	Noteworthy Findings
<i>Determination of the optimal fixed-dose ratio and necessity for co-administration of bupivacaine and meloxicam</i>						
33-12	POP	Saline	NA	NA	NA	<u>Cross-comparison between Studies 33-12 and 33-14:</u> Analgesic effect of HTX-002-01 was greater than that of liposomal bupivacaine. The analgesic effect diminished for HTX-002-01 from 48 to 96 hours and returned by 120 hours. The analgesic effect for liposomal bupivacaine diminished from 24 hours but did not return. <u>Cross-comparison among Studies 33-12, 33-13, and 33-14:</u> In contrast to HTX-002-01 and liposomal bupivacaine, HTX-011-03 resulted in a sustained analgesic effect that lasted from the time of administration through 144 hours <u>Cross-comparison among Study 33-12, 33-14, and 33-24:</u> The analgesic effect of HTX-002-01 started to diminish after 24 hours. In contrast, the analgesic effect of HTX-011-10 was at its maximal level at 24 and 48 hours with the benefit maintained through 72 hours. HTX-009-03 did not result in a meaningful analgesic effect.
		HTX-002-01	BPV 15%	NA	316/0	
33-14	POP	HTX-011-10	BPV 10% + MLX 0.38%	NA	234/8.9	
		Exparel (bupivacaine liposome injectable suspension)	BPV 1.3%	NA	106.4/0	
33-13	POP	HTX-011-03 ^d	BPV 15% + MLX 3.5%	0.233	351/81.9	
33-24	POP	HTX-009-03	MLX 0.15%	NA	0/3	

Table from Page 3 of the Applicant's Pharmacology Tabulated Summary (2.6.3)

Table 87: Pharmacodynamic Studies Submitted-Part 2

Heron Study No. ^{a,b}	Type of Study	Test Article ^c	Composition (w/w%)	MLX:BPV Ratio	BPV Dose/MLX Dose (mg)	Noteworthy Findings
33-61	POP	Saline	NA	NA	NA	<i>Cross-comparison among Studies 33-61, 33-68, and 33-71:</i> The lowest effective MLX:BPV ratio in the extended-release combination formulation was determined to be (b) (4).
		HTX-011-56 ^e	BPV 2.5% + MLX 0.075%	0.030	100/3	
		HTX-011-62	BPV 2.5% + MLX 0.038%	0.015	100/1.5	
		HTX-011-63	BPV 2.5% + MLX 0.019%	0.008	100/0.75	
33-68	POP	HTX-011-56 ^e	BPV 2.5% + MLX 0.075%	0.030	100/3	<i>Cross-comparison between Studies 33-61 and 33-71:</i> HTX-011-56 resulted in a greater analgesic effect compared with that for HTX-002-13 with SC administered meloxicam at most evaluation timepoints.
		HTX-011-72	BPV 2.5% + MLX 0.1125%	0.045	100/4.5	
		HTX-011-74	BPV 2.5% + MLX 0.15%	0.060	100/6	
33-71	POP	HTX-011-74	BPV 2.5% + MLX 0.15%	0.060	100/6	
		HTX-002-13 + SC meloxicam ^f	BPV 2.5% + 3 mg MLX	NA	100/3	
<i>Determination of the mechanism by which meloxicam enhances effectiveness of bupivacaine</i>						
33-87	POP incision model	Saline	NA	NA	NA	The pH of the tissues at the incisions treated with HTX-011-56 or left untreated (sham control) dropped after the surgery and was approximately 6.0 at 8 hours. Afterwards, the pH of the tissues at the incisions treated with HTX-011-56 started to increase and was at the baseline level by 48 hours after the surgery. In contrast, the pH of the tissues at the incisions left untreated (sham control) or treated with saline remained approximately 6.0 and further decreased after 30 hours.
		HTX-011-56 ^e	BPV 2.5% + MLX 0.075%	0.030	100/3	
		Sham control	NA	NA	NA	

Table from Page 4 of the Applicant's Pharmacology Tabulated Summary (2.6.3)

Table 88: Pharmacodynamic Studies Submitted-Part 3

Heron Study No. ^{a,b}	Type of Study	Test Article ^c	Composition (w/w%)	MLX:BPV Ratio	BPV Dose/MLX Dose (mg)	Noteworthy Findings
<i>Synergistic Effect of Bupivacaine and Meloxicam Co-Formulated in HTX-011</i>						
33-23	POP	HTX-011-19	BPV 5% + MLX 0.15%	0.030	105.3/3.2	<i>Cross-comparison among Studies 33-23, 33-25, 33-29, 33-61, 33-68, and 33-71:</i> Bupivacaine and meloxicam in the extended-release combination formulation are acting synergistically.
33-25	POP	HTX-011-16	BPV 5% + MLX 0.3%	0.060	117/7	
		HTX-011-17	BPV 5% + MLX 0.19%	0.038	117/4.4	
		HTX-011-18	BPV 5% + MLX 0.08%	0.016	117/1.9	
33-29	POP	HTX-011-56 ^e	BPV 2.5% + MLX 0.075%	0.030	100/3	
		HTX-011-19	BPV 5% + MLX 0.15%	0.030	100/3	

Abbreviations: BPV, bupivacaine; MLX, meloxicam; NA, not applicable; No., number; POP, postoperative pain; w/w, weight per weight.

Note: % concentrations for test articles refer to % w/w.

^a Postoperative pain was assessed using the Von Frey method at 1, 3, and 5 hours following the surgery on Study Day 0 and on Study Days 1, 2, 3, 4, 5, and 6. In Studies 33-68 and 33-71, postoperative pain using the Von Frey method was also assessed at 8 hours following surgery on Study Day 0.

^b Test articles, except ELOXIJECT in Study 33-71, were administered by local administration into the incision site. ELOXIJECT in Study 33-71 was administered subcutaneously.

^c Other formulations were studied but only those relevant to HTX-011-56 are presented in this table.

^d In the study report for 33-13, the formulation was identified as HTX-011. The complete name of the formulation is HTX-011-03.

^e HTX-011-056 refers to the HTX-011 formulation.

^f Eloxiject™ (meloxicam) 5 mg/mL Solution for Injection.

Sources: Module 4, Section 4.2.1.1, Study Report 33-12, Study Report 33-14, Study Report 33-13, Study Report 33-24, Study Report 33-61, Study Report 33-68, Study Report 33-71, Study Report 33-87, Study Report 33-23, Study Report 33-25, Study Report 33-29.

Table from Page 5 of the Applicant's Pharmacology Tabulated Summary (2.6.3)

Table 89: Safety Pharmacology Studies Submitted

Study	Type of Study	Species	Test Article	Concentration (µM)	Noteworthy Findings
33-62	In vitro	In vitro (CHO cells)	Meloxicam	0.03, 0.3, 3, 30	MLX did not cause a meaningful inhibition of the hERG channel.

Abbreviations: CHO, Chinese hamster ovary; hERG, human ether-a-go-go related gene; MLX, meloxicam.

Source: Module 4, Section 4.2.1.3, Study Report 33-62.

Table from Page 7 of the Applicant's Pharmacology Tabulated Summary (2.6.3)

Table 90: Pharmacokinetics Studies Submitted

Overview					
Type of Study	Species	Method of Administration (Test Article)	Testing Facility	Heron Study No.	
Absorption					
	Rat	Subcutaneous (HTX-011-56)	(b) (4)	33-79	
	Dog	Subcutaneous (HTX-011-56)		33-36	
Excretion					
	Minipig sow and neonate piglet	Subcutaneous (HTX-011-56)		33-77	
Pharmacokinetic Drug Interactions					
	Minipig	Subcutaneous (lidocaine/ HTX-011-56)		33-78	
Other					
	Dog	Subcutaneous (HTX-002-13)		33-47	
	Dog	Subcutaneous (HTX-009-05)		33-69	
	Rat	Oral (DMSO)		35-25	
	Rabbit	Oral (DMSO)	35-26		
	Rabbit	Oral (maleic acid)	35-27		
	Rat	Oral (triacetin)	35-34		

Table from Page 4 of the Applicant's Pharmacokinetics Tabulated Summary (2.6.5)

Table 91: Pharmacokinetic Studies Submitted (Continued)

			Test Article: APF18A (TEG-POE Polymer)
Distribution			
	Rat	Subcutaneous	(b) (4) 24-21 ^a
Excretion			
	Rat	Subcutaneous	24-21 ^a

Abbreviations: No., number.

^a Study 24-21 of the vehicle control APF18A was performed as part of the NDA for SUSTOL[®] (granisetron) extended-release injection (NDA 022445).

(b) (4) The vehicle control data from the SUSTOL NDA were submitted to the HTX-011-56 IND (IND 125927).

Sources: Module 4, Section 4.2.2.2, [Study Report 33-79](#), [Study Report 33-36](#); Module 4, Section 4.2.2.5, [Study Report 33-77](#); Module 4, Section 4.2.2.6, [Study Report 33-78](#); Module 4, Section 4.2.2.7, [Study Report 33-47](#), [Study Report 33-69](#), [Study Report 24-21](#).

Table from Page 5 of the Applicant's Pharmacokinetics Tabulated Summary (2.6.5)

Table 92: Toxicology Studies Submitted-Part 1

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Single Dose Toxicity							
Single Dose Toxicity (HTX-011-56, HTX-002-14)	Rat (SD)	IP	Single Dose	BPV: 0, 15 mg/kg (M) MLX: 0, 0.5 mg/kg (M)	GLP	(b) (4)	33-37
Single Dose (HTX-002)	Rat (SD)	SC	Single Dose	BPV: 0, 7, 42, 83 mg/kg (M); 0, 11, 63, 126 mg/kg (F) MLX: 0 mg/kg (M/F)	GLP		33-48
Extended Single Dose (HTX-009-005)	Rat (SD)	SC	Single Dose	BPV: 0 mg/kg (M/F) MLX: 0, 0.2, 0.6, <u>1.2</u> mg/kg (M); 0, 0.3, 0.8, <u>1.6</u> mg/kg (F)	GLP		33-55
Repeated Dose Toxicity							
28-Day Toxicity Study (HTX-011-56)	Rat (SD)	SC	28 Days (every 48 hours)	BPV: 0, 0, 12.5, <u>25</u> mg/kg MLX: 0, 0, 0.38, <u>0.75</u> mg/kg	GLP		33-85
28-Day Toxicity Study (HTX-011-56)	Dog (Beagle)	SC	28 Days (every 48 hours)	BPV: 0, 0, 10, <u>13</u> mg/kg MLX: 0, 0, 0.3, <u>0.39</u> mg/kg	GLP		33-86
14-Day Toxicity Study (HTX-002-14, HTX-002-19)	Rat (SD)	SC	14 Days (every 48 hours)	BPV: 0, 100, <u>150</u> mg/kg	GLP		34-84

Table from Page 6 of the Applicant's Toxicology Tabulated Summary (2.6.7)

Table 93: Toxicology Studies Submitted-Part 2

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Genotoxicity							
Ames Assay (HTX-011-15)	S. typhimurim and E. coli strains	In vitro	Incubated for 48 – 72 hours	Up to 5,000 µg/plate	GLP	(b) (4)	33-11
Local Tolerance							
Bone Healing Model (HTX-011-19)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 0, 17.80, 44.45, 88.98 mg/kg (M) 0, 0, 21.21, 53.04, 106 mg/kg (F) MLX: 0,0, 0.55, 1.37, 2.74 mg/kg (M) 0, 0, 0.65, 1.63, 3.26 mg/kg (F)	Non-GLP		33-15
Bone Healing Model (HTX-11-56, HTX-002-14)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 50 mg/kg (F) MLX: 0, 1.5 mg/kg (F)	Non-GLP		33-39
Bone Healing Model (HTX-011-56)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 14.6 mg/kg (F) MLX: 0, 0.44 mg/kg (F)	GLP		33-83
Hernia Repair Model (HTX-11-56, HTX-002-14)	Rabbit (NZW)	Test article was administered onto the mesh prior to closure of the SC tissues and skin at the incision site	Single Dose	BPV: 0, 10 (M); 0, 9 mg/kg (F) MLX: 0, 0.3 (M); 0, 0.3 mg/kg (F)	Non-GLP		33-38

Table from Page 7 of the Applicant's Toxicology Tabulated Summary (2.6.7)

Table 94: Toxicology Studies Submitted-Part 3

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Hernia Repair Model (HTX-011-56)	Rabbit (NZW)	Test article was administered onto the mesh prior to closure of the SC tissues and skin at the incision site	Single Dose	1.0 mL/site BPV: 29.25 mg/mL MLX: 0.88 mg/mL	GLP	(b) (4)	33-80
Wound Healing Model (HTX-11-56, HTX-002-14)	Minipig (Göttingen)	Dermal	Single Dose	BPV: 0, 2.3, 4.6 mg/kg (M); 0, 2.3, 4.5 mg/kg (F) MLX: 0, 0.1 mg/kg (M); 0, 0.1 mg/kg (F)	GLP		33-41
Wound Healing Model (HTX-002-13), HTX-009-05)	Minipig (Göttingen)	Dermal	Single Dose	BPV: 0, 4.6 mg/kg (M/F) MLX: 0, 0.1 mg/kg (M/F)	GLP		33-54
Brachial Plexus Nerve Block Model (HTX-024-02, HTX-002-14, HTX-011-56)	Dog (Beagle)	Intra-plexus	Single Dose	BPV: 0, 1.4, 8.3 (M); 0, 1.8, 10.5 (F) MLX: 0, 0.2 (M); 0, 0.3 (F)	GLP		33-42
Perineural Toxicity Study (HTX-011-56)	Dog (Beagle)	Perineural injection to femoral nerve	Single Dose	BPV: 0, 2, 7, 14.1 (M) MLX: 0, 0.2, 0.4 (M)	GLP		33-63
Other Toxicity Studies Conducted with APF18A Supporting the TEG-POE Polymer							
Repeat Dose Toxicity (APF18A)	Rat/Crl:CD [®] (SD)	SC	Up to 93 days	APF18A: 0, 295 mg/dose	Yes		25-07
Repeat Dose Toxicity (APF18A)	Dog/Beagle	SC	Up to 93 days	APF18A: 0, 1180 mg/dose	Yes		25-08

Table from Page 8 of the Applicant's Toxicology Tabulated Summary (2.6.7)

Table 95: Toxicology Studies Submitted-Part 4

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Genotoxicity (APF18A)	S. typhimurium strains TA98, TA100, TA1535 and TA1537 E. coli strain WP2uvrA	In vitro	52±4 hour incubation	APF18A: ± S9: 0 (DMSO), 100, 333, 1000, 3330 and 5000 µg APF18A/plate	Yes	(b) (4)	24-17
Genotoxicity (APF18A)	Mouse lymphoma L5178Y cells	In vitro	Initial assay: 4 hour incubation Confirmatory assay: 24-hour treatment period	APF18A: ±S9: 0 (DMSO), 78.5, 157, 313, 625, 1250, 2500, 4000 and 5000 µg APF18A/mL	Yes		24-19
Genotoxicity (APF18A)	Rat/Crl:CD (SD)BR	SC	Single dose Animals sacrificed 24 and 48 hours post dose	APF18A: 0 (saline), 750, 1500 and 3000 mg/kg	Yes		24-16
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Males: beginning 28 days before cohabitation through cohabitation Females: beginning 15 days before cohabitation till Gestation Day 7	APF18A: 0 (0.9% NaCl), 0.142 and 0.295 g APF18A/day	Yes		27-02
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Gestation Days 7-17 (0, 0.14 and 0.295 g/day) Gestation Days 7, 10, 13 and 16 (0.295 g/day)	APF18A: 0 (0.9% NaCl), 0.14 and 0.295 g APF18A/day and 0.295 g APF18A/every 72 hours)	Yes		25-09

Table from Page 9 of the Applicant's Toxicology Tabulated Summary (2.6.7)

Table 96: Toxicology Studies Submitted-Part 5

Test Article: HTX-011-56 or Supporting Formulations								
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses*	GLP Compliance	Testing Facility (b) (4)	Study Number	
Reproductive and Developmental Toxicity (APF18A)	Rabbit/New Zealand white	SC	Gestation Days 7-19 (0.59 and 1.18 g/day) Gestation Days 7, 10, 13, 16 and 19 (1.18 g/day)	APF18A: 0 (0.9% NaCl), 0.59 and 1.18 g APF18A/day and 1.18 g APF18A/every 72 hours)	Yes		25-10	
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Gestation Day 7 to Lactation Day 20	APF18A: 0 (0.9% NaCl), 0.142 and 0.295 g APF18A/day	Yes		27-03	
Other Toxicity Studies Conducted With Nonpolymeric Excipients Supporting HTX-011-56								
Toxicokinetics to support OECD SIDS Dossier 2000 studies (DMSO)	Rat (SD)	Oral	Males and non-pregnant females: once a day for 5 days Pregnant females: Gestation Day 6 to 17	DMSO: 1000 mg/kg/day	Yes		35-30	
Toxicokinetics to support OECD SIDS Dossier 2000 study (DMSO)	Rabbit/New Zealand white	Oral	Gestation Day 6 to 19	DMSO: 1000 mg/kg/day	Yes		35-31	
Embryo-Fetal Developmental Toxicity (Maleic Acid)	Rabbit/New Zealand white	Oral	Gestation Day 6 to 18	Maleic Acid: 0, 2, 5, 20 mg/kg/day	Yes	34-74		
Toxicokinetics to support Study 34-74 (Maleic Acid)	Rabbit/New Zealand white	Oral	Gestation Day 6 to 18	Maleic Acid: 20 mg/kg/day	Yes	35-32		

Table from Page 10 of the Applicant's Toxicology Tabulated Summary (2.6.7)

Table 97: Toxicology Studies Submitted-Part 6

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
(Q)SAR Assessment for HTX-011-56 Drug Product Impurities							
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No	(b) (4)	33-98
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No		33-99
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No		34-10
Other Studies Conducted with Early HTX-011 Formulations Containing Higher Concentrations of Bupivacaine and Meloxicam than HTX-011-56							
Single Dose Toxicity (HTX-011-12, HTX-011-14, HTX-011-15)	Rat (SD)	SC	Single Dose	BPV: 0, 97.2, 146.7, 195.0 mg/kg (M); 0, 127.7, 193.3, 258.8 mg/kg (F) MLX: 0, 7.4, 14.7, 29.3 (M); 0, 9.7, 19.3, 38.8 mg/kg (F)	Yes	(b) (4)	33-09

Abbreviations: BPV, bupivacaine; DMSO, dimethyl sulfoxide; F, female; GLP, Good Laboratory Practice; IP, intraperitoneal; M, male; MLX, meloxicam; NA, not applicable; NZW, New Zealand White; (Q)SAR, Quantitative Structure Activity Relationship; SC, subcutaneous; SD, Sprague Dawley; TEG-POE, tri(ethylene glycol)-based poly(orthoester) polymer.

^a Unless otherwise specified. For Repeated Dose Toxicity, the highest No Observed Adverse Effect Level (NOAEL) is underlined. Doses listed specify the approximate mg/kg doses of the active pharmaceutical ingredients (APIs), bupivacaine and meloxicam, which incorporates the correction for density of 1.17 g/mL. Studies utilized a variety of vehicles for HTX-011.

Table from Page 11 of the Applicant's Toxicology Tabulated Summary (2.6.7)

3.1 Studies Reviewed

The Applicant conducted multiple nonclinical studies, many with previous formulations of the drug product. The following studies were reviewed in the original submission: Studies 33-85 and 33-86 were formally reviewed as these were the pivotal studies for justifying the safety of the clinical HTX-011 formulation, including the levels of bupivacaine and excipients. Special toxicity studies for hernia repair (Study 33-38), bone healing (Study 33-39), and wound healing (Study 33-54) were also reviewed as these studies supported the use of HTX-011 in the various surgeries used clinically. In addition, Studies 33-48, 33-55, 24-17, 24-19, 24-16, 27-02, 25-09, 25-07, 25-08, 25-10, and 25-03 were reviewed to justify the excipients, especially the AP135 polymer. While a formal review of the studies in the previous sentence is not included below, summaries of the results of these studies can be found in the excipients section (Section 2.4) of this review.

The following studies were reviewed as part of the Applicant's first resubmission dated September 26th, 2019: Study 34-84 was a 14-day subcutaneous injection study in rats to support the safety of exposure to systemic levels of bupivacaine that are equivalent to clinical doses of 400 mg bupivacaine or higher. Study 34-74 was a developmental study in rabbits with maleic acid to support the safety of this excipient in the formulation.

The following studies were reviewed as part of the Applicant's second resubmission dated November 12th, 2020: The Applicant submitted new toxicokinetic studies for the excipients (Studies: 35-25, 35-26, 35-27, 35-34, 35-30, 35-31, and 35-32) which were reviewed. While a formal review of the studies is not included below, summaries of the results of these studies can be found in the excipients section (Section 2.4) of this review.

3.2 Studies Not Reviewed

The remaining studies in the Applicant's tables above were not reviewed formally but summaries of relevant studies are provided in some of the sections below.

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

HTX-011 contains bupivacaine, an amide-type local anesthetic, and meloxicam, an NSAID. (b) (4)

[Redacted]

[Redacted] (b) (4)

4.2 Secondary Pharmacology

Secondary pharmacology studies with HTX-011 were not conducted.

4.3 Safety Pharmacology

The only safety pharmacology study conducted by the Applicant was an in vitro study on cardiovascular targets, including the hERG channel. Meloxicam did not cause a meaningful inhibition of any cardiovascular targets. A 20.5% inhibition of the hERG channel was detected at 30 mcM meloxicam.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Exposure to bupivacaine and meloxicam was assessed following a single subcutaneous administration of HTX-011 in rats and dogs (Study 33-79 and 33-36) and the pharmacokinetic parameters from those studies are included below. In rats, bupivacaine was observed in plasma for 72 to 144 hours with the low dose (25 mg/kg) and for 144 to 168 hours with the high dose (50 mg/kg). In dogs, bupivacaine was observed in the plasma for 96 to 120 hours.

Excretion was measured in minipig sows following a single subcutaneous dose of HTX-011 (Study 33-77). Concentrations of bupivacaine, meloxicam, and the water-soluble end products of AP135 ((b) (4))

(b) (4)) were measured in milk, plasma, and urine. The same compounds were measured in the plasma and urine of untreated neonatal piglets. Bupivacaine and meloxicam were found in the plasma, milk, and urine of the sows, while only bupivacaine was found in the urine and plasma of piglets. The water-soluble end products were found in the milk, plasma, and urine of sows, while only (b) (4) was present in piglet plasma. All four products were found in pooled urine from piglets.

Table 98: Highest Concentrations of Bupivacaine and Meloxicam in Plasma, Milk, and/or Urine of Minipig Sows and Neonate Piglets Following a Single SC Administration of HTX-011 to Sows

Matrix	Species	N	Bupivacaine (µg/mL)	Meloxicam (µg/mL)
Milk	Sow	3	0.577	0.0632
Plasma	Sow	3	2.07	0.132
	Piglet	24	0.330	BLQ ^a
Urine	Sow	3	3.70	0.0226
	Piglet ^b	24	0.0552	BLQ ^a

Abbreviation: BLQ, below the limit quantitation.

^a Lower limit of quantitation for meloxicam was 0.010 µg/mL for plasma and urine.

^b Urine samples were pooled for each litter for determination of bupivacaine and meloxicam concentrations.

Source: [Module 4, Section 4.2.2.5, Study Report 33-77, Table 6.](#)

Table from page 28 of the Applicant's Pharmacokinetics Written Summary (2.6.4)

The potential for drug interactions between bupivacaine, meloxicam, and lidocaine were assessed in minipigs (Study 33-78). There was no meaningful impact on the pharmacokinetic profiles of any of the compounds when co-administered.

Table 99: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Rats

Dose of HTX-011-56 (mg/kg)	Analyte	Sex	N	t _{1/2} (h)	T _{max} (h)	C _{max} (ng/mL)	AUC _{inf} (h·ng/mL)
<i>Low dose</i>							
25	Bupivacaine	M	9	20.8	6.00	276	7,260
		F	9	13.6	0.50	321	6,240
0.75	Meloxicam	M	9	26.3	24.00	1,860	175,000
		F	9	NC ^a	72.00	3,770	NC ^a
<i>High dose</i>							
50	Bupivacaine	M	9	18.1	4.00	474	16,400
		F	9	16.2	1.00	510	15,000
1.5	Meloxicam	M	9	36.5	72.00	3,480	340,000
		F	9	40.5	72.00	7,180	962,000

Abbreviations: AUC_{inf}, area under the concentration-time curve from time 0 to infinity; C_{max}, maximum observed concentration; F, female; M, male; NC, not calculated; PK, pharmacokinetic; SC, subcutaneous; t_{1/2}, apparent terminal half-life; T_{max}, time of maximum observed concentration.

^a Not calculated due to insufficient number of samples.

Source: [Module 4, Section 4.2.2.2, Study Report 33-79, Appendix 5, Table 5.](#)

Table from page 37 of the Applicant's Nonclinical Overview (2.4)

Table 100: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Dogs

Dose of HTX-011-56 (mg/kg)	Analyte	Sex	N	t _{1/2} (h)	T _{max} (h)	C _{max} (ng/mL)	AUC _{last} (h·ng/mL)	AUC _{inf} (h·ng/mL)
6.96	Bupivacaine	M	5	35.6	1.80	321	9,527	10,495
6.66		F	4 ^a	36.5	2.50	228	8,230	9,458
0.209	Meloxicam	M	5	154	36.0	301	28,474	69,649
0.200		F	4 ^a	94.1	48.0	257	23,778	44,894

Abbreviations: AUC_{inf}, area under the concentration-time curve from time 0 to infinity; AUC_{last}, area under the concentration-time curve from time 0 to last quantifiable sampling time point; C_{max}, maximum observed concentration; F, female; M, male; PK, pharmacokinetic; SC, subcutaneous; t_{1/2}, apparent terminal half-life; T_{max}, time of maximum observed concentration.

Note: the dose level in mg/kg for the active ingredients was calculated based on the nominal concentration of bupivacaine or meloxicam including the density of 1.17 g/mL, the dose volume administered, and an average body weight for each sex.

^a One female received only half of the targeted dose. The plasma concentration-time data from this female was excluded from the analysis.

Source: [Module 4, Section 4.2.2.2, Study Report 33-36, Table 1.](#)

Table from page 38 of the Applicant's Nonclinical Overview (2.4)

6 General Toxicology

Over the course of development, the toxicity studies tested various formulations of HTX and treatment groups employed code numbers for the formulations. The table below, reproduced from the submission, summarizes many of the compositions of the formulations tested compared to the to-be-marketed formulation coded HTX-011-56.

Table 101: Composition of Test Articles Used in Nonclinical Studies Supporting Development of HTX-011-56

Test Article	Active Ingredient		Excipient			
	Bupivacaine (mg/mL)	Meloxicam (mg/mL)	DMSO (mg/mL)	TEG-POE (mg/mL)	Maleic Acid (mg/mL)	Triacetin (mg/mL)
<i>Extended Release Formulations of Bupivacaine and Meloxicam</i>						
HTX-011-56	29.25	0.88	117.0	729.8	0.59	292.5
HTX-011-12 ^a	58.50	4.45	NA	924.5	7.02	NA
HTX-011-14 ^a	87.75	8.78	NA	887.3	10.6	NA
HTX-011-15 ^a	117.0	17.55	NA	848.1	11.70	NA
HTX-011-19 ^a	58.50	1.76	NA	927.2	7.02	NA
HTX-011-49	29.25	0.88	93.60	635.0	1.76	409.5
<i>Extended Release Formulations of Bupivacaine Only</i>						
HTX-002-13	29.25	NA	117.0	730.7	0.59	292.5
HTX-002-14	29.25	NA	93.60	635.9	1.76	409.5
<i>Extended Release Formulation of Meloxicam Only</i>						
HTX-009-05	NA	0.88	117.0	759.6	NA	292.5
<i>Vehicles for HTX-011-56</i>						
HTX-024-01	NA	NA	117.0	643.5	NA	409.5
HTX-024-02	NA	NA	117.0	760.5	NA	292.5
<i>Vehicle for HTX-011-19</i>						
HTX-018-02 ^a	NA	NA	NA	850	NA	NA
<i>Controls</i>						
Bupivacaine HCl	5	NA	NA	NA	NA	NA
Saline	NA	NA	NA	NA	NA	NA

Abbreviations: DMSO, dimethyl sulfoxide; NA, not applicable; TEG-POE, tri(ethylene glycol) poly(orthoester).

Notes: the mg/mL concentrations of bupivacaine, meloxicam, and excipients, except for HTX-018-02, were calculated based on the density of 1.17 g/mL. In the nonclinical toxicology studies additional formulations were evaluated. They are not listed in this table because they are not relevant for HTX-011-56.

^a Formulation contained (b) (4)

Sources: Module 4, Section 4.2.3.1, Study Report 33-37, Study Report 33-09; Study Report 33-48, Study Report 33-55; Module 4, Section 4.2.3.2, Study Report 33-85, Study Report 33-86; Module 4, Section 4.2.3.3.1, Study Report 33-11; Module 4, Section 4.2.3.6, Study Report 33-15, Study Report 33-39, Study Report 33-83, Study Report 33-38, Study Report 33-38 Amendment 1, Study Report 33-80, Study Report 33-41, Study Report 33-54, Study Report 33-42, Study Report 33-63.

Table from page 36 of the Applicant's Toxicology Written Summary (2.6.6)

6.1 Single-Dose Toxicity

Single-dose studies with the HTX-011 vehicle were summarized as part of the Comments on Novel Excipients Section (Section 2.4).

A total of four single-dose toxicity studies were conducted, as summarized in the Applicant's table below.

Table 102: Overview of Single-dose Toxicity Studies Conducted by Applicant

Heron Study No./ GLP	Species	Route	Necropsy Day Postdose	Test Articles	TK Assessed
33-37/ Yes	Rat	IP	3, 8, 15	Saline HTX-024-01 (vehicle for HTX-011-56) HTX-002-14 (bupivacaine only comparator) ^a HTX-011-56 ^b	Yes
33-09/ Yes	Rat	SC	3, 14	Saline HTX-011-12 ^c HTX-011-14 ^d HTX-011-15 ^e	Yes
33-48/ Yes	Rat	SC	3, 14	Saline HTX-024-02 (vehicle for HTX-011-56) Bupivacaine HCl HTX-002-14 (bupivacaine only comparator) ^a	Yes
33-55/ Yes	Rat	SC	4, 15	Saline HTX-024-02 (vehicle for HTX-011-56) HTX-009-05 (meloxicam only comparator) ^f	Yes

Abbreviations: GLP, Good Laboratory Practice; IP, intraperitoneal; No., number; SC, subcutaneous; TK, toxicokinetics.

^a HTX-002-14 is an extended-release formulation of bupivacaine only and contains the same amount of bupivacaine as HTX-011-56.

^b HTX-011-56 refers to HTX-011, the Phase 3, Phase 2b, and intended commercial formulation.

^c HTX-011-12 contains 2-times higher concentration of bupivacaine and 5-times higher concentration of meloxicam than HTX-011-56.

^d HTX-011-14 contains 3-times higher concentration of bupivacaine and 10-times higher concentration of meloxicam than HTX-011-56.

^e HTX-011-15 contains 4-times higher concentration of bupivacaine and 20-times higher concentration of meloxicam than HTX-011-56.

^f HTX-009-05 is an extended-release formulation of meloxicam only and contains the same amount of meloxicam as HTX-011-56.

Sources: [Module 4, Section 4.2.3.1](#), [Study Report 33-37](#), [Study Report 33-09](#), [Study Report 33-48](#), [Study Report 33-55](#).

Table from page 39 of the Applicant's Toxicology Written Summary (2.6.6)

Only one of the studies actually tested HTX-011-56 and that study tested the drug product via intraperitoneal dosing. As such, these studies are not further reviewed here as relative comparisons to the referenced product cannot be made nor are the formulations directly applicable to the proposed product. The exception was Study number 33-48. This study is briefly reviewed here because it is included treatment with bupivacaine alone as a control, which is useful in evaluating if the local tissue effects are related to the excipients used in the drug product.

Study title: HTX-002: A Single Dose Subcutaneous Injection Toxicity Study in Sprague-Dawley Rats

Study no.: 33-48
Study report location: SDN 1, Module 4
\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4231-single-dose-tox\33-48\33-48.pdf
Conducting laboratory and location: (b) (4)
Date of study initiation: 2/26/2016
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Purity vales are for bupivacaine
HTX-002-014, Lot 15055, purity 101.6%
HTX-002-013, Lot 16007, purity 101.3%
HTX-024-002, Lot 1273098, purity negative
HTX-024-003, Lot 1273100, purity negative
bupivacaine, Lot 55211DD and 58201DD,
purity not provided

Key Study Findings

- Treatment of animals with saline or bupivacaine resulted in similar local injection site histopathological findings.
- Treatment with HTX (HTX-002-013) caused an increased incidence and/or severity of subcutaneous inflammation, hemorrhage, necrosis (female only), and edema (female only) compared to the saline control 3 days after treatment. An increased incidence and/or severity of subcutaneous inflammation and hemorrhage compared to the saline control was also seen in recovery animals on Day 14.
- Treatment with the HTX vehicle (HTX-024-002) caused an increased incidence and/or severity of subcutaneous inflammation, hemorrhage (females only), necrosis, and edema (female only) compared to the saline control 3 days after treatment. In addition, an increased incidence and/or severity of epidermal crust was observed. An increased incidence and/or severity of subcutaneous inflammation and hemorrhage compared to the saline control was also seen in recovery animals on Day 14.

- In recovery animals only, treatment with HTX (HTX-002-013) caused an increased incidence and/or severity of fibroplasia and epidermal hyperplasia/regeneration (females only).
- In recovery animals only, treatment with the HTX vehicle (HTX-024-002) caused an increased incidence and/or severity of fibroplasia (males only) and epidermal hyperplasia/regeneration (females only).
- The local histopathological findings appear to be related to the excipients (DMSO, AP135, and triacetin) since similar findings were seen with both the HTX vehicle and HTX formulation and the incidence and /or severities were greater than the bupivacaine alone. However, the results were not always consistent making definitive conclusions impossible.

Methods

Doses:	Bupivacaine 2.5 mg, low dose of HTX: 0.585 mg bupivacaine, high dose of HTX: 1.17 mg bupivacaine
Frequency of dosing:	Single injection
Route of administration:	Subcutaneous injection
Dose volume:	See experimental design
Formulation/Vehicle:	See formulation table
Species/Strain:	Sprague-Dawley rat
Number/Sex/Group:	10 for the main experiment, 5 for recovery, and 3 (saline and vehicle) or 27 (low and high dose) for toxicokinetics
Age:	8-9 weeks old
Weight:	193-227 g (male) and 148-193 g (female)
Satellite groups:	Toxicokinetic and recovery groups
Unique study design:	For dose volumes of 2 mL (saline and the vehicle controls), 1 mL was injected into two separate injection sites
Deviation from study protocol:	None that impacted study results

Table 103 Formulations Tested in Study Number 33-48

Test and Control Items	Lot Number	Active Pharmaceutical Ingredients (APIs)	Excipients			
		Bupivacaine w/w% (mg/g)	Maleic Acid w/w% (mg/g)	DMSO w/w% (mg/g)	Triacetin w/w% (mg/g)	AP135 w/w% (mg/g)
HTX-024-002	1273098	NA	NA	10.0 (100.0)	25.0 (250.0)	65.0 (650.0)
HTX-024-003	1273100	NA	NA	8.0	35.0	57.0
HTX-002-013	16007	2.5 (25.0)	0.15 (1.5)	10.0 (100.0)	25.0 (250.0)	62.35 (623.5)
HTX-002-014	15055	2.5 (25.0)	0.15 (1.5)	8.0 (80.0)	35 (350.0)	54.35 (543.5)

Table from page 14 of the Applicant's study report

Experimental design

Table 104 Experimental Design of Study Number 33-48

Group Numbers	Group Designation	Dose Volume (mL)	Toxicology Animals				Toxicokinetic Animals	
			Main		Recovery		Male	Female
			Male	Female	Male	Female		
1	Saline Control	2.0	10	10	5	5	3	3
2	Vehicle Control HTX-024-002	2.0	10	11*	5	4*	3	3
3	Vehicle Control HTX-024-003	2.0	10	10	5	5	3	3
4	Bupivacaine comparator	0.5	10	9*	5	6*	6	6
5	HTX-002-013 Low dose	0.5	10	10	5	5	6	6
6	HTX-002-013 High dose	1.0	10	10	5	5	6	6
7	HTX-002-014 Low dose	0.5	10	10	5	5	6	6
8	HTX-002-014 High dose	1.0	10	10	5	5	6	6

Table from page 14 of the Applicant's study report

Only results from HTX-024-002, referred to as HTX vehicle, and HTX-002-013, referred to as HTX, are presented in this review as these are the closest to the clinical formulation. Furthermore, only the histopathological results are described in detail below as this was the key data for assessing whether the local effects were potentially related to the excipients as opposed to bupivacaine. As the data from this study was not with the clinical formulation and the safety of the clinical formulation was addressed in the pivotal repeat dose studies, detailed review of the remaining data was not deemed critical for this review.

Histological Findings

The incidence and severity of subcutaneous inflammation was increased in males following treatment with the HTX vehicle, the low dose of HTX-002-013, and the high dose of HTX-002-013 compared to both the saline control and bupivacaine. In females, only a small increase in severity was observed with the vehicle, the low dose of HTX-002-013, and the high dose of HTX-002-013 compared to both the saline control and bupivacaine because the saline and bupivacaine groups had a higher incidence and severity of findings. The incidence and severity of this finding was also increased following recovery in both sexes, except in female animals receiving the HTX vehicle.

The incidence and severity of subcutaneous hemorrhage was increased in males following treatment with the low and high dose of HTX-002-013, and the high dose of HTX-002-013 compared to both the saline control and bupivacaine. In females, the incidence and severity of subcutaneous hemorrhage was increased with both doses of HTX and the HTX vehicle. Hemorrhages were still found at a higher incidence/severity following recovery in both sexes with both doses of HTX but not the HTX vehicles.

The incidence and severity of subcutaneous necrosis was increased in males following treatment with the HTX vehicle compared to both the saline control and bupivacaine. In females, the incidence and severity of subcutaneous necrosis was increased in animals receiving HTX or the HTX vehicle compared to both the saline control and bupivacaine. Necrosis was not seen in the recovery animals.

A higher incidence or severity of epidermis crust was observed in both males and females who received the HTX vehicle compared to both the saline control and bupivacaine. An increase was also seen in males receiving the low dose of HTX, but in no other groups receiving HTX. Epidermis crust was seen in some recovery animals but there did not appear to be a pattern to the findings.

The incidence and severity of subcutaneous edema was increased in female animals treated with the HTX vehicle or HTX compared to both the saline control and bupivacaine. Edema was not observed in recovery animals.

Fibroplasia was only observed in recovery animals. There was an increased incidence and severity of this finding in males receiving the HTX vehicle and HTX compared to both the saline control and bupivacaine. In females, the increased incidence and severity were only seen with HTX.

Epidermal hyperplasia/regeneration was only seen in female recovery animals who received HTX or the HTX vehicle.

Table 105 Histopathological Findings on Day 3 at the Primary Injection Site, Males

	Male				
	Saline	Bupivacaine	HTX-024-02 (vehicle)	HTX-002-013 LD	HTX-002-013 HD
Inflammation, Subcutaneous	60% (1.5)	60% (1.2)	90% (1.9)	90% (1.9)	100% (2.0)
Inflammation, epidermal/dermal	50% (1.0)	10% (1.0)	40% (1.0)	50% (1.4)	30% (1.0)
Hemorrhage Subcutaneous	60% (1.2)	70% (1.0)	70% (1.0)	80% (1.4)	90% (1.7)
Necrosis Subcutaneous	0%	10% (1.0)	70% (1.7)	20% (1.0)	10% (1.0)
Crust Epidermis	30% (1.0)	10% (1.0)	40% (1.0)	50% (1.6)	20% (1.0)
Edema Subcutaneous	20% (1.0)	0%	30% (1.3)	0%	20% (1.5)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe
Table prepared by Reviewer based on data from study report

Table 106 Histopathological Findings on Day 3 at the Secondary Injection Site, Males

	Male	
	Saline	HTX-024-02 (vehicle)
Inflammation, Subcutaneous	70% (1.3)	100% (1.7)
Inflammation, epidermal/dermal	20% (1.0)	40% (1.3)
Hemorrhage Subcutaneous	40% (1.0)	80% (1.1)
Necrosis Subcutaneous	0%	60% (1.3)
Crust Epidermis	10% (1.0)	70% (1.1)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe
Table prepared by Reviewer based on data from study report

Table 107 Histopathological Findings on Day 14 (Recovery) at the Primary Injection Site, Males

	Male				
	Saline	Bupivacaine	HTX-024-02 (vehicle)	HTX-002-013 LD	HTX-0020013 HD
Inflammation, Subcutaneous	40% (1.0)	20% (1.0)	60% (1.3)	100% (1.6)	80% (1.5)
Inflammation, epidermal/dermal	0%	20% (2.0)	0%	0%	20% (3.0)
Hemorrhage Subcutaneous	0%	0%	0%	40% (1.5)	40% (2.0)
Crust Epidermis	0%	20% (2.0)	0%	0%	0%
Fibroplasia	0%	0%	20% (2.0)	40% (1.5)	60% (2.3)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 108 Histopathological Findings on Day 14 (Recovery) at the Secondary Injection Site, Males

	Male	
	Saline	HTX-024-02 (vehicle)
Inflammation, Subcutaneous	0%	20% (1.0)
Hemorrhage Subcutaneous	0%	0%
Fibroplasia	0%	0%

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 109 Histopathological Findings on Day 3 at the Primary Injection Site, Females

	Female				
	Saline	Bupivacaine	HTX-024-02 (vehicle)	HTX-002-013 LD	HTX-0020013 HD
Inflammation, Subcutaneous	100% (1.9)	78% (1.9)	91% (2.5)	100% (2.1)	90% (2.7)
Inflammation, epidermal/dermal	70% (1.3)	89% (1.3)	45% (1.6)	60% (1.3)	70% (1.1)
Hemorrhage Subcutaneous	90% (1.0)	67% (1.2)	91% (1.8)	100% (1.3)	90% (1.7)
Necrosis Subcutaneous	10% (1.0)	0%	54% (1.7)	20% (1.0)	60% (1.3)
Crust Epidermis	60% (1.0)	100% (1.3)	54% (1.7)	70% (1.0)	50% (1.4)
Edema Subcutaneous	0%	0%	40% (1.8)	40% (1.0)	70% (1.4)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 110 Histopathological Findings on Day 3 at the Secondary Injection Site, Females

	Female	
	Saline	HTX-024-02 (vehicle)
Inflammation, Subcutaneous	80% (2.3)	100% (2.3)
Inflammation, epidermal/dermal	30% (1.3)	64% (1.6)
Hemorrhage Subcutaneous	80% (1.1)	100% (1.5)
Necrosis Subcutaneous	20% (1.0)	54% (1.2)
Crust Epidermis	40% (1.0)	72% (1.6)
Edema, Subcutaneous	10% (1.0)	9% (1.0)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 111 Histopathological Findings on Day 14 (Recovery) at the Primary Injection Site, Females

	Female				
	Saline	Bupivacaine	HTX-024-02 (vehicle)	HTX-002-013 LD	HTX-0020013 HD
Inflammation, Subcutaneous	0%	50% (1.0)	25% (1.0)	80% (1.5)	80% (1.5)
Inflammation, epidermal/dermal	0%	0%	25% (2.0)	0%	0%
Hemorrhage Subcutaneous	0%	0%	0%	40% (1.0)	40% (1.5)
Crust Epidermis	0%	17% (3.0)	25% (3.0)	20% (2.0)	0%
Fibroplasia	0%	17% (1.0)	0%	40% (1.5)	40% (2.0)
Hyperplasia/rege neration Epidermal	0%	0%	25% (3.0)	40% (1.5)	20% (2.0)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 112 Histopathological Findings on Day 14 (Recovery) at the Secondary Injection Site, Females

	Female	
	Saline	HTX-024-02 (vehicle)
Inflammation, Subcutaneous	20% (1.0)	100% (1.5)
Inflammation, epidermal/dermal	0%	25% (1.0)
Hemorrhage Subcutaneous	0%	0%
Crust Epidermis	0%	50% (2.5)
Fibroplasia	0%	50% (1.5)
Hyperplasia/rege neration Epidermal	0%	25% (1.0)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

6.2 Repeat-Dose Toxicity

Study title: HTX-011: 28-Day Intermittent Subcutaneous Toxicity and Toxicokinetic Study in Sprague-Dawley Rats (with a 2-week Recovery)

Study no.: 33-85
Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\33-85\33-85.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/17/2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: HTX-011-056, Lot 026517, 99.9% bupivacaine and 97.3% meloxicam

Key Study Findings

- All animals survived until necropsy.
- No treatment-related effects were observed on body weight, food consumption, ophthalmic parameters, clinical chemistry parameters, organ weights, or urinalysis parameters.
- An increased incidence of scabbing was seen at the injection site of some animals receiving HTX-011 or the vehicle at some injection sites. The incidence of scabbing was reduced following recovery.
- In males, increased platelets, neutrophils, eosinophils, and reticulocytes were observed in all treated groups (including the vehicle) compared to saline. Increases in hematology parameters were no longer present following recovery.
- Histopathological findings that appeared to be related to systemic exposure were limited to minimal mononuclear cell infiltrates in the liver of high dose males and minimal accumulation of erythrocytes in the thymus of low and high dose males.
- Injection site histopathological findings included chronic inflammation, hemorrhage, and presence of test material following treatment with the vehicle and HTX-011. Treatment with HTX-011 resulted in an increase in the severity or incidence of some findings. Chronic inflammation was still present in recovery animals, although the overall incidence was reduced in animals that had a longer recovery time.

- Bupivacaine and meloxicam toxicokinetics were measured on Days 1, 5, and 27. For both compounds, AUC increased linearly with dose. AUCs also appeared to increase with time suggesting some accumulation. Bupivacaine T_{max} values were generally short (0.1-1 hours), while meloxicam T_{max} values were longer (0.5-48 hours) but with more variance. C_{max} values for bupivacaine were highly variable and no trends could be deciphered. In contrast, meloxicam C_{max} values followed a linear trend with dose and increased with time.
- Polymer breakdown products were evaluated in urine on Day 20. (b) (4)
[REDACTED]
were all detected at levels above the calibration range.
- In terms of systemic effects, findings were limited to males and included effects on hematology parameters in all treated animals (vehicle and both doses of HTX-011) and potentially associated histopathological findings of infiltration of mononuclear cells into the liver (high dose of HTX-011) and accumulation of erythrocytes in the thymus (low and high dose of HTX-011). Changes in hematology parameters were small and did not follow a dose response. The observed histopathological findings in the liver and thymus were of minimal severity and had a small incidence (30%). They were not considered toxicologically relevant.
- A local inflammatory reaction was observed following treatment with the vehicle and HTX-011. The effects are consistent with a biological response to injection of foreign material. The majority of the effects resolved as indicated by the lack of findings in recovery animals. However, chronic inflammation was still present in recovery animals up to 26 days following the last injection. Effects were at times worse (increased incidence and/or severity) with HTX-011 compared to the vehicle suggesting that bupivacaine or meloxicam may have exacerbated the effects of the vehicle. There was no dose response, except for the presence of test material, which is expected since the dose was controlled by injecting different volumes of the clinical formulation (same concentration of drug substance and excipients). The Applicant claims that the effects are non-adverse and therefore the NOAEL is the highest dose tested. While the effects observed are not unexpected, it is difficult to ascertain the adversity and clinical relevance of the observed persisting chronic inflammation. Therefore, in terms of local effects, this study demonstrates that the clinical formulation causes chronic inflammation up to 26 days that is likely primarily the result of the vehicle.

Methods

Doses:	Saline, vehicle control, 12.5/0.38 mg/kg bupivacaine/meloxicam, and 25/0.75 bupivacaine/meloxicam (see table below)
Frequency of dosing:	Once every 48 hours (see injection site rotation below)
Route of administration:	Subcutaneous injection
Dose volume:	0.85 mL/kg (saline, vehicle, and 25/0.75 mg/kg) or 0.43 mL/kg (12.5/0.38 mg/kg)
Formulation/Vehicle:	HTX-011-056 (HTX-011) : 25% triacetin, 10% DMSO, 62.375% AP135 polymer, 0.05% maleic acid, 2.5% bupivacaine, and 0.075% meloxicam HTX-024-002 (vehicle) : 25% triacetin, 10% DMSO, and 65% AP135 polymer
Species/Strain:	Sprague-Dawley rat
Number/Sex/Group:	10 for the main experiment, 5 for recovery, and 3 (saline and vehicle) or 27 (low and high dose) for toxicokinetics
Age:	8-9 weeks old
Weight:	193-227 g (male) and 148-193 g (female)
Satellite groups:	Toxicokinetic and recovery groups
Unique study design:	None
Deviation from study protocol:	None that impacted study results

Experimental Design

Table 113 Experimental Design of Study 33-85

Group ^a	Description	Number of Rats/Sex/Group			Dose Level		Dose Volume (mL/kg)	Dose Concentration ^d	
		Core	Recovery	TK	bupivacaine (mg/kg/day)	meloxicam (mg/kg/day)		bupivacaine (mg/mL)	meloxicam (mg/mL)
1 ^b	Saline Control	10	5	3	0	0	0.85	0	0
2 ^c	Vehicle Control	10	5	3	0	0	0.85	0	0
3	Low Dose	10	5	27	12.5	0.38	0.43	29.25	0.88
4	High Dose	10	5	27	25	0.75	0.85	29.25	0.88

^aAnimals were dosed once every 48 hours ($\pm$ 1 hour) for a total of 14 doses. Animals were dosed on Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27.

^bGroup 1 was administered saline control (sterile saline) only.

^cGroup 2 was administered vehicle control (HTX-024-002) only.

^dConcentrations of bupivacaine and meloxicam were 2.5% and 0.075% w/w (or 25 mg/g and 0.75 mg/g), respectively. When corrected for density (1.17 g/mL) the final concentrations of bupivacaine and meloxicam were 29.25 mg/mL and 0.88 mg/mL, respectively.

Table from page 15 of the Applicant's study report

Injection Site Rotations

Dose Site	Location	Injection Day	Dose Free Period to Terminal Necropsy
A	Left Cranial Back	1, 13, and 25	4
B	Right Cranial Back	3, 15, and 27	2
C	Left Mid Back	5 and 17	12
D	Right Mid Back	7 and 19	10
E	Left Caudal Back	9 and 21	8
F	Right Caudal Back	11 and 23	6

Observations and Results

Mortality

All animals survived until scheduled necropsies.

Clinical Signs

Dosing sites were scored using a modified Draize scoring system prior to dosing and 2-4 hours post dose. Detailed clinical examinations were performed weekly during the study and cage-side observations were performed at the time of dose site observations.

No evidence of edema or erythema was observed during the study. The most remarkable finding was scabbing at the injection sites. Findings were seen with both the vehicle and HTX-011 in males and females, with an increasing incidence of findings in the low- and high-dose groups in some cases. Scabs were most prevalent at the injection sites that were injected three times (A and B). However, Site C also had a large amount of scabbing and it was only injected a total of two times. Furthermore, Site A and B both had scabbing occur prior to the third injection and in some cases prior to the second injection. The incidence of scabbing was greatly reduced in recovery animals with only a few sporadic findings.

Table 114: Incidence of Clinical Observations in Males

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Scab, A, small, 1-10 mm	1	2	5	8
Scab, B, small, 1-10 mm	-	6	6	6
Scab, C, small, 1-10 mm	-	-	4	6
Sore, C, small 1-10 mm	-	-	-	3

N = 15

Table 115: Incidence of Clinical Observations in Females

	Female			
	Saline	Vehicle	12.5/0.38	25/0.75
Scab, A, small, 1-10 mm, multiple	-	1	-	3
Scab, A, small, 1-10 mm	-	9	7	9
Scab, B, small, 1-10 mm	-	5	5	10
Scab, B, medium, >10-20 mm	-	1	1	3
Scab, C, small, 1-10 mm	-	1	5	5
Scab, D, small, 1-10 mm, multiple	-	1	4	5
Scab, D, small, 1-10 mm	-	1	5	8

N = 15

Body Weights

Body weights were recorded prior to randomization, prior to treatment, and weekly for the remainder of the study.

There were no differences in body weights between groups.

Feed Consumption

Food consumption was measured in conjunction with body weight.

Rats of all groups consumed a similar amount of food.

Ophthalmoscopy

Indirect funduscopic examinations were performed pretest and on the main experiment animals one week prior to the end of treatment.

There were no treatment-related ophthalmic changes observed.

Hematology

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of hematology and coagulation parameters was evaluated.

Changes in hematology parameters compared to the saline control were limited to male animals and included increased absolute platelets (all treated groups), increased neutrophils as a percentage of red blood cells (high dose), increased absolute neutrophils (all treated groups), increased absolute eosinophils (all treated groups), increased absolute and as a percentage of red blood cells reticulocytes (all treated groups), and increased fibrinogen (all treated groups). Following recovery, all hematology parameters were similar between the saline control and treated groups. Overall, the changes were relatively small and the fact that the changes were seen in all treated groups indicated that the effects are likely secondary to the local inflammatory reaction.

Table 116: Notable Changes in Hematology Parameters in Male Rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Platelets (x 10 ³ cells/mcL)	1170 ± 107	1312 ± 135* (12)	1285 ± 112 (10)	1331 ± 131* (14)
Neutrophils (%)	13.4 ± 4.4	14.0 ± 5.8 (4)	14.2 ± 4.9 (6)	16.5 ± 4.4 (23)
Neutrophils (x 10 ³ cells/mcL)	1.11 ± 0.34	1.33 ± 0.56 (20)	1.52 ± 0.60 (37)	1.45 ± 0.47 (31)
Eosinophils (x 10 ³ cells/mcL)	0.10 ± 0.02	0.12 ± 0.04 (20)	0.14 ± 0.04* (40)	0.11 ± 0.04 (10)
Reticulocytes (x 10 ⁹ cells/L)	196.5 ± 28.3	228.8 ± 29.0 (16)	223.5 ± 33.5 (14)	257.8 ± 47.0* (31)
Reticulocytes (%)	2.37 ± 0.41	2.72 ± 0.43 (15)	2.81 ± 0.51 (19)	3.18 ± 0.55* (34)
Fibrinogen (mg/dL)	327 ± 31	390 ± 28* (19)	391 ± 31* (20)	394 ± 19* (20)

* Significantly different from saline control, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10

Clinical Chemistry

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of clinical chemistry parameters was evaluated.

A statistically significant decrease in albumin was reported in males receiving the vehicle and high dose of HTX-011. The magnitude of change was small and there was no difference following the recovery period, therefore the change is not likely toxicologically relevant.

A large increase in aspartate aminotransferase (AST) was noted in one male receiving the low dose of HTX-011, which caused the average AST level in this group to be elevated. Since the increase in AST was only seen in one animal across the entire study, this was considered an incidental finding not related to treatment.

Table 117: Notable Changes in Clinical Chemistry Parameters in Rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Albumin (g/dL)	2.8 ± 0.1	2.6 ± 0.1* (7)	2.7 ± 0.1 (4)	2.6 ± 0.1* (7)
Aspartate aminotransferase (%)	119 ± 20	133 ± 25 (12)	168 ± 110 (41)	130 ± 13 (9)

* Significantly different from saline control, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10

Urinalysis

Urine was collected from fasted main study and recovery rats the day prior to terminal sacrifice. A complete battery of urinalysis parameters was evaluated.

There were no treatment-related changes in urinalysis parameters.

Gross Pathology

Following sacrifice, all main study and recovery animals received a complete necropsy.

Gross observations were primarily at the injection site and consisted of scabs and accumulations in animals receiving HTX-011 or the vehicle. In males, findings were primarily at Injection Site A and B, which were the injection sites with the least time between the last injection and sacrifice (4 and 2 days, respectively). In females, findings were also seen at Injection Site C, which had the longest time between the last injection and sacrifice (12 days). There was no clear dose-response to the findings, although the findings were overall more prevalent in animals who received HTX-011 than the vehicle. The lack of a dose response between the two HTX-011 doses is not unexpected since the gross observations represent a local effect and dose was controlled by injecting different volumes of the same concentration of HTX-011. Scabbing and accumulation were not observed in recovery animals.

Table 118: Gross Observations in Male Rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Accumulation (A)	-	-	-	1
Accumulation (B)	-	1	3	1
Scab (A)	-	-	2	-
Scab (B)	-	1	2	-

Table 119: Gross Observations in Female Rats

	Female			
	Saline	Vehicle	12.5/0.38	25/0.75
Accumulation (A)	-	-	-	2
Accumulation (B)	-	-	3	2
Accumulation (C)	-	1	2	-
Scab (A)	-	2	-	1
Scab (B)	-	-	1	3
Scab (C)	-	-	3	-

N = 10

Organ Weights

The following tissues were weighed:

Adrenal Glands	Liver	Testes
Brain	Ovaries	Thymus
Epididymides	Pituitary gland	Thyroid
Heart	Prostate gland	Uterus
Kidneys	Spleen	

There were no treatment-related changes in organ weights.

Histopathology**Adequate Battery: Yes****Peer Review: Yes**

Tissue Collection Table

Adrenals*	Pancreas
Aorta	Pituitary*
Brain* (entire)	Prostate* (with seminal vesicles/ coagulating gland/empty bladder)*
Epididymides*	Salivary glands (mandibular)
Esophagus	Sciatic nerve
Eyes with optic nerve	Skeletal muscle
Femur with marrow	Skin (with mammary gland for females)
Harderian gland	Spinal cord (cervical, thoracic, lumbar)
Heart*	Spleen*
Intestines (gut-associated lymphoid tissue, examined only if present in plane of section)	Sternum (bone with marrow)
Cecum	Stomach
Colon	Testes*
Duodenum	Thymus*
Ileum	Thyroid* (parathyroids, examined if present in plane of section)
Jejunum	Tongue
Rectum	Trachea
Kidneys*	Uterus* (with cervix/empty bladder)
Larynx	Vagina
Liver*	Gross lesions/masses
Lungs with bronchi	Identification (ear tag)
Lymph nodes (mandibular and mesenteric)	Injection sites A, B, C, D, E, F (full thickness at each site; including underlying muscle)
Nasal Cavity	
Ovaries* (with oviducts)	

Histological Findings

Histopathological findings related to systemic exposure were only seen in male animals and included minimal mononuclear cell infiltrates in the liver at the high dose and minimal accumulation of erythrocytes in the thymus at the low and high doses. These effects were not seen in recovery animals. These findings were not considered adverse due to the minimal severity and low incidence.

The remaining histopathological findings were seen at the injection sites with the vehicle and HTX-011. While not consistent, treatment with HTX-011 did result in a greater incidence and or severity of some findings compared to the vehicle control. Observations included chronic inflammation, granulomatous inflammation, hemorrhage, and the presence of test material. Granulomatous inflammation was seen at all injection sites with an average severity between minimal and mild but was no longer seen in the

recovery groups indicating that it resolved by 14 days post treatment. Findings of hemorrhage were more prevalent at the injection sites that were more recently injected although there were some exceptions. Hemorrhage was not seen in the recovery animals suggesting it resolves after 16 days.

Chronic inflammation was described in the report as either “active” or having no additional description. Active chronic inflammation appeared to be most prevalent at Injection Site B, where there was only 2 days following injection before necropsy. In general, the incidence of active chronic inflammation decreased with time after injection, although there were some exceptions. Active chronic inflammation was not observed in recovery animals. In contrast, chronic inflammation was most prevalent at Injection Site C, where there was 12 days following injection before necropsy. Based on the incidence data for both types of chronic inflammation, it appears that it is active following injection and eventually transitions over to chronic inflammation, although it is difficult to determine the precise time course based on the available data. What is clear is that for up to 12 days post injection there is some form of chronic inflammation at the injection site. Chronic inflammation, but not active chronic inflammation, was observed in recovery animals. While still present, the incidence was reduced in the animals which had the longest recovery time (26 days).

There did not appear to be a clear difference between the low and high dose of HTX-011 in terms of incidence or severity for histopathological findings, except for the presence of test material. This is not unexpected since the histopathological observations represent a local effect and the dose was controlled by injecting a different volume of the same concentration of HTX-011. However, the presence of test material would be expected to be related to the volume injected as a higher volume would result in a larger absolute amount of test material.

Table 120: Systemic Histopathological Observations

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Liver- mononuclear cell infiltrate	1 (1)	1 (1)	1 (1)	3 (1)
Thymus- accumulation of erythrocyte	0	0 (1)	2 (1)	3 (1)

N = 10

Table 121: Chronic Inflammation (Incidence)

Inj Site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	2 (2)	1 (1)	2 (1.5)	2 (1)	1 (1)	2 (1)	1 (1)	3 (1.3)
A [4]	2 (1)	1 (2)	3 (2)	4 (1.3)	1 (1)	4 (1)	6 (1.7)	6 (1.5)
F [6]	0	2 (1)	2 (2)	6 (2)	0	4 (1)	4 (1)	2 (2.5)
E [8]	0	4 (1.5)	4 (2)	2 (2.5)	1 (1)	4 (1.3)	4 (1.5)	2 (2.5)
D [10]	0	2 (1.5)	6 (2)	4 (1.5)	0	4 (1.8)	6 (1.7)	4 (2.3)
C [12]	0	8 (1.1)	7 (1.7)	7 (1.4)	0	4 (1)	4 (1.3)	9 (1.4)

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 122: Chronic Inflammation, Active (Incidence)

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	3 (1)	7 (1.7)	8 (2.1)	8 (2)	1 (1)	5 (1.6)	9 (1.7)	7 (1.9)
A [4]	0	5 (2.2)	5 (2.4)	3 (1.7)	0	3 (2)	2 (1.5)	4 (1.5)
F [6]	0	3 (2.7)	5 (2.6)	3 (2.3)	0	0	1 (3)	3 (3)
E [8]	0	2 (1.5)	4 (2.5)	6 (2)	0	2 (1.5)	1 (1)	5 (2.2)
D [10]	0	2 (2)	1 (2)	5 (2.4)	0	0	3 (2)	2 (1)
C [12]	0	0	1 (3)	2 (1.5)	0	1 (2)	0	1 (1)

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 123: Granulomatous Inflammation (Incidence)

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	2 (1)	1 (2)	0	0	0	0	1 (1)
A [4]	0	3 (1)	2 (1.5)	1 (1)	0	2 (1)	0	1 (1)
F [6]	0	2 (1.5)	2 (1.5)	2 (1)	0	2 (1)	1 (1)	3 (1.7)
E [8]	1 (2)	1 (1)	4 (1)	5 (1)	0	1 (1)	1 (1)	5 (1.4)
D [10]	0	3 (1)	2 (1.5)	4 (1)	0	1 (1)	2 (1)	3 (1.7)
C [12]	0	0	4 (1)	4 (1)	0	2 (1.5)	0	3 (1)

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 124: Hemorrhage (Incidence)

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	1 (2)	8 (2.9)	6 (1.8)	0	2 (1)	3 (2)	4 (1.8)
A [4]	0	2 (1.5)	2 (3)	2 (1)	0	2 (1.5)	5 (1.2)	2 (1)
F [6]	0	1 (4)	1 (2)	5 (1.8)	0	0	1 (3)	1 (2)
E [8]	1 (2)	0	2 (2)	4 (1)	0	0	0	2 (1)
D [10]	0	1 (3)	1 (1)	1 (1)	0	1 (1)	1 (1)	1 (2)
C [12]	1 (1)	1 (3)	0	2 (3)	0	1 (2)	0	0

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 125: Presence of Test Material (Incidence)

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	0	4 (2.8)	5 (3)	0	2 (1)	3 (1.7)	3 (1.3)
A [4]	0	1 (1)	1 (3)	3 (2.7)	0	2 (2)	2 (1.5)	1 (1)
F [6]	0	1 (1)	0	2 (2.5)	0	0	0	3 (3)
E [8]	0	0	2 (2)	3 (2.7)	0	0	1 (1)	2 (3.5)
D [10]	0	0	0	3 (2)	0	0	2 (1)	0
C [12]	0	0	0	1 (3)	0	1 (2)	0	0

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 126: Chronic Inflammation, Recovery Animals

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [16]	0	1 (1)	3 (1.3)	2 (1.5)	0	1 (1)	1 (1)	2 (1.5)
A [18]	0	0	2 (1)	5 (1.4)	0	2 (1)	2 (2)	0
F [20]	0	0	0	0	0	1 (1)	1 (1)	1 (1)
E [22]	0	4 (1)	4 (1.5)	4 (1.3)	0	3 (1.3)	0	3 (1.7)
D [24]	0	2 (1.5)	2 (1)	1 (1)	0	2 (1)	3 (1)	1 (1)
C [26]	0	2 (2)	1 (1)	1 (2)	0	2 (1)	1 (1)	0

N = 5/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Toxicokinetics

Toxicokinetic animals (3 rats/sex/timepoint) were bled via the sublingual vein on Days 1, 5, and 27. Urine was collected from vehicle and high-dose toxicokinetic animals in Week 3 for analysis of polymer breakdown products.

Bupivacaine

Bupivacaine AUC followed a nearly linear dose response on Day 1 and Day 5, but not on Day 27. It appeared that there was some accumulation of bupivacaine over time as AUC increased with time in males, but not females, with both doses. By Day 27, the AUC was 1.8-2.3 times higher in males compared to AUC on Day 1. In addition, there were still small, but detectable, amounts of bupivacaine in plasma at 48 hours after dosing in both males and females. Bupivacaine appeared to release quickly with T_{max} s ranging from 0.5 – 1 hour. Bupivacaine C_{max} values were highly variable and it is difficult to interpret any patterns. In general, C_{max} , T_{max} , and AUC were comparable between males and females.

Table 127: Bupivacaine Toxicokinetic Parameters

Dose (mg/kg/dose)	Day	Sex		C_{max} (ng/mL)	t_{max} (h)	AUC _{0-48h} (ng·h/mL)	R ^a	$C_{max}/Dose$	AUC _{0-48h}/Dose}
12.5	1	Male	Mean	348	0.5	2570	NA	27.8	206
			SE	235		278			
		Female	Mean	303	0.5	3220	NA	24.3	258
			SE	45.2		420			
	5	Male	Mean	1250	0.5	3640	1.42	100	291
			SE	852		498			
Female		Mean	326	1	3000	0.930	26.1	240	
		SE	94.4		302				
27	Male	Mean	470	0.5	5860	2.28	37.6	469	
		SE	149		938				
	Female	Mean	487	1	4650	1.44	38.9	372	
		SE	188		605				
25	1	Male	Mean	254	6	4580	NA	10.1	183
			SE	86.5		478			
		Female	Mean	718	0.5	6600	NA	28.7	264
			SE	184		574			
	5	Male	Mean	328	0.5	6360	1.39	13.1	254
			SE	104		487			
		Female	Mean	558	1	7290	1.10	22.3	292
			SE	80.1		1250			
	27	Male	Mean	948	1	8120	1.77	37.9	325
			SE	367		753			
		Female	Mean	566	1	6490	0.980	22.6	260
			SE	211		465			

NA: Not Applicable; SE: standard error (C_{max} and AUC_{0-48h} only)

$C_{max}/Dose$ units: [(ng/mL)/(mg/kg/dose)]

AUC_{0-48h}/Dose units: [(ng·h/mL)/(mg/kg/dose)]}

^a R (Accumulation Ratio) = Day 5 or Day 27 AUC_{0-48h}/Day 1 AUC_{0-48h}}}

Taken from page 552 of Study report 33-85

Meloxicam

Meloxicam AUC followed a linear dose response on all days for both sexes. Meloxicam AUC increased with time; by Day 27 AUC was 2.7-3.9 times higher than on Day 1. There were still high levels of meloxicam in plasma 48 hours after dosing, which is reflected by the high T_{max} values ranging from 0.5-48 hours. C_{max} values also followed a

linear dose response and increased with time. For both C_{max} and AUC, the values were higher in females compared to males. The T_{max} values were highly variable in both sexes but generally seemed comparable.

Table 128: Meloxicam Toxicokinetic Parameters

Dose (mg/kg/dose)	Day	Sex		C_{max} (ng/mL)	t_{max} (h)	AUC _{0-48h} (ng•h/mL)	R ^a	$C_{max}/Dose$	AUC _{0-48h}/Dose}
0.38	1	Male	Mean	597	48	25600	NA	1570	67400
			SE	75.8		989			
		Female	Mean	1200	48	42300	NA	3170	111000
			SE	119		4540			
	5	Male	Mean	1340	6	50100	1.96	3510	132000
			SE	473		3240			
		Female	Mean	3080	12	112000	2.65	8110	295000
			SE	457		11200			
	27	Male	Mean	1810	12	70200	2.74	4770	185000
			SE	219		4040			
		Female	Mean	4190	12	163000	3.85	11000	429000
			SE	280		22400			
0.75	1	Male	Mean	1280	24	49000	NA	1710	65300
			SE	159		2520			
		Female	Mean	2740	48	79500	NA	3660	106000
			SE	198		8840			
	5	Male	Mean	2770	6	109000	2.22	3700	145000
			SE	318		6370			
		Female	Mean	5200	36	209000	2.63	6930	279000
			SE	1520		28800			
	27	Male	Mean	3680	6	142000	2.90	4900	189000
			SE	89.9		8190			
		Female	Mean	7710	0.5	289000	3.64	10300	385000
			SE	647		39900			

NA: not applicable; SE: standard error (C_{max} and AUC_{0-48h} only)

$C_{max}/Dose$ units: [(ng/mL)/(mg/kg/dose)]

AUC_{0-48h}/Dose} units: [(ng•h/mL)/(mg/kg/dose)]

^a R (Accumulation Ratio) = Day 5 or Day 27 AUC_{0-48h}/Day 1AUC_{0-48h}}

Taken from page 556 of Study report 33-85

Polymer breakdown products

Urine collected on Day 20 was analyzed for the presence of breakdown products of the polymer (AP135) using a non-validated method for exploratory purposes. The breakdown products measured were (b) (4)

For all compounds, the levels were over the calibration range and extrapolated values were reported when available. For (b) (4) the values were above the calibration range (b) (4) mcg/mL) but estimated values were not reported. Excluding (b) (4) the most prevalent breakdown product was (b) (4) followed by (b) (4) and then (b) (4). Levels of (b) (4) were similar between males and females while levels of (b) (4) and (b) (4) were higher in males.

Table 129: Levels of Polymer Breakdown Products in the Urine

Dose Group	Animal ID	Sex
Group 2 Vehicle Control	507	Male
	508	Male
	509	Male
	510	Female
	511	Female
	512	Female
Group 4 High Dose	567	Male
	568	Male
	569	Male
	594	Female
	595	Female
	596	Female

(b) (4)

^a The assays used to measure the concentrations were not validated, and all samples were above the calibration range. The extrapolated values are shown for informational purposes only, when available.

Taken from page 28 of Study 33-85

Dosing Solution Analysis

The vehicle was analyzed at the end of the study (March 6, 2018) to compare to the results from the Certificate of Analysis provided by the Applicant dated March 19th, 2018. There were no differences between the CoA and the analysis. Meloxicam and bupivacaine were not detected in the vehicle.

HTX-011 was not analyzed. The Applicant provided characterization of the test substance including stability data. The CoA for Lot 026517 indicated that the test substance had 99.9% bupivacaine and 97.3% meloxicam with a retest date of November 2018 (24 months after manufacture), that is later than the end of Study 33-85.

Study title: HTX-002: 14-Day Intermittent Subcutaneous Toxicity and Toxicokinetic Study in Sprague-Dawley Rats (with a 2-week Recovery)

Study no.: 34-84
Study report location: SDN 48, Module 4
<\\cdsesub1\levsprod\nda211988\0048\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\34-84\34-84.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: 9/17/2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: **HTX-002-14**, Lot 082719, 100.9%
bupivacaine
HTX-002-19, Lot 090519, 99.8%
bupivacaine
HTX-024-03, Lot 083019, 0% bupivacaine

Key Study Findings

- No treatment related mortalities were observed.
- No toxicologically significant effect was seen on body weight, ophthalmoscopy, clinical chemistry parameters, urinalysis parameters, or organ weights.
- An increased incidence of clinical signs compared to the vehicle were observed at the injection site. These included raised areas, scabbing, and sores (females only). These effects were consistent with other repeat dose studies with HTX formulations.
- Hematology parameters were altered compared to the control group in animals receiving bupivacaine formulations. These effects were consistent with other repeat dose studies with HTX formulations.
- Gross observations were limited to the injection site in animals receiving the bupivacaine formulations and included accumulations, crust (females only), and scabbing (females only). These effects were consistent with other repeat-dose studies with HTX formulations.
- Histopathological findings were limited to the injection site. Increased incidences of mononuclear cell infiltration, fibrosis, edema, erythrocyte accumulation, ulcer (females at Injection Site A), and acute inflammatory exudate (females at Injection Site A) were seen in animals who received the bupivacaine

formulations. These effects were consistent with other repeat-dose studies with HTX formulations.

- Bupivacaine AUC was nearly dose proportional on Days 1 and 13 with an expected fold difference of 1.5 (100 vs. 150 mg/kg) but with an actual dose ratio of 1.6-1.8 fold. It appeared that there was some accumulation of bupivacaine over time as AUC increased with time in both sexes and doses. Bupivacaine AUC and C_{max} was similar between males and females.
- The goal of the study was to assess the systemic safety of high-dose bupivacaine delivered via depot formulations. In that regard, no systemic toxicity was seen up to a dose of 150 mg/kg (HED of 1451.6 mg bupivacaine for a 60 kg individual). The corresponding AUC_{inf} at this dose was 52,900 ng*h/mL using the combined data from males and females. This value is greater than the highest human AUC_{inf} for bupivacaine in the Applicant's clinical trials (Study 209, total knee arthroplasty, 31,173 h*ng/mL) by a factor of ~1.7.
- A local inflammatory reaction was seen in the animals who received the bupivacaine in vehicle formulation which was more pronounced compared to the vehicle alone which showed a very mild response. This is interesting because a similar vehicle was used in the pivotal 28-day studies in rat and dog (Study 33-85 and 33-86) and caused a rather strong local immune response similar to the HTX formulation containing bupivacaine. Since the vehicle in this study has a different excipient profile compared to the bupivacaine formulations it is unclear whether the increased local response was due to the presence of bupivacaine or other excipients.

Methods

Doses: Vehicle control (HTX-024-03, 0 mg/kg bupivacaine), HTX-002-14 (100 mg/kg bupivacaine), and HTX-002-19 (150 mg/kg bupivacaine). See table below.

Frequency of dosing: Once every 48 hours

Route of administration: Subcutaneous injection

Dose volume: 1 mL/kg (vehicle), 3.4 mL/kg (HTX-002-14), and 2.6 mL/kg (HTX-002-19)

Formulation/Vehicle: **HTX-002-14**: 29.25 mg/mL bupivacaine, 93.6 mg/mL DMSO, 635.9 mg/mL AP135, 1.755 mg/mL maleic acid, and 409.5 mg/mL triacetin)
HTX-024-003: 93.6 mg/mL DMSO, 666.9 mg/mL AP135, 409.5 mg/mL triacetin).
The chemical composition of **HTX-002-19** is unknown. The toxicology written summary states that HTX-002-19 contains bupivacaine in only the TEG-POE polymer.

Species/Strain: Sprague-Dawley rat

Number/Sex/Group: 10 for the main experiment, 5 for recovery, and 3 (vehicle) or 9 (bupivacaine) for toxicokinetics

Age: 8-9 weeks old

Weight: 218-355 g (male) and 164-261 g (female)

Satellite groups: Toxicokinetic and recovery groups

Unique study design: None

Deviation from study protocol: None that impacted study results

Experimental Design

Table 130 Experimental Design of Study 34-84

Group ^a	Description	Number of Rats/Sex/Group			Dose Level	Dose	Dose Concentration ^c
		Core	Recovery	TK	bupivacaine	Volume	bupivacaine
					(mg/kg/day)	(mL/kg)	(mg/mL)
1 ^b	HTX-024-03 Vehicle Control	10	5	3	0	1	0
2	HTX-002-14	10	5	9	100	3.4	29.25
3	HTX-002-19	10	5	9	150	2.6	58.5

^aAnimals were dosed once every 48 hours ($\pm$ 2 hours), except on TK collection days when it was 48 hours ($\pm$ 1 hour), for a total of 7 doses. Animals will be dosed on Days 1, 3, 5, 7, 9, 11 and 13.

^bGroup 1 was administered vehicle control (HTX-024-03) only.

^cConcentrations of bupivacaine were 2.5% w/w for HTX-002-14 and 5.0% w/w for HTX-002-19. When corrected for density (1.17 g/mL) the final concentration of bupivacaine were 29.25 and 58.5 mg/mL, respectively.

From page 14 of the Applicant's Study Report 34-84

Injection Site Rotations (from page 15 of the Applicant's Study Report 34-84)

Dose Site	Locations	Days
A	Left cranial back	1 and 13
B	Right cranial back	3
C	Left mid back	5
D	Right mid back	7
E	Left caudal back	9
F	Right caudal back	11

Observations and Results

Mortality

One high-dose male was found dead following TK blood collection on Day 1. No test-article related lesions were observed during gross necropsy.

One high-dose female had declining health status and was sacrificed on Day 15. Clinical signs included body weight loss, discolored paws and mouth, discolored inguinal fur, and red material around the eyes/nose. Necropsy revealed red, mottled, and fluid filled lungs. The cause of death was labeled as acute pneumonia.

Clinical Signs

Dosing sites were scored using a modified Draize scoring system prior to dosing and at least once following dosing. Detailed clinical examinations were performed weekly

during the study and cage-side observations were performed at the time of dose site observations.

An increased incidence of clinical signs at the injection site compared to the vehicle control was observed. In both sexes, a raised area and scabbing was seen at the injection site. Sores at the injection site were only seen in female animals. Clinical signs at the injection sites were still present following the recovery period.

Table 131: Incidence of Clinical Observations in Males

Males			
	Vehicle	100 mg/kg	150 mg/kg
Raised area, left mid back	0	5	9
Raised area, right mid back	0	5	8
Raised area, left caudal back	0	3	11
Scab, left cranial back	0	7	6
Scab, right cranial back	2	7	4

N = 15

Table 132: Incidence of Clinical Observations in Females

Females			
	Vehicle	100 mg/kg	150 mg/kg
Raised area, left mid back	0	1	3
Raised area, left caudal back	0	2	4
Scab, left cranial back	4	11	14
Scab, right cranial back	5	9	14
Scab, left mid back	1	3	11
Scab, right mid back	1	6	7
Scab, left caudal back	0	3	3
Scab, right caudal back	0	1	3
Sore, left cranial back	1	5	2
Sore, right cranial back	1	3	2

N = 15

Body Weights

Body weights were recorded prior to randomization, prior to treatment, and weekly for the remainder of the study.

There were no differences in body weights between groups based on the mean body weight measured during the treatment period. However, there was a statistically significant 7% decrease in fasted body weight compared to the vehicle control in males of the 150 mg/kg group on the final day of sacrifice. Male body weights in the 150 mg/kg group were higher (11%) than the vehicle control following recovery. The decrease in body weight is not considered toxicologically relevant by the reviewer since it was only seen at the end of the study, was less than 10%, and recovered.

Food Consumption

Food consumption was measured in conjunction with body weight.

Rats of all groups consumed a similar amount of food except males receiving 150 mg/kg during the recovery period. This increase in food consumption corresponds to the observed increase in body weight during the recovery period.

Ophthalmoscopy

Indirect fundoscopic examinations were performed pretest and near the end of the treatment period.

There were no treatment-related ophthalmic changes observed.

Hematology

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of hematology and coagulation parameters was evaluated.

Hematology parameters were altered in both males and females receiving bupivacaine compared to the vehicle controls. Some changes were small in magnitude but statistically significant. There did not appear to be a dose response for many parameters suggesting these effects could be secondary to the local inflammation reaction at the injection site, although it could also be related to the narrow dose spacing in the study.

The following changes were observed at one or both doses: increased white blood cells (M/F, no recovery), decreased red blood cells (M/F, recovery except males at 100 mg/kg), decreased hemoglobin (M/F, males did not recover), decreased hematocrit (M/F, males did not recover), increased platelets (M/F, recovered), increased neutrophils (absolute and %, M/F, recovery except females at 100 mg/kg), decreased

eosinophils (absolute and %, M only, recovered), increased reticulocytes (M only, recovered), increased fibrinogen (M/F, recovered), and decreased MCHC (F only, recovered).

Table 133: Notable Changes in Hematology Parameters in Male Rats

	Males			
	Vehicle	100 mg/kg	150 mg/kg	Recovery
WBC (x10 ³ cells/mcL)	7.86	10.40* (32%)	9.62 (22%)	Still elevated:
RBC (x10 ⁶ cells/mcL)	7.65	7.25* (-5%)	7.53 (-2%)	Still decreased:
HGB (g/dL)	14.6	14.1* (-3%)	14.2 (-3%)	Still decreased:
HCT (%)	43.7	42.6 (-3%)	42.5 (-3%)	Still decreased:
MCHC (g/dL)	33.4	33.1 (-1%)	33.4 (0%)	Similar to control
PLAT (x10 ³ cells/mcL)	1364	1493 (9%)	1564* (15%)	Similar to control
NEUT (%)	20.0	22.2 (11%)	25.9* (30%)	Decreased
NEUT (x10 ³ cells/mcL)	1.58	2.27* (44%)	2.47* (56%)	Similar to control
EOS (%)	1.3	0.8* (-38%)	0.7* (-46%)	Similar to control
EOS (x10 ³ cells/mcL)	0.11	0.08 (-28%)	0.07 (-36%)	Similar to control
Retic (x10 ⁹ cells/L)	290.1	381.2* (31%)	323.0 (11%)	Similar to control
Retic (%)	3.80	5.27* (39%)	4.32 (14%)	Similar to control
FIB (mg/dL)	390	455* (17%)	461* (18%)	Similar to control

* Significantly different from vehicle control, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10

Table 134: Notable Changes in Hematology Parameters in Female Rats

	Females			
	Vehicle	100 mg/kg	150 mg/kg	Recovery
WBC (x10 ³ cells/mcL)	5.18	6.62 (28%)	7.14 (38%)	Still elevated
RBC (x10 ⁶ cells/mcL)	7.97	7.40* (-7%)	7.44* (-7%)	Similar to control
HGB (g/dL)	15.0	13.9* (-7%)	13.7* (-9%)	Similar to control
HCT (%)	43.4	41.1* (-5%)	40.3* (-7%)	Similar to control
MCHC (g/dL)	34.6	33.7* (-3%)	33.9* (-2%)	Similar to control
PLAT (x10 ³ cells/mcL)	1349	1495 (11%)	1471 (9%)	Similar to control
NEUT (%)	15.4	19.9 (29%)	21.9 (42%)	Similar to control
NEUT (x10 ³ cells/mcL)	1.20	1.72 (43%)	1.44 (20%)	100 mg/kg: still elevated 150 mg/kg: similar to control
EOS (%)	1.1	1.1 (0%)	1.1 (0%)	Similar to control
EOS (x10 ³ cells/mcL)	0.07	0.08 (14%)	0.08 (14%)	Similar to control
Retic (x10 ⁹ cells/L)	202.3	190.0 (-6%)	219.7 (9%)	100 mg/kg: elevated but large SD 150 mg/kg: similar to control
Retic (%)	2.51	2.41 (-4%)	2.87 (14%)	100 mg/kg: elevated but large SD 150 mg/kg: similar to control
FIB (mg/dL)	255	260 (2%)	279 (9%)	Similar to control

* Significantly different from vehicle, $p \leq 0.05$

Values in () represent % change compared to saline.
N = 10, except for the 150 mg/kg females where N = 9

Clinical Chemistry

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of clinical chemistry parameters was evaluated.

Clinical chemistry parameters were altered in both males and females receiving bupivacaine compared to the vehicle controls. Changes were small in magnitude but statistically significant. Based on the small magnitude of change and lack of a clear dose response the findings are not likely toxicologically relevant. In addition, most parameters were similar to the vehicle following the recovery phase.

Table 135: Notable Changes in Clinical Chemistry Parameters in Male Rats

	Males			Recovery groups
	Vehicle	100 mg/kg	150 mg/kg	
ALB (g/dL)	2.8	2.6*	2.6*	No difference
GLU (mg/dL)	99	84*	88	No difference
T. PRO (g/dL)	5.2	4.9*	4.9*	No difference
K (mmol/L)	4.9	5.0	5.2*	Slight decrease

* Significantly different from vehicle, $p \leq 0.05$

N = 10

Table 136: Notable Changes in Clinical Chemistry Parameters in Female Rats

	Females			Recovery groups
	Vehicle	100 mg/kg	150 mg/kg	
ALB (g/dL)	3.2	2.9*	2.8*	No difference
GLU (mg/dL)	99	105	100	100 mg/kg: slight elevation 150 mg/kg: No difference
T. PRO (g/dL)	5.4	5.1*	4.9*	No difference
Na (mmol/L)	141	143*	142*	No difference
K (mmol/L)	4.9	5.0	5.2*	100 mg/kg: no difference 150 mg/kg: Elevated
Cl (mmol/L)	105	106	107*	No difference

* Significantly different from vehicle, $p \leq 0.05$

N = 10, except for the 150 mg/kg females where N = 9

Urinalysis

Urine was collected the day prior to terminal sacrifice. A complete battery of urinalysis parameters was evaluated.

The only treatment related finding was a small but statistically significant decrease in urine pH of males receiving bupivacaine. Due to the small magnitude of the change, the fact it was seen in one sex and that values were similar to the control following recovery suggests the finding is likely not toxicologically relevant.

Table 137: Urine pH in Male and Female Rats

	Vehicle	100 mg/kg	150 mg/kg	Recovery groups
pH (males)	6.7	6.2*	6.4*	No difference
pH (females)	6.3	6.4	5.9	No difference

* Significantly different from vehicle, $p \leq 0.05$

N = 10, except for the 150 mg/kg females where N = 9

Gross Pathology

Following sacrifice, all main study and recovery animals received a complete necropsy.

Gross observations were limited to the injection sites. In males, accumulations were observed at the injection site while, in females, accumulations, crust, and scabbing were present. Findings were similar to the vehicle-treated group following a recovery period.

Table 138: Gross Observations in Male Rats (Incidence)

Males			
	Vehicle	100 mg/kg	150 mg/kg
Injection site-subcutaneous fluid filled accumulation	0	3	1
Injection site-subcutaneous yellow accumulation	0	1	3
Injection site-subcutaneous accumulation	0	4	1

N = 10

Table 139: Gross Observations in Female Rats (Incidence)

Females			
	Vehicle	100 mg/kg	150 mg/kg
Injection site-crust	1	3	2
Injection site-scab	0	1	3
Injection site-subcutaneous accumulation	0	4	1

N = 10, except for the 150 mg/kg females where N = 9

Organ Weights

The following tissues were weighed:

Adrenal Glands
Brain

Ovary
Spleen

Heart	Testes
Kidneys	Thymus
Liver	

Statistically significant changes in organ weights were observed in males but were generally small in magnitude. This included decreased heart weight (abs), increased liver weight relative to body weight, increased brain weight (relative to body weight), and increased testes weight (relative to body weight). Effects on the brain and testis seemed to follow somewhat of a dose response. The effects were no longer present following the recovery phase. Due to the small magnitude of change and lack of histopathological correlates the effects seem to not be toxicologically relevant.

In females the only organ weight change was increased spleen weight relative to body weight at 150 mg/kg compared to the vehicle treated animals. Spleen weight was still slightly elevated (7%) in the recovery animals but this small difference is likely not toxicologically relevant.

Table 140: Organ Weights in Male Rats

	Males			
	Vehicle	100 mg/kg	150 mg/kg	Recovery
Heart (g)	1.182	1.082* (-8%)	1.073* (-9%)	Similar to control
Liver (rel)	3.15	3.38* (7%)	3.36 (7%)	Similar to control
Brain (rel)	0.612	0.632 (3%)	0.660* (8%)	Similar to control
Testis (rel)	0.924	0.986 (7%)	1.040* (13%)	Similar to control

* Significantly different from vehicle, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10

Table 141: Organ Weights in Female Rats

	Females			
	Vehicle	100 mg/kg	150 mg/kg	Recovery
Spleen (rel)	0.235	0.248 (6%)	0.270* (15%)	Slight elevation

* Significantly different from vehicle, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10, except for the 150 mg/kg females where N = 9

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Tissue Collection Table

Adrenals*	Pancreas
Aorta	Pituitary
Brain* (entire)	Prostate (with seminal vesicles and coagulating gland)
Epididymides	Salivary glands (mandibular)
Esophagus	Sciatic nerve
Eyes with optic nerve	Skeletal muscle
Femur with marrow	Skin (untreated; with mammary gland for females)
Harderian gland	Spinal cord (cervical, thoracic, lumbar)
Heart*	Spleen*
Intestines (gut-associated lymphoid tissue, examined only if present in plane of section)	Sternum (bone with marrow)
Cecum	Stomach
Colon	Testes*
Duodenum	Thymus*
Ileum	Thyroid (parathyroids, examined if present in plane of section)
Jejunum	Tongue
Rectum	Trachea
Kidneys*	Urinary bladder
Larynx	
Liver*	Uterus (with cervix)
Lungs with bronchi	Vagina
Lymph nodes (mandibular and mesenteric)	Gross lesions/masses
Ovaries* (with oviducts)	Identification (ear tag)
Regional Lymph Nodes (axillary)	Injection sites A, B, C, D, E, F (full thickness at each site; including underlying muscle)

While all tissues were collected, only the brain, heart, liver, lungs, kidneys, and injection sites underwent histopathological evaluation. This was considered adequate because the largest concern with bupivacaine is cardiotoxicity.

Histological Findings

There were no systemic histopathological findings identified following treatment with bupivacaine. An increased incidence of lesions was observed at the injection site of animals who received bupivacaine including mononuclear cell infiltration, fibrosis, edema, erythrocyte accumulation, ulcer (females at Injection Site A), and acute inflammatory exudate (females at Injection Site A). Findings at the injection site were mostly resolved following the recovery period.

Table 142: Infiltrate of Mononuclear Cells (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	3 (1.3)	7 (1.4)	8 (1.3)	3 (1.7)	3 (1.3)	6 (1.3)
F [3]	2 (2)	8 (1.5)	7 (1.1)	2 (1.0)	8 (1.5)	6 (2)
E [5]	5 (1.2)	9 (1.6)	9 (1.9)	5 (1.4)	10 (1.9)	8 (1.5)
D [7]	4 (2)	10 (1.6)	7 (1.3)	7 (1.6)	9 (1.7)	7 (1.4)
C [9]	7 (1)	9 (1.6)	9 (1.2)	1 (2)	4 (1.3)	7 (1.6)
B [11]	3 (1)	3 (1.3)	5 (1.2)	5 (1.2)	8 (1.4)	7 (1.1)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Table 143: Fibrosis (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	1 (2.0)	5 (2.0)	4 (2.3)	0	0	1 (1.0)
F [3]	2 (2.5)	2 (2.5)	2 (2.5)	0	2 (1.5)	2 (2.5)
E [5]	3 (2.7)	7 (2.3)	6 (1.7)	1 (2.0)	7 (2.4)	4 (1.8)
D [7]	3 (2.7)	8 (3.0)	5 (2.0)	3 (1.0)	5 (2.2)	6 (2.0)
C [9]	4 (2.3)	9 (2.6)	7 (2.6)	0	3 (2.3)	5 (2.4)
B [11]	1 (2.0)	3 (1.7)	4 (1.8)	0	2 (3.0)	3 (1.0)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Table 144: Edema (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	2 (2.5)	4 (3.0)	6 (2.5)	0	1 (3.0)	1 (1.0)
F [3]	0	5 (4.2)	3 (4.7)	0	2 (2.0)	3 (4.7)
E [5]	0	3 (3.3)	5 (3.8)	0	5 (3.2)	2 (3.0)
D [7]	0	6 (3.7)	6 (3.5)	0	1 (3.0)	3 (4.0)
C [9]	0	4 (3.0)	2 (4.0)	0	1 (3.0)	2 (2.5)
B [11]	0	0	1 (3.0)	0	5 (3.0)	5 (3.4)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Table 145: Erythrocyte Accumulation (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	2 (1.5)	6 (3.2)	9 (3.7)	0	2 (4.5)	2 (4.5)
F [3]	2 (2.0)	6 (3.5)	4 (2.8)	0	2 (2.0)	3 (2.3)
E [5]	1 (3.0)	7 (3.0)	6 (3.8)	1 (3.0)	5 (3.2)	3 (3.0)
D [7]	2 (1.0)	7 (3.6)	2 (3.0)	0	4 (4.0)	2 (2.0)
C [9]	1 (1.0)	3 (3.0)	5 (1.8)	0	4 (3.3)	4 (3.5)
B [11]	0	2 (3.5)	1 (3.0)	4 (2.0)	6 (3.0)	6 (3.7)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Table 146: Ulcer (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	0	1 (3.0)	0	1 (4.0)	2 (3.0)	6 (2.7)
F [3]	0	1 (4.0)	0	0	1 (3.0)	2 (4.5)
E [5]	0	0	0	0	1 (3.0)	0
D [7]	0	0	0	0	0	1 (3.0)
C [9]	0	0	0	0	0	2 (4.0)
B [11]	1 (3.0)	0	0	1 (1.0)	3 (3.0)	2 (3.0)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 147: Acute Inflammatory Exudate (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	0	1 (4.0)	1 (3.0)	1 (3.0)	5 (3.2)	8 (3.9)
F [3]	0	1 (4.0)	0	0	1 (3.0)	2 (4.5)
E [5]	0	0	0	0	1 (4.0)	1 (1.0)
D [7]	0	0	0	0	0	2 (4.0)
C [9]	0	0	0	0	0	2 (4.0)
B [11]	1 (3.0)	0	0	1 (3.0)	4 (4.0)	2 (3.5)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Toxicokinetics

Toxicokinetic animals (3 rats/sex/timepoint) were bled via the sublingual vein on Days 1 and 13. Vehicle control animals had blood collected at 6 hours post dose on both days. Bupivacaine treated animals had blood collection at the following timepoints: pre dose, and 0.5, 1, 4, 6, 12, 24, 36, 48, 60, and 72 hours post dose on Day 13; pre dose, and 0.5, 1, 4, 6, 12, 24, 36, and 48 hours post dose on Day 1.

Bupivacaine

Bupivacaine AUC was nearly dose proportional on Days 1 and 13 with an expected fold difference of 1.5 (100 vs. 150 mg/kg) but with an actual dose ratio of 1.6-1.8 fold. It appeared that there was some accumulation of bupivacaine over time as AUC increased with time in both sexes and doses. Bupivacaine AUC and C_{max} were similar between males and females.

Table 148: Bupivacaine Toxicokinetic Parameters

Treatment	BPV Dose (mg/kg)	Day	Sex	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC ₀₋₂₄ ^a (ng•h/mL)	AUC ₀₋₄₈ ^b (ng•h/mL)	AUC _{last} (ng•h/mL)	AUC _{inf} (ng•h/mL)	R _c ^c
HTX-002-14	100	1	Male	684	4	15.8	10200	14300	14300	16100	NA
			Female	686	6	15.3	11400	15500	15500	17400	NA
			Combined	655	4	15.2	10800	14900	14900	16700	NA
		13	Male	1350	4	28.8	17300	26000	28400	31200	1.8
			Female	1180	4	16.8	19900	26600	29500	30800	1.7
			Combined	1260	4	17.2	18600	26300	28900	30500	1.8
HTX-002-19	150	1	Male	1120	6	14.6	18000	23700	23700	26500	NA
			Female	1050	12	16.1	18900	24400	24400	27000	NA
			Combined	1010	6	13.2	18400	24000	24000	26400	NA
		13	Male	2060	12	ND	34100	43600	48300	ND	1.8
			Female	3030	1	16.3	36200	48600	53800	55700	2.0
			Combined	2180	1	16.4	35200	46100	51000	52900	1.9

BPV = bupivacaine; NA = Not Applicable; ND = Not Determined.

^a AUC₀₋₂₄ is area under the plasma concentration-time from time zero to 24 h post-dose.

^b AUC₀₋₄₈ is area under the plasma concentration-time from time zero to 48 h post-dose.

^c R_c is accumulation ratio Day 13 AUC₀₋₄₈/Day 1 AUC₀₋₄₈.

Taken from page 469 of Study report 34-84

Dosing Solution Analysis

Dosing solution analysis was not performed. End-of-use stability analysis of the test article formulations was supposed to be conducted and appended to the final study report, but it does not appear to have been completed yet at the time of review.

Study title: HTX-011: 28-day intermittent subcutaneous toxicity and toxicokinetic study in Beagle dogs (with a 2-week recovery)

Study no.: 33-86
Study report location: SDN 1, Module 4
<\\cdsesub1\levsprod\nda211988\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\33-86\33-86.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/22/2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: HTX-011, Lot 026517, 99.9% bupivacaine and 97.3% meloxicam

Key Study Findings

- All animals survived until necropsy.
- No treatment-related effects were observed on ophthalmic parameters, electrocardiography parameters, or urinalysis parameters.
- There was an increased incidence of edema at the injection site following vehicle and HTX-011 treatment. Incidence and severity were highest with the vehicle, while both doses of HTX-011 caused similar incidence and severity scores that were on average lower than the vehicle. Edema was not seen in recovery animals.
- Treatment with the vehicle also resulted in hypoactivity, dermal scabs, and swollen sites. These were not observed in recovery animals.
- Decreased body weight was observed following treatment with the vehicle. This did not appear to be toxicologically relevant in males due to lower weight prior to treatment and similar body weight gain to saline control. However, the effect was toxicologically relevant in females where body weight gain was greatly reduced over the course of the study.
- Treatment with the vehicle affected multiple hematology parameters while treatment with HTX-011 only resulted in increased reticulocytes. There was no difference in any hematology parameters in recovery animals.
- Treatment with the vehicle affected multiple clinical chemistry parameters while treatment with HTX-011 only resulted in increased glucose at the interim sacrifice. There was no difference in any clinical chemistry parameters in recovery animals.

- Gross pathologic inspection revealed thickening at the injection site in vehicle-treated animals and accumulation in HTX-011 and vehicle-treated animals. No treatment-related macroscopic findings were present in recovery animals.
- Changes in organ weights were observed in vehicle-treated animals compared to saline control. This included increased absolute and relative liver weights, increased absolute and relative thymus weights (males only), increased absolute and relative adrenal weights (females only), and increased absolute and relative kidney weights (females only). Most of these changes were also observed in recovery animals.
- Injection site histopathological findings included chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage, and presence of test material following treatment with the vehicle and HTX-011. In general, findings were more prevalent or more severe in female animals and vehicle-treated animals. Multifocal chronic inflammation, multifocal granulomatous inflammation, and multifocal fibrosis were still present in recovery animals at reduced severity and/or incidence up to around 28 days.
- Bupivacaine and meloxicam toxicokinetics were measured on Days 1, 9, and 27. Bupivacaine and meloxicam C_{max} and AUC increased with time and were similar between the two dose groups. T_{max} values for both compounds were highly variable.
- Polymer breakdown products were detected in urine on Day 22. (b) (4)
[REDACTED]
were all detected at levels above the calibration range.
- Effects that could be potentially related to systemic exposure included hypoactivity, decreased body weight, altered hematology parameters, altered clinical chemistry parameters, and increased organ weights. All of these effects were observed following treatment with the vehicle and not saline or HTX-011. The majority of findings were similar in both males and females (except for effects on body weight). No histopathological effects on any organs were observed and most effects (except organ weight changes) were reversible. Overall, the data support that the excipients in the vehicle cause a systemic response.
- The injection volume used for the vehicle was larger than that for HTX-011; therefore, vehicle animals received higher exposure to certain excipients which may have caused the systemic effects. The vehicle was not a perfect match for the drug product as it does not contain maleic acid and contains more AP135 (650 mg/g vs. 623.75 mg/g) than HTX-011. Compared to HTX-011, the vehicle resulted in about twice as much exposure to DMSO (117 vs. 51.5 mg/kg), triacetin (292.5 vs. 128.7 mg/kg), and AP135 (760.5 vs. 321.1 mg/kg). Compared

to human exposure at the MRHD of 400 mg/12 mg, HTX-011 showed limited systemic effects (increased reticulocytes in females) with an exposure margin of ~1.1x for DMSO, triacetin, and AP135, while the vehicle showed systemic effects with an exposure margin of ~2.5x for DMSO, triacetin, and AP135 based on BSA and a 60 kg individual.

- A local inflammatory reaction was observed following treatment with the vehicle and HTX-011. The effects are consistent with a biological response to injection of foreign material. The majority of the effects resolved following a recovery period as indicated by the lack of those findings in recovery animals. However, multifocal chronic inflammation, granulomatous inflammation, and fibrosis were still present in recovery animals up to 28 days following the last injection. Local effects were predominantly in females and were worse with vehicle compared to HTX-011, likely due to the increased injection volume used for the vehicle. In addition, there appeared to be more effects with the high dose of HTX-011 compared to the low dose. The increased local findings with the vehicle and HTX-011 high dose is slightly unusual as generally the concentration of the formulation is considered the dose metric for local effects. However, the results in dogs support that the absolute amount of the excipients delivered to the local tissue seems to be the driving force to the local effects.
- The Applicant claims that the effects are non-adverse and therefore the NOAEL is the highest dose tested. While the effects observed are not unexpected, it is difficult to ascertain the adversity and clinical relevance of the observed persisting chronic inflammation, granulomatous inflammation, and fibrosis. Therefore, in terms of local effects, this study demonstrates that the clinical formulation causes a local inflammatory response up to 28 days that is likely the result of the vehicle. The total amount of excipients seems to be a driving force in the local reaction. However, if that is the case, it is difficult to compare the local effects in animals to humans since the dosing in the animal study is different from the clinical dosing regimen and it is unclear how to translate the volume of injection into a HED dose.

Methods

Doses: Saline, vehicle control, 10/0.30 mg/kg bupivacaine/meloxicam, and 13/0.39 bupivacaine/meloxicam

Frequency of dosing: Once every 48 hours

Route of administration: Subcutaneous injection

Dose volume: 0.44 mL/kg (saline and 13/0.39 mg/kg), 1 mL/kg for vehicle (start of study), 0.5 mL/kg for vehicle (after seeing decreased body weight and injection site findings), or 0.34 mL/kg (10/0.30 mg/kg)

Formulation/Vehicle: HTX-011-056 (aka HTX-011): 25% triacetin, 10% DMSO, 62.375% AP135 polymer, 0.05% maleic acid, 2.5% bupivacaine, and 0.075% meloxicam
HTX-024-002 (vehicle): 25% triacetin, 10% DMSO, and 65% AP135 polymer

Species/Strain: Beagle dog

Number/Sex/Group: 4 for the main experiment, and 2 for recovery

Age: 5-6 months old

Weight: 6.4-8.9 kg (male) and 5.2-7.2 kg (female)

Satellite groups: Recovery groups

Unique study design: None

Deviation from study protocol: None that impacted study results

Experimental Design

Table 149 Experimental Design of Study 33-86

Group ^a	Description	Bupivacaine Dose Level (mg/kg/day)	Meloxicam Dose Levels (mg/kg/day)	Dose Volume (mL/kg)	Bupivacaine Conc. ^d (mg/mL)	Meloxicam Conc. ^d (mg/mL)	Number of Dogs/Group	
							Core	Recovery
1 ^b	Saline Control	0	0	0.44	0	0	4/sex	2/sex
2 ^c	Vehicle Control	0	0	1/0.5 ^e	0	0	4/sex	2/sex
3	Low Dose	10	0.30	0.34	29.25	0.88	4/sex	2/sex
4	High Dose	13	0.39	0.44	29.25	0.88	4/sex	2/sex

^aAnimals will be dosed once every other day for a total of 14 doses, on Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27.

^bGroup 1 will be administered saline control (sterile saline) only.

^cGroup 2 will be administered vehicle control (HTX-024) only.

^dConcentrations of bupivacaine and meloxicam will be 2.5 and 0.075% w/w (or 25 mg/g and 0.75 mg/g), respectively. When corrected for density (1.17 g/mL) the final concentrations of bupivacaine and meloxicam will be 29.25 mg/mL and 0.88 mg/mL, respectively.

^eDue to decreased body weights and adverse injection site findings, the dose volume for the Vehicle Control group was decreased to 0.5 mL/kg for Days 21, 23, 25, and 27.

From page 16 of the Applicant's Study Report 33-86

Injection Site Rotations

Dose Site	Location	Injection Day	Dose Free Days to Terminal Necropsy
A	Left Cranial Back	1 and 13	16
B	Right Cranial Back	3 and 15	14
C	Left Mid Back	5 and 17	12
D	Right Mid Back	7 and 19	10
E	Left Caudal Back	9, 21, and 25	4
F	Right Caudal Back	11, 23, and 27	2

Observations and Results

Mortality

All animals survived until scheduled necropsies.

Clinical Signs

Dosing sites were scored using a modified Draize scoring system prior to dosing and two hours post dose. Detailed clinical examinations were performed weekly during the study in conjunction with body weight. Cage-side observations were performed at the time of dose site/dermal observations.

The primary finding at the dermal site was increased edema. The largest effect was seen with the vehicle treatment resulting in edema scores of four (severe) at most time points. Treatment with either HTX-011 dose resulted in less frequent and less severe observations of edema than the vehicle, but they were still increased compared to the saline control. Edema scores were highest two days after dosing. There was no dose response to the incidence or severity; however, this is not unexpected since the doses are very similar and similar volumes were injected. At the end of the recovery period, the incidence and severity of edema was greatly reduced and no difference between treatment groups was observed.

The dose volume of the vehicle control was reduced due to the observation of injection site findings. After reducing the volume from 1 mL/kg to 0.5 mL/kg (similar to the high-dose group), the edema scores were reduced as well.

Some of the animals receiving the vehicle control exhibited serosanguinous fluid with expulsion from the injection site. The fluid was tested for bacteria and did not contain any. Additional findings only seen in the vehicle control animals were hypoactivity, dermal scabs, and swollen sites.

Table 150: Incidence of Edema at the Injection Site

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
A	0	6 (4)	6 (2)	5 (2)	0	6 (4)	6 (2)	6 (2)
B	0	6 (4)	6 (1)	6 (1)	0	6 (4)	6 (1)	6 (1)
C	0	6 (4)	3 (1)	3 (1)	0	6 (4)	2 (1)	6 (1)
D	0	6 (4)	5 (1)	6 (2)	0	6 (4)	5 (1)	5 (1)
E	0	6 (4)	6 (2)	6 (2)	0	6 (4)	4 (2)	6 (2)
F	0	6 (4)	6 (2)	6 (2)	0	6 (4)	6 (3)	6 (2)

N = 6

Values in () represent average severity: 1 = very slight, 2 = well-defined, 3 = moderate, 4 = severe.

Table 151: Average Severity of Edema in Vehicle Control Group

Inj site	Day 11 (1 ml/kg) M/F	Day 13 (1 ml/kg) M/F	Day 23 (0.5 ml/kg) M/F	Day 25 (0.5 ml/kg) M/F
E	3/3.8	-	2.8/2.3	-
F	-	3.7/3.7	-	2.7/2.7

Body Weights

Body weights were recorded prior to randomization, prior to treatment, and weekly for the remainder of the study.

Body weights in male vehicle treated animals were less than saline control. However, it appears that this group of animals weighed less than the saline control prior to treatment (Day 0). Furthermore, the group as a whole had a similar level of body weight gain over the first 19 days of treatment (0.28 kg for both the saline and vehicle control group) compared to the saline control group.

Body weight was also decreased in female vehicle treated animals compared to the saline control. Similar to males, it appears that this group of animals weighed less than the saline control prior to treatment (Day 0). In contrast to males, there did appear to be an effect on body weight by the vehicle treatment as the % difference between the two groups increased over the first 19 days of treatment. This can also be seen by comparing body weight gain over the 19 days between the saline control (0.31 kg) and the vehicle control (-0.15 kg).

No differences between the HTX-011 groups and the saline control were observed.

Table 152: Body Weights

Day	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
0	7.78	91.9%	98.2%	96.5%	6.32	96.2%	98.6%	101.3%
1	7.69	91.7%	98.5%	97.1%	6.18	96.4%	97.6%	100.8%
7	7.90	89.4%	98.7%	99.0%	6.32	94.3%	102.5%	106.3%
13	7.81	92.2%	102.2%	100.5%	6.50	90.0%	99.2%	103.2%
19	8.06	92.2%	102.7%	100.4%	6.62	89.6%	99.2%	104.8%
25	8.34	91.7%	100.2%	99.4%	6.74	93.6%	100.3%	103.0%
27	8.00	95.0%	103.4%	100.8%	6.67	95.8%	100.9%	105.4%

N = 6

Ophthalmoscopy

Indirect fundoscopic examinations were performed pretest, at the end of the treatment period, and during recovery.

There were no treatment-related ophthalmic changes observed.

Electrocardiography

A six-lead electrocardiogram was performed on each dog prior to treatment, on Day 27, and during the second week of recovery. ECGs were evaluated by a qualified veterinary cardiologist.

There were no treatment-related electrocardiographic effects.

Hematology

Blood was collected from the jugular vein prior to treatment, at about 3 weeks, at end of the treatment period, and the end of recovery. A complete battery of hematology and coagulation parameters was evaluated.

A number of hematology parameters were affected by treatment with the vehicle in both males and females. Consistently effected parameters were increased white blood cells, decreased red blood cells, decreased hemoglobin, decreased hematocrit, increased platelets, increased absolute and relative neutrophils, decreased relative lymphocytes, decreased relative basophils, increased absolute monocytes, increased absolute leukocytes, increased red blood cell distribution width, increased absolute eosinophils, and increased absolute and relative reticulocytes. All parameters were similar to the saline control after a two-week recovery period.

In contrast, there were very few effects on hematology with the drug product. In males an absolute and relative increase in reticulocytes were seen at the interim blood collection with both doses but values were similar at the terminal blood collection. In females, absolute and relative reticulocytes were increased at both the interim and terminal blood collections. All parameters were similar to the saline control after a two-week recovery period.

Table 153: Hematology Parameters in Male Dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
WBC (x10 ³ cells/mcL)	11.05	433.9%*	107.9%	111.9%
	7.99	301.1%*	110.4%	115.5%
RBC (x10 ³ cells/mcL)	6.71	75.3%*	100.4%	99.3%
	6.24	78.5%*	101.0%	96.6%
HGB (g/dL)	15.2	73.7%*	98.0%	95.4%
	13.9	79.1%*	100.7%	94.2%
HCT (%)	46.2	76.6%*	98.7%	95.7%
	43.2	80.0%*	99.1%	93.5%
RDW (%)	14.3	103.5%	104.9%	105.6%
	14.0	111.4%*	104.3%	105.0%
PLAT (x10 ³ cells/mcL)	321	144.2%	101.9%	114.6%
	269	208.9%*	105.9%	110.0%
NEUT (%)	56.5	151.2%*	111.7%	110.3%
	54.9	135.2%*	107.5%	104.6%
LYMP (%)	34.5	20.6%*	84.6%	87.5%
	34.5	47.8%*	93.6%	93.0%
EOS (%)	1.4	28.6%*	114.3%	114.3%
	2.1	104.8%	95.2%	133.3%
MONO (%)	6.7	97.0%	80.6%	79.1%
	7.4	86.5%	77.0%	93.2%
LUC (%)	0.5	100.0%	80.0%	80.0%
	0.7	85.7%	85.7%	85.7%
Retic (%)	0.71	129.6%	163.4%	171.8%
	0.78	239.7%*	101.3%	125.6%
NEUT (x10 ³ cells/mcL)	6.25	657.3%*	120.6%	125.8%
	4.47	410.5%*	116.8%	119.9%
LYMPH (x10 ³ cells/mcL)	3.80	85.8%	91.1%	95.0%
	2.69	133.1%*	105.6%	107.4%
EOS (x10 ³ cells/mcL)	0.15	113.3%	120.0%	126.7%
	0.16	281.3%*	112.5%	168.8%
MONO (x10 ³ cells/mcL)	0.76	415.8%*	84.2%	85.5%
	0.58	256.9%*	86.2%	108.6%
LUC x10 ³ cells/mcL	0.05	460.0%	100.0%	80.0%
	0.05	280.0%*	100.0%	100.0%
Retic (x10 ⁹ cells/L)	47.4	96.6%	165.0%	171.5%*
	48.0	188.1%*	104.6%	123.1%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Table 154: Hematology Parameters in Female Dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
WBC (x10 ³ cells/mcL)	12.82	256.9%*	83.5%	83.5%
	9.47	276.2%*	107.5%	92.8%
RBC (x10 ³ cells/mcL)	7.04	78.6%*	99.3%	98.7%
	6.65	84.1%*	99.7%	98.8%
HGB (g/dL)	15.7	75.8%*	94.9%	100.0%
	14.9	81.9%*	95.3%	98.7%
HCT (%)	48.5	77.5%*	94.8%	98.8%
	45.7	83.6%*	95.6%	99.3%
RDW (%)	14.7	103.4%	104.1%	101.4%
	14.2	109.2%*	107.7%*	102.8%
PLAT (x10 ³ cells/mcL)	392	114.3%	91.8%	91.8%
	333	148.0%*	109.9%	103.0%
NEUT (%)	55.9	143.1%*	106.1%	107.5%
	51.5	146.0%*	121.7%*	112.0%
LYMP (%)	36.1	33.5%*	91.1%	89.2%
	39.5	38.2%*	74.9%*	87.3%
EOS (%)	1.4	71.4%	135.7%	157.1%
	2.2	104.5%	83.4%	127.3%
MONO (%)	5.8	110.3%	87.9%	82.8%
	5.8	117.2%	86.2%	72.4%
LUC (%)	0.5	100.0%	80.0%	80.0%
	0.5	80.0%	80.0%	80.0%
Retic (%)	0.96	94.8%	124.0%	157.3%*
	0.70	198.6%	171.4%	162.9%
NEUT (x10 ³ cells/mcL)	7.24	369.8%*	87.8%	89.1%
	4.92	404.1%*	134.4%	103.9%
LYMPH (x10 ³ cells/mcL)	4.56	78.9%*	77.2%*	75.0%*
	3.71	103.0%	75.2%*	81.1%
EOS (x10 ³ cells/mcL)	0.17	152.9%	117.6%	141.2%
	0.21	261.9%*	85.7%	109.5%
MONO (x10 ³ cells/mcL)	0.73	287.7%*	75.3%	69.9%
	0.54	325.9%*	100.0%	68.5%
LUC x10 ³ cells/mcL	0.06	233.3%*	66.7%	66.7%
	0.05	200.0%*	80.0%	60.0%
Retic (x10 ⁹ cells/L)	67.5	75.6%	123.6%	155.3%*
	46.5	164.7%	173.3%	163.9%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Clinical Chemistry

Blood was collected from the jugular vein prior to treatment, at about 3 weeks, at end of the treatment period, and at the end of recovery. A complete battery of hematology and coagulation parameters was evaluated.

Treatment with the vehicle caused multiple changes in clinical chemistry parameters in male and female animals. Consistent changes in both sexes at both blood collection time points were decreased albumin, decreased ALT, decreased creatinine, decreased glucose, increased LDH, increased globulin, and decreased A/G ratio. Other less

consistent changes included decreased total bilirubin (males at both time points; females at interim only), decreased calcium (males at interim only; females at both time points), increased cholesterol (both sexes at interim only), decreased blood urea nitrogen (males at interim only; females at both time points), decreased potassium (both sexes at interim only), increased CPK (males at terminal only), increased triglycerides (males at terminal only), decreased chloride (males at terminal only), and decreased GGT (females at terminal only). All parameters were similar to the saline control animals following a 2-week recovery period.

The only change observed in animals treated with the drug product was an increase in glucose. This change was seen in both sexes and only at the interim time point and similar to saline treated animals after the 2-week recovery period.

Table 155: Clinical Chemistry Parameters in Male Dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Alb (g/dL)	2.9	72.4%	96.6%	96.6%
	2.8	82.1%	100.0%	96.4%
Alt (U/L)	29	34.5%	93.1%	93.1%
	27	48.1%	111.1%	103.7%
Crea (mg/dL)	0.83	43.4%	90.4%	101.2%
	0.59	54.2%	91.5%	98.3%
Glu (mg/dL)	80	55.0%	120.0%	120.0%
	88	71.6%	102.3%	98.9%
LDH (U/L)	72	370.8%	90.3%	102.8%
	112	313.4%	100.9%	127.7%
GLB (g/dL)	2.5	128.0%	96.0%	92.0%
	2.5	124.0%	92.0%	92.0%
A/G	1.2	58.3%	100.0%	100.0%
	1.2	66.7%	100.0%	100.0%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Table 156: Clinical Chemistry Parameters in Female Dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Alb (g/dL)	3.1	74.2%	93.5%	96.8%
	3.0	80.0%	96.7%	96.7%
Alt (U/L)	25	48.0%	112.0%	112.0%
	24	54.2%	112.5%	104.2%
Crea (mg/dL)	0.87	51.7%	80.5%	90.8%
	0.59	54.2%	89.8%	84.7%
Glu (mg/dL)	84	79.8%	111.9%	119.0%
	92	80.4%	97.8%	106.5%
LDH (U/L)	78	174.4%	85.9%	91.0%
	100	194.0%	131.0%	93.0%
GLB (g/dL)	2.4	137.5%	95.8%	91.7%
	2.3	130.4%	100.0%	95.7%
A/G	1.3	53.8%	100.0%	107.7%
	1.3	61.5%	100.0%	107.7%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Urinalysis

Urine was collected directly from the bladder of animals at scheduled necropsy. A complete battery of urinalysis parameters was evaluated.

There were no treatment-related changes in urinalysis parameters.

Gross Pathology

Following sacrifice, all main study and recovery animals received a complete necropsy.

Macroscopic observations were limited to the injection sites. Accumulations were observed in animals treated with the vehicle and HTX-011. The incidence of accumulations appeared to be higher in HTX-011 dosed animals but a clear dose response was not apparent. Thickening of the injection site was primarily observed in vehicle treated animals with the highest incidences occurring at Injection Sites E and F, the injection sites treated closest to the end of the study. No macroscopic findings were present following the 2-week recovery period.

Table 157: Gross Observations in Male Dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Accumulation (A)	-	1	-	2
Accumulation (B)	-	-	-	1
Accumulation (D)	-	2	-	1
Accumulation (E)	-	1	2	2
Accumulation (F)	-	1	1	3
Thickened (E)	-	3	1	1
Thickened (F)	-	3	-	-

N = 4

Table 158: Gross Observations in Female Dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Accumulation (A)	-	1	-	2
Accumulation (B)	-	1	-	1
Accumulation (D)	-	2	-	-
Accumulation (E)	-	1	3	3
Accumulation (F)	-	-	1	2
Thickened (E)	-	3	1	-
Thickened (F)	-	4	2	2

N = 4

Organ Weights

The following tissues were weighed:

Adrenal Glands	Liver	Testes
Brain	Ovaries	Thymus
Epididymides	Pituitary gland	Thyroid
Heart	Prostate gland	Uterus
Kidneys	Spleen	

Changes in organ weights were primarily seen in the vehicle control group. In males, absolute and relative liver weights were increased. Following recovery, absolute liver weights were still increased but relative liver weights were similar to the saline control. Absolute and relative thymus weights were increased at terminal sacrifice and following recovery.

In females, altered organ weights were observed in the adrenal, liver, and kidney. Absolute and relative adrenal weights were increased but only absolute adrenal weight was increased following recovery. In the main study animals, only relative liver weight was increased but following recovery an increase in absolute liver weight was also observed. Increased absolute and relative kidney weights were observed in both main study and recovery animals.

Table 159: Organ Weights in Male Dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Liver	225	278 (23%)	246 (9%)	237 (5%)
	2.94	3.88 (32%)*	3.29 (11%)	3.12 (9%)
Thymus	8.7	10.28 (18%)	8.94 (3%)	9.41 (8%)
	0.116	0.143 (23%)	0.118 (2%)	0.125 (8%)
Liver- recovery	204	254 (24%)	228 (12%)	242 (19%)
	2.88	3.00 (4%)	2.91 (1%)	3.08 (7%)
Thymus- recovery	9.63	14.60 (52%)	11.64 (21%)	6.88 (-29%)
	0.131	0.173 (32%)	0.151 (15%)	0.089 (-32%)

For each row, the top value is for absolute weight and the bottom value is for organ weight relative to body weight

N = 4 for main study and N = 2 for recovery

Table 160: Organ Weights for Female Dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Adrenal	1.12	1.48 (32%)*	1.02 (-9%)	1.16 (4%)
	0.017	0.026 (52%)*	0.016 (-6%)	0.018 (6%)
Liver	190	203 (7%)	184 (-3%)	207 (9%)
	2.95	3.54 (20%)*	2.92 (-1%)	3.24 (10%)
Kidney	31.8	37.9 (19%)	33.7 (6%)	38.6 (21%)
	0.494	0.660 (34%)*	0.533 (8%)	0.605 (22%)
Adrenal- recovery	1.08	1.24 (15%)	0.98 (-9%)	0.95 (-12%)
	0.017	0.018 (6%)	0.015 (-12%)	0.013 (-24%)
Liver-recovery	180	224 (24%)	190 (6%)	194 (8%)
	2.85	3.17 (11%)	2.88 (1%)	2.67 (-6%)
Kidney-recovery	28.8	46.0 (60%)	34.9 (21%)	36.2 (26%)
	0.454	0.651 (43%)	0.528 (16%)	0.495 (9%)

For each row, the top value is for absolute weight and the bottom value is for organ weight relative to body weight

N = 4 for main study and N = 2 for recovery

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Tissue Collection Table

Adrenals*	Pancreas
Aorta	Pituitary*
Brain* (entire)	Prostate*
Epididymides*	Salivary glands (mandibular and parotid)
Esophagus	Sciatic nerve
Eyes with optic nerve	Skeletal muscle (biceps femoris)
Femur with marrow	Skin (with mammary gland for females)
Gallbladder	Spinal cord (cervical, thoracic, lumbar)
Harderian gland	Spleen*
Heart*	Sternum (bone with marrow)
Intestines	Stomach
Cecum	Testes*
Colon	Thymus*
Duodenum	Thyroid* (parathyroids if present in the section)
Ileum (with Peyer's patches)	Tongue
Jejunum	Trachea
Rectum	Urinary bladder
Kidneys*	Uterus* (with cervix)
Larynx	Vagina
Liver*	Gross lesions
Lungs with bronchi	Identification (ear tattoo)
Lymph nodes (mandibular and mesenteric)	Injection sites A, B, C, D, E, and F (full thickness at each site, including underlying muscle)
Ovaries* (with oviducts)	

A number of histopathological findings were observed at the injection sites with treatment of either the vehicle or HTX-011 as compared to saline control where few or no findings were observed. These included chronic inflammation, granulomatous inflammation, fibrosis, presence of test material, and hemorrhage. Recovery animals were included who were treated for the same amount of time as the main study animals except they were sacrificed 14 days after treatment was stopped. It is important to note that the decreased number of animals used for recovery (two versus four) made it difficult to compare incidence back to the main experiment animals since a single finding represented 50% of the animals studied.

Chronic inflammation was described as focal, multifocal, or active/multifocal with multifocal and multifocal/active being the more prevalent types of chronic inflammation observed. For active multifocal chronic inflammation, the highest incidences and most severe findings were seen at Injection Sites E and F, the injection sites treated closest to the end of the study, with both the vehicle and HTX-011. Overall, the highest incidences and average severity was observed with treatment of the vehicle and overall incidence for all treatments was higher in females. The overall severity and incidence were lower at injection sites that had a longer time between treatment and necropsy for all treatments. Active chronic multifocal inflammation was not observed at a significant level in recovery animals.

In contrast, multifocal chronic inflammation incidence and severity seemed to be higher at injection sites where there was a longer time between the last injection and necropsy with both the vehicle and HTX-011. As with active chronic multifocal inflammation a higher incidence of findings were observed in females but in this case it appeared that the greatest incidence and severity was seen with the high dose of HTX-011. Chronic multifocal inflammation was also observed in recovery animals. Again, findings were primarily in females and with HTX-011 (both doses). The incidence and severity seemed to decrease at injection sites that had longer time since last injection such that findings were not really seen in either sex around 28-30 days.

Taken together, it appears that active chronic multifocal inflammation is seen early following treatment, which transitions to a chronic multifocal inflammation. Based on the female data, where findings were more prevalent, this occurs around 12 days following injection and the chronic active multifocal inflammation lasts until 28 days following injection.

Granulomatous inflammation was primarily seen in female animals treated with the vehicle (all injection sites) and the high dose of HTX-011 (Injection Sites A, B, E, and F). It was also observed in males receiving the high dose at Sites A, B, C, and D. Overall, the average severity was minimal to mild. It was also observed in recovery females at all injection sites except A, although the overall severity was decreased. Taken together, the data suggests that the incidence and severity of granulomatous inflammation peaks around Day 16, decreases with time since injection, and was completely resolved by Day 30.

Fibrosis was described as either focal or multifocal with multifocal being the more prevalent form. Multifocal fibrosis was observed in both sexes with both the vehicle and HTX-011. The overall severity of fibrosis was high at multiple injection sites with average severity values of moderate to marked. Fibrosis was observed at all injection sites in main study animals, suggesting it lasts at least 16 days following injection. Findings of fibrosis were still observed in some recovery animals. Based on the observed injection sites, fibrosis with an average severity of mild to marked was observed in female animals up to 26 days after the last injection. No observations of fibrosis were seen at Injection Sites A or B indicating that fibrosis was completely resolved 28-30 days post injection.

Hemorrhage was observed in both males and females receiving the vehicle and HTX-011. In both sexes, findings were more prevalent with the vehicle and the high dose. The majority of the findings were seen at Injection Sites E and F suggesting that hemorrhage resolved by Day 10-12. Hemorrhage was not observed at a significant level in recovery animals.

The presence of test material at the injection site was seen in some animals and defined as either focal or multifocal, with multifocal being more prevalent. In males, the presence of multifocal test material was seen with an incidence of 50% or more with the vehicle at Injection Site F, and the high dose at Injection Site F and D. In females, the presence of multifocal test material was seen with an incidence of 50% or more with the vehicle at Injection Site E and F, the low dose at Injection Site E, and the high dose at Injection Site E and F. This suggests that the test material was only present at the injection site for up to 10 days. However, test material was seen in individual animals with severity scores ranging from minimal to marked up to 16 days after injection. The test material was not observed in recovery animals.

In summary, treatment with the vehicle lead to an increase in incidence/severity of chronic active inflammation, granulomatous inflammation, hemorrhage, and fibrosis compared to the saline control. These findings were primarily in female animals and were mostly not present in recovery animals, with some exceptions. In some cases, findings with the vehicle were worse than with HTX-011 (active chronic inflammation, and fibrosis). This is interesting since the vehicle had a similar concentration of excipients as HTX-011 (the vehicle did not contain maleic acid and has slightly less AP135). The major difference was a much larger injection volume of the vehicle at the beginning of the study (1 vs. 0.44 mL/kg). Thus, despite being local effects, the increased absolute amount of the excipients could have played a role in these findings.

Treatment with HTX-011 lead to an increase in incidence/severity of chronic active inflammation, granulomatous inflammation, hemorrhage, and fibrosis compared to the saline control. In general, females were more affected than males but it is unclear why there would be a sex difference for these local effects. For many of the observed findings there was a slight increase in the severity and incidence with the high dose of HTX-011 compared to the low dose of HTX-011. It is unclear why this would be the case since the dose was controlled by injecting different volumes of the same concentration solution. It appears that the local effects are somewhat related to the absolute amount of the solution present which is consistent with the increased findings with the vehicle which had a much larger injection volume. Taken together, it appears that the majority of the findings are likely related to the presence of one or more excipients in the drug product.

Table 161: Percentage of Main Study Animals with Chronic Multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	25 (1)	0	0	25 (2)	25 (1)	0	25 (1)	25 (1)
E (4)	0	0	0	75 (1.3)	25 (1)	0	50 (1)	50 (1)
D (10)	0	25 (1)	25 (1)	25 (1)	0	25 (1)	0	50 (1)
C (12)	0	25 (1)	25 (1)	25 (2)	25 (1)	50 (1)	50 (1)	75 (1.3)
B (14)	0	0	50 (1.5)	0	0	0	25 (1)	75 (1.7)
A (16)	0	0	75 (1)	0	0	50 (1.5)	25 (1)	100 (2)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 162: Percentage of Recovery Animals with Chronic Multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	0	50 (1)	50 (2)	0	50 (1)	50 (2)	50 (1)
E (18)	0	50 (1)	0	50 (1)	0	0	50 (2)	50 (2)
D (24)	0	50 (1)	0	0	0	0	50 (1)	50 (2)
C (26)	0	0	0	50 (1)	0	0	50 (1)	50 (1)
B (28)	0	0	0	0	0	0	50 (1)	0
A (30)	0	0	50 (1)	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 163: Percentage of Main Study Animals with Active Chronic Multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	75 (3.7)	75 (1.3)	50 (2)	25 (1)	75 (3.3)	25 (3)	75 (3.3)
E (4)	0	75 (1.7)	25 (1)	25 (2)	0	100 (3.8)	50 (2)	50 (2.7)
D (10)	0	25 (2)	0	50 (2.5)	0	50 (2.5)	50 (2)	0
C (12)	0	0	50 (2)	25 (1)	0	25 (3)	0	25 (1)
B (14)	0	0	0	50 (1.5)	0	75 (2.3)	25 (1)	0
A (16)	0	0	0	50 (1.5)	0	75 (2.3)	25 (1)	0

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 164: Percentage of Main Study Animals with Multifocal Granulomatous Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	0	25 (1)	0	0	50 (1)	0	50 (1.5)
E (4)	0	0	0	0	0	50 (1)	25 (1)	50 (1.5)
D (10)	0	0	0	25 (1)	0	75 (1.7)	25 (1)	0
C (12)	0	0	50 (1)	50 (1)	0	50 (1.5)	0	0
B (14)	0	0	0	25 (1)	0	75 (2)	25 (1)	75 (1.3)
A (16)	0	0	0	25 (2)	0	50 (1)	0	75 (1.7)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 165: Percentage of Recovery Animals with Multifocal Granulomatous Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	0	0	0	0	100 (1.5)	50 (1)	0
E (18)	0	0	0	0	0	0	50 (1)	0
D (24)	0	0	0	0	0	50 (1)	50 (1)	0
C (26)	0	0	0	0	0	50 (1)	50 (1)	50 (1)
B (28)	0	0	0	0	0	0	50 (1)	50 (1)
A (30)	0	0	0	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 166: Percentage of Main Study Animals with Multifocal Fibrosis

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	100 (3.8)	0	0	0	75 (4.3)	50 (3.5)	25 (4)
E (4)	0	100 (4.5)	25 (1)	0	0	100 (3)	25 (2)	75 (2.7)
D (10)	0	100 (1.8)	0	75 (1.3)	0	75 (2.7)	25 (2)	25 (1)
C (12)	0	50 (3)	50 (1.5)	50 (2)	0	75 (1.7)	50 (1.5)	50 (1.5)
B (14)	0	25 (3)	25 (2)	0	0	50 (3.5)	0	75 (2.7)
A (16)	0	0	25 (2)	25 (2)	0	75 (2.3)	0	25 (3)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 167: Percentage of Recovery Animals with Multifocal Fibrosis

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	50 (1)	0	0	0	0	50 (1)	0
E (18)	0	0	0	0	0	50 (4)	0	50 (2)
D (24)	0	50 (1)	0	0	0	100 (2.5)	0	0
C (26)	0	0	0	0	0	0	0	50 (2)
B (28)	0	0	0	0	0	0	0	0
A (30)	0	0	0	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 168: Percentage of Main Study Animals with Multifocal Hemorrhage

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	25 (3)	0	50 (3)	0	75 (1.7)	50 (1.5)	75 (2)
E (4)	0	75 (1.7)	0	0	0	50 (2.5)	0	50 (1.5)
D (10)	0	25 (1)	0	50 (1.5)	0	0	0	0
C (12)	0	0	25 (1)	25 (1)	0	0	0	25 (1)
B (14)	0	0	0	25 (2)	0	0	0	0
A (16)	0	0	0	0	0	0	0	0

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 169: Percentage of Main Study Animals with Multifocal Presence of Test Material

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	50 (3.5)	25 (2)	75 (4.3)	0	50 (4)	25 (3)	75 (4)
E (4)	0	0	25 (4)	25 (4)	0	50 (4)	50 (3)	50 (3.5)
D (10)	0	25 (2)	0	75 (1.7)	0	25 (4)	25 (1)	0
C (12)	0	0	25 (2)	0	0	0	0	25 (4)
B (14)	0	0	0	0	0	0	0	0
A (16)	0	0	25 (2)	0	0	0	0	25 (4)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Toxicokinetics

All animals were bled via the jugular vein on Days 1, 9, and 27. Urine was collected from vehicle and high-dose animals in Week 3 for analysis of polymer breakdown products.

Bupivacaine

Bupivacaine C_{max} increased with time; a large increase was seen between Day 1 and Day 9 and a smaller increase between Day 9 and Day 27. C_{max} values were similar between the low- and high-dose groups, which isn't surprising considering how closely spaced the doses were. There was a good deal of variability to the T_{max} with values ranging from 1-8 hours across the study. There did not appear to be any time or dose trend to the T_{max} values. Over the course of the experiment, females generally had higher T_{max} values. The AUC values increased with time; again a larger increase was seen between Day 1 and Day 9 compared to Day 9 and Day 27. AUC values were generally comparable between the low- and high-dose groups in females. In males, AUC in the high-dose group tended to be higher (27-61%) and was generally in line with the increased amount of bupivacaine in the high dose (30%).

Table 170: Bupivacaine PK Parameters

Dose (mg/kg/dose)	Day	Sex		C _{max} (ng/mL)	t _{max} (h) ^a	AUC _{0-48h} (ng•h/mL)
10	1	Male	Mean	289	1	7460
			SD	160	1-4	2010
			n	6	6	6
		Female	Mean	315	5	11100
			SD	68.7	1-12	3010
			n	6	6	6
	9	Male	Mean	514	1	13900
			SD	152	1-4	3120
			n	6	6	6
		Female	Mean	567	1	15100
			SD	126	0.5-6	3700
			n	6	6	6
27	Male	Mean	424	2.5	11600	
		SD	152	1-4	4050	
		n	6	6	6	
	Female	Mean	736	1	18400	
		SD	693	0.5-4	13000	
		n	6	6	6	
13	1	Male	Mean	280	4	10500
			SD	80.1	1-24	2910
			n	6	6	6
		Female	Mean	271	8	9890
			SD	53.5	1-48	1760
			n	6	6	6
	9	Male	Mean	581	1	17600
			SD	142	1-1	4330
			n	6	6	6
		Female	Mean	498	4	15600
			SD	106	1-24	2710
			n	6	6	6
	27	Male	Mean	639	1	18700
			SD	183	1-4	6500
			n	6	6	6
		Female	Mean	622	2.5	17500
			SD	312	1-4	8230
			n	6	6	6

^a t_{max} is reported as median and minimum-maximum for group summary statistics instead of mean and standard deviation (SD).

Meloxicam

Meloxicam C_{max} increased with time; a large increase was seen between Day 1 and Day 9 and there was essentially no difference between Day 9 and Day 27. C_{max} values were slightly higher with the high dose, which is in line with the small difference between the two dose groups. There was a good deal of variability to the T_{max} with values ranging

from 0.75-48 hours across the study. The majority of the T_{max} values were on the higher end (18-48 hours). There did not appear to be any time, sex, or dose trend to the T_{max} values. The AUC values increased with time; again a larger increase was seen between Day 1 and Day 9 compared to Day 9 and Day 27. AUC values in the high dose group tended to be higher (15-44%) and were generally in line with the increased amount of meloxicam in the high dose (30%).

Table 171: Meloxicam PK Parameters

Dose (mg/kg/dose)	Day	Sex		C _{max} (ng/mL)	t _{max} (h) ^a	AUC _{0-48h} (ng•h/mL)
0.30	1	Male	Mean	359	36	13100
			SD	57.0	24-48	2200
			n	6	6	6
		Female	Mean	370	48	12700
			SD	78.1	24-48	2750
			n	6	6	6
	9	Male	Mean	760	18	30900
			SD	146	4-24	6010
			n	6	6	6
		Female	Mean	749	0.75	28700
			SD	212	0.5-24	8100
			n	6	6	6
27	Male	Mean	876	8	35500	
		SD	118	4-24	5480	
		n	6	6	6	
	Female	Mean	728	3.5	30400	
		SD	228	0.5-48	9300	
		n	6	6	6	
0.39	1	Male	Mean	412	36	15200
			SD	33.8	24-48	1630
			n	6	6	6
		Female	Mean	470	48	15500
			SD	95.0	24-48	3480
			n	6	6	6
	9	Male	Mean	1050	24	43100
			SD	342	4-24	15500
			n	6	6	6
		Female	Mean	895	18	33700
			SD	343	0-24	11700
			n	6	6	6
27	Male	Mean	1150	4	45500	
		SD	272	4-24	13000	
		n	6	6	6	
	Female	Mean	1050	36	45400	
		SD	207	0.5-48	8790	
		n	6	6	6	

^a t_{max} is reported as median and minimum-maximum for group summary statistics instead of mean and standard deviation (SD).

Polymer breakdown products

Urine collected on Day 22 was analyzed for the presence of breakdown products of the polymer (AP135) using a non-validated method for exploratory purposes. The breakdown products measured were (b) (4)

(b) (4) For all compounds, the levels were over the calibration range and extrapolated values were reported when available. For (b) (4) the values were above the calibration range (b) (4) mcg/mL) but estimated values were not reported. Excluding (b) (4) the most prevalent breakdown product was (b) (4) followed by (b) (4) and then (b) (4)

Dosing Solution Analysis

The vehicle was analyzed at the end of the study (March 6, 2018) to compare to the results from the Certificate of Analysis provided by the Applicant dated March 19th 2018. There were no differences between the CoA and the analysis. Meloxicam and bupivacaine were not detected in the vehicle.

HTX-011 was not analyzed. The Applicant provided characterization of the test substance including stability data. The CoA for Lot 026517 indicated that the test substance had 99.9% bupivacaine and 97.3% meloxicam with a retest date of November 2018 (24 months after manufacture) that is later than the end of Study 33-86.

7 Genetic Toxicology

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

Study title:

Study no.: 33-11
 Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\33-11\33-11.pdf>

Conducting laboratory and location:

(b) (4)

Date of study initiation: July 18, 2014
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: HTX-011-15 (10.0% bupivacaine, 1.5% meloxicam, 72.29% APF135, 1.21% maleic acid, 15.0% N-methylpyrrolidone) Lot 1257021, not applicable since it is a mixture

Key Study Findings

The study was considered invalid due to a very low response from the positive controls that was well below the laboratories historical control values.

Methods

Strains:	TA1535, TA1537, TA98, TA100, and WP2 <i>uvrA</i>
Concentrations in definitive study:	50, 158, 500, 1581, and 5000 mcg/plate
Basis of concentration selection:	Based on maximum recommended dose in ICH S2 (R1)
Negative control:	DMSO
Positive control:	Sodium azide (TA1535 and TA100, without metabolic activation), 9-aminoacrididine hemihydrate (TA1537 without metabolic activation), 2-nitrofluorene (TA98 without metabolic activation), 4-nitroquinoline N-oxide (WP2 <i>uvrA</i> without metabolic activation), 2-aminoanthracene (TA 1535 and WP2 <i>uvrA</i> , with metabolic activation), and benzo[a]pyrene (TA1537, TA98, and TA100, with metabolic activation)
Formulation/Vehicle:	HTX-011-15 was dissolved in DMSO
Incubation & sampling time:	An aliquot of 0.5 mL of S9 mix (+S9) or phosphate buffer 0.1 M pH 7.4 (0S9) was combined with 0.1 mL bacterial culture in a sterile container. Appropriate volume, as indicated in the study design, of the test/negative and positive control items was added. Molten top agar (approximately 2 mL) supplemented with 0.05 mM biotin and minimal (0.05 mM) histidine and minimal (0.05 mM) tryptophan was added to the sample. The solution was mixed and overlaid on a minimal glucose plate (1.5% agar, Vogel-Bonner medium E, 2% glucose). After the overlay solidified, the plates were inverted and placed in an incubator set to maintain 37°C for 48 to 72 hours. The sterile containers and the plates were labeled with the ^{(b) (4)} study number and plate identification number.

Study Validity

The following criteria were determined to be valid:

- Selection of bacterial tester strains was adequate based upon Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (ICH-S2(R1); June 2012).
- Historical data were provided.
- Cytotoxicity was properly evaluated.
- The appropriate positive and negative controls were employed according to OECD guideline no. 471
- All strains contain the appropriate genetic markers.
- Dose selection for the plate incorporation were adequate based upon use of the limit dose (i.e., 5000 mcg/plate).
- The S9 concentration was within acceptable limits.

However, the assay was determined to be invalid because the responses from the positive controls were well below the historical averages for the laboratory (see tables below).

Results

Overall there was not a positive response with the HTX-011-15 formulation in the presence and absence of S9. The positive controls did cause a small increase in the number of revertant colonies but in many cases they were barely 2-fold above the vehicle control. Generally a positive response is a doubling or tripling over the negative control. The laboratory included historical control data. The positive controls were far below the historical control values for the laboratory. Therefore, the assay is considered invalid.

Table 172: Plate Incorporation in Absence of S9

Strains	Dose level (µg/plate of RYR)	Number of revertants			Mean	SD	Background observations			Fold response†
		<i>x1</i>	<i>x2</i>	<i>x3</i>			<i>x1</i>	<i>x2</i>	<i>x3</i>	
TA1535	DMSO	1	1	1	1.0	0.0				
	50	2	1	0	1.0	1.0				1.0
	158	0	2	4	2.0	2.0				2.0 ^b
	500	2	3	1	2.0	1.0				2.0 ^b
	1581	1	2	1	1.3	0.6				1.3
	5000	1	1	1	1.0	0.0				1.0
TA1537	DMSO	12	8	8	9.3	2.3				
	50	10	8	8	8.7	1.2				0.9
	158	7	6	11	8.0	2.7				0.9
	500	9	13	15	12.3	3.1				1.3
	1581	5	5	9	6.3	2.3				0.7
	5000	22	12	12	15.3	5.8				1.6
TA98	DMSO	9	12	6	9.0	3.0				
	50	8	7	10	8.3	1.5				0.9
	158	1	6	7	4.7	3.2				0.5
	500	5	6	10	7.0	2.7				0.8
	1581	7	5	1	4.3	3.1				0.5 ^a
	5000	5	9	6	6.7	2.1				0.7
TA100	DMSO	44	50	32	42.0	9.2				
	50	41	46	66	51.0	13.2				1.2
	158	36	27	54	39.0	13.8				0.9
	500	36	30	41	35.7	5.5				0.9
	1581	20	24	42	28.7	11.7				0.7
	5000	31	21	34	28.7	6.8				0.7
WP2 uvrA	DMSO	4	4	3	3.7	0.6				
	50	3	8	4	5.0	2.7				1.4
	158	5	5	5	5.0	0.0				1.4
	500	4	5	4	4.3	0.6				1.2
	1581	3	6	7	5.3	2.1				1.5
	5000	2	3	2	2.3	0.6				0.6

Comments on plate and/or background lawn (as appropriate) : Normal (no comment), MC: Methylcellulose

†: Fold response in mean revertants compared to concurrent vehicle; SD: Sample standard deviation

^a: Not biologically relevant decrease; ^b: Not biologically relevant positive response

The table above is from page 18 of the study report.

Table 173: Plate Incorporation in Presence of S9

Strains	Dose level (μ g/plate of RZR)	Number of revertants			Mean	SD	Background observations			Fold response [†]
		<i>x1</i>	<i>x2</i>	<i>x3</i>			<i>x1</i>	<i>x2</i>	<i>x3</i>	
TA1535	DMSO	4	8	2	4.7	3.1				
	50	3	4	3	3.3	0.6				0.7
	158	1	1	3	1.7	1.2				0.4
	500	3	1	7	3.7	3.1				0.8
	1581	3	5	6	4.7	1.5				1.0
	5000	2	3	3	2.7	0.6				0.6
TA1537	DMSO	7	15	13	11.7	4.2				
	50	13	10	9	10.7	2.1				0.9
	158	5	10	10	8.3	2.9				0.7
	500	9	8	7	8.0	1.0				0.7
	1581	13	11	6	10.0	3.6				0.9
	5000	6	5	5	5.3	0.6				0.5T
TA98	DMSO	16	27	23	22.0	5.6				
	50	12	23	16	17.0	5.6				0.8
	158	14	14	18	15.3	2.3				0.7
	500	18	16	18	17.3	1.2				0.8
	1581	12	11	9	10.7	1.5				0.5 ^a
	5000	8	15	13	12.0	3.6				0.6
TA100	DMSO	35	48	51	44.7	8.5				
	50	55	36	54	48.3	10.7				1.1
	158	51	62	43	52.0	9.5				1.2
	500	44	42	66	50.7	13.3				1.1
	1581	39	54	46	46.3	7.5				1.0
	5000	44	38	35	39.0	4.6				0.9
WP2 <i>uvrA</i>	DMSO	10	7	8	8.3	1.5				
	50	6	9	10	8.3	2.1				1.0
	158	5	5	9	6.3	2.3				0.8
	500	6	4	10	6.7	3.1				0.8
	1581	8	6	1	5.0	3.6				0.6
	5000	2	2	4	2.7	1.2				0.3T

Comments on plate and/or background lawn (as appropriate) : Normal (no comment)

[†]: Fold response in mean revertants compared to concurrent vehicle; SD: Sample standard deviation

T: Toxic; ^a: Non biologically relevant decrease

The table above is from page 19 of the study report.

Table 174: Positive Control Data

Strains	S9	Compound	Dose level (µg/plate)	Number of revertants			Mean	SD	Fold response†
				x1	x2	x3			
TA1535	-	SAZ	0.5	71	78	91	80.0	10.1	80.0
TA1537	-	9AC	20	7	22	26	18.3	10.0	2.0
TA98	-	2NF	1.25	11	29	30	23.3	10.7	2.6
TA100	-	SAZ	0.5	111	117	72	100.0	24.4	2.4
WP2 <i>uvrA</i>	-	NQO	0.5	9	5	11	8.3	3.1	2.3
TA1535	+	2AA	5	35	46	52	44.3	8.6	9.4
TA1537	+	B[a]P	5	34	29	36	33.0	3.6	2.8
TA98	+	B[a]P	2	167	165	174	168.7	4.7	7.7
TA100	+	B[a]P	5	568	586	518	557.3	35.2	12.5
TA100	+	B[a]P	10	497	481	521	499.7	20.1	11.2
WP2 <i>uvrA</i>	+	2AA	15	28	16	27	23.7	6.7	2.9

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

The table above is from page 20 of the study report

Table 175: Historical Control Data

Vehicle controls, without S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	18.0	11.7	25.0	141.6	26.8
Standard deviation	12.9	3.0	6.4	24.9	7.2
Minimum	6	7	14	105	16
Maximum	64	19	38	185	40
Number of experiments	6	6	6	6	6
Vehicle controls, with S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	15.7	13.5	34.0	141.4	29.2
Standard deviation	5.3	5.7	9.0	25.1	9.2
Minimum	6	4	22	90	14
Maximum	25	28	56	196	52
Number of experiments	6	6	6	6	6
Positive controls, without S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	489.2	327.7	182.6	611.9	140.2
Standard deviation	88.9	166.5	38.5	189.5	51.4
Minimum	400	152	137	400	84
Maximum	626	697	237	875	222
Number of experiments	3	3	3	3	3
Positive controls, with S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	229.3	-	134.0	-	107.9
Standard deviation	98.7	-	28.2	-	28.8
Minimum	91	-	100	-	69
Maximum	347	-	191	-	163
Number of experiments	3	-	3	-	3

Positive controls without S9 Mix

TA1535 SAZ (0.5 µg/plate)
 TA1537 9AC (20 µg/plate)
 TA98 2NF (1.25 µg/plate)
 TA100 SAZ (0.5 µg/plate)
 WP2 *uvrA* NQO (0.5 µg/plate)

Positive controls with S9 Mix

TA1535 2AA (5 µg/plate)
 TA98 B[a]P (2 µg/plate)
 WP2 *uvrA* 2AA (5 µg/plate)

Table above is from page 22 of the study report.

The Applicant is also relying upon the data for the two drug substances as described in the referenced drug product labels.

The referenced Marcaine label (NDA 16964) contains the following information:

The mutagenic potential and the effect on fertility of bupivacaine hydrochloride have not been determined.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

Meloxicam was not mutagenic in an Ames assay, or clastogenic in a chromosome aberration assay with human lymphocytes and an in vivo micronucleus test in mouse bone marrow.

Although the exposure to bupivacaine via this drug product technically exceeds that of the referenced drug product, the increase in exposure is not considered to be of the magnitude that would warrant requiring an Applicant to conduct these studies for an older drug product.

8 Carcinogenicity

Carcinogenicity studies with HTX-011 are not required for an acute-use drug.

The referenced Marcaine label (NDA 16964) contains the following information:

Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine hydrochloride have not been conducted.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

No carcinogenic effect of meloxicam was observed in rats given oral doses up to 0.8 mg/kg/day (approximately 0.4-fold the human dose at 15 mg/day for a 50 kg adult based on body surface area conversion) for 104 weeks or in mice given oral doses up to 8.0 mg/kg/day (approximately 2.2-fold the human dose, as noted above) for 99 weeks.

Exposure margins will be adjusted for this drug product maximum daily dose of meloxicam.

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicity studies were conducted with HTX-011. The Applicant is relying upon the Agency previous finding of safety of bupivacaine and

meloxicam via the 505(b)(2) pathway. A combination embryo-fetal development study was not required because the existing data on meloxicam and bupivacaine, as described in the referenced product labeling, already report significant adverse effects at doses below the clinical dose and the pharmacological and systemic toxicological profile of the two drugs do not suggest the potential for additive toxicity.

As part of the requirement under the Pregnancy Lactation and Labeling Rule (PLLR), the Applicant conducted a review of the nonclinical literature for bupivacaine (1972 to 2018) and meloxicam (1990 to 2018). Many of the articles referenced by the Applicant were already previously reviewed during recent label updates for meloxicam and bupivacaine containing products. As such, these articles were already taken into consideration as part of the labeling of other meloxicam and bupivacaine products and the labeling for HTX-011 will reflect the existing data in the referenced labels adjusted for dose plus any relevant information identified in the PLLR updates for meloxicam and bupivacaine products. The following section describes the literature data submitted by the Applicant that was not evaluated for other products and the relevance of these studies for labeling.

In addition, the Applicant submitted an embryo-fetal developmental study with maleic acid to justify the safety of this excipient and is reviewed below in Section 9.2.

The referenced Marcaine label (NDA 16964) contains the following information:

The mutagenic potential and the effect on fertility of bupivacaine hydrochloride have not been determined.

Pregnancy

Bupivacaine hydrochloride produced developmental toxicity when administered subcutaneously to pregnant rats and rabbits at clinically relevant doses.

Bupivacaine hydrochloride was administered subcutaneously to rats at doses of 4.4, 13.3, & 40 mg/kg and to rabbits at doses of 1.3, 5.8, & 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). The high doses are comparable to the daily maximum recommended human dose (MRHD) of 400 mg/day on a mg/m² body surface area (BSA) basis. No embryo-fetal effects were observed in rats at the high dose which caused increased maternal lethality. An increase in embryo-fetal deaths was observed in rabbits at the high dose in the absence of maternal toxicity with the fetal No Observed Adverse Effect Level representing approximately 1/5th the MRHD on a BSA basis.

In a rat pre- and post-natal development study (dosing from implantation through weaning) conducted at subcutaneous doses of 4.4, 13.3, & 40 mg/kg, decreased pup survival was observed at the high dose. The high dose is comparable to the daily MRHD of 400 mg/day on a BSA basis.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

Meloxicam did not impair male and female fertility in rats at oral doses up to 9 and 5 mg/kg/day, respectively (4.9-fold and 2.5-fold the human dose, as noted above). However, an increased incidence of embryoletality at oral doses ≥ 1 mg/kg/day (0.5-fold the human dose, as noted above) was observed in rats when dams were given meloxicam 2 weeks prior to mating and during early embryonic development.

Exposure margins will be adjusted for this drug product based on the maximum daily dose of meloxicam.

Bupivacaine and Pregnancy/Lactation – Animals literature review of studies not previously reviewed for other bupivacaine and meloxicam products

Intravenous injection of bupivacaine in the pregnant ewe. (Craft et al., 1979)

Objective: To evaluate the effect of bolus intravenous injections of bupivacaine on maternal and fetal cardiovascular dynamics, catecholamines, uterine blood flow and fetal acid-base status

Method: Bupivacaine (10, 20, 30, or 40 mg) was injected *intravenously* into eight pregnant ewes 24 hours following hysterotomy under anesthesia. Maternal, uterine and fetal vascular dynamics were monitored continuously. Maternal and fetal arterial blood samples were obtained at 1, 3, 5, 15, 30, 45, and 60 minutes after injection for acid-base, local anesthetic, and catecholamine determination.

Key Findings:

- 10 mg of bupivacaine increased maternal cardiac output but did not affect maternal or fetal acid-base status.
- 40 mg of bupivacaine resulted in sustained fetal hypoxemia resulting from decreased uterine blood flow, increased uterine tone, and decreased maternal PaO₂.

Reviewer's note: The study data are from a meeting abstract with limited information. The results are mechanistic in nature. While they describe an effect on the fetus at 40 mg in terms of decreased PaO₂, it is unclear what the toxicological significance of this finding would be. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Transfer of bupivacaine, lignocaine, and pethidine across the rabbit placenta: influence of maternal protein binding and fetal flow. (Hamshaw-Thomas et al., 1984)

Objective: To evaluate the effect of umbilical flow on the placental transfer of compounds (including bupivacaine) with differing lipid solubility and protein binding

Method: Bupivacaine, lignocaine, pethidine (1.25 mg/mL each), and antipyrine (4.0 mg/mL) were infused intravenously into a neck vein of 17 anesthetized pregnant New Zealand white rabbits (3.44 to 4.95 kg) within three days of term (30 days) at a rate of 12.0 mL/h for 20 minutes and 6.0 mL/h thereafter. The rabbit placentas were perfused in situ under different flow rates. Placental perfusions and maternal blood samples were analyzed for bupivacaine, lignocaine, pethidine, and antipyrine. Maternal and fetal plasma were analyzed for protein binding.

Key Findings:

- Transfer rates across the placenta at flow rates of 0.5 to 2.0 mL/min reflected the compounds protein binding not their lipid solubilities.
- Bupivacaine, which had the highest % drug bound (76.5%), had the lowest transfer rates of the compounds studied.

Reviewer's note: The study is mechanistic in nature and only describes the ability of bupivacaine to cross the placenta. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Placental transfer of bupivacaine, pethidine and lignocaine in the rabbit. Effect of umbilical flow rate and protein content. (Hamshaw-Thomas et al., 1985)

Objective: To evaluate the effects of protein binding, fetal perfusate glycoprotein content, and umbilical flow on placental transfer of bupivacaine, lignocaine and pethidine with antipyrine

Method: Bupivacaine, lignocaine, pethidine (1.25 mg/mL each), and antipyrine (4.0 mg/mL) were infused intravenously into 11 anesthetized pregnant New Zealand white rabbits (4.4 to 5.5 kg) within three days of term (30 days) at a rate of 12.0 mL/h for 20 minutes and 6.0 mL/h thereafter. The rabbit placentas were perfused in situ under different flow rates. Placental perfusions and maternal blood samples were analyzed for bupivacaine, lignocaine, pethidine, and antipyrine. Maternal and fetal plasma were analyzed for protein binding.

Key Findings:

- Protein on the fetal side enhanced transfer of highly bound drugs, such as bupivacaine.
- Clearance of the drugs was flow-dependent.

Reviewer's note: The study is mechanistic in nature and only describes the ability of bupivacaine to cross the placenta. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal

development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

The placental transfer of bupivacaine and lidocaine and their myocardial and cerebral uptake in the rabbit. (Hollmen et al., 1973)

Objective: To compare the extent of binding of lidocaine and bupivacaine to fetal heart and brain in the rabbit when injected intravenously

Method: Tritiated bupivacaine (0.3 mg/kg) or lidocaine (0.6 mg/kg) were administered IV to 40 near-term anesthetized pregnant rabbits. Caesarian section was performed at 2, 5, 8, 20, and 45 minutes after injection and fetuses were sacrificed. Maternal heart and arterial blood were also collected. Maternal heart, maternal blood, fetal heart, fetal brain, and fetal ventricular blood were all analyzed for bupivacaine and lidocaine.

Key Findings:

- Fetal heart/blood and brain/blood ratios were lower for bupivacaine as compared to lidocaine.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine can cross the placenta and reach the fetal heart and brain to some extent. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

In vivo comparison of the effects of bupivacaine and levobupivacaine on the pregnant rat myometrium using electrohysterogram. (Kaynak et al., 2012)

Objective: To compare the effects of bupivacaine and levobupivacaine on rat uterine contractility in an in vivo setting

Method: 20 rats (23 days pregnant) weighing 150-200 g where anesthetized and underwent laparotomy. Electrical activity of the uterus was measured every five minutes for a total of 35 minutes. Bupivacaine or levobupivacaine were administered intramyometrially at a dose of 2 mg/kg after the first recording (at five minutes).

Key Findings:

- Both compounds led to lower frequencies of uterine contractions compared to the controls.
- Levobupivacaine led to less muscle relaxation as compared to bupivacaine.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine can decrease uterine contractions. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development

animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Uptake and distribution of bupivacaine in fetal lambs. (Kennedy et al., 1986)

Objective: To determine the uptake and distribution of bupivacaine in fetal lambs

Method: Bupivacaine (3 mg/kg) was infused into a jugular vein at a constant rate over 1 hour into five pregnant ewes. It is unclear at what time of pregnancy the ewes were, but single pregnancy was confirmed by x-ray. Following injection of bupivacaine, blood was sampled from the maternal femoral artery, the umbilical vein, and the fetal distal aorta. After five hours the fetuses were killed, removed, and dissected.

Key Findings:

- Fetal blood concentrations were less than maternal blood concentrations.
- PPX was not found in urine or tissue samples suggesting that neither the mother nor fetus was metabolizing bupivacaine.
- Bupivacaine diffused back from the fetus to the mother as evidenced by a lower umbilical vein concentration compared to the fetal aorta concentration.
- Fetal uptake was linear over the course of the infusion.
- The high correlation of mean fetal bupivacaine uptake to the maternal dose suggests a flow-independent fetal uptake.
- The highest levels of fetal bupivacaine were in the lung (~0.09 nmoles/g tissue). Bupivacaine was also detected in the brain (~0.06 nmoles/g tissue), kidney (~0.08 nmoles/g tissue), heart (~0.06 nmoles/g tissue), and liver (~0.06 nmoles/g tissue).

Reviewer's note: The study is mechanistic in nature and describes placental transfer of bupivacaine in sheep, including distribution data in the fetus. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Effects of spinal blockade with bupivacaine on parturition in rats. (Li et al., 1985)

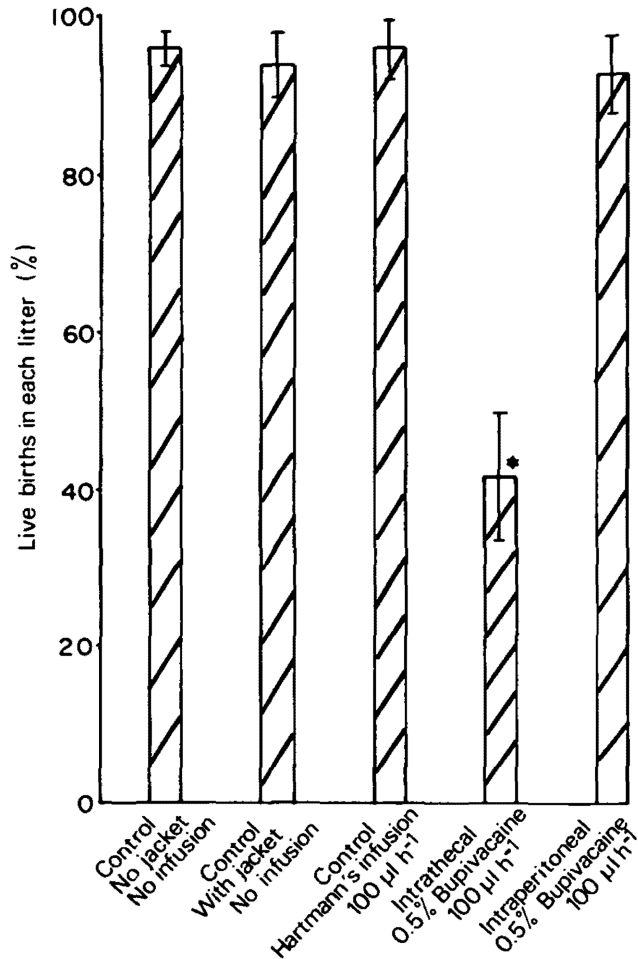
Objective: To observe the effects of differential degrees of motor and sensory blockade on the parturition process

Method: Pregnant Wistar rats (180-220 g prior to pregnancy) were anesthetized on the 16-18th day of gestation and fitted with a catheter in the intrathecal space. The animals were divided into five groups: 1) control with no jacket or infusion, 2) control with jacket

but no infusion, 3) 100 mcL/h infusion of Hartmann's solution, 4) 100 mcL/h infusion of 0.5% bupivacaine intrathecal, 5) 100 mcL/h infusion of 0.5% bupivacaine intraperitoneal. The rats were then left until delivery was complete monitored by video.

Key Findings:

- There was no difference in the number of pups per litter.
- Delivery was ~3 times longer in the group receiving intrathecal blockade with 0.5% bupivacaine. This appeared to be due to a lack of maternal effort.
- The mean percentage of live births per litter surviving the first 6 hours was reduced in the group receiving intrathecal blockade with 0.5% bupivacaine. (42% vs. 94-96% in the control groups). There was no reduction in the group receiving IP bupivacaine (93%).



Reviewer's note: The study describes an effect on fetal mortality following intrathecal administration of bupivacaine. A similar effect was not observed following IP administration. The authors attributed the effects on perinatal mortality to the prolonged

labor and not to a direct effect on the pups, which is supported by the lack of an effect following IP dosing. The information in this report is supportive of the embryo-fetal mortality but does not add any additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Effects of levobupivacaine and bupivacaine on rat myometrium. (Li et al., 2006)

Objective: To study the effect of levobupivacaine and bupivacaine on the contractility of isolated muscle strips from pregnant and non-pregnant rats.

Method: Pregnant rats (18-21 days) and non-pregnant female rats (~200 g) were treated with 40 mcg diethylstilbestrol by SC injection for two days to induce estrus. Rats were killed and myometrial strips were prepared. Once contractions of the strips became regular, baseline frequency and amplitude were measured followed by addition of either bupivacaine or levobupivacaine.

Key Findings:

- In non-pregnant rat myometrial strips, both bupivacaine and levobupivacaine (at cumulative concentrations ranging from 10^{-8} to 10^{-4} mol/L) decreased contractile activity. Levobupivacaine was stronger than bupivacaine.
- In pregnant rat myometrial strips, bupivacaine and levobupivacaine did not have an effect at lower cumulative concentrations but did reduce contractile activity at higher cumulative concentrations.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine can reduce muscle contractility. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Comparative effects of sodium channel blockers in short term rat whole embryo culture. (Nilsson et al., 2013)

Objective: To examine the effect on the rat embryonic heart of two experimental drugs (AZA and AZB)

Method: Pregnant rats were euthanized on Gestational Day 13. The uterus was removed and opened so that the decidual swellings could be removed. From the swellings, the embryo was removed and cultured in Dulbecco's Modified Eagle Medium. After 1 hour, embryos were examined for a normal heart rate of 140 beats per minute. Then 20 mcL of test compound was added. Embryos were inspected 1, 2, and 3 hours after addition of compound. Bupivacaine was tested as a control in this experiment at a concentration of 10 mcM.

Key Findings:

- Bupivacaine caused severe bradycardia that increased in severity with time. This effect was reversed by a washout.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine can adversely affect the fetal heart in vitro. Due to the in vitro nature of the experiment it is unclear the clinical significance of the findings. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

The effect of foetal acidosis on bupivacaine levels in utero. (Pickering et al., 1981)

Objective: To determine if the highly protein bound local anesthetic bupivacaine demonstrates ion-trapping in the acidotic fetus

Method: Six near term (mean gestational age of 135 days) underwent hysterectomy to insert catheters for blood collection. Animals were given 24 hours to recover before dosing. The experiment consisted of three phases: 1) maternal bupivacaine infusion (120 minutes; 0.08 mg/kg/min bupivacaine), 2) maternal bupivacaine infusion plus fetal acid infusion (45 minutes; 2.5 M lactic acid), and 3) maternal bupivacaine infusion and fetal bicarbonate infusion (45 minutes; 0.1M sodium bicarbonate). Measurements included maternal and fetal arterial blood pressure, pulse, blood gases, pH, and bupivacaine levels.

Key Findings:

- Infusion of acid into the fetus caused an increase in the fetal arterial concentration of bupivacaine and the fetal/maternal ratio.
- Infusion of bicarbonate to correct the pH reduced the fetal arterial concentration of bupivacaine and the fetal/maternal ratio back to control values.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine concentration in the fetus is pH dependent. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

The electrophysiologic effects of bupivacaine on adult, neonatal, and fetal guinea pig papillary muscles. (Sun et al., 1991)

Objective: To study developmental changes in the effects of bupivacaine on the transmembrane potentials of adult, neonatal, and fetal guinea pig papillary muscles using standard microelectrode techniques

Method: Neonatal (postnatal age of 1-10 days), nonpregnant adult, and pregnant adult guinea pigs were used in this experiment. Hysterotomies were performed on pregnant

animals and fetuses (gestational age of 62-66 days) were removed. Sternotomies were performed on all animals and the hearts were collected. Papillary muscles from either ventricle were dissected for measurement of transmembrane potentials. Tonic block was achieved using bupivacaine concentrations of 0.2, 1.0, and 1.5 mcg/mL for 30 mins each.

Key Findings:

- 1.5 mcg/mL bupivacaine hyperpolarized maximum diastolic potential in adult and neonatal guinea pig muscles, but not fetal muscles.
- Bupivacaine did not change the action potential duration (APD) in adult or neonatal tissue. In the fetal tissue, 1.0 and 1.5 mcg/mL bupivacaine shortened APD₅₀ and APD₁₀₀.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine may affect heart muscle APD to a greater extent than in adults or neonates. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Epidural anesthesia worsens uterine blood flow and fetal oxygenation during hemorrhage in gravid ewes. (Vincent et al., 1992)

Objective: To determine whether epidural anesthesia alters maternal and fetal hemodynamic and acid-base responses to hemorrhage in gravid ewes

Method: Pregnant ewes of 120 days of gestation underwent laparotomy and hysterotomy for inserting catheters and allowed to recover for at least three days. On the day of the experiment, each animal received an intravenous bolus of 500 mL saline. 15 minutes later 6-20 mL of 0.5% bupivacaine or saline was delivered through the epidural catheter. At 30 mins after the first injection, an additional 0.5% of bupivacaine was delivered if the sensory level of analgesia was lower than T10. Also at 30 mins, each animal received 15,000 units of heparin intravenously. At 45 minutes after the first injection, each animal was bled for 20 ml/kg via the femoral arterial catheter. At 110 minutes after the first injection, the collected maternal arterial blood was reinfused over 55 minutes. Hemodynamic measurements were continued until the end of the experiment (170 minutes after the first injection). Parameters measured included maternal arterial blood pressure, maternal central venous pressure, maternal pulmonary artery pressure, fetal arterial blood pressure, maternal heart rate, fetal heart rate, uterine artery blood flow, maternal/fetal arterial blood gas, maternal/fetal arterial pH, and plasma norepinephrine and epinephrine concentrations.

Key Findings:

- Two fetal deaths occurred in the epidural group.

- Maternal heart rate was lower during hemorrhage in the epidural group.
- Maternal mean arterial blood pressure was lower in the epidural group before and during hemorrhage.
- Maternal cardiac output was lower in the epidural group before and during hemorrhage.
- Maternal systemic vascular resistance in the epidural group increased during hemorrhage.
- Uterine blood flow was lower during hemorrhage in the epidural group.
- Fetal PO₂ and pH were lower during hemorrhage in the epidural group.
- Plasma epinephrine concentrations dropped below baseline in the epidural group.

Reviewer's note: The study indicates that bupivacaine may adversely affect the compensatory response to untreated hemorrhage. Given the long clinical history of epidural anesthesia, the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Meloxicam

Effect of meloxicam on pregnancy rate of recipient heifers following transfer of in vitro produced embryos (Aguar et al., 2013)

Objective: To determine if administration of meloxicam is beneficial in heifers in which embryo transfer is more difficult

Method: In vitro embryo production blastocysts were transferred non-surgically. Recipients were divided into two groups depending on the degree of difficulty in passing the catheter through the cervix; easy (< 60 seconds) or difficult (> 80 seconds). Immediately after embryo transfer the recipients received an IM injection of 200 mg meloxicam.

Key Findings:

- Meloxicam increased the overall pregnancy rate (66.67% vs. 49.02% in control).
- When stratified by difficulty, meloxicam did not increase the pregnancy rate of easy heifers but did increase the pregnancy rate of difficult heifers (66.13% vs. 23.91% in controls).

Reviewer's note: The study indicates that meloxicam improves the pregnancy rate of difficult heifers receiving embryo transfer. This is not a toxicological finding and the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Combined administration of gonadotropin-releasing hormone, progesterone, and meloxicam is an effective treatment for the repeat-breeder cow. (Amiridis et al., 2009)

Objective: To test the hypothesis that a combined treatment would benefit the conception rate of repeat-breeder cows

Method: 402 cows in their second to fifth lactation, with a body condition score of 2.75 to 3.5 (scale of 1 to 5), producing more than 25 kg milk per day, with at least four unsuccessful inseminations, and were 128 to 285 days in milk were used in this study. Animals received either 0.012 mg IM buserelin 4 to 6 hours before AI, 100 mg P4 gelatin capsules administered intravaginally every 12 hours on Days 4, 5, and 6, 0.5 mg/kg meloxicam subcutaneously on Days 16, 17, and 18, or the combination of all three.

Key Findings:

- Pregnancy rate was increased by each individual treatment, but the highest effect was from the combination of all three treatments.
- Conception rate was increased by P4 and meloxicam but a larger effect was observed with the combination of all three treatments.

Reviewer's note: The study indicates that meloxicam slightly improves the pregnancy and conception rates in the cows treated. This is not a toxicological finding and the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Analysis of the nonsteroidal anti-inflammatory drug literature for potential developmental toxicity in rats and rabbits. (Cook et al., 2003)

Objective: To determine whether NSAIDs other than aspirin induce developmental anomalies similar to those elicited by aspirin.

Method: Developmental toxicity studies were identified through literature searches of PubMed and TOXNET, and pregnancy outcome data were extracted and tabulated. By using a set of defined criteria, each study was evaluated for quality and assigned to one of five tiers. The relation between certain malformations and NSAID treatment was analyzed for the best studies (tiers 1–4) by using concurrent control data (Mantel–

Haenszel and permutation tests) and by combining the concurrent control data with historical control data (w2 test and permutation tests).

Key Findings:

- In rats, the incidence of ventricular septal defects and midline defects in non-aspirin treated NSAID treated rats was about 1.5 times that of controls.
- In rabbits, the incidence of diaphragmatic hernia, ventricular septal defects and midline defects in non-aspirin treated NSAID treated rabbits were greater than the controls.

Reviewer's note: The study indicates that non-aspirin NSAIDs are possibly associated with developmental anomalies based on a literature review. The data are not specific to meloxicam and only suggests that there is a potential risk for all non-aspirin NSAIDs. In that regard, the developmental toxicity of meloxicam has already been characterized by nonclinical studies in rats and rabbits and is described in the labeling. This includes the finding of septal heart defects in rabbits treated with high doses of meloxicam. As such, this study does not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Hot topic: Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield. (Carpenter et al., 2016)

Objective: To replicate the results of a previous study showing that postpartum administration of sodium salicylate can increase 305-day milk yield in older dairy cattle by using meloxicam in place of sodium salicylate

Method: Multiparous cows (12 to 36 hours after calving) received either an oral drench of 125 g/d of sodium salicylate (~185 mg/kg) for three consecutive days or 675 mg of meloxicam (~1 mg/kg) as a bolus treatment on Day 1.

Key Findings:

- Both treatments increased daily and 305-day milk production compared to control.
- Both treatments increased the protein yield of the milk.

Reviewer's note: The study indicates that meloxicam can increase milk yield in dairy cows. This is not a toxicological finding and the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Prostaglandins regulate conceptus elongation and mediate effects of interferon tau on the ovine uterine endometrium. (Dorniak et al., 2011)

Objective: To test the hypothesis that prostaglandin (PG)s from the conceptus and perhaps the endometrium itself have autocrine, paracrine, and perhaps intracrine effects on conceptus development, endometrial function, and endometrial responses to progesterone and interferon tau (IFNT) in ruminants

Method: Ewes used in this study only if they exhibited at least two estrous cycles of normal duration (16-18 days). Mated ewes underwent a mid-ventral laparotomy on day 8 post-mating for insertion of a catheter and osmotic pump. The osmotic pumps contained either 2% ethanol saline or meloxicam (612.5 ng/mL). The pumps delivered 10 mcL/hour for seven days. Ewes were euthanized on Day 14 post-mating for evaluation of the blastocyst/conceptus development, uterine flushing, and processing of the uteri.

A second experiment was conducted with unmated ewes on day 10 postestrous. Ewes underwent a mid-ventral laparotomy for insertion of a catheter and osmotic pump. The osmotic pumps contained either 2% ethanol saline with 101 mcg ovine serum proteins, 101 mcg of recombinant ovine IFNT, meloxicam with 101 mcg ovine serum proteins, or recombinant ovine IFNT plus meloxicam. Ewes were euthanized on Day 14 post-mating for uterine flushing, and processing of the uteri.

Key Findings:

- Meloxicam inhibits conceptus elongation, reduces endometrial PG synthase activity, and reduces PGs in the uterine lumen.
- IFNT increases endometrial production of PGs and PGG/H synthase and cyclooxygenase 2 activity in cyclic ewes.
- Meloxicam impaired the ability of IFNT to modulate expression of genes in the endometrium.

Reviewer's note: The study is mechanistic in nature. It indicates that when meloxicam is given by intrauterine infusion, it has an inhibitory effect on prostaglandin synthase activity resulting in reduced prostaglandins and this results in elongation of the conceptus. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Comparison of milk and plasma pharmacokinetics of meloxicam in postpartum versus mid-lactation Holstein cows. (Gorden et al., 2018)

Objective: To determine whether differences occurred in meloxicam pharmacokinetics between postpartum cows and mid-lactation cows

Method: Cows were divided into two groups of either < 26 hours postpartum or in mid-lactation (>90 days in milk). Both groups received meloxicam. Cows were milked three times daily. Treatment with meloxicam was given following milking as a gelatin bolus dose of 1 mg/kg. Blood samples were collected from the jugular or coccygeal vein prior to dosing and at 2, 4, 6, 8, 12, 16, and 24 hours. Blood samples were collected every 8 hours past the 24-hour sample until the drug was cleared from the system. Milk samples were collected prior to dosing at every 8 hours after until meloxicam was below the detection limit.

Key Findings:

- Plasma levels of meloxicam were lower in mid-lactation cows as compared to postpartum cows, starting at around 8 hours post dosing.
- The milk concentration of meloxicam was lower in mid-lactation cows as compared to postpartum cows, starting at around 8 hours post dosing.
- Postpartum cows had a higher mean plasma C_{max} , T_{max} , AUC_{inf} , AUC_{last} and mean residence time compared to mid-lactation cows.
- The relative bioavailability in postpartum cows was 210% that of mid-lactation cows.

Reviewer's note: The study is a PK study as opposed to a toxicity study. It indicates that in cows, meloxicam stays in the milk longer at postpartum as opposed to mid-lactation. The clinical significance of this finding is unknown. In terms of the label, there is already animal data suggesting that meloxicam can be found in milk of rats and pigs. The addition of data that it is also in the milk of cows does not really enhance the label in a meaningful way. This reviewer does not consider the study data necessary to be included in the label.

Effects of meloxicam on reproduction parameters in dairy cattle. (Hirsch and Philipp 2009)

Objective: To investigate possible drug effects on different reproductive parameters as well as on the progeny

Method: 38 healthy lactating cows were used in the study (18 meloxicam and 20 control). Day 0 was defined as the day of artificial insemination. Cows received a single IV injection of 1.5 mg/kg meloxicam or placebo into the jugular vein on Days -12, 9, 35, 105, 203, and 259. The following indices were calculated: insemination index, pregnancy rate, embryo-fetal death rate, live birth rate, and rate of obstetrics. The calves were examined at birth and at 28 days.

Key Findings:

- There was no difference between meloxicam treated and control cows for the parameters listed in the methods.
- There were no differences observed in terms of the calves at either birth or at Day 28 between the two groups, including live birth rate and survival rate.

Reviewer's note: The study reports that there were no effects on fertility, pregnancy, or other reproductive parameters in cows who received meloxicam. The current labeling for meloxicam products indicates that there are potential reproductive and developmental effects identified in nonclinical studies. As such, this reviewer does not consider the lack of findings in this study need to be included in the label.

Effects of Meloxicam on Implantation and Parturition of Rat. (Jaffal et al., 2006)

Objective: To study the anti-implantation and parturition effects of meloxicam on pregnant rats

Method: For effects on early implantation, 0, 7.5, or 10 mg/kg meloxicam was administered orally to rats with a positive detection of a vaginal plug on Days 1, 2, and 3 of gestation. For effects on late implantation, 0, 7.5, or 10 mg/kg meloxicam was administered orally to rats with a positive detection of a vaginal plug on Days 3, 4, and 5 of gestation. For effects on parturition, 0, 7.5, or 10 mg/kg meloxicam was administered orally to rats with a positive detection of a vaginal plug on Days 20, 21, and 22 of gestation. One group was laparotomized on Day 23 while the second group went through natural delivery. For the implantation experiments, uteri were examined visually and implants were counted and scored as resorptions, dead, or live fetuses. Fresh uteri tissue was stored for histopathological evaluation. For parturition experiments, the number of live and dead pups were counted, body weights of pups were measured, and pups underwent macrophotography. Rats that were sacrificed on Day 23 were inspected for the number of live, dead, or resorbed fetuses.

Key Findings:

- All doses of meloxicam caused an increase in resorptions, a decrease in viable implanted sites, and a decrease in the number of rats with viable implantation sites. The effects appeared to be worse when the animals were dosed during late implantation (Days 3-5) as opposed to early implantation (Days 1-3).
- All doses of meloxicam caused a decrease in the number of viable fetuses and an increase in the number of dead fetuses.
- The high dose (10 mg/kg) of meloxicam caused an increase in maternal plasma levels of oxytocin.

Reviewer's note: The study reports that there were adverse developmental effects of meloxicam in rats, including embryo-fetal lethality. The lowest dose used in this study

was 7.5 mg/kg. The current labeling for meloxicam products includes nonclinical data in rats and rabbits which describe the developmental effects of meloxicam. These studies indicated similar findings as seen in the Jaffal et al., 2006 study. The rat study in the label for meloxicam reported these effects at a lower dose (1 mg/kg) than tested in the Jaffal et al., 2006 study. As such, the findings from Jaffal et al., 2006 study are already covered by the information in the label and this reviewer does not consider the study data necessary to be included in the label.

Pregnancy rates and serum 13,14-dihydro-15-keto-PGF₂alpha concentrations in recipient Nelore heifers treated with meloxicam after the transfer of in vitro-produced embryos. (Lopes et al., 2018)

Objective: To determine if the administration of meloxicam to Nelore heifers would improve the pregnancy rates by decreasing serum 13,14-dihydro-15-keto-PGF₂alpha (PGFM) concentrations

Method: Cycling Nelore heifers of 33 months of age with a body score of 6 or 7 (1-9 scale) were used as recipients for embryo transfer of in vitro-produced blastocysts. Recipients were classified one month before the experiment based on the degree of difficulty in passing a catheter through the cervix (easy, < 80 seconds or difficult, >80 seconds). One hour before embryo transfer, animals received either a 200 mg meloxicam IM injection or an equal amount of saline. Blood samples were collected from all animals 1 hour before embryo transfer and at 4 and 8 hours after transfer to determine PGFM serum concentrations. Pregnancy was confirmed by ultrasound 30 days after embryo transfer.

Key Findings:

- Meloxicam increased the pregnancy rates of Grade I (easy) Nelore heifers but had no effect on Grade II (difficult) Nelore heifers.
- Grade II (difficult) animals receiving meloxicam had lower serum PGFM concentrations 4 and 8 hours after treatment compared to control and Grade I (easy) meloxicam animals.

Reviewer's note: The study indicates that meloxicam can increase the pregnancy rate of Nelore heifers graded as easy based on the difficulty to pass a catheter through the cervix. This is not a toxicological finding and the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. (Malreddy et al., 2013)

Objective: To determine the depletion of gabapentin and meloxicam into milk

Method: Twelve Holstein-Friesian cows (34-62 months old and 543-891 kg) in their 1st-3rd lactation were used. Six cows received 10 mg/kg gabapentin and 1 mg/kg meloxicam in a gelatin capsule. The remaining six cows received 20 mg/kg gabapentin and 1 mg/kg meloxicam in a gelatin capsule. Milk was collected prior to drug administration and then every 8 hours for 7 days. At each milk sampling time, 10 mL of blood was collected by venipuncture of the jugular vein.

Key Findings:

- The concentration of meloxicam in plasma was higher than in milk.
- Milk concentrations of meloxicam depleted below detectable levels within 80 hours.

Reviewer's note: The study is a PK study as opposed to a toxicity study. It indicates that in cows, meloxicam can be found in the milk of lactating cows. In terms of the label, there is already animal data suggesting that meloxicam can be found in milk of rats and pigs. The addition of data that it is also in the milk of cows does not really enhance the label in a meaningful way. This reviewer does not consider the study data necessary to be included in the label.

Altered fetal pituitary-adrenal function in the ovine fetus treated with RU486 and meloxicam, an inhibitor of prostaglandin synthase-II. (McKeown et al., 2000)

Objective: To examine the effects of specific PGHS-II inhibition in sheep during induced labor.

Method: Catheters and electromyogram electrodes were placed into pregnant ewes of known gestational age. A 5-day period of recovery was allowed after the operation. All animals received a SC injection of 10 mg/kg RU486 to induce labor on Day 127 of gestation. Once in labor, animals received either 1) IV saline (only 18-hour infusion due to animals delivering), 2) IV infusion of 2 mcg/kg meloxicam per minute for 2 hours then 4 mcg/kg per minute for 2 hours, then increased by 4 mcg/kg per minute until the uterine activity pattern returned to baseline. Meloxicam was continued at this dose until termination., 3) meloxicam infusion as in 2 except that once contractions returned to baseline, the dose was decreased by 4 mcg/kg per minute until 2 mcg/kg per minute. Meloxicam was continued at this dose until termination. A second injection of RU486 was given within 12 hours of starting the saline or meloxicam infusions. Meloxicam infusions were terminated at 48 hours and the animals were killed. Maternal and fetal arterial blood samples were collected at 8-hour intervals starting 48 hours prior to the first injection of RU486.

Key Findings:

- Both infusions of meloxicam significantly decreased uterine contraction frequency.

- Both infusions of meloxicam significantly decreased maternal PGFM concentrations and fetal PGE₂ concentrations.
- Both infusions of meloxicam significantly decreased fetal ACTH and cortisol concentrations.

Reviewer's note: The study is mechanistic in nature. It indicates that meloxicam can attenuate the effects of RU486, a progesterone receptor agonist, in sheep. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Effects of meloxicam on milk production, behavior, and feed intake in dairy cows following assisted calving. (Newby et al., 2013)

Objective: To evaluate the effect of meloxicam on behavior, health, and production in cows with assisted calving

Method: Holstein cows (42) and heifers (61) that experienced assisted parturition were enrolled in the study. Calving difficulty was scored as either: 1) easy pull by one person, or 2) a difficult pull with more than one person and/or with mechanical assistance. Animals were blocked into primiparous and multiparous groups and into calving difficulty and randomly assigned within each block to receive either meloxicam (0.5 mg/kg) subcutaneously 25.4 hours following calving or a similar volume of placebo. Dry matter intake, milk yield, body weight, rectal temperatures, and clinical health events were measured. In addition, blood samples were obtained by coccygeal venipuncture after calving and at 1, 3, 6, 9, and 12 days in milk. Feeding and lying behaviors were also measured.

Key Findings:

- Animals who received meloxicam visited the feed bunk more often than the placebo group and spent a longer time feeding.
- No other differences (including in dry matter intake, milk production, or metabolic indicators) were seen between the meloxicam and placebo groups.

Reviewer's note: The study did not indicate any toxicological effect in cows or heifers undergoing assisted calving. There was a change in feeding behavior, but the clinical significance of this finding is unknown. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

The potential danger of COX-2 inhibitors. (Norman and Wu, 2004)

Objective: None. Article is an editorial.

Method: None

Key Findings:

- None

Reviewer's note: The study is an editorial opinion. The Authors believe that "normal women of childbearing age attempting to have a baby need to pay more attention to COX inhibitors generally and COX-2 inhibitors specifically. They should avoid taking these drugs or reduce their dose while seeking pregnancy. Women who require these drugs for chronic inflammatory diseases should consider the benefit–risk profile before trying to become pregnant. Fertility experts should ensure that they take an adequate drug history and give appropriate advice."

The author's present some nonclinical data for meloxicam, but they do not cite a reference. The label for meloxicam products already includes nonclinical data to characterize the potential developmental and reproductive risks of meloxicam. This editorial does not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Evaluation of the contraceptive effects of carprofen, flunixin meglumine and meloxicam in rats. (Paksoy and Kirbas, 2017)

Objective: To determine the suitability of carprofen, flunixin meglumine, and meloxicam for use in emergency contraception

Method: Mated female Sprague-Dawley rats were divided into six groups: 1) carprofen 10 mg/kg SC, 2) carprofen 10 mg/kg SC given twice 12 hours apart, 3) flunixin meglumine 5 mg/kg IM, 4) flunixin meglumine 5 mg/kg IM given twice 12 hours apart, 5) meloxicam 2 mg/kg SC, and 6) saline. Administrations were given on the third day after mating. Rats were euthanized on the 7th day post mating to determine pre-implantation loss.

Key Findings:

- Meloxicam resulted in a significant decrease in implantation sites (3.00 vs. 11.38 in the controls).
- Only 3 of 8 animals treated with meloxicam became pregnant. All 8 control animals became pregnant.

Reviewer's note: The study indicated that meloxicam can result in a decrease in implantation sites leading to pre-implantation loss and decreased pregnancy in rats following a single dose on the 3rd day after mating. The labels for meloxicam products already contain information from animal studies describing pre- and post-implantation

loss. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Dose-dependent effects of meloxicam administration on cyclooxygenase-1 and cyclooxygenase-2 protein expression in intrauterine tissues and fetal tissues of a sheep model of preterm labor. (Rac et al., 2007)

Objective: To determine the effect of different dosage regimens of meloxicam on COX-1 and COX-2 protein expression in ovine endometrium, myometrium, amnion, placenta, and fetal tissues in a model of RU486-induced preterm labor.

Method: Polled Dorset time-pregnant ewes (12 for Study 1 and 6 for Study 2) were surgically prepared under anesthesia on Day 121 of gestation to insert catheters for blood monitoring and drug infusion and electrodes to measure uterine contractions. Animals were allowed to recover 5 days before the experiment began.

In Study 1, labor was introduced by maternal SC injection of 10 mg/kg RU486 on Day 127. The animals were divided into three groups: 1) IV infusion of saline (control), 2) IV infusion of meloxicam at 2 mcg/kg/min for 2 hours doubled every 2 hours until uterine contractile activity reversed back to baseline (~17 mcg/kg/min) and maintained until the end of the protocol, and 3) same as 2) except when contractions returned to baseline, meloxicam dose was reduced by 4 mcg/kg/min until 2 mcg/kg/min was achieved and this dose was maintained until the end of the protocol.

In Study 2, labor was introduced by maternal SC injection of 10 mg/kg RU486 on Day 127. The animals were divided into two groups: 1) IV infusion of saline (control), 2) IV infusion of meloxicam at 16 mcg/kg/min increased by 4 mcg/kg/min every 2 hours until contractile activity was inhibited (average dose of 16-20 mcg/kg/min) and maintained for 10 hours. After that the dose was dropped to 4 mcg/kg/min until the end of the protocol.

For both studies, the duration was 48 hours. Also, a second injection of RU486 was given within 12 hours of starting the infusions. Maternal and fetal blood were collected starting 48 hours prior to the first RU486 injection. At the end of the studies, animals were euthanized and tissue samples were collected.

Key Findings:

- Meloxicam treatment (Study 1) did not affect maternal or fetal arterial: pH, pCO₂, pO₂, or hemoglobin.
- Meloxicam (Study 1) reduced COX-2 protein expression in the endometrium, myometrium, and amnion. No change was observed for COX-2 in the placenta or in COX-1 in any of the tissues. No effect was observed on either COX-1 or COX-2 in any tissue with treatment of meloxicam in Study 2.

- Meloxicam treatment (Study 1) did not affect fetal tissue expression of COX-1 or COX-2 protein.

Reviewer's note: The study is mechanistic in nature. The study did indicate that meloxicam can stop preterm labor contractions and prolong pregnancy. However, the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Effects of non-steroidal anti-inflammatory drugs on blood pressure in dogs. (Montoya et al., 2012)

Objective: To evaluate the potential effects on blood pressure of a 2-month course of orally administered meloxicam or firocoxib in healthy dogs.

Method: Six cross-bred dogs (Age 2-6 years, weighing 17-24 kg) were used in the study. Each dog received 5 mg/kg firocoxib and 1 mg/kg orally for 60 days with a 90-day washout between treatments.

Key Findings:

- Treatment with meloxicam had no effect on mean, diastolic, or systolic blood pressure was observed.

Reviewer's note: The study is only a meeting poster, did not evaluate any developmental or reproduction parameters and did not find a toxicological effect of meloxicam. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Risk factors, microbiological findings and outcome of necrotizing fasciitis in New Zealand; a retrospective chart review. (Das et al., 2012)

Objective: To investigate the relationship between the incidence of necrotizing fasciitis (NF) in New Zealand and traditional Samoan tattooing and other risk factors, and to document the microbiology, complications, treatment and outcomes of NF.

Method: 209 hospital charts of patients discharged with a NF diagnosis in eight hospitals in New Zealand from 2000 to 2006 were retrospectively reviewed.

Key Findings:

- ~25% of the patients were taking NSAIDs but the role of NSAIDs was unclear.

Reviewer's note: This study did not specify what NSAIDs were used and it was unclear if usage was a risk factor for NF. Furthermore, it is unclear what such an association

would have with developmental or reproductive parameters. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Diverse roles of prostaglandins in blastocyst implantation. (Salleh, 2014)

Reviewer's note: This article is a review article which summaries the effects of prostaglandins on blastocyst implantation. Meloxicam or NSAIDs are not mentioned at all. The label already includes a brief description of the potential effects of prostaglandin inhibition and its potential developmental effects. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

9.2 Embryonic and Fetal Development*

9.2.1 Study Title: Maleic Acid: An Oral Developmental Toxicity Study in Pregnant New Zealand White Rabbit

Study no.: 34-74
Study report location: Module 4:
<\\cdsesub1\evsprod\nda211988\0053\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\34-74\34-74-audited-draft.pdf>
Conducting laboratory and location: (b) (4)
GLP compliance: Yes
Drug, lot #, and % purity: Maleic acid, 2HH0135, 100.5%

Key Study Findings

- New Zealand White rabbits were administered 0, 2, 5, and 20 mg/kg/day maleic acid by oral gavage once daily from Gestational Day 6 to 18.
- No evidence of test article-related maternal toxicity was observed in the study.
- There was no treatment related effect on litter viability based on the following parameters: pre- and post-implantation loss, number of corpora lutea, number of

live fetuses, total number of implants, early/late resorption, total resorptions, early/late death, total deaths, and total non-live resorptions and deaths

- There was a small decrease in the male to female pup ratio in treated groups compared to the vehicle. The magnitude of the effect was small and did not follow a dose response so it was not considered significant.
- There was no treatment-related effect on fetal malformations/ variations of any kind. All observed effects did not follow a dose response, were similar to control, or within historical control ranges.
- The Applicant identified 20 mg/kg/day as the NOAEL for maternal and embryo-fetal development. The reviewer agrees. The NOAEL of 20 mg/kg is a human equivalent dose of 387.1 mg based on body surface area and assuming a 60 kg person. The amount of maleic acid at the MRHD is 8.0 mg resulting in a margin of exposure of 48.4x.

Methods

Doses:	2, 5, and 20 mg/kg/day
Frequency of dosing:	Once daily
Number/Sex/Group:	23 females/group
Dose volume:	10 mL/kg
Formulation/Vehicle:	Maleic acid was dissolved in deionized water.
Route of administration:	ORAL
Species:	Rabbit
Strain:	New Zealand White
Comment on Study Design and Conduct:	
Dosing Solution Analysis:	Dose solution analysis was conducted on the first day of preparation and at the end of the dosing period. All maleic acid formulations were between -0.5 and + 0.5% of the target concentration. The vehicle control did not have any detectable maleic acid (below LOQ of 50 mcg/mL).

Observations and Results

F₀ Dams

Mortality

All does were observed twice daily on weekdays and once daily on weekends/holidays.

All animals survived until scheduled C-section on GD 29.

Clinical Signs

Each doe was evaluated during dosing for overt signs of toxicity. A detailed clinical examination was performed pre-test and daily during the study. Clinical observations were performed ~1-hour post dosing.

No treatment related clinical signs were observed.

Body Weight

Does were weighed upon arrival and on GD 6, 9, 12, 15, 17, 19, 21, 23, 25, 27, and 29.

Treatment with maleic acid did not appear to affect the body weight or body weight gain of the rabbits.

Food Consumption

Food consumption was measured on the same days as body weight measurements.

Food consumption was generally similar across the groups except for between GD 12-15 where all treated groups had a lower food consumption compared to the vehicle treated group. There was no dose response relationship to the observation and decreased food consumption during this time period was not considered treatment related or adverse.

Necropsy/ Histopathology

All surviving rabbits were euthanized with a euthanasia solution on GD 29. A gross necropsy was performed and the uterus of each doe was weighed with the ovaries. Corpora lutea were counted for both ovaries. Each uterine horn was inspected for tissue resorptions and fetal death. Uteri of does that appeared to be non-gravid were stained with 10% ammonium sulfide. Tissue masses and suspected lesions were dissected and fixed in 10% neutral buffered formalin.

No gross abnormalities were observed during the necropsy in maternal animals. All animals were found to be pregnant except two control animals who were confirmed to be non-gravid by ammonium sulfide staining and one 5 mg/kg animal which was gravid with one implantation site.

Corpora Lutea/Preimplantation Loss

Litter viability was assessed by the following parameters: pre- and post-implantation loss, number of corpora lutea, number of live fetuses, total number of implants,

early/late resorption, total resorptions, early/late death, total deaths, and total non-live resorptions and deaths.

There were no treatment related effects on any of the parameters measured. An increase in the % of preimplantation loss was observed in animals receiving 2 mg/kg maleic acid compared to the control. This effect was not observed at higher doses and appeared to be driven by an increased average number of corpora lutea in this group as opposed to a decrease in the number of implantations.

Table 176: Litter Viability

	Vehicle	2 mg/kg	5 mg/kg	20 mg/kg
% preimplantation loss	5.9	15.4	3.2	4.9
% post implantation loss	10.5	6.7	11.5	7.2
# of corpora lutea	9.6	10.2	9.0	9.1
Total # of implants	9.0	8.7	8.7	8.7
Live fetuses	8.0	8.0	8.0	8.0
Resorptions, early	0.9	0.4	0.5	0.5
Resorptions, late	0	0.1	0.1	0.1
Resorption, total	0.9	0.5	0.6	0.6
Deaths, early	0	0.1	0.1	0
Deaths, late	0	0.1	0.1	0
Deaths, total	0	0.1	0.1	0.1
Resorptions and death, total	1.0	0.6	0.7	0.7

N = 21 for the vehicle control group and 23 for all other groups.

Values presented as means

F₁ Offspring

Litter body weights were similar amongst all groups. There was a slight shift in the male:female pup ratio in treated groups compared to the control group with an increase in the number of females born and a decrease in the number of males born. There did not appear to be a dose response as all maleic acid treated groups had a similar male:female ratio. This effect was not considered treatment related or meaningful.

Table 177: Sex and Sex Ratio of Litter

	Vehicle N = 20	2 mg/kg N = 21	5 mg/kg N = 22	20 mg/kg N = 23
# of Males (mean)	4.6	4.1	4.0	3.5
# of females (mean)	3.7	4.3	4.4	4.4
M/F ratio	1.2	0.9	0.9	0.8

Terminal Observations

Fetal Malformations/ Variations (external, visceral, skeletal)

Removed fetuses were given a gross morphological examination. Fetuses were euthanized by induction of hypothermia. Visceral, cephalic, and skeletal examinations were performed on the fetuses.

During the external examinations, one fetus from the 2 mg/kg group was found with multiple malformations: arthrogryposis of both forelimbs, cleft palate, club foot, protruding tongue, short snout, and small upper jaw. In addition, one fetus from the 5 mg/kg group was identified with a malformation of arthrogryposis. The malformations were considered sporadic due to the low incidence and lack of findings at the highest dose tested.

No test-article related visceral or cephalic findings were noted. Overall there was a low number of variations and malformations observed across all groups. Any variations and malformations observed were either also seen in the vehicle treated animals or did not follow a dose response.

Only the vehicle control group and high-dose group were evaluated for skeletal variations/malformations. The overall rate of skeletal variations in vertebrae, skull, sternbrae, and ribs were similar across all groups. Skeletal malformations were limited to a single fetus in the 20 mg/kg group (fused rib). The fetal and litter incidence of this finding was within the historical control range reported by the (b) (4)

10 Special Toxicology Studies

10.1 Wound Healing and Bone Studies

Wound and bone healing studies were conducted with HTX-011 and were reviewed during the IND. In summary, there did not appear to be any effect on bone healing or adverse interaction with the Prolene mesh with HTX-011-56. From the nonclinical perspective, no additional testing to assess these parameters are necessary. There was some evidence of a potential effect on wound healing in the rabbit study. However, the minipig study (33-54), which used a higher dose, supported that there was only a very slight difference in wound healing with HTX-011-56 compared to control treatments. However, it must be kept in mind that when the dose volume was normalized to the size of the incision site (estimated incision sizes for the surgeries proposed in the clinical studies were provided by the clinical team) the minipig study (1 mL/cm) was lower than the proposed clinical surgeries (herniorrhaphy, 3-4 cm, 2.5-3.4 mL/cm (b) (4)

Study title: HTX-011: A Hernia Repair Model with Mesh in New Zealand White Rabbit

Study no.: 33-38
Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-to\33-38\33-38.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: September 17, 2015
GLP compliance: No
QA statement: No (Study Director Statement only stating the study was conducted with (b) (4) SOPs)
Drug, lot #, and % purity: HTX-011-019, Lot 14072, 97.7% Meloxicam and 101.0% Bupivacaine
HTX-011-49, Lot 15011, 97.9% Meloxicam and 101.5% Bupivacaine
HTX-011-056, Lot 025774, 101.4% Meloxicam and 103.6% Bupivacaine

Key Study Findings

- New Zealand White Rabbits were administered a single application of 25 mg bupivacaine formulation or 25 mg bupivacaine and 0.75 mg meloxicam formulations onto Prolene mesh in a hernia repair model. There was no bupivacaine alone control arm to permit comparison to the approved drug products.
- No treatment-related mortalities were observed.
- There were no treatment-related effects on body weight or food consumption.
- Fecal output was reduced in males receiving the bupivacaine formulation comparator and all three HTX formulations. Fecal output was primarily reduced in females receiving HTX-011-19. This finding was not considered adverse.
- Treatment with the vehicle, bupivacaine comparator formulation, and all three HTX formulations resulted in increased incidence/severity of erythema and edema at the injection site in males and females on Days 3 and 15 although there did not appear to be a consistent pattern across formulation or sex. Overall, there were more findings on the last day of the study in animals treated with the bupivacaine comparator and the HTX formulations than in the saline and vehicle

treated groups.

- Treatment with the vehicle, bupivacaine comparator formulation, and all three HTX formulations resulted in increased incidence/severity of wound observations at the injection site in males and females on Days 3 and 15 although there did not appear to be a consistent pattern across formulation or sex. Overall, the most commonly observed effect, hemorrhage, was no longer present in most animals by Day 15 regardless of treatment.
- On Day 3, various effects on hematology parameters were seen following treatment with the various formulations used compared to the saline control, including increased white blood cells, increased neutrophils, decreased monocytes, decreased reticulocytes, and decreased platelets. These changes were less pronounced by Day 15, suggesting recovery.
- Changes in clinical chemistry parameters noted were decreased triglycerides (20-30%) in male animals and decreased phosphorous (13-22%) in female animals. The toxicological significance of both findings is unknown.
- Occasionally, elevations in the number of gross findings at the injection site were increased in some treated groups compared to the saline control.
- On the Day 3 sacrifice, injection site histopathology findings were of similar incidence and severity across the saline control and all treatments.
- On Day 15, there appeared to be some differences compared to the saline control animals in histopathological lesions of the injection site. This included increased incidences/severity of cell debris, cystic change, hemorrhage, epidermal hyperplasia, dermal granulation, necrosis, epidermal ulcer, and subcutaneous/muscular/intermuscular granulation.
- Taken together, the various formulations, including the vehicle control, bupivacaine formulation comparator, and the three HTX formulations, caused increased incidences/severities in dermal, wound site, gross, and histopathological findings throughout the study. The observations at the wound site appeared to be similar to saline control by the end of the study and are likely not adverse. In contrast, the presence of erythema and edema at the end of the study was only seen with the bupivacaine formulation comparator and HTX formulations. Similarly, increased incidence and severity of various histopathological findings were seen at the end of the study with the majority of them occurring with the HTX formulations. This suggests that some shared component of the different formulations may be interfering with wound healing in this study.
- It is unclear if the treatments caused an interaction with the Prolene mesh as no

clear evidence of the mesh was seen in any sections, including on Day 3. The Applicant attributed the lack of mesh to loss of the fibers during tissue processing. The similar incidence and severity of histopathological effects on Day 3, especially in cystic change, suggest that the mesh performed similarly in all groups.

Methods

Doses: 25 mg bupivacaine and 0.75 mg meloxicam
 Frequency of dosing: Once prior to closing the subcutaneous tissues and the skin.
 Route of administration: Injection
 Dose volume: 0.5 (HTX-011-19) – 1.0 mL (HTX-024-001, HTX-002-014, HTX-011-49, and HTX-011-56)
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-011-19, HTX-011-49, and HTX-011-56). The vehicle control was HTX-024-001. See table below for further details.
 Species/Strain: Rabbit/New Zealand White, Crl:KBL (NZW) BR
 Number/Sex/Group: 4/sex/group (Main)
 Age: ~4 months
 Weight: 2.5-3.4 kg (m) and 2.6-3.9 kg (f)
 Satellite groups: none
 Unique study design: Hernia repair model using Prolene mesh
 Deviation from study protocol: None that impacted the results of the study.

Table 178: Treatment Groups Hernia Repair Study

Group	Test and Control Items	Active Pharmaceutical Ingredients (APIs)		Excipients				
		Bupivacaine w/w% ¹ (mg/g)	Meloxicam w/w% (mg/g)	Maleic Acid w/w% (mg/g)	(b) (4) w/w% (mg/g)	DMSO w/w% (mg/g)	Triacetin w/w% (mg/g)	API35 w/w% (mg/g)
1	Saline Control	NA	NA	NA	NA	NA	NA	NA
2	HTX-024-001 Vehicle Control	NA	NA	NA	NA	10.00 (100.0)	35.00 (350.0)	55.00 (550.0)
3	HTX-002-014 Comparator	2.50 (25.0)	NA	0.15 (1.5)	NA	8.00 (80.0)	35.00 (350.0)	54.35 (543.5)
4	Test Item HTX-011-19	5.0 (50.0)	0.15 (1.5)	0.60 (6.0)	(b) (4)	NA	NA	79.25 (792.5)
5	Test Item HTX-011-49	2.50 (25.0)	0.075 (0.75)	0.15 (1.5)	NA	8.00 (80.0)	35.00 (350.0)	54.275 (572.75)
6	Test Item HTX-011-56	2.50 (25.0)	0.075 (0.75)	0.05 (0.5)	NA	10.00 (100.0)	25.00 (250.0)	62.375 (623.75)

Data from page 8 of the study report.

Observations and Results

Mortality

One female was found dead during the surgery procedures prior to being dosed and was replaced by a spare animal. Since the animal wasn't dosed this death was not considered treatment related.

All other animals survived until scheduled termination of the study.

Clinical Signs

Cage side observations were conducted once daily throughout the study except on days with detailed clinical observations. Detailed clinical observations were conducted once before treatment, weekly throughout the study period, and before necropsy.

In males, the number of animals with and the total number of occurrences of reduced fecal output were increased slightly in animals who received the bupivacaine comparator, HTX-011-19, HTX-011-49, and HTX-011-56 compared to the vehicle and saline controls. In females, reduced fecal output was seen in all groups except the saline control. The incidence of this finding was higher in the bupivacaine comparator and HTX-011-19 formulation. This finding was not considered adverse due to the sporadic nature, observation in the control animals, and unclear toxicological significance.

All other clinical signs observed were not considered treatment related.

Table 179: Clinical Signs Rabbit Hernia Repair Study

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Reduced fecal output (total # of observations)	3	3	6	7	6	6
Reduced fecal output (# of animals)	3/8	2/8	5/8	4/8	5/8	3/8
Females						

Reduced fecal output (total # of observations)	0	2	3	6	1	1
Reduced fecal output (# of animals)	0/8	1/8	2/8	4/8	1/8	1/8

Dermal Observations

Dermal observations were made daily for the first seven days post-surgery (except Day 1), weekly thereafter, and prior to necropsy. Dermal observations were recorded using a modified Draize scoring system.

In animals sacrificed on Day 3, there were minor increases in the incidence and severity of erythema in males and females compared to the saline control. In males, increased severity was seen with the vehicle, HTX-011-19 and HTX-011-56 formulations while in females increases in severity were seen with the bupivacaine formulation comparator and all three HTX formulations. In animals sacrificed on Day 3, there were also increases in the incidence and severity of edema compared to the saline control in males and females. In males, all treated groups showed an increased incidence compared to saline with the highest incidence and severity in animals who received the vehicle, bupivacaine formulation comparator, and HTX-011-56. In females, all treated groups showed an increased incidence compared to saline, with the highest severity seen following treatment with the vehicle and HTX-011-56.

In animals sacrificed on Day 15, all groups in males showed an increased severity in erythema compared to the saline treated animals with HTX-011-49 and HTX-011-56 showing the highest increases in severity. In females, an increased incidence of erythema was seen in all treated groups compared to the saline control with the highest severity seen with HTX-011-19. The incidence and severity of edema in males was similar across all treatment groups compared to the saline control, while in females an increase in severity was seen in all groups compared to the saline control. In the saline control groups, findings of erythema (females only) and edema resolved by the middle of the study (Day 6). This was also true for the vehicle treated groups except for one case of edema in a female animal with edema from Day 8-15. For the groups treated with the bupivacaine formulation comparator and the HTX formulations, erythema and edema were more frequently seen on the last day of treatment suggesting a lack of recovery or a prolonged effect.

Overall, the vehicle resulted in more skin observations than the saline control. The bupivacaine formulation comparator displayed a pattern of skin findings very similar to the vehicle control. The three formulations of HTX appeared similar to both the comparator and the vehicle control, although there were some instances where the formulations appeared slightly worse. Taken together, the formulations appeared slightly worse than the saline control, although there did not appear to be a consistent pattern across formulation or sex. In addition, there were more findings at the end of the

study with the bupivacaine formulation comparator and the HTX formulations.

Table 180: Dermal Observations of Day 3 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	2 (1)	3 (1)	2 (1)	3 (1.3)	1 (1)	4 (1.5)
Edema	0	3 (1.7)	3 (1.3)	2 (1)	1 (1)	3 (1.7)
Females						
Erythema	2 (1)	1 (1)	3 (1)	2 (1.5)	2 (1.5)	3 (1.3)
Edema	1 (1)	4 (1.5)	3 (1)	3 (1.3)	3 (1.3)	3 (1.7)

N=4

Bold = increased incidence/severity over saline control

Table 181: Dermal Observations of Day 15 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	3 (1.3)	4 (1.3)	4 (1.3)	2 (1.5)	4 (1.8)	3 (2)
Edema	4 (1.8)	4 (2)	4 (2)	3 (2.3)	4 (2)	4 (1.5)
Females						
Erythema	0	3 (1.3)	4 (1.5)	4 (1.8)	4 (1.5)	2 (1.5)
Edema	4 (1.3)	4 (1.8)	4 (2)	3 (1.7)	4 (1.5)	4 (1.5)

N=4

Bold = increased incidence/severity over saline control

Table 182: Dermal Observations on Day 15

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	0	0	0	0	1	1
Edema	0	0	1	3	3	2
Females						
Erythema	0	0	2	3	2	0
Edema	0	1	2	1	1	1

N=4

Wound Observations

Wound observations were made daily for the first seven days post-treatment, weekly thereafter, and prior to necropsy.

In animals sacrificed on Day 3, there were no clear differences between any treatments in males. In females, a minor increase in severity of hemorrhage was observed in animals receiving the bupivacaine comparator with larger increases in severity in animals receiving the vehicle and HTX-011-56. In addition, females receiving the bupivacaine comparator and HTX-011-49 had increased incidence and severity of

exudate.

In animals sacrificed on Day 15, there were increases in the severity of hemorrhage at the wound site in females receiving all treatments compared to the saline control. In males, the severity of hemorrhage was similar across all groups. In females, there was increased severity in exudate in animals treated with the vehicle, HTX-011-19, and HTX-011-49 compared to controls. The severities for the presence of exudate were similar across all groups in males. Males did have an increased incidence of granulation in animals who received the bupivacaine formulation comparator, HTX-011-19 and HTX-011-49.

Since there were several findings in the saline control animals, only the severity of the findings was increased with some treatments and a lack of clear effects across sex and time, it is difficult to make a clear conclusion from the wound observations. The primary finding at the wound site was hemorrhage which was no longer present by Day 15 in most animals, although it was still present in half the female animals who received HTX-011-56. Therefore, while there were slight differences in wound healing during the study, wound healing was on average similar to saline treated animals by the end of the study.

Table 183: Wound Observations of Day 3 Sacrifice Group (Incidence)

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
hemorrhage	4 (2.5)	4 (2.8)	4 (2)	4 (1.3)	3 (1.7)	3 (3)
exudate	1 (1)	1 (1)	0	1 (1)	2 (1)	2 (1)
granulation	0	0	0	0	0	0
Females						
hemorrhage	4 (2)	3 (3)	4 (2.5)	4 (1.3)	3 (2)	4 (2.8)
exudate	1 (1)	1 (1)	2 (2)	2 (1)	3 (1.7)	3 (1)
granulation	0	0	0	0	0	0

N=4

Values in () represent average area affected: 1 = 1 mm, 2 = 2 mm, 3 = 1/2 of wound, 4 = whole wound
 Bold = increased incidence/severity over saline control

Table 184: Wound Observations of Day 15 Sacrifice Group (Incidence)

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
hemorrhage	4 (2.8)	4 (1.8)	4 (3)	3 (3)	4 (3.3)	4 (2.5)
exudate	3 (1.3)	2 (1.5)	4 (1.5)	3 (1)	3 (1.3)	2 (1)
granulation	0	0	1 (2)	2 (2)	1 (3)	0
Females						

hemorrhage	4 (2.3)	4 (3.5)	4 (2.8)	4 (3.3)	4 (3)	4 (2.8)
exudate	3 (1.3)	4 (2)	4 (1)	4 (1.8)	3 (2)	3 (1)
granulation	0	0	1 (1)	1 (1)	0	0

N=4

Values in () represent average area affected: 1 = 1 mm, 2 = 2 mm, 3 = 1/2 of wound, 4 = whole wound
 Bold = increased incidence/severity over saline control

Table 185: Last Day of Wound Hemorrhage

Group	Saline	Vehicle	Bup comparator	HTX-011- 19	HTX-011- 49	HTX-011- 56
Males						
Hemorrhage- last day of finding	8, 6, 6, 7	8, 8, 7, 6	15, 8, 8, 7	6, 5, 6	8, 6, 6, 7	7, 4, 6, 6
Females						
Hemorrhage- last day of finding	6, 6, 8, 8	8, 8, 7, 8	8, 8, 8, 7	8, 8, 8, 7	15, 8, 5, 8	15, 15, 8, 7

Body Weights

Body weights were recorded once prior to group assignment, one week prior to treatment, and on Days -1, 4, 8, and 15.

No treatment-related changes in absolute body weight or body weight gain were observed.

Food Consumption

Food consumption was measured daily during the last week of the pre-treatment period and throughout the treatment and observation periods.

No treatment-related changes in food consumption were observed.

Hematology

Blood samples were collected from all animals (fasted) on prior to the start of treatment and on necropsy days via the central ear artery. A complete battery of hematology and coagulation parameters was evaluated.

In animals sacrificed on Day 3, there were several effects on hematology parameters with different treatments. In males, treatment with the vehicle lead to decreased platelets, monocytes, and reticulocytes, as well as, increased white blood cells and neutrophils. Similar changes were seen in females, except there was no difference in reticulocyte counts. The bupivacaine formulation comparator caused similar changes as

the vehicle control, except that white blood cell counts were only elevated in females. Overall, the three HTX formulations seemed to cause the same effects as the vehicle and the bupivacaine formulation comparator, except that the effects were less consistently observed in both sexes.

Of note, differences of similar magnitude for some parameters were also seen prior to treatment. For example, decreased platelets were seen in males receiving the vehicle, the bupivacaine formulation comparator, HTX-011-19, and HTX-011-56, decreased reticulocytes were seen in males receiving vehicle and the HTX formulations and increased white blood cells were seen in males receiving HTX-011-19. The pretreatment differences could not explain all the changes observed with treatment of the various formulations. Taking the pretreatment changes into consideration, the following effects were still observed:

Vehicle: Increased WBC (male/female), increased neutrophils (male/female), decreased monocytes (males)

Bupivacaine formulation comparator: Decreased reticulocytes (male), increased neutrophils (male), decreased monocytes (male/female), decreased platelets (female), increased WBC (female)

HTX-011-19: Increased neutrophils (male/female), decreased reticulocytes (female), increased WBC (female), decreased monocytes (female)

HTX-011-49: Decreased platelets (male), increased neutrophils (male), increased WBC (female)

HTX-011-56: Increased WBC (male/female), decreased monocytes (male/female), decreased platelets (female)

Across all formulations and the vehicle, the most consistent findings were increased WBCs, increased neutrophils, and decreased monocytes suggesting these are likely related to the shared excipients.

In animals sacrificed on Day 15, the effects on hematology seen on Day 3 were not as apparent, possibly indicating these effects are recoverable. Still, there were isolated changes in hematological parameters that were consistent with those on Day 3, such as decreased platelets in males receiving HTX-011-56 and females receiving HTX-011-19. Overall, on Day 15, hematology parameters were similar between treated and saline control animals.

Table 186: Hematology Findings on Day 3, Percentage of Saline Control

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						

Platelets	100	76.7	87.5	90.6	77.5	75.1
Retic (%)	100	76.5	83.8	70.6	75.0	80.9
WBC	100	123.3	102.4	117.6	104.6	112.8
Neutrophil	100	149.9	121.2	127.2	115.4	84.6
Monocytes	100	50.0	61.1	111.1	0	33.3
Females						
Platelets	100	89.4	80.0	97.6	99.5	76.9
Retic (%)	100	102.3	88.6	70.5	100.0	127.3
WBC	100	135.9	115.4	132.3	115.1	113.2
Neutrophil	100	150.0	124.3	141.4	101.7	94.9
Monocytes	100	70.0	55.0	65.0	80.0	45.0

Table 187: Hematology Findings on Day 15, Percentage of Saline Control

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Platelets	100	94.5	105.0	95.0	106.5	88.4
Retic (%)	100	113.3	100.0	100.0	82.2	75.6
WBC	100	92.3	86.6	89.2	105.1	100.8
Neutrophil	100	108.7	104.8	111.9	130.9	130.2
Monocytes	100	58.8	111.8	88.2	123.5	129.4
Females						
Platelets	100	90.9	102.8	84.4	106.7	101.2
Retic (%)	100	77.3	122.7	79.5	93.2	90.9
WBC	100	92.5	91.0	88.7	73.3	85.6
Neutrophil	100	115.5	109.2	119.1	75.3	92.4
Monocytes	100	125.0	216.7	158.3	91.7	216.7

Clinical Chemistry

Blood samples were collected from all animals (fasted) prior to the start of treatment and on necropsy days via the central ear artery. A complete battery of clinical chemistry parameters was evaluated.

The only changes in clinical chemistry parameters noted were decreased triglycerides (20-30%) in male animals treated with the HTX formulations compared to the saline control and decreased phosphorous (13-22%) in females of all groups compared to the saline controls. The toxicological significance of both findings is unknown.

Gross Pathology

A complete necropsy was performed on all animals.

Numerous lesions were observed at the incision site consisting of adhesion, dark area, firm, gelatinous material, mass, soft, pale area, raised area, and thickening. Occasionally, these findings were elevated compared to the saline control in the treated

groups as indicated in the tables below.

Table 188: Gross Findings of Day 3 Sacrifice Group

Groups	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f
Number of animals INCISION SITE	Males						Females					
	4	4	4	4	4	4	4	4	4	4	4	4
Adhesion	0	0	0	0	0	1	0	0	0	0	0	0
Dark area	4	3	3	4	3	2	3	4	4	4	4	3
Firm	0	1	3	0	0	0	1	1	0	1	0	0
Gelatinous material	0	1	0	0	0	0	0	0	0	0	0	0
Mass	0	1	0	0	0	0	1	0	0	0	0	0
Pale area	0	1	0	2	1	1	1	1	2	2	1	1
Raised area	1	0	0	0	0	0	0	0	0	0	0	0
Soft	3	0	0	0	0	0	0	2	1	0	0	0
Thickening	3	2	1	3	3	2	2	1	3	2	4	4

^a – Saline Control; ^b – Vehicle Control; ^c – Comparator (HTX-002-014); ^d – HTX-011-19;
^e – HTX-011-49; ^f – HTX-011-56

Table 189: Gross Findings of Day 15 Sacrifice Group

Groups	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f
Number of animals INCISION SITE	Males						Females					
	4	4	4	4	4	4	4	4	4	4	4	4
Adhesion	1	0	0	0	0	0	0	0	0	0	0	0
Dark area	0	0	1	0	2	1	0	1	0	1	0	1
Firm	0	1	1	0	0	1	0	2	2	0	0	1
Gelatinous material	0	0	0	0	1	0	0	0	0	0	0	0
Mass	0	0	1	1	0	0	0	0	0	0	0	0
Pale area	0	1	1	0	1	0	1	0	2	1	1	0
Raised area	1	0	0	0	0	0	0	0	0	0	1	0
Scab	0	0	0	0	0	0	0	0	0	0	1	2
Soft	0	0	0	0	1	0	1	0	1	1	0	0
Thickening	3	4	1	3	3	2	4	4	2	4	1	3

^a – Saline Control; ^b – Vehicle Control; ^c – Comparator (HTX-002-014); ^d – HTX-011-19;
^e – HTX-011-49; ^f – HTX-011-56

Histopathology

Adequate Battery: Yes

Peer Review: No.

Histopathological examinations were only performed on incision sites and gross lesions

identified during necropsy.

Histological Findings

On the Day 3 sacrifice, injection site findings consisted of minimal to mild cell debris, minimal to moderate cystic change, minimal to mild epidermal erosion/ulcer, minimal to moderate hemorrhage, minimal epidermal hyperplasia, minimal to moderate subcutaneous acute inflammation, and minimal to mild necrosis. These findings were of similar incidence and severity across the saline control and all treatments. No clear evidence of the mesh material was seen in the sections. However, cystic change was related to the space created by the mesh and since this finding was similar amongst all treatment groups it is unlikely that the mesh performed differently amongst the groups.

On Day 15, similar findings were present at the injection site except both dermal granulation and subcutaneous/muscular/intermuscular granulation were also present. At this time point, there appeared to be some differences compared to the saline control animals. In males, there was an increased incidence of cell debris in animals receiving the HTX formulations, increased incidences of cystic change and hemorrhage in animals of all treatment groups, increased incidence/severity of epidermal hyperplasia with HTX-011-19 and HTX-011-49, and an increased incidence of dermal granulation in animals receiving HTX-011-19. In females, an increased incidence of epidermal ulcer was seen in animals receiving all treatments except the vehicle control, an increased severity of subcutaneous/muscular/intermuscular granulation was observed in animals receiving all treatments except HTX-011-49, an increased incidence of hemorrhage in animals receiving the vehicle, the bupivacaine formulation comparator, and HTX-011-56, and an increased incidence of necrosis in the bupivacaine formulation comparator. Taken together, it appears there was some impact on wound healing on Day 15 following treatment with the various formulations, primarily in males who received the HTX formulations.

Table 190: Histological Findings of Day 15 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Cell debris	0	0	0	1 (2)	1 (2)	1 (2)
Cystic change	1 (2)	3 (2)	4 (2)	4 (2)	4 (2)	4 (2)
Epidermal ulcer	0	0	0	2 (1)	0	0
Hemorrhage	2 (1.5)	3 (1.7)	3 (1.7)	3 (1.7)	3 (1.3)	4 (1.5)
Epidermal hyperplasia	1 (1)	2 (1)	2 (1)	2 (1.5)	1 (2)	2 (1)
Dermal granulation	2 (1.5)	4 (1)	3 (1.3)	4 (1.3)	2 (1.5)	3 (1)
Granulation subcutaneous/muscular/intermuscular	4 (2.8)	4 (3)	4 (3)	4 (2.3)	4 (2.8)	4 (2.5)
Necrosis	1 (2)	1 (2)	3 (1.1)	2 (1.5)	2 (1)	2 (1)
Females						

Cell debris	2 (2)	0	1 (1)	2 (1.5)	1 (3)	1 (2)
Cystic change	3 (2.3)	4 (1.8)	4 (2.3)	4 (2)	3 (2)	4 (1.8)
Epidermal ulcer	0	0	1 (1)	3 (1.3)	1 (3)	1 (2)
hemorrhage	0	4 (1.5)	2 (1.5)	1 (1)	1 (1)	2 (1.5)
Epidermal hyperplasia	4 (2.3)	3 (1)	1 (2)	2 (2)	2 (1)	3 (1.7)
Dermal granulation	4 (2)	3 (1.7)	2 (1)	3 (1.3)	3 (2)	4 (2)
Granulation subcutaneous/muscular/intermuscular	4 (1)	4 (1.5)	4 (2)	4 (1.8)	3 (1.3)	4 (1.5)
necrosis	2 (1.5)	3 (1)	4 (1.5)	3 (1.3)	3 (1.7)	3 (1.3)

Bold = increased incidence/severity over saline control

Dosing Solution Analysis

Dosing solution analysis was not conducted by the testing laboratory. Instead, data on the achieved concentrations of the test and control items was provided by the Applicant in the form of certificates of analysis and stability data to cover the period of dosing.

Study title: Single Subcutaneous Dose Effect of HTX-002-014, HTX-001-49 and HTX-011-56 on Bone Healing in the Cortical Defect Model in Female Sprague Dawley Rats

Study no.: 33-39
 Study report location: SDN 1, Module 4
<\\cdsesub1\levsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-to\33-39\33-39.pdf>

Conducting laboratory and location:

(b) (4)

Date of study initiation: September 26, 2015
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: HTX-024-001, Lot 1237047
 HTX-002-14, Lot 1273049
 HTX-011-49, Lot 15011
 HTX-011-056, Lot 025774
 Purities were not provided

Key Study Findings

- SD rats were administered a single application of 12.5 mg bupivacaine or 12.5 mg bupivacaine and 0.375 mg meloxicam by subcutaneous injection following surgery to induce cortical defect.
- At least six animals were found to have lameness following surgery. Of these, six

animals were sacrificed early due to associated fractures. Since none of these animals were from the control group, the treatment-related nature of this finding could not be ruled out.

- Skin lesions (scabs and ulceration) were seen in animals receiving the bupivacaine comparator and HTX formulations.
- There were no treatment-related effects on body weight.
- No treatment-related effects on bone mineral content, bone mineral density, or the healing stage of the bone were identified throughout the study.
- The study should have been conducted in accordance with Good Laboratory Practices (GLPs). Although this study was not conducted according to GLPs, according to the Applicant the only deviation from GLP was that the study was not quality assured. The results were reviewed, and the study was designed reasonably well. The Division concluded that based on the results obtained, repeating the study in accordance with full GLPs was not likely to impact the clinical assessment of this critical endpoint and would not be required in this unusual circumstance.

Methods

Doses: 12.5 mg bupivacaine and 0.375 mg meloxicam
 Frequency of dosing: Once
 Route of administration: Subcutaneous Injection
 Dose volume: 0.5 mL
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-002-014, HTX-011-49, and HTX-011-56). The vehicle control was HTX-024-001. See table below for further details.

Species/Strain: Rat/Sprague-Dawley
 Number/Sex/Group: 8/females/group
 Age: 15-16 weeks
 Weight: 293.59 ± 10.93 g
 Satellite groups: none
 Unique study design: Cortical defect surgery and CT scans.
 Deviation from study protocol: Part way through the study, animals began being housed individually to minimize the occurrence of fractures.
 Husbandry checks were only performed once daily instead of twice daily.
 Macroscopic observations were not collected automatically in a way that allowed cross referencing with histological observations.

Group	Test and Control Items	Active Pharmaceutical Ingredients			Excipients		
		Bup	Mel	Maleic Acid	DMSO	Triacetin	AP135
		w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)
1	Saline Control	NA	NA	NA	NA	NA	NA
2	Vehicle Control HTX-024-001	NA	NA	NA	10.00 (100.0)	35.00 (350.0)	55.00 (550.0)
3	Comparator HTX-002-014	2.5 (25.0)	NA	0.15 (1.5)	8.00 (80.0)	35.00 (350.0)	54.35 (543.5)
4	Test Item HTX-011-49	2.5 (25.0)	0.075 (0.75)	0.15 (1.5)	8.00 (80.0)	35.00 (350.0)	54.275 (572.75)
5	Test Item HTX-011-56	2.5 (25.0)	0.075 (0.75)	0.05 (0.5)	10.00 (100.0)	25.00 (250.0)	62.375 (623.75)

Abbreviations: Bup = Bupivacaine; Mel = Meloxicam

Data from page 7 of the study report.

Observations and Results

Mortality

Six animals were identified by CT scan as having fractures. These animals were sacrificed early. All other animals survived to scheduled sacrifice.

Clinical Signs

Animals were monitored daily for lameness, death, and morbidity. Animals that showed signs of lameness were x-rayed and/or CT scanned and sacrificed if a fracture was detected.

As described above, at least six animals were found to have lameness and underwent a subsequent CT scan. A total of six animals were identified with a fracture and they consisted of two animals from the vehicle control group, two animals from the bupivacaine comparator group, one animal from the HTX-011-49 group, and one animal from the HTX-011-56 group. Fractures were primarily identified on the right femur (5/6) which is the leg that underwent injection. Since the fractures only occurred in animals receiving one of the formulations and not the saline control, the treatment-related nature of this finding could not be ruled out.

Skin lesions at the injection site, consisting of scabs and ulceration, were observed in six animals receiving the bupivacaine comparator, four animals receiving HTX-011-49, and four animals receiving HTX-011-56. These lesions were found during cage side observations on the day of necropsy.

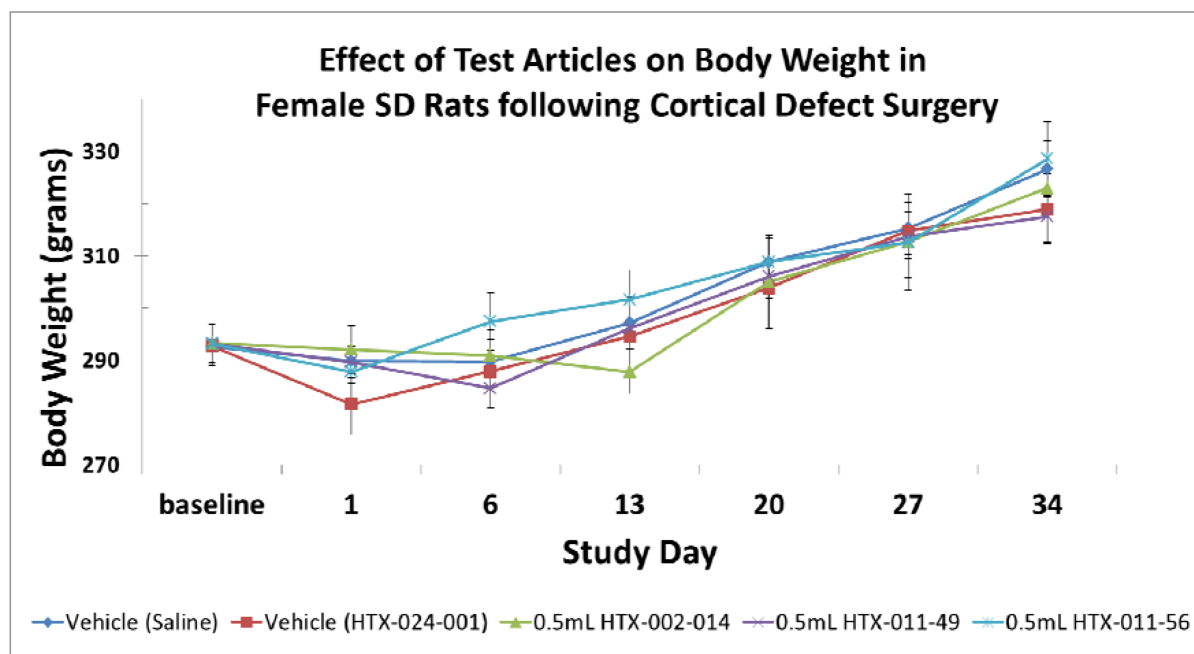
Table 191: Clinical Signs

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Fracture	0/8	2/8 (right, both)	2/8 (right, both)	1/8 (left)	1/8 (right)
Skin lesions	0/8	0/8	6/8	4/8	4/8

Body Weights

Body weights were recorded on Day 5 of the predose phase and on Days 1, 6, 13, 20, and 34 of the dosing phase.

No treatment-related differences in absolute body weight were observed.

Figure 7: Body Weight in Rat Bone Defect Study

Data from page 12 of the study report.

CT Imaging

Animals underwent CT imaging on Days 1, 6, 13, 20, and 34 of the dosing phase. Total bone mineral density (BMD) and bone mineral content (BMC) of the callus were measured for both the left and right femur.

No significant differences were noted in total BMD for the left and right femurs and total BMC in the left femurs from Days 1-34. No significant change was observed in total BMC of the right femurs from Days 6 to 34. A significant increase in total BMC was observed in the right femurs of animals receiving HTX-011-56 on Day 1 only. The toxicological significance of this finding is unknown.

Table 192: Bone Mineral Content of Left Femur (Percent of Saline Control)

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	87.6	84.8	113.8	117.1
Day 6	100	97.7	107.1	95.2	111.7
Day 13	100	112.7	117.8	112.3	147.7
Day 20	100	113.0	123.0	115.6	131.9
Day 34	100	105.6	116.8	108.5	117.5

Table 193: Bone Mineral Content of Right Femur (Percent of Saline Control)

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	104.5	97.4	116.5	128.8
Day 6	100	104.7	96.6	107.9	119.2
Day 13	100	113.2	83.5	95.3	125.9
Day 20	100	106.1	86.2	91.8	108.9
Day 34	100	99.9	96.8	96.2	103.9

Table 194: Bone Mineral Density of Left Femur (Percent of Saline Control)

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	89.6	86.4	111.3	110.2
Day 6	100	100.2	108.7	93.0	106.0
Day 13	100	115.6	119.8	110.1	140.5
Day 20	100	116.1	125.7	113.4	125.9
Day 34	100	108.6	119.4	106.5	112.2

Table 195: Bone Mineral Density of Right Femur (Percent of Saline Control)

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	103.7	96.3	109.5	117.4
Day 6	100	103.9	95.7	101.5	109.2
Day 13	100	114.3	82.1	90.8	116.8
Day 20	100	106.3	85.0	86.6	100.6
Day 34	100	100.1	96.0	90.8	96.0

Gross Pathology

A complete necropsy was performed on all animals.

Due to an error, no macroscopic findings were recorded.

Histopathology

Adequate Battery: Not for a full toxicology study, because only left and right femur were analyzed. As this study was intended to characterize the effects on bone, this is acceptable.

Peer Review: No.

Histopathological examinations were only performed on the left and right femur. The stage of the healing process was scored by the pathologist according to a scoring system described by Xu et al., 2011.

Histological Findings

There was no significant difference in the mean healing score for any group compared to the saline control. All animals had a healing score of 4-5, except one animal receiving the vehicle control which had a healing score of 2.

Table 196: Healing Score of Femur

Group	Saline	Vehicle	Bup comp	HTX-011-49	HTX-011-56
Healing score-left	4.00	4.50	4.00	4.00	4.00
Healing score-right	4.13	3.67	4.00	4.14	4.14

2.1 Scoring System: Healing Stages

- Stage 1: Predominance of soft tissue reaction (mixture of hematoma and granulation tissue) with a small amount of intramedullary woven bone located adjacent to the bone defect.
- Stage 2: Intramedullary woven bone predominates, mixed with smaller amounts of soft tissue reaction composed of immature mesenchymal tissue. Bone defect is not completely bridged by the new bone formation.
- Stage 3: Complete bridging of the bone defect on the endosteal surface and moderate amount of remodeled intramedullary trabecular bone remaining in the medullary cavity with inter-trabecular spaces containing varying amounts of immature connective tissue and/or re-established marrow population.
- Stage 4: Complete bridging and partial filling of bone defect with remodeled woven bone, reduced amount of intramedullary woven bone as compared with Stage 3, and reestablishment of marrow population.
- Stage 5: All cortical surfaces bridged by the remodeled bone with the absence of the intramedullary bone and complete resolution of soft tissue reaction and bone defect.

From page 74 of Study 33-39

Dosing Solution Analysis

Dosing solution analysis was not conducted.

Study title: HTX-002, HTX-009 and HTX-011: A Single Dose 15-Day Wound Healing Dermal Toxicity Study in the Gottingen Minipig

Study no.: 33-54
Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-to\33-54\33-54.pdf>

Conducting laboratory and location:  (b) (4)

Date of study initiation: June 20, 2016
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: HTX-002-013, Lot 16007, 2.5% meloxicam, >99.9% purity
HTX-009-005, Lot 15011, 0.075% meloxicam, purity not reported
HTX-011-056, Lot 026126, 0.075% meloxicam and 2.5% bupivacaine, purity not reported

Key Study Findings

- HTX-011 caused slightly worse Draize scoring and wound observations compared to saline and sham control.
- Wounds were not completely healed by Day 15 in any group. A slight increase in the severity of histopathological findings was observed at incision sites treated with HTX-011 compared to saline and sham controls.
- Overall the impact on wound healing appeared to be minimal since only small increases in severity were observed by histopathology.
- The doses used in this study are lower than those used in the clinical studies based on normalization to the incision size. The volume per incision size used in this study was 1 mL/cm. Clinical studies are expected to be herniorrhaphy; 2.5-3.4 mL/cm,  (b) (4) based on information provided by the clinical team. Because clinical studies were permitted to proceed, the reader is referred to the clinical review for definitive information on the impact of the drug product on soft tissue wound healing in humans.

Methods

Doses: 87.75 mg bupivacaine and 2.63 mg meloxicam
 Frequency of dosing: Once by wound surface application into a surgical incision.
 Route of administration: Wound surface application
 Dose volume: 3 mL
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-002-013, HTX-009-005, and HTX-011-56). The vehicle control was HTX-024-002. See table below for further details.
 Species/Strain: Gottingen Minipig, *Sus scrofa domestica*
 Number/Sex/Group: 3/sex/group (Main)
 Age: 9-10 months
 Weight: 17.8-20.5 kg (m) and 17.9-21.5 kg (f)
 Satellite groups: None
 Unique study design: Wound healing following surgical incision. Each animal received six incisions and received all treatments: saline, HTX-011-056, vehicle, HTX-009-005, HTX-002-013, and sham; one treatment per incision.
 Deviation from study protocol: None that impacted the results of the study.

Table 197 Experimental Design of Study 33-54

Incision	Test and Control Items	Active Pharmaceutical Ingredients (APIs)		Excipients			
		Bupivacaine w/w% ¹ (mg/g)	Meloxicam w/w% (mg/g)	Maleic Acid w/w% (mg/g)	DMSO w/w% (mg/g)	Triacetin w/w% (mg/g)	AP135 w/w% (mg/g)
1	Saline Control	NA	NA	NA	NA	NA	NA
2	Vehicle Control HTX-024-002	NA	NA	NA	10.00 (100.00)	25.00 (250.00)	65.00 (650.00)
3	HTX-002-013	2.50 (25.00)	NA	0.05 (0.50)	10.00 (100.00)	25.00 (250.00)	62.45 (624.5)
4	HTX-011-056	2.50 (25.00)	0.075 (0.75)	0.05 (0.50)	10.00 (100.00)	25.00 (250.00)	62.375 (623.75)
5	HTX-009-005	NA	0.075 (0.75)	NA	10.00 (100.00)	25.00 (250.00)	64.925 (649.25)
6	Sham Control	NA	NA	NA	NA	NA	NA

Data from page 10 of the study report.

Observations and Results

Mortality

No mortalities were observed.

Clinical Signs

Cage side observations were conducted once daily throughout the study except on days with detailed clinical observations. Detailed clinical observations were conducted once before treatment, weekly throughout the study period, and before necropsy.

Bruising, crust, and decreased activity were seen sporadically throughout the study. Since each animal received all treatments, these findings could not be ascribed to any of the test articles.

Dermal Observations

Dermal observations were assessed post-surgery on Days 2-8 and Day 15 for animals being sacrificed on Day 15. Dermal observations were assessed post-surgery on Days 2-4 for animals being sacrificed on Day 4.

In animals sacrificed on Day 4, increased number of days with erythema was observed with treatment of HTX-011 compared to the saline and sham controls in both males and females. Increased number of days with erythema were also observed with treatment of the bupivacaine formulation comparator (male and female) and vehicle (female). The average severity was similar between all groups. For edema, there did not appear to be a major difference in the number of days observed. There was a small increase in the average severity of edema for females at incision sites treated with HTX-011.

In animals sacrificed on Day 15, there did not appear to be a difference in the number of days edema was observed between the different treatments. However, there did appear to be an increase in the incidence of observations on Day 15 compared to the saline and vehicle control with HTX-011.

Taken together, HTX-011 appeared to result in a slightly worse Draize scoring across the study.

Table 198: Dermal Observations in Day 4 Sacrifice Animals, Male

Group	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
Erythema 3 animals	-	1 (1)	3 (1)	3 (1)	2 (1)	5 (1)
Edema 3 animals	8 (1.2)	8 (1)	8 (1.3)	9 (1)	9 (1)	8 (1.2)

Total # of days observed (average severity); n=3 animals/group

Table 199: Dermal Observations in Day 4 Sacrifice Animals, Female

Group	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
Erythema 3 animals	2 (1)	6 (1)	4 (1)	2 (1)	2 (1)	4 (1)
Edema 3 animals	9 (1.1)	9 (1.3)	9 (1.1)	9 (1.1)	9 (1.2)	9 (1.5)

Total # of days observed (average severity); n=3 animals/group

Table 200: Dermal Observations in Day 15 Sacrifice Animals, Male

Animal	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
M1		5 (1.2)	6 (1)	7 (1.2)	5 (1.2)	6 (2) Day 15 (2)
M2		6 (1.2) Day 15 (1)	5 (1)	5 (1)	6 (1)	5 (1) 6 (2) Day 15 (1)
M3		6 (2)	1 (2)	1 (1)	5 (1)	5 (1) 6 (1.3) Day 15 (1)

Total # of days observed (average severity); n=3 animals/group

Table 201: Dermal Observations in Day 15 Sacrifice Animals, Female

Animal	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
F1	6 (1.8)	6 (1.8)	6 (2)	6 (1.8)	5 (2)	6 (2.2) Day 15 (3)
F2	6 (1.5)	6 (1.8) Day 15 (1)	6 (1.8) Day 15 (1)	6 (1.8)	7 (1.5)	6 (2) Day 15 (1)
F3	6 (2)	6 (2)	6 (1.8)	6 (1.8)	6 (1.8)	6 (2) Day 15 (1)

Total # of days observed (average severity); n=3 animals/group

Wound Observations

Wound observations were made on Day 4 (all animals), and on Days 8 and 15 for animals in the Day 15 sacrifice group.

On Day 4, there was a slight increase in the appearance of the wound (erythema), condition of the skin surrounding the wound (erythema), and the presence of hemorrhage in males at incision sites treated with HTX-011 compared to saline and sham controls. In females, this was only true for the condition of skin surrounding the wound.

In animals sacrificed on Day 15, increased severity was observed in males on Day 8 for presence of hemorrhage only. No difference in severity for any findings was observed on Day 15.

Table 202: Wound Observations on Day 4

Group	Saline	Vehicle	Bup comp	Meloxicam	Sham	HTX-011-56
Males						
Appearance of Wound and Wound Edge n = 6	6 (1.5)	6 (1.3)	6 (1.8)	6 (1.5)	5 (1.6)	6 (1.8)
Condition of skin surrounding the wound n = 6	5 (1)	5 (1)	5 (1.6)	4 (1)	4 (2)	5 (1.8)
Presence of hemorrhage n = 6	5 (1.8)	5 (1.6)	6 (2.0)	3 (2.3)	4 (1.8)	6 (2.3)
Presence of exudates n = 6	0	1 (2)	1 (3)	0	0	2 (1)
Females						

Appearance of Wound and Wound Edge n = 6	6 (2.2)	6 (2.3)	6 (1.7)	6 (2.0)	6 (2.3)	5 (2.2)
Condition of skin surrounding the wound n = 6	6 (1.5)	6 (1.2)	4 (1.5)	4 (1.5)	5 (1.6)	6 (1.8)
Presence of hemorrhage n = 6	6 (2.8)	6 (2.3)	6 (2.7)	6 (2.5)	6 (2.7)	6 (2.5)
Presence of exudate n = 6	4 (2.5)	1 (2)	4 (2.8)	3 (2)	1 (3)	4 (2.3)

of animals with finding (average severity); n = 6/sex/group

Table 203: Wound Observations on Day 8

Group	Saline	Vehicle	Bup comp	Meloxicam	Sham	HTX-011-56
Males						
Appearance of Wound and Wound Edge (1/3)	3 (1)	3 (1)	3 (1.3)	2 (1.5)	3 (1.3)	2 (1.5)
Condition of skin surrounding the wound (1/3)	3 (1.3)	2 (1)	2 (2.5)	1 (1)	3 (2)	3 (2)
Presence of hemorrhage (3/3)	3 (1.3)	2 (1.5)	3 (2.3)	2 (2)	3 (1)	3 (2.7)

of animals with finding (average severity); n = 6/group

Gross Pathology

Only the dosing sites were evaluated for gross lesions. There did not appear to be any difference between groups in terms of gross pathological findings at the surgical site.

Table 204: Gross Pathology of the Incision Site, Day 4

Sex	Male						Female					
Incisions *	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Clear fluid	0	1	0	0	1	0	2	2	3	2	0	0
Crepitus	0	0	0	0	0	2	0	0	0	0	0	2
Dark area	2	3	2	3	2	3	3	3	3	3	3	3
Dark material	0	0	0	0	0	0	0	0	1	1	0	0
Gelatinous material	1	1	2	3	3	1	1	0	0	1	0	1
Pale material	0	0	1	1	0	0	0	2	1	0	0	0
Scar	3	2	2	3	3	2	1	1	1	1	1	2
Wound	0	1	1	0	0	1	2	2	2	2	2	1

* Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 29 of the study report

Table 205: Gross Pathology of the Incision Site, Day 15

Sex	Male						Female					
Incisions *	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Crepitus	0	0	0	0	0	0	0	0	0	0	0	1
Dark area	0	0	3	0	0	0	1	2	2	2	1	1
Mass	0	0	0	1	0	0	0	0	0	1	0	0
Pale material	0	0	0	0	0	0	0	0	0	0	1	0
Scar	3	3	3	3	3	3	3	3	3	3	3	3
Thickening	0	0	0	0	0	0	0	0	1	1	0	0

* Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 30 of the study report

Histopathology

Adequate Battery: Yes**Peer Review: No.**

Histopathological examinations were only performed on incision sites and gross lesions identified during necropsy.

Histological Findings

Overall, there was a similar incidence of histopathological findings across the different treatments. The exception was that one male in HTX-011-56 (Group 4) on Day 15 had a mild abscess that was not seen with any other treatment group. There were some minor increases in the severity of findings with HTX-011 for a couple of findings, including serro-cellular crust on Day 4, inflammatory cell infiltrates on Day 15, and granulomatous inflammation of the subcutis on Day 15.

Table 206: Summary of Histopathological Findings, Day 4

Sex	Male						Female					
Incisions*	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Cell infiltrate, inflammatory	3	3	3	3	3	3	3	3	3	3	3	3
minimal	0	0	0	0	0	0	2	1	0	1	2	1
mild	3	1	3	2	3	2	1	2	3	2	1	2
moderate	0	2	0	1	0	1	0	0	0	0	0	0
Eosinophilic material	3	3	3	3	3	3	3	3	3	3	3	3
minimal	3	2	2	1	2	2	1	1	0	0	2	1
mild	0	1	1	2	1	1	2	2	3	2	1	1
moderate	0	0	0	0	0	0	0	0	0	1	0	1
Sero-cellular crust	3	3	3	3	2	3	3	3	3	3	3	3
minimal	2	1	2	1	2	1	1	1	1	1	3	2
mild	1	2	0	2	0	2	2	1	1	1	0	1
moderate	0	0	1	0	0	0	0	1	1	1	0	0
Ulcer	3	3	3	2	2	3	3	3	3	3	2	2
minimal	2	2	2	1	2	2	2	3	2	2	2	2
mild	1	1	1	1	0	1	1	0	1	1	0	0

*Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 31 of the study report

Table 207: Summary of Histopathological Findings, Day 15

Sex	Male						Female					
	1	2	3	4	5	6	1	2	3	4	5	6
Incisions*	1	2	3	3	3	3	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Abscess	0	0	0	1	0	0	0	0	0	0	0	0
mild	0	0	0	1	0	0	0	0	0	0	0	0
Cell infiltrate, inflammatory	1	0	0	1	0	0	1	0	0	1	0	0
minimal	1	0	0	0	0	0	1	0	0	0	0	0
mild	0	0	0	1	0	0	0	0	0	1	0	0
Contraction dermal (present)	3	1	1	2	1	3	3	3	3	2	3	2
Inflammation, granulomatous, dermis	3	3	3	3	3	3	3	3	3	3	3	3
minimal	2	2	0	3	2	2	0	1	0	0	0	0
mild	1	1	3	0	1	1	3	0	2	3	2	3
moderate	0	0	0	0	0	0	0	2	1	0	1	0
Inflammation, granulomatous subcutis	3	3	3	3	3	3	3	3	3	3	3	3
minimal	1	2	0	0	1	3	0	0	1	0	0	3
mild	2	1	3	1	2	0	3	2	1	1	3	0
moderate	0	0	0	2	0	0	0	1	1	2	0	0
Sero-cellular crust	3	2	2	3	3	3	3	3	1	1	3	2
minimal	1	1	2	2	2	1	1	3	1	1	3	2
mild	2	1	0	1	1	2	2	0	0	0	0	0

*Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 32 of the study report

10.2 Juvenile Animal Studies

As noted in the agreed iPSP, the Applicant will be completing two juvenile animal toxicology studies. The first is a study in the rat model to evaluate the impact of DMSO on brain development. This study is being completed because the Division raised concerns with the potential impact of DMSO via this product on brain development based on published report describing widespread neuronal apoptosis in young animals (Hanslick et al., 2009). (b) (4)

(b) (4). Likewise, because kidney, liver, lung, and testes are known target organs of toxicity for NSAIDs, a juvenile animal study will be completed (b) (4) (b) (4)

(b) (4). Feedback on the protocols has already been provided by the Division. According to the currently agreed amended IPSP, these studies are to be completed in accordance with the following timelines.

Table 208 Timeline of the Pediatric Development Plan

(b) (4)

Table from the Applicant's agreed iPSP filed under IND 125927

11 Integrated Summary and Safety Evaluation

The Applicant's original NDA submission was issued a Complete Response with multiple nonclinical deficiencies which are summarized as follows: the Applicant did not provide adequate justification for the safety of excipients (DMSO, triacetin, and maleic acid) in terms of reproductive and developmental toxicity, the Applicant did not provide adequate justification for the specification of the impurities (b) (4) or (b) (4) and the Applicant did not provide adequate extractable/leachable information for toxicological evaluation of the safety of the container closure system. In addition, to the deficiencies, the Applicant was reminded that they did not have nonclinical support for their proposed dose of 400 mg bupivacaine and that the safety of this dose was not adequately supported in their submission. In the Applicant's first resubmission, they provided adequate data to address the specification for (b) (4) the deficiencies regarding the extractable leachable testing, and the safety of 400 mg bupivacaine. However, a second Complete Response was issued due to the Applicant not adequately addressing the specification for (b) (4) and the safety of the excipients in

terms of reproductive and developmental toxicity. The following sections describe the adequacy of the Applicant's attempts to address the deficiencies in the second resubmission and the overall pharm/tox conclusions regarding the drug product based on data from the original submission and the Applicant's resubmissions.

Excipients

In the original submission, the Applicant did not provide adequate justification for the safety of excipients (dimethyl sulfoxide, triacetin, and maleic acid) in terms of reproductive and developmental toxicity. The first resubmission included new data to address these deficiencies but was still considered inadequate and a second Complete Response was issued. Additional data were provided in the second resubmission.

To support the reproductive and developmental safety of dimethyl sulfoxide (DMSO), the Applicant referenced the Organization for Economic Cooperation and Development's (OECD) Screening Information Dataset (SIDS) dossier for DMSO, which contained information regarding GLP reproductive and developmental toxicity studies. No evidence of reproductive or developmental toxicity was reported in an oral reproduction/developmental toxicity screening study in rats, an oral developmental toxicity study in rats, or an oral developmental toxicity study in rabbits up to 1000 mg/kg. Developmental toxicity associated with maternal toxicity (decreased body weight gain in dams manifesting as decreased fetal weight and reduced/delayed ossification of the ribs) was observed in the rat developmental study at 5000 mg/kg. The overall NOAEL for reproductive and developmental toxicity was 1000 mg/kg, which is a human equivalent dose of 9,677.4 mg based on body weight scaling and a 60 kg individual.

The Applicant performed PK studies in animals and humans to bridge exposure to DMSO at the MRHD in patients undergoing TKA. Rats and rabbits were dosed with HTX-011 at 1000 mg/kg to mimic the conditions of the nonclinical toxicology studies and PK parameters were compared to humans undergoing TKA and receiving the MRHD of HTX-011 (400 mg/12 mg). Both AUC_{0-inf} and C_{max} were higher in animals receiving oral doses of 1000 mg/kg than in TKA patients receiving the MRHD. AUC_{0-inf} was 47 (nonpregnant) to 55 (pregnant) times higher in rats receiving oral doses of 1000 mg/kg compared to TKA patients receiving the MRHD and 72 times higher in pregnant rabbits receiving oral doses of 1000 mg/kg compared to TKA patients receiving the MRHD. C_{max} was ~125 times higher in rats and ~146 times higher in rabbits as compared to TKA patients receiving the MRHD. Since the nonclinical reproductive and developmental toxicity studies cited by the Applicant did not show any evidence of reproductive or developmental effects with oral doses of DMSO up to 1,000 mg/kg and the systemic exposure to DMSO in these studies was estimated to be higher than DMSO exposure from the drug product at the MRHD, the nonclinical data support that there is minimal risk for developmental or reproductive toxicity from the DMSO in the drug product.

To support the reproductive and developmental safety of triacetin, the Applicant cited an unpublished Japanese GLP oral combined reproduction/developmental toxicity study in

rats and provided a translated copy of the study report. This study was also evaluated by the Organization for Economic Cooperation and Development (OECD 2002a). No findings of reproductive or developmental toxicity were observed in the study, which tested up to 1000 mg/kg (human equivalent dose of 9,677.4 mg based on body weight scaling and a 60 kg individual).

Developmental data in a second species were not available. To address this data gap, the Applicant cited developmental data located in OECD SIDS documents for the major expected metabolites of triacetin, glycerol and acetic acid. Glycerol showed no evidence of developmental toxicity when tested in the rat (up to 1310 mg/kg), mouse (1280 mg/kg), or rabbit (up to 1180 mg/kg). Acetic acid showed no evidence of developmental toxicity when tested in the rat, mouse, and rabbit (all up to 80 mg/kg). It appeared from the data in the SIDS document that higher doses of acetic acid could not be tested due to stomach irritation. The OECD documents also contained reproductive toxicity data for the metabolites. Glycerol was tested in a two-generation reproduction study administered orally as a 20% solution in water each day and no effects were observed up to a dose of 2000 mg/kg. Fertility was evaluated in an oral study with sodium acetate (as a surrogate for acetic acid) in mice to assess fertility up to a dose of 60 mg/kg and no effects on fertility were observed.

The Applicant performed PK studies in rats and humans to bridge exposure to triacetin at the MRHD in patients undergoing TKA. In rats, triacetin was rapidly metabolized and primarily only detectable above the limit of quantitation (LOQ) at the first two time points (5 and 15 minutes). Overall, the data suggested that orally administered triacetin is rapidly metabolized in rats and that systemic exposure is primarily to metabolites of triacetin. In humans, sampling was limited by the fact that an esterase inhibitor needed to be added at the time of blood collection so that further metabolism would not occur before measurement. This resulted in a small number ($n = 9$) of samples with sparse PK measurements being available by the time of the second resubmission. Of the nine patients, only two had detectable levels above the LOQ (2 ng/mL), suggesting that for the most part triacetin was not detectable in human circulation following dosing with HTX-011.

Based on the animal and human data, the Applicant concluded that there was low to no exposure in humans and that the exposure in rats was substantially higher. This was based on a comparison of the estimated C_{max} between rats and humans since non compartmental analysis for a determination of AUC could not be performed on the human data. However, there are some uncertainties in the data supporting the Applicant's conclusion. First, the animal data are primarily driven by an individual animal who had a triacetin value that was substantially higher (>200 fold) than any other measurement and above the upper LOQ. The estimated C_{max} with this value removed would be much closer to the human value (29.4 ng/mL in rats vs. 15.8 ng/mL in humans). Second, the first PK time point in humans was at 30 minutes. While the in vitro release data suggests that significant triacetin should be released at this time point, the limited human PK data indicated that triacetin was highest at the first time point (30 mins). Thus, it is unclear if higher values of triacetin would be observed at earlier

timepoints. Considering the uncertainties in the data, it is the FDA reviewer's opinion that the data are not robust enough to make a direct comparison of systemic exposure to triacetin between animals and humans.

The Applicant also cited literature review articles to support that triacetin would not cross the placenta. The review articles and the data summarized within did not directly state that triacetin would not cross the placenta. Instead, the references focused on how the body utilizes triglycerides during pregnancy. Since triacetin is a relatively simple triglyceride molecule, it seems reasonable that the metabolic pathways described in the article would apply to triacetin as well. The consensus across the review articles was that triglycerides do not cross the placenta intact and are first metabolized by lipoprotein lipases to produce fatty acids, glycerol, and other glycerides, which can then enter the placental cells.

Due to the very low levels of triacetin in humans following administration of HTX-011, the Applicant estimated the steady state concentrations of glycerol and acetic acid from HTX-011 assuming the entire amount of triacetin in the drug product at the MRHD (4 g) was released over 12 hours and converted into the two metabolites. The assumption of 12-hour release is based on the results of in vitro release data. Based on the limited PK data, it is unclear if the assumption of triacetin being released over 12 hours is an accurate portrayal of in vivo release since triacetin was only seen in two patients; one with a single value at 30 minutes and a second with the highest value at 30 minutes with a gradual decrease. Using published clearance values for glycerol and acetic acid, the estimated steady state concentrations of glycerol and acetic acid from the drug product were 1.7-8.2 times lower than reported endogenous levels. Since both glycerol and acetic acid are endogenous molecules that are involved in a number of important physiological processes, the body would be expected to be able to utilize the metabolites and they would be unlikely to disrupt normal biological processes.

When taken together, the data support a low risk for reproductive or developmental toxicity from the level of triacetin in the drug product. Triacetin has a short half-life in rat and human blood and the animal/human PK data suggest that systemic exposure to triacetin is low and transient. Further, any systemic triacetin is not expected to cross the placenta. Thus, it appears that exposure will primarily be to the metabolites of triacetin, which are expected to be glycerol and acetic acid. Neither triacetin, nor its metabolites, showed any evidence of reproductive or developmental toxicity in animal studies following oral exposure. For triacetin and glycerol, it appears that exposure in the animal studies was sufficient to support the safety of the levels of triacetin in the drug product. This was not the case for acetic acid since dosing in animals by the oral route is limited by stomach toxicity due to the corrosiveness of acetic acid. However, it is likely that sufficient levels of acetic acid were generated from triacetin in the rat combined reproductive/developmental study based on the Applicant's rat PK experiment to support the levels expected from triacetin in the drug product dosed at the MRHD. Further, the estimated human exposure to acetic acid and glycerol from the drug at the MRHD was below endogenous values reported in the literature. Since both molecules are important for multiple physiological processes, it is expected that the body will be able to utilize the

metabolites without consequence. Based on a weight of the evidence approach, the levels of triacetin in the drug product are not expected to result in reproductive or developmental toxicity.

To support the reproductive and developmental safety of maleic acid, the Applicant provided rat oral reproduction and developmental toxicity data from an EPA action memorandum in the original submission. No reproductive or developmental effects were seen at human equivalent doses of 532 mg (reproductive) and 1354.8 mg (teratogenic), which is 67x and 169x times the levels a patient would be exposed to at the MRHD, respectively, based on body surface area and a 60 kg individual. The oral bioavailability in rats was about 30% based on a reference provided by the Applicant. The safety margins would still be sufficiently high even if it is assumed that instillation into the wound would result in 100% bioavailability of maleic acid with adjusted safety margins of 20x and 51x.

In the first resubmission, the Applicant submitted an oral developmental study in rabbits with maleic acid (Study 34-74). No evidence of developmental toxicity was observed in the study up to an oral dose of 20 mg/kg, which is a human equivalent dose of 387.1 mg based on body surface area and a 60 kg person. Higher doses could not be tested due to the potential for stomach irritation. This dose produced no evidence of maternal toxicity and therefore was not ideally designed as a toxicology study but could be justified as a sufficiently high enough dose as it was expected to provide ~50-fold exposure compared to the MRHD by BSA scaling.

For the second resubmission, the Applicant performed PK studies in pregnant rabbits and humans to bridge exposure to maleic acid following oral exposure in animals to exposure at the MRHD in patients undergoing TKA. In pregnant rabbits receiving the same dose as in the developmental toxicity study (20 mg/kg), maleic acid was detectable in the blood and AUC_{0-24h} and C_{max} were calculated from the data. In TKA patients receiving the MRHD of HTX-011, only 12/50 of the patients had quantifiable concentrations of maleic acid at any time point. Due to the small number of quantifiable time points, noncompartmental analysis could not be completed to calculate an AUC. Thus, to compare exposure between animals and humans the C_{max} was assumed to be less than the lower limit of quantitation (0.05 mcg/mL) and the AUC_{0-24h} was assumed to be $<1.2 \text{ h} \cdot \text{mcg/mL}$ (assuming the LLOQ for 24 hrs). Using the LLOQ as the worst case estimate of the AUC_{0-24h} and C_{max} results in the animal study having higher exposure by 5.6-times for AUC_{0-24h} and 56-times for C_{max} . Since most of the human samples had detectable peaks despite being below the LLOQ, the Applicant also extrapolated the values of maleic acid below the standard curve. Based on the extrapolated values, the estimated C_{max} was 0.038 mcg/mL.

Despite the low detectable levels of maleic acid in humans, comparing the animal and human data seems reasonable for the following reasons: 1) the shape of the PK curve was very similar to DMSO suggesting a similar release profile for maleic acid, 2) the level of maleic acid in the drug product is relatively low compared to other excipients so low exposure is reasonable, and 3) the AUC comparison is a very conservative estimate

and still provides a reasonable safety margin. Still, low exposure to maleic acid could indicate that maleic acid is being rapidly metabolized and that exposure is predominantly to metabolites of maleic acid. The Applicant provided data to support that maleic acid is eliminated by conversion to malic acid and excreted as CO₂. The Applicant reasons that malic acid does not possess a concern for reproductive or developmental safety since it is an intermediary of the citric acid cycle.

Since the nonclinical rabbit developmental toxicity study conducted by the Applicant did not show any evidence of developmental effects with oral doses of maleic acid up to 20 mg/kg and the systemic exposure to maleic acid in these studies would be higher than exposure from the drug product at the MRHD, the oral developmental study is adequate to support that the levels of maleic acid in the drug product at the MRHD is not expected to result in developmental toxicity.

In terms of local toxicity of the excipients, treatment with the HTX-011 vehicle (containing DMSO and triacetin) resulted in an inflammatory reaction that is consistent with a reaction to foreign material. This was characterized as chronic inflammation that lasted for up to 28 days. A similar reaction was seen with the HTX-011 drug product (containing DMSO, triacetin, and maleic acid). Based on studies with APF18A (a similar polymer (b) (4)), the inflammatory reaction is very likely driven by the presence of AP135, although a contribution of the other excipients cannot be completely ruled out. In addition to the local inflammatory reaction, more severe findings, such as necrosis, were seen in the single-dose rat studies but this is possibly due to the increased injection volume used in these studies (0.5-2 mL in single dose studies as compared to 0.13-0.19 mL in repeat dose studies).

In terms of systemic effects of the excipients, the primary findings with the HTX-011 vehicle (containing DMSO and triacetin) were changes in hematology parameters with similar effects observed with HTX-011 (containing DMSO, triacetin, and maleic acid). Again, it is likely that these changes are the result of AP135 as similar findings were seen with APF18A, although a contribution of the other excipients cannot be completely ruled out. More severe effects (decreased body weights and decreased activity) were seen in the repeat-dose dog study with the HTX-011 vehicle (Study 33-86). The more robust systemic reaction to the HTX-011 vehicle in dogs is likely related to the large dose of vehicle in this study as the effects were not observed with the clinical formulation in the same study, which delivered about ½ the level of excipients.

In conclusion, all of the previous deficiencies regarding the excipients were fully addressed by the Applicant. The existing data suggest that there is minimal risk for reproductive or developmental toxicity from the excipients in the drug product when given at the MRHD. Based on the local tissue assessment conducted with various formulations, the data clearly suggest that the excipients contribute to the observed local inflammatory reaction and the systemic hematology findings. However, we acknowledge that there is clinical experience with the excipients from the Phase 2 and 3 studies and defer to the clinical review team regarding if the effects observed in non-

clinical studies translate into clinical adverse effects. If the clinical team concludes that the nonclinical findings do not translate into clinical adverse effects, then the excipients are characterized for systemic safety at a margin of 1.5x for AP135, 1.4x for DMSO and triacetin, and 1.1x for maleic acid and locally at a margin of ~1x (clinical concentration used in experiments) at the MRHD of 400 mg bupivacaine/12 mg meloxicam.

Impurities

In the original submission, the Applicant did not provide adequate justification for two impurities that were above the ICH Q3B(R2) qualification threshold, (b) (4) and (b) (4). (b) (4) was adequately addressed in the Applicant's first submission and (b) (4) was adequately addressed in their second resubmission.

The Applicant reduced the specification of (b) (4) in the drug product to NMT (b) (4)%, which is acceptable based on the qualification thresholds in ICH Q3B(R2).

For (b) (4) the Applicant justified the levels using a combination of unpublished nonclinical studies with (b) (4) to address systemic toxicity and nonclinical experience with HTX-011 to address local toxicity. (b) (4) has been reported to be negative in an Ames bacterial reverse mutation assay, a chromosomal aberration assay, and a sister chromatid exchange assay. In an unpublished GLP 13-week dietary repeat-dose toxicity study in rats, decreased body weight was seen at a dose of (b) (4) mg/kg with a NOAEL of (b) (4) mg/kg. This would correspond to an HED of (b) (4) mg based on body weight scaling and 60 kg individual. The Applicant also cited a report from the Cosmetic Ingredient Review (CIR, 2006) which included data in rats indicating that (b) (4) is well absorbed by males following oral administration, with (b) (4)% of the administered dose found in urine after 5 days. Thus, based on the high oral bioavailability of (b) (4) the dietary study is appropriate for use to support the clinical route of administration.

The maximum amount of (b) (4) in the drug product based on the Applicant's proposed specification of NMT (b) (4)% would be (b) (4) mg at the MRHD ((b) (4) mg), which is covered by the dosing in the above study. (b) (4)

(b) (4). The maximum amount of (b) (4) from the drug product would be (b) (4) mg), (b) (4)

(b) (4). The results of the repeat-dose study support that even the overestimate of (b) (4) g of (b) (4) would not be expected to result in any systemic toxicological effects.

In terms of local exposure, the Applicant reduced the specification to NMT (b) (4) % based on the amount of (b) (4) in the nonclinical batches used in the 6- (b) (4) % and 9- (b) (4) % month nonclinical studies at the 6- and 9-month timepoints. While the amount of (b) (4) in the nonclinical studies is not quite (b) (4) %, an increase of (b) (4) to (b) (4) % compared to the amount tested in the nonclinical studies would not be expected to result in a worse local reaction to the drug product.

The specifications of all other impurities were either within ICH guidelines or adequate justification was available. Other than (b) (4) there are no nonclinical deficiencies regarding drug substance impurity or drug substance/product degradant specifications.

Extractables/Leachables

In the original submission, the Applicant did not provide adequate leachable data to permit substantive toxicological evaluation of the safety of the container closure system and the (b) (4) of the drug product. Specifically, the Applicant did not test at least three drug product stability batches over the entire course of proposed expiry, did not perform leachable studies for all compounds identified above the safety concern threshold (b) (4) mcg/day), did not adequately identify the compounds they detected, and did not provide adequate justification for the use of a surrogate approach for (b) (4) found in the drug product.

The Applicant addressed these deficiencies in their resubmission. This included conducting new more comprehensive extractable and leachable studies, updating the results of their previous studies, and providing a detailed risk assessment for the leachables identified. In their new leachable studies ((b) (4) Leachable Study 3 and 4), the Applicant evaluated 24 or more lots of drug product aged between 2 and 38 months, which allowed evaluation of trends over time. The range of samples tested covers the entire proposed expiry of the drug product (36 months) and includes samples at early, mid, and late timepoints. In total, the Applicant conducted five leachable studies and when considered together they cover all of the extractables identified over (b) (4) mcg/day. A number of previously unidentified compounds had updated designations in the resubmission including further identification of (b) (4) identifying some compounds as (b) (4) and the identification of (b) (4). Finally, the Applicant provided detailed risk assessments for each compound to support the PDEs they chose and the data they relied on. This included an updated justification for the use of (b) (4) for (b) (4).

The Applicant conducted four extractable studies (b) (4). All four extractable studies resulted in very similar extractable profiles. However, there was less agreement in terms of the leachables identified above the AET and the quantitative levels of the leachables across the five leachable studies. For example, the leachables identified by the (b) (4) leachable study above the AET ((b) (4) and (b) (4)) were not found above the AET in any of the (b) (4) leachable studies. It is not clear why the studies gave different results, but possible reasons could include differential targeting of leachables, differences in the samples evaluated, and differences in

analytical methods. Due to the discrepancies, the highest level of any leachable identified above the AET from any of the leachable studies was considered to require safety justification.

In total, eight individual leachables were identified above the AET across the five leachable studies: (b) (4)

(b) (4). In addition, the combined level of (b) (4) was reported above the AET in one leachable study.

The safety of the (b) (4) was addressed with a surrogate approach. The Applicant chose (b) (4) as a surrogate compound and the Division of Applied Regulatory Science Computational Toxicology Consultation Service (CTCS) was consulted to assist in evaluating the adequacy of this approach. The CTCS agreed with the use of (b) (4) but also suggested (b) (4) as a surrogate based on its structure, more comparable molecular weight, and availability of data (see Appendix). The combined maximum detected levels of the (b) (4) were below the PDEs derived from both (b) (4) and (b) (4).

The (b) (4) were identified as potential leachables in the original submission (b) (4). The Applicant performed root cause analysis to determine the source of the (b) (4) as part of the resubmission. Their analysis supported that the primary source of the (b) (4) was the drug substances and the excipients. This is in good agreement with their extraction studies; the (b) (4) were only identified as a potential leachable from the (b) (4) in (b) (4) Extractable Study 2 where the (b) (4) used were in contact with the drug product prior to analysis. Regardless of the source, (b) (4) Leachable Study 2 and 4 identified (b) (4) in the drug product above the AET and the safety of the levels of the (b) (4) needed to be justified.

In the original submission, (b) (4) were identified above the AET in (b) (4) Leachable Study 2 based on a conservative correction factor for quantitation. For the resubmission, the Applicant recalculated the values for the (b) (4) using response factors that were specific to the (b) (4) and a more accurate maximum dose volume (b) (4). While (b) (4) mL is technically below the MRHD of (b) (4) mL, it was determined that the difference would not significantly alter the Applicant's results. Following the recalculation, the levels of (b) (4) were lower than originally determined and only three (b) (4) (b) (4) and (b) (4) were above the AET. The results are consistent with the Applicant's new leachable study where only (b) (4) but not the other (b) (4) was observed above the AET.

The Applicant used a surrogate approach to address the potential for toxicity of the (b) (4) because toxicological data was not available for (b) (4) and the toxicity profiles of (b) (4) and (b) (4) were similar. The available data for both (b) (4) and (b) (4) suggested that the liver was the major target organ and the compounds caused similar irritation effects following

inhalation exposure. PDEs calculated by the FDA reviewer were also similar (b) (4) and using the more conservative value for both (b) (4) and (b) (4) is reasonable. Since no toxicological data are available for (b) (4) the Division of Applied Regulatory Science Computational Toxicology Consultation Service (CTCS) was consulted to assist in evaluating the adequacy of the Applicant's approach to use (b) (4) and (b) (4) as surrogates. The CTCS concluded that the Applicant's approach was acceptable. Since all of the (b) (4) were predicted to result in similar toxicological effects, the highest values of (b) (4) (b) (4) and (b) (4) reported in the leachables studies were combined and compared to the most conservative PDE, (b) (4) mg/day. The highest level reported for each (b) (4) when combined was below the PDE by a factor of 15x. Therefore, the levels of (b) (4) detected in the drug product are not of a toxicological concern.

The Applicant did not directly measure the levels of (b) (4) in the leachable studies despite detecting it in extractable studies above the SCT because they did not consider it a leachable from the container closure (b) (4). Since (b) (4) could end up in the final drug product (b) (4) (based on it being identified in the extraction studies), the Division felt that the total amount in the final drug product needed to be justified for safety, which was communicated to the Applicant as an information request during the second cycle. To address the safety of (b) (4) in the drug product, the Applicant provided a worst-case estimate of the level of (b) (4) and performed a risk assessment based on their estimated worst-case levels.

The Applicant's worst-case levels were based on the specification for (b) (4) at the MRHD and the maximal level extracted from (b) (4) with (b) (4) in the (b) (4) Extractable study. The level of (b) (4) at the MRHD (b) (4) was estimated as (b) (4) mg and the level reported in the extraction study was (b) (4) (b) (4) resulting in a total worst-case estimate of (b) (4) mg. The amount of (b) (4) reported in the (b) (4) extractable study was less than the levels reported in other extraction studies, such as (b) (4) Extractable Study 2 where (b) (4) mcg was extracted from (b) (4) with bulk drug product. This suggests that (b) (4) mcg may be an underestimate of the amount of (b) (4) from (b) (4). The FDA reviewer believed that since no (b) (4) was detected above the minimum quantifiable limit of (b) (4) mcg/mL (or (b) (4) mcg based on the MRHD) in (b) (4) leachable study after subtracting for background (b) (4) in the drug product that (b) (4) mcg would be a more reasonable estimate of the worst-case level of (b) (4) in the drug product from (b) (4). This would make the final worst-case estimate of (b) (4) in the drug product as (b) (4) mg. The Applicant provided publicly available toxicity data for (b) (4) which was deemed to be adequate to assess its toxicity. The worst-case estimate of the levels of (b) (4) were below the PDE calculated from the data.

(b) (4) and (b) (4) were addressed by published toxicology studies and deriving PDEs. The data for both compounds were very limited with only two studies identified for (b) (4) and one study identified for (b) (4) (b) (4) is a reactive metabolite of (b) (4) and was found to cause hemorrhaging and reduce

coagulation factors more potently than (b) (4) (b) (4) is also a metabolite of (b) (4) but it was much less potent in causing hemorrhage and effects on blood coagulation as compared to (b) (4). The highest levels of each compound detected in the drug product were below the calculated PDEs and are likely not of a toxicological concern.

The (b) (4) were addressed by referencing published risk assessments from the Environmental Protection Agency, and the Massachusetts Department of Environmental Protection. These regulatory agencies have taken a fraction approach to (b) (4) dividing them into categories (b) (4)

The highest level of the summed (b) (4) in the drug product were below the calculated PDEs for (b) (4) by a factor of (b) (4) and are likely not of a toxicological concern.

In summary, the Applicant has adequately addressed the safety of all the identified leachables.

400 mg Bupivacaine dose

In the original submission, it was determined that the exposure (AUC) to bupivacaine via the drug product at 400 mg bupivacaine exceeded that of the referenced product. The pivotal 28-day nonclinical studies in rats and dogs included exposure data at 24 and 48 hours but not AUC_{inf}. Compared to human exposures in clinical study 209 (total knee arthroplasty), the rat exposures were ~ 0.5x and 0.3x for AUC₀₋₂₄ and AUC₀₋₄₈, respectively, and the dog exposures were ~ 1.0x and 0.8 x for AUC₀₋₂₄ and AUC₀₋₄₈, respectively. It was communicated to the Applicant in the CR letter as a non-hold comment that the nonclinical data did not fully support the safety of bupivacaine above a dose of 300 mg and that further data would be needed to support the 400 mg dose of bupivacaine.

Table 209 Comparison of Bupivacaine Exposure in Nonclinical Studies to Clinical Study 206

	28-day rat (33-85)	28-day dog (33-86)	Total knee arthroplasty (209) ¹	Exposure margin
AUC ₀₋₂₄ (h * ng/mL)	5710 (M, 25 mg/kg)	11700 (F, 10 mg/kg)	11223 (n = 50)	0.5x, rat 1.0x, dog
AUC ₀₋₄₈ (h * ng/mL)	8120 (M, 25 mg/kg)	18700 (M, 13 mg/kg)	23580 (n = 50)	0.3x, rat 0.8x, dog

¹Value calculated by the FDA clinical pharmacology team and differs from the values reported by the Applicant. See clinical pharmacology review for more information.

To address the lack of coverage in the rat, the Applicant conducted a 14-day study in rats by subcutaneous injection (dosing every 48 hours) of HTX formulations containing only bupivacaine (not the to be marketed formulation). The highest dose of bupivacaine

used was 150 mg/kg, which is equivalent to a human dose of ~1450 mg based on body surface area and a 60 kg individual. Results from the study were similar to those in other nonclinical studies with HTX formulations or the APF18A polymer; the only effects that could potentially be due to systemic exposure were changes in hematology parameters. The most prominent effects were increased WBC, increased platelets, increased neutrophils, increased reticulocytes and increased fibrinogen. It is difficult to assess if the magnitudes of the changes are more severe in this study compared to previous studies which used lower doses of bupivacaine due to differences in dosing volumes, formulations, and durations of treatment. No other significant adverse effects, beyond local site reactions, were observed suggesting that the overall impact on the health of the animals was minor. Since the findings appear similar to those observed with lower doses of bupivacaine and are likely caused by the excipients since multiple HTX vehicle formulations also showed effects on hematology parameters, we defer to the clinical team to determine if the effects observed are acceptable from a risk-benefit perspective. If so, then the 14-day rat study supports the 400 mg dose of bupivacaine as the highest systemic exposure (AUC_{inf}) of bupivacaine achieved in the study (55,700 ng*h/mL for females) was higher than the highest reported bupivacaine exposure reported in the Applicants clinical trials (209, total knee arthroplasty, 31,173 h*ng/mL) by a factor of ~1.8x.

It was communicated to the Applicant during a Type A meeting on August 7th, 2019 that if they planned to rely on the nonclinical data to support the 400 mg bupivacaine dose that they would need adequate data in two species. The Applicant did not submit any additional studies in dogs in the resubmission. While the data from the 28-day dog study (Study 33-86) included in the original submission is not ideal (AUC_{inf} was not calculated) it does provide data in a second species that had similar exposure to bupivacaine over 24 hours as humans exposed to 400 mg bupivacaine (~1x). The only systemic finding in dogs at this exposure level was an increase in reticulocytes. Taken together with the results from the 14-day rat study which achieved a higher exposure multiple, the safety of the 400 mg dose of bupivacaine is supported by the nonclinical data as long as the clinical team does not believe that changes in hematology parameters are of a clinical concern considering the human experience.

(b) (4)

(b) (4)

Wound and bone healing

The rat bone healing study and the rabbit hernia repair studies were not conducted in accordance to good laboratory practices (GLP). The Applicant claims that the only deviations from GLP were the lack of quality assurance statements and involvement of the Quality Assurance department during study conduct. In addition, the Applicant provided the standard operating procedures of the conducting laboratories, which have been quality assured. Based on the data provided by the Applicant and the study reports, it is this reviewers opinion that while the studies were not conducted under ideal GLP conditions, the results of the studies appear to be reliable and repeating the study would be an inappropriate use of animals based on the findings to date. The Applicant was instructed to complete any future studies intended to support human safety in accordance with GLPs and was required to include enhanced monitoring in the clinical setting to fully evaluate the potential impact on bone healing.

The main goal of the rat bone healing study was to determine if the clinical formulation would have an adverse impact on bone healing to support the proposed bunionectomy clinical study. In that regard, bone mineral density, bone mineral content, and histopathological evaluation of the bone healing stage were evaluated. No treatment related effects on bone mineral content, bone mineral density, or the healing stage of the bone were identified in this study. Based on the conditions used in this study, there was no difference in bone healing with the clinical formulation compared to the control.

The main goal of the rabbit hernia repair model was to determine the effects of the clinical formulation on Prolene mesh to support its use in the herniorrhaphy clinical study, which also included the use of mesh as part of the surgery. The interaction with

the mesh was evaluated by histopathological evaluation. It was difficult to determine how the formulation interacted with the Prolene mesh as no clear evidence of the mesh was seen in any of the sections, including on Day 3, the earliest sacrifice time point. As noted above, the Applicant attributed the lack of evidence of the mesh in the histopathology slides as likely due to loss of the fibers from the mesh during tissue processing. They note that the open spaces in the tissue where the mesh was present were noted, suggesting recent loss. However, the similar incidence of histopathological findings, including in cystic change, on Day 3 suggests that there was no adverse interaction with the mesh.

Across the two studies there was limited evidence of systemic effects following treatment with the HTX-formulations. In the rat bone healing study, no effects related to systemic exposure were observed, although the number of parameters assessed was limited. In the rabbit hernia repair study, the primary systemic effect observed was changes in hematology parameters on Day 3 following treatment with the vehicle, the bupivacaine comparator (HTX-011 without meloxicam), and the different HTX formulations. The most consistent finding across all treatments were increased white blood cells, increased neutrophils, and decreased monocytes, which is consistent with other studies with HTX-011. Additionally, decreased platelets and reticulocytes were seen with some treatments. Most of these changes were not seen on Day 15 suggesting partial to complete recovery. Since effects were seen in the vehicle treated group and across multiple formulations, this suggests that effects on hematology parameters could be related to one or more shared excipients. In this study, the proposed clinical formulation was used at a dose of ~8.3 mg/kg bupivacaine and 0.25 mg/kg meloxicam which is a human equivalent dose of 160.6 mg and 4.8 mg, respectively. Therefore, the effects were observed at a human equivalent dose that is ~2.7 times higher than lowest proposed human dose (60 mg/1.8 mg) and ~2.5 times lower than the highest proposed human dose (400 mg/12 mg).

Dermal and wound site effects were the prominent findings in the two studies. In the rat bone healing study, skin lesions (scab and ulceration) were seen at the injection site on Day 33 in the groups receiving the bupivacaine comparator and both HTX formulations. These were the only local findings reported in this study.

A more detailed analysis of local effects was conducted in the rabbit hernia repair model. In this study, dermal observations, wound observations and histopathological effects were reported. Dermal observations were reported according to the Draize scoring system with the severity and incidence of edema and erythema reported. Increased incidences and or severity of both findings compared to saline control were seen across the vehicle, bupivacaine comparator, and HTX formulations, although there did not appear to be a consistent pattern across formulation or sex.

At the wound site, the most common observation was hemorrhage and was primarily observed in the middle of the study in animals who were sacrificed on Day 15. The severity of hemorrhage was increased in females receiving the vehicle, bupivacaine comparator, and all three HTX formulations. Hemorrhage was not present at the end of

the study in most animals, except for 1 female receiving HTX-011-49 and 2 females receiving HTX-011.

Finally, histopathological analysis of the injection sites also revealed increased incidences and severity of findings in treated groups compared to the saline control for animals undergoing necropsy on Day 15. These included the findings of cell debris, cystic change, epidermal ulcer, hemorrhage, epidermal hyperplasia, dermal granulation, and subcutaneous/muscular/intermuscular granulation. There did not appear to be a consistent pattern across formulation or sex making it difficult to interpret if any formulation adversely affected wound healing.

Taken together, it did appear that the vehicle, bupivacaine formulation comparator, and the HTX formulations had an impact at the local injection site compared to the saline control. The effect seemed to be most pronounced at the end of the study (Day 15). Based on the observation of findings with multiple formulations, it suggests that the effects were potentially related to one or more shared excipients. The dose of the proposed clinical formulation used in this study was 25 mg bupivacaine and 0.75 mg meloxicam, which is less than the lowest proposed human dose of 60 mg bupivacaine and 1.8 mg meloxicam. The size of the incisions in the rat and rabbit studies were not reported, so a comparison in terms of volume per cm of incision between the nonclinical and the clinical studies could not be made.

The clinical formulation was also tested for wound healing in minipigs (Study 33-54). In this study, male and female minipigs were given a single 3 mL dose of saline, vehicle, bupivacaine alone, meloxicam alone, and HTX-011. In addition, a sham control was included where an incision was made but no treatment was given. Minipigs were sacrificed on Day 15, the same duration as the rabbit study. There were limited increases in the severity of wound site observations on Day 4 compared to the sham and vehicle control. Observations included increased redness around the wound site, increased severity of hemorrhage, and increased severity of exudate. These findings were no longer elevated in severity compared to the vehicle or sham control on Day 15. In terms of histopathological findings, there was increased severity of inflammatory cell infiltrate and granulomatous inflammation of the sub cutis on Day 15. Taken together, there was a very slight difference in wound healing in this study. The dose used in this study was 3 mL (75/2.25 mg bupivacaine/meloxicam) with a 3 cm incision or 1 mL/cm. Based on estimated incision sizes provided by the clinical team, for herniorrhaphy (3-4 cm), (b) (4) the dose used in this study is lower than those proposed in the clinical studies (herniorrhaphy; 2.5-3.4 mL/cm, (b) (4) .

In summary, there did not appear to be any effect on bone healing or overt adverse interaction with the Prolene mesh with HTX-011. From the nonclinical perspective, no additional testing to assess these parameters are necessary. There was some evidence of a potential effect on wound healing in the rabbit study. However, the minipig study, which used a higher dose, supported that there was only a very slight difference in

wound healing with HTX-011 compared to control treatments. Even the minipig study did not test local doses as high as those in the Phase 3 studies and therefore the nonclinical data does not fully support these studies. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in terms of further evaluation of the potential effect on wound healing at clinically relevant doses.

Local and systemic toxicity of the drug product

Nonclinical studies were submitted to justify the safety of the drug product formulation which contains novel excipients, and two previously approved drug products. These studies were designed to address the local safety of meloxicam as this is a new route of administration and the systemic safety of bupivacaine since exposure of bupivacaine from the drug product exceeded the reference product both in terms of AUC_{0-24h} and overall duration of exposure.

The local tissue effects of this drug product when dosed in accordance with the proposed indication are not well characterized from a nonclinical perspective. The development program included many changes to the formulation which precluded a clear delineation of the local tissue response of the to-be-marketed drug product except under exaggerated conditions. However, these studies were considered acceptable to inform the toxicological effects of the materials in the drug product formulations to be tested during development. Specifically, the Division leveraged these data to inform the clinical monitoring program and the informed consent for the early clinical studies. However, pivotal repeat-dose studies testing the to-be-marketed drug product formulation were required to support the NDA. The pivotal studies were two 28-day repeat-dose studies (rat and dog by subcutaneous injection, Studies 33-85 and 33-86) conducted with the final clinical formulation, HTX-011. These two studies provided the only clear product-specific information characterizing the local tissue effects of the to-be-marketed formulation. Local effects observed throughout development consisted of a prolonged inflammatory response, including areas of tissue necrosis. The findings appear to be related primarily to the excipients as similar findings were often observed with the vehicles used in the studies. The local tissue findings were discussed with the clinical review team during development and the clinical team concluded that clinical studies could proceed with adequate monitoring and informed consent to determine if the adverse effects translated into adverse clinical findings.

In terms of local toxicity, the primary finding following treatment with HTX-011 and the HTX-011 vehicles (similar levels of excipients but no active drug products) in both rats and dogs in the pivotal studies was an inflammatory response that is consistent with a foreign body reaction. This was primarily characterized by persistent chronic inflammation that was still present up to 28 days following injection. The clinical significance of prolonged chronic inflammation is unclear. Based on a minipig study (33-54) by wound instillation, HTX-011 appears to only have a very minor impact on soft tissue wound healing consistent with herniorrhaphy procedures. However, it must be kept in mind that when the dose volume was normalized to the size of the incision site (estimated incision sizes for the surgeries proposed in the clinical studies were provided

by the clinical team) the minipig study (1 mL/cm) was lower than the proposed clinical surgeries (herniorrhaphy, 3-4 cm, 2.5-3.4 mL/cm; (b) (4)

The local effects appear to be primarily driven by the presence of the tri(ethylene glycol)-based poly(orthoester) polymer (AP135) since similar local effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4)

Since the clinical formulation was used in the repeat-dose studies, the studies adequately characterize the local toxicity of the drug product, including meloxicam and the excipients, at a margin of 1x. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in determining if the chronic inflammation noted in the animals translates to clinically meaningful adverse effects.

While the 28-day studies were conducted under exaggerated conditions (each injection site received 2-3 injections) similar dermal effects were observed after single injections with the HTX-011 vehicle in rats (Studies 33-48 and 33-55) suggesting the findings in the repeat-dose studies are relevant for a single administration. The findings in the single-dose studies with the HTX-011 vehicle were more severe (e.g., necrosis) than in the repeat-dose studies. This is likely due to the increased volume of injection used in these studies. Increased volume of injection was also linked to increased severity in the 28-day dog study where the HTX-011 vehicle (5.2-8.9 mL) showed increased severity and incidence of local findings compared to the HTX-011 clinical formulation (2.3-3.9 mL). It is difficult to translate the increased volumes into a clinically meaningful dose except to say that increased amounts of locally applied HTX-011 increases the risk for local toxicity.

In terms of systemic safety, the primary finding following treatment with HTX-011 and the HTX-011 vehicle in the pivotal studies was a change in hematology parameters, primarily an increase in reticulocytes, although increases in white blood cells, platelets, leukocytes, eosinophils, neutrophils, monocytes and lymphocytes were also observed. The effects seem to be derived from the AP135 polymer since similar local effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4) This is likely due to breakdown of the polymer into smaller fragments that are then systemically distributed.

More severe effects were seen in the 28-day dog study where a larger volume of injection was used for the HTX-011 vehicle which resulted in higher exposure to the excipients (2.4-2.5x at the MRHD based on BSA scaling). These effects included decreased activity, decreased body weight, and increased organ weights (liver, thymus, adrenal, and kidney). It is likely that these effects are not relevant for a single-dose exposure since no effects were seen in single-dose studies using the HTX-011 vehicle in rats at exposure levels of the excipients of ~7.2x those of the MRHD based on BSA scaling. Increased hematology parameters were seen in one single-dose rat study using the HTX-011 vehicle at excipient exposures of 0.9-1.5x those of the MRHD based on BSA scaling. Based on all of the studies, increased hematology parameters occur at excipient exposures of ~0.5-1.5x those of the MRHD based on BSA scaling. We acknowledge that there is clinical experience with the drug product that included

monitoring for changes in hematology parameters and defer to the clinical review team in determining if these increases also occur in the clinical setting and are clinically relevant.

12 Appendix/Attachments

12.1 Consults from DARS

The Division of Applied Regulatory Science Computational Toxicology Consultation Service consultation review of surrogates for (b) (4)

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/s/

JAIME R D'AGOSTINO
05/10/2021 06:09:48 PM

JAY H CHANG
05/10/2021 06:54:33 PM

RICHARD D MELLON
05/11/2021 07:47:21 AM
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 211988

Supporting document/s: 1, 11, 26, 40, 48, 50, 51, 53, 59

Applicant's letter date: 9/21/2018, 12/26/2018, 2/12/2019, 9/26/2019,
12/23/2019, 12/31/2019, 1/2/2020, 1/24/2020,
5/4/2020

CDER stamp date: 9/21/2018, 12/26/2018, 2/12/2019, 9/26/2019,
12/23/2019, 12/31/2019, 1/2/2020, 1/24/2020,
5/4/2020

Product: ZYNRELEF (bupivacaine and meloxicam)
solution

Indication: Post-operative pain management

Applicant: Heron Therapeutics, Inc.

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

Reviewer: Jaime D'Agostino, PhD

Supervisor/Team Leader: R. Daniel Mellon, PhD/Jay H. Chang, PhD

Division Director: Rigoberto Roca, MD

Project Manager: Allison Meyer

Template Version: September 1, 2010

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TABLE OF CONTENTS

1 EXECUTIVE SUMMARY.....	8
1.1 INTRODUCTION	8
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS	8
1.3 RECOMMENDATIONS	12
2 DRUG INFORMATION.....	22
2.1 DRUG	22
2.2 RELEVANT INDS, NDAs, BLAs AND DMFs.....	23
2.3 DRUG FORMULATION	24
2.4 COMMENTS ON NOVEL EXCIPIENTS	25
2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	41
2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN.....	128
2.7 REGULATORY BACKGROUND	128
3 STUDIES SUBMITTED.....	133
3.3 PREVIOUS REVIEWS REFERENCED.....	143
4 PHARMACOLOGY	143
4.1 PRIMARY PHARMACOLOGY	143
4.2 SECONDARY PHARMACOLOGY	143
4.3 SAFETY PHARMACOLOGY	143
5 PHARMACOKINETICS/ADME/TOXICOKINETICS	144
5.1 PK/ADME	144
6 GENERAL TOXICOLOGY.....	146
6.1 SINGLE-DOSE TOXICITY	147
6.2 REPEAT-DOSE TOXICITY	154
7 GENETIC TOXICOLOGY.....	207
7.1 <i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES)	207
8 CARCINOGENICITY.....	214
9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	214
9.2 EMBRYONIC AND FETAL DEVELOPMENT+	236
10 SPECIAL TOXICOLOGY STUDIES	240
10.1 WOUND HEALING AND BONE STUDIES.....	240
10.2 JUVENILE ANIMAL STUDIES.....	269
11 INTEGRATED SUMMARY AND SAFETY EVALUATION.....	270
12 APPENDIX/ATTACHMENTS.....	282

Table of Tables

Table 1: Relevant INDs, NDAs, and DMFs.....25
Table 2: Composition of HTX-011 26
Table 3: Amount of AP135 in Different Formulations 27
Table 4: Reproductive Toxicology Studies with APF18A 28
Table 5: Summary of Local Effects Following AP135 Treatment 30
Table 6: Summary of Systemic Effects Following AP135 Treatment 33
Table 7: DMSO FDA-approved Products as Per IID 34
Table 8: DMSO Literature Cited by Applicant.....35
Table 9: Triacetin in FDA-approved Products as per CDER IID 36
Table 10: Maleic Acid FDA-approved Products as Per CDER IID 39
Table 11: Summary of Local Effects Potentially Associated with Excipients.....42
Table 12: Summary of Systemic Effects Potentially Associated with Excipients.....42
Table 13: Bupivacaine Drug Substance Specifications 45
Table 14: Meloxicam Drug Substance Specifications 47
Table 15: HTX-011 Drug Product Specifications (Part 1).....51
Table 16: HTX-011 Drug Product Specifications (Part 2).....52
Table 17: Specifications for AP135 55
Table 18: Primary Container Closure 56
Table 19: Preparation and Administration Components.....56
Table 20: List of Extractable Studies 57
Table 21: Components Tested in Extraction Study 1 58

(b) (4)



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Table 76: Pharmacology Studies Submitted	137
Table 77: Pharmacodynamic Studies Submitted-Part 1	138
Table 78: Pharmacodynamic Studies Submitted-Part 2	138
Table 79: Pharmacodynamic Studies Submitted-Part 3	139
Table 80: Safety Pharmacology Studies Submitted	139
Table 81: Pharmacokinetics Studies Submitted	140
Table 82: Pharmacokinetic Studies Submitted (Continued)	140
Table 83: Toxicology Studies Submitted-Part 1	141
Table 84: Toxicology Studies Submitted-Part 2	142
Table 85: Toxicology Studies Submitted-Part 3	143
Table 86: Toxicology Studies Submitted-Part 4	144
Table 87: Toxicology Studies Submitted-Part 5	144
Table 88: Toxicology Studies Submitted-Part 6	145
Table 89: Highest Concentrations of Bupivacaine and Meloxicam in Plasma, Milk, and/or Urine of Minipig Sows and Neonate Piglets Following a Single SC Administration of HTX-011 to Sows.....	147
Table 90: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Rats	148
Table 91: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Dogs	148
Table 92: Composition of Test Articles Used in Nonclinical Studies Supporting Development of HTX-011-56	149
Table 93: Overview of Single-dose Toxicity Studies Conducted by Applicant	150
Table 94 Formulations Tested in Study Number 33-48	153
Table 95 Histopathological Findings on Day 3 at the Primary Injection Site, Males.....	155
Table 96 Histopathological Findings on Day 3 at the Secondary Injection Site, Males	155
Table 97 Histopathological Findings on Day 14 (Recovery) at the Primary Injection Site, Males	156
Table 98 Histopathological Findings on Day 14 (Recovery) at the Secondary Injection Site, Males.....	156
Table 99 Histopathological Findings on Day 3 at the Primary Injection Site, Females	157
Table 100 Histopathological Findings on Day 3 at the Secondary Injection Site, Females	157
Table 101 Histopathological Findings on Day 14 (Recovery) at the Primary Injection Site, Females.....	158
Table 102 Histopathological Findings on Day 14 (Recovery) at the Secondary Injection Site, Females.....	158
Table 103: Incidence of Clinical Observations in Males	162
Table 104: Incidence of Clinical Observations in Females	163
Table 105: Notable Changes in Hematology Parameters in Male Rats	164
Table 106: Notable Changes in Clinical Chemistry Parameters in Rats	165
Table 107: Gross Observations in Male Rats	166
Table 108: Gross Observations in Female Rats.....	166
Table 109: Systemic Histopathological Observations	168
Table 110: Chronic Inflammation (Incidence).....	169
Table 111: Chronic Inflammation, Active (Incidence)	169

Table 112: Granulomatous Inflammation (Incidence).....	169
Table 113: Hemorrhage (Incidence).....	170
Table 114: Presence of Test Material (Incidence).....	170
Table 115: Chronic inflammation, Recovery Animals.....	170
Table 116: Bupivacaine Toxicokinetic Parameters.....	171
Table 117: Meloxicam Toxicokinetic Parameters.....	172
Table 118: Levels of Polymer Breakdown Products in the Urine.....	173
Table 119: Incidence of Clinical Observations in Males.....	178
Table 120: Incidence of Clinical Observations in Females.....	178
Table 121: Notable Changes in Hematology Parameters in Male Rats.....	180
Table 122: Notable Changes in Hematology Parameters in Female Rats.....	180
Table 123: Notable Changes in Clinical Chemistry Parameters in Male Rats.....	181
Table 124: Notable Changes in Clinical Chemistry Parameters in Female Rats.....	181
Table 125: Urine pH in Male and Female Rats.....	182
Table 126: Gross Observations in Male Rats (Incidence).....	182
Table 127: Gross Observations in Female Rats (Incidence).....	182
Table 128: Organ Weights in Male Rats.....	183
Table 129: Organ Weights in Female Rats.....	183
Table 130: Infiltrate of Mononuclear Cells (Incidence).....	185
Table 131: Fibrosis (Incidence).....	185
Table 132: Edema (Incidence).....	185
Table 133: Erythrocyte Accumulation (Incidence).....	186
Table 134: Ulcer (Incidence).....	186
Table 135: Acute Inflammatory Exudate (Incidence).....	186
Table 136: Bupivacaine Toxicokinetic Parameters.....	187
Table 137: Incidence of Edema at the Injection Site.....	193
Table 138: Average Severity of Edema in Vehicle Control Group.....	193
Table 139: Body Weights.....	194
Table 140: Hematology Parameters in Male Dogs.....	195
Table 141: Hematology Parameters in Female Dogs.....	196
Table 142: Clinical Chemistry Parameters in Male Dogs.....	197
Table 143: Clinical Chemistry Parameters in Female Dogs.....	198
Table 144: Gross Observations in Male Dogs.....	199
Table 145: Gross Observations in Female Dogs.....	199
Table 146: Organ Weights in Male Dogs.....	200
Table 147: Organ Weights for Female Dogs.....	200
Table 148: Percentage of Main Study Animals with Chronic Multifocal Inflammation.....	204
Table 149: Percentage of Recovery Animals with Chronic Multifocal Inflammation....	204
Table 150: Percentage of Main Study Animals with Active Chronic Multifocal Inflammation.....	204
Table 151: Percentage of Main Study Animals with Multifocal Granulomatous Inflammation.....	205
Table 152: Percentage of Recovery Animals with Multifocal Granulomatous Inflammation.....	205
Table 153: Percentage of Main Study Animals with Multifocal Fibrosis.....	205
Table 154: Percentage of Recovery Animals with Multifocal Fibrosis.....	206

Table 155: Percentage of Main Study Animals with Multifocal Hemorrhage.....	206
Table 156: Percentage of Main Study Animals with Multifocal Presence of Test Material	206
Table 157: Bupivacaine PK Parameters.....	208
Table 158: Meloxicam PK Parameters	210
Table 159: Plate Incorporation in Absence of S9	214
Table 160: Plate Incorporation in Presence of S9	215
Table 161: Positive Control Data.....	216
Table 162: Historical Control Data.....	217
Table 163: Litter Viability	243
Table 164: Sex and Sex Ratio of Litter	243
Table 165: Treatment Groups Hernia Repair Study	247
Table 166: Clinical Signs Rabbit Hernia Repair Study	248
Table 167: Dermal Observations of Day 3 Sacrifice Group.....	250
Table 168: Dermal Observations of Day 15 Sacrifice Group.....	250
Table 169: Dermal Observations on Day 15	250
Table 170: Wound Observations of Day 3 Sacrifice Group (Incidence)	251
Table 171: Wound Observations of Day 15 Sacrifice Group (Incidence)	251
Table 172: Last Day of Wound Hemorrhage	252
Table 173: Hematology Findings on Day 3, Percentage of Saline Control	253
Table 174: Hematology Findings on Day 15, Percentage of Saline Control	254
Table 175: Gross Findings of Day 3 Sacrifice Group	255
Table 176: Gross Findings of Day 15 Sacrifice Group	255
Table 177: Histological Findings of Day 15 Sacrifice Group	256
Table 178: Clinical Signs	260
Table 179: Bone Mineral Content of Left Femur (Percent of Saline Control)	262
Table 180: Bone Mineral Content of Right Femur (Percent of Saline Control).....	262
Table 181: Bone Mineral Density of Left Femur (Percent of Saline Control).....	262
Table 182: Bone Mineral Density of Right Femur (Percent of Saline Control)	262
Table 183: Healing Score of Femur.....	263
Table 184: Dermal Observations in Day 4 Sacrifice Animals, Male	267
Table 185: Dermal Observations in Day 4 Sacrifice Animals, Female	267
Table 186: Dermal Observations in Day 15 Sacrifice Animals, Male	267
Table 187: Dermal Observations in Day 15 Sacrifice Animals, Female	267
Table 188: Wound Observations on Day 4.....	269
Table 189: Wound Observations on Day 8.....	270
Table 190: Gross Pathology of the Incision Site, Day 4	271
Table 191: Gross Pathology of the Incision Site, Day 15	271
Table 192: Summary of Histopathological Findings, Day 4	272
Table 193: Summary of Histopathological Findings, Day 15.....	273

Table of Figures

Figure 1: Levels of [redacted] (b) (4) in [redacted] (b) (4) Leachable Study 3.....109

Figure 2: Levels of [redacted] in [redacted] Leachable Study 3 119

Figure 3: Levels of [redacted] (b) (4) ([redacted] (b) (4) in [redacted] (b) (4) Leachable Study 4
.....124

Figure 4: Levels of [redacted] (b) (4) ([redacted] (b) (4) in [redacted] (b) (4) Leachable Study 4
.....126

Figure 5: Body Weight in Rat Bone Defect Study.....261

1 Executive Summary

1.1 Introduction

The Applicant, Heron Therapeutics Inc, has developed a fixed-dose combination product containing bupivacaine and meloxicam, which they referred to as HTX-011 during development. HTX-011 is a viscous solution that is formulated in a proprietary tri(ethylene glycol)-based poly(orthoester) polymer. The Applicant developed HTX-011 for application into a surgical site to reduce postoperative pain for 72 hours and reduce the need for opioid analgesics. The Applicant has submitted a 505(b)(2) application relying on the Agency's previous finding of safety for MARCAINE® (bupivacaine HCl injection; NDA 16964) and MOBIC® (meloxicam; NDA 20938) with additional support from published literature. However, the latest 356h form indicates that the Applicant is also relying on two other bupivacaine NDAs (18053 and 18304). From a nonclinical perspective, these three FDA-approved bupivacaine labels contain the same data. In addition, the Applicant proposed reliance upon an Agency finding for Luvox (luvoxamine maleate; NDA 20243) to support the safety of the excipient maleic acid.

NDA 211988 was submitted on September 21st, 2018 and a complete response was issued on April 30th, 2019, which included nonclinical deficiencies. The Applicant resubmitted on September 26th, 2019.

1.2 Brief Discussion of Nonclinical Findings

The drug product formulation contains several "new" excipients as defined by the FDA guidance document as excipients used at higher doses, concentrations, or routes of administration than previously approved drug products. Specifically, the proposed doses of AP135, dimethyl sulfoxide (DMSO), triacetin, and maleic acid exceeded levels present in FDA-approved drug products as per the CDER Inactive Ingredients Database (IID). The Applicant was informed of the need to adequately qualify the safety of these new excipients in their NDA early in development. In the original submission, adequate data were provided to characterize the reproductive and developmental safety profile of AP135 at the maximum recommended human dose (MRHD). However, the Applicant did not adequately justify the safety of DMSO, maleic acid, and triacetin with respect to the standard reproductive and developmental toxicology battery endpoints. The Applicant submitted additional reproductive and developmental toxicity data in the resubmission which addressed some of the concerns; however, adequate bridging data were not provided nor data to support a waiver of embryofetal studies in a second species for one excipient.

General toxicology studies were submitted to characterize the toxicological potential of the drug product formulation which contains new excipients. These studies were also specifically designed to address the local safety of meloxicam as this is a new route of administration and the systemic safety of bupivacaine since exposure of bupivacaine from the drug product exceeded the reference product both in terms of AUC_{0-24h} and overall duration of exposure.

The pivotal studies were two 28-day repeat-dose studies (rat and dog by subcutaneous injection, Studies 33-85 and 33-86) conducted with the final clinical formulation, HTX-011. These two studies provided the only clear product-specific information characterizing the local tissue effects of the to-be-marketed formulation. Local effects observed throughout development consisted of a prolonged inflammatory response, including areas of tissue necrosis. The findings appear to be related primarily to the vehicle. The local tissue findings were discussed with the clinical review team during development and the clinical team concluded that clinical studies could proceed with adequate monitoring and informed consent to determine if the adverse effects translated into adverse clinical findings.

In terms of local toxicity, the primary finding following treatment with HTX-011 and the HTX-011 vehicle (similar levels of excipients but no active drug products) in both rats and dogs in the pivotal studies was an inflammatory response that is consistent with a foreign body reaction. This was primarily characterized by persistent chronic inflammation that was still present up to 28 days following injection. The clinical significance of prolonged chronic inflammation is unclear. Based on a minipig study (33-54) by wound instillation, HTX-011 appears to only have a very minor impact on soft tissue wound healing consistent with herniorrhaphy procedures. However, it must be kept in mind that when the dose volume was normalized to the size of the incision site (estimated incision sizes for the surgeries proposed in the clinical studies were provided by the clinical team) the minipig study (1 mL/cm) was lower than the proposed clinical surgeries (herniorrhaphy, 3-4 cm, 2.5-3.4 mL/cm; (b) (4)

The local effects appear to be primarily driven by the presence of the tri(ethylene glycol)-based poly(orthoester) polymer (AP135) since similar local effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4)

Since the clinical formulation was used in the repeat-dose studies, the studies adequately characterize the local toxicity of the drug product, including meloxicam and the excipients, at a margin of 1x. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in determining if the chronic inflammation noted in the animals translates to clinically meaningful adverse effects.

Given the prolonged and rather robust local tissue response, the potential impact of the drug product on a variety of surgical meshes was tested in nonclinical models. The results suggest that the drug product did not appear to impact the integrity of the surgical meshes tested nor translate into clinically meaningful changes in wound healing. In addition, to support the proposed bunionectomy study and indication, the impact of the drug product on bone healing and integrity was studied in an animal model. The study did not suggest a clear clinically meaningful delay in bone healing, a finding that was further and carefully monitored in clinical studies.

While the 28-day studies were conducted under exaggerated conditions (each injection site received 2-3 injections) similar dermal effects were observed after single injections with the HTX-011 vehicle in rats (Studies 33-48 and 33-55) suggesting the findings in

the repeat-dose studies are relevant for a single administration. The findings in the single-dose studies with the HTX-011 vehicle were more severe (e.g., necrosis) than in the repeat-dose studies. This is likely due to the increased volume of injection used in these studies. Increased volume of injection was also linked to increased severity in the 28-day dog study where the HTX-011 vehicle (5.2-8.9 mL) showed increased severity and incidence of local findings compared to the HTX-011 clinical formulation (2.3-3.9 mL). It is difficult to translate the increased volumes into a clinically meaningful dose except to say that increased amounts of locally applied HTX-011 increases the risk for local toxicity.

In terms of systemic safety, the primary finding following treatment with HTX-011 and the HTX-011 vehicle in the pivotal studies was a change in hematology parameters, primarily an increase in reticulocytes, although increases in white blood cells, platelets, leukocytes, eosinophils, neutrophils, monocytes, and lymphocytes were also observed. The effects seem to be derived from the AP135 polymer since similar local effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4)). This is likely due to breakdown of the polymer into smaller fragments that are then systemically distributed.

More severe effects were seen in the 28-day dog study where a larger volume of injection was used for the HTX-011 vehicle which resulted in higher exposure to the excipients (2.4-2.5x at the MRHD based on BSA scaling). These effects included decreased activity, decreased body weight, and increased organ weights (liver, thymus, adrenal, and kidney). It is likely that these effects are not relevant for a single-dose exposure since no effects were seen in single-dose studies using the HTX-011 vehicle in rats at exposure levels of the excipients of ~7.2x those of the MRHD based on BSA scaling. Increased hematology parameters were seen in one single-dose rat study using the HTX-011 vehicle at excipient exposures of 0.9-1.5x those of the MRHD based on BSA scaling. Based on all of the studies, increased hematology parameters occur at excipient exposures of ~0.5-1.5x those of the MRHD based on BSA scaling. We acknowledge that there is clinical experience with the drug product that included monitoring for changes in hematology parameters and defer to the clinical review team in determining if these increases, consistent with an inflammatory reaction, also occur in the clinical setting and are clinically relevant.

In clinical bioavailability studies, the exposure (AUC) to bupivacaine via the drug product formulation at the proposed maximum daily dose of 400 mg bupivacaine exceeded that of the referenced drug products (e.g., Marcaine), and therefore additional data were needed to qualify the systemic safety of bupivacaine for the proposed clinical use. The pivotal 28-day nonclinical studies in rats and dogs included exposure data at 24 and 48 hours but not AUC_{inf} . Compared to human exposures in Clinical Study 209 (total knee arthroplasty), the rat exposures were ~ 0.5x and 0.3x for AUC_{0-24} and AUC_{0-48} , respectively, and the dog exposures were ~ 1.0x and 0.8x for AUC_{0-24} and AUC_{0-48} , respectively for bupivacaine. Since the exposure in rats was lower than in humans, the Applicant conducted a 14-day study in rats by subcutaneous injection (dosing every 48 hours) of HTX formulations containing only bupivacaine (not the to-be-marketed

formulation) and used a higher dose than in the previous rat study. The toxicological findings in the 14-day rat study were similar to the results of the 28-day studies, which included an inflammatory response that is consistent with a foreign body reaction and changes in hematology parameters. It is difficult to assess if the magnitudes of the changes are more severe in this study compared to previous studies which used lower doses of bupivacaine due to differences in dosing volumes, formulations, and durations of treatment. The combined male and female systemic exposure (AUC_{inf}) of bupivacaine achieved in the study (52,900 ng*h/mL) was higher than the highest reported bupivacaine exposure reported in the Applicant's clinical trials (Study 209, total knee arthroplasty, 31,173 h*ng/mL) by a factor of ~1.7x. Of note, while the data from the 28-day dog study is not ideal (AUC_{inf} was not calculated) it does provide data in a second species that had similar exposure to bupivacaine over 24 hours as humans exposed to 400 mg bupivacaine (~1x). The only systemic finding in dogs at this exposure level was an increase in reticulocytes. Taken together with the results from the 14-day rat study which achieved a higher exposure multiple, the safety of the 400 mg dose of bupivacaine is supported by the nonclinical data as long as the clinical team does not believe that changes in hematology parameters are of a clinical concern considering the human experience.

To justify the safety of the container closure system, the Applicant conducted multiple extraction and leachable studies to identify potential leachables from the (b) (4) and the container closure system. The extractable and leachable testing in the original submission did not provide adequate leachable data to permit substantive toxicological evaluation of the safety of the container closure system and the (b) (4) of the drug product. Specifically, the Applicant did not test at least three drug product stability batches over the entire course of proposed expiry, did not perform leachable studies for all compounds identified above the safety concern threshold (b) (4) mcg/day), did not adequately identify the compounds they detected, and did not provide adequate justification for the use of a surrogate approach for (b) (4) found in the drug product. The Applicant addressed these deficiencies in their resubmission and based on discussions with the chemistry, manufacturing, and controls (CMC) team, the extractable and leachable testing was considered adequate.

In total, eight individual leachables were identified above the AET across the five leachable studies: (b) (4)

The Applicant provided a detailed risk assessment for the leachables and PDEs were derived for the compounds based on the data available. The maximum detected levels of the leachables were all below the calculated PDEs and are not expected to be of a safety concern.

The drug substance impurity and drug product degradant specifications are acceptable with the exception of the drug product specification for (b) (4). The deficiency related to the drug product degradant (b) (4) has been

addressed by reducing the specification to NMT (b) (4)%, which is acceptable based on the qualification thresholds in ICH Q3B(R2). The Applicant did not adequately address the specification for (b) (4) in the drug product. Although they provide a hypothesis (b) (4)

they did not provide data to support this hypothesis.

From the nonclinical perspective, the Applicant has not adequately addressed all of the deficiencies identified in the original submission. In summary, the Applicant did not adequately justify the safety of the excipients DMSO, maleic acid, and triacetin with respect to the standard reproductive and developmental toxicology battery endpoints. In addition, the Applicant did not provide adequate data to qualify the proposed drug product specification for (b) (4) at NMT (b) (4)%. In terms of the safety of the drug product, the nonclinical data indicated that a local inflammatory response and changes in hematology parameters occurs at clinically relevant doses. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in determining if these effects also occur in the clinical setting and are clinically relevant.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical pharmacology toxicology perspective, adequate data have not been submitted to permit a recommendation of approval at this time and a complete response is recommended. The following outstanding deficiencies are recommended:

1. You have not provided adequate data to support your request for a waiver of reproductive and developmental studies for triacetin. Although you have concluded that the primary metabolites of triacetin do not represent a risk for reproductive and developmental effects, you have provided no data to support the conclusion that triacetin instilled into a wound at the maximum daily dose will not result in systemic exposure to the triacetin molecule. Provide data to support the conclusion that triacetin levels are not present in the systemic circulation via your drug product or conduct reproductive and developmental toxicology studies with triacetin via a route that mimics the clinical exposure to this excipient. If you elect to continue to rely upon the cited oral rat reproductive and developmental study, provide justification that the animals in that study were exposed to triacetin at levels comparable to those that will occur following clinical use of your drug product at the maximum recommended human dose.
2. You have not provided an adequate scientific bridge to the referenced oral reproductive and developmental studies submitted to qualify the safety of DMSO exposure via your drug product. Instillation of the drug product may result in higher C_{max} and AUC compared to oral studies and therefore it is not clear if the referenced studies adequately address the safety of your product. Provide data to support your conclusion that these referenced oral studies resulted in

exposures to DMSO that exceed the exposure to DMSO via your drug product or conduct studies using an appropriate route of administration.

3. You have not provided adequate data to support the proposed drug product specification for (b) (4) at NMT (b) (4)%. The nonclinical and clinical lots tested to date in your development program did not contain this high of a percentage of (b) (4) and therefore does not qualify this specification. Further, you did not provide data to support your conclusion that (b) (4) (b) (4) could not contribute to the local tissue effects of the drug product. Either conduct a local tissue toxicity study that tests drug product that contains at least the maximum specified level of (b) (4) reduce the specification for (b) (4) to that which was tested in the nonclinical toxicology studies, or provide data to support your hypothesis that the (b) (4) in the product does not contribute to local tissue effects of the drug product.
4. Your embryofetal development study in rabbits tested maleic acid via an oral route of administration rather than the proposed route of administration of your drug product and you did not provide an adequate scientific bridge to support your conclusion that the study provides adequate characterization of the developmental effects of maleic acid exposures via installation into a wound. Submit justification that the oral toxicology study resulted in exposures (C_{max} and AUC) that provide adequate coverage for the exposure via your drug product when the product is instilled into a wound. In addition, submit the final study report for the oral maleic acid embryofetal development study in the rabbit to the NDA.

1.3.2 Additional Nonclinical Recommendations

Although not listed as a deficiency in the first cycle, your proposed specification for DMSO of (b) (4) has an upper limit that has never been tested in your clinical or nonclinical development program. As DMSO may contribute to local tissue toxicity, either revise the specification to reflect the testing completed by your development program, conduct a new study to characterize the local effects of your to-be-marketed drug product containing DMSO at the upper limit of your proposed specification, or justify why DMSO at (b) (4)% will not contribute to the local tissue effects of the drug product.

Once approved, the following PREA PMRs will be required:

1. Conduct a juvenile animal study in the rodent model to characterize the impact of DMSO on the developing brain to support clinical studies in pediatric patients under three years of age.

2. Conduct a juvenile animal study in an appropriate model to characterize the impact of meloxicam on the developing kidney, liver, lung, and testes to support clinical studies in pediatric patients under three years of age.

1.3.3 Labeling

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

Applicant's Proposed Labeling	Reviewer's Recommended Changes	Rationale for Changes/Comments

(b) (4)



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2 Drug Information

2.1 Drug

CAS Registry Number: 38396-39-3 (bupivacaine); 71125-38-7 (meloxicam)

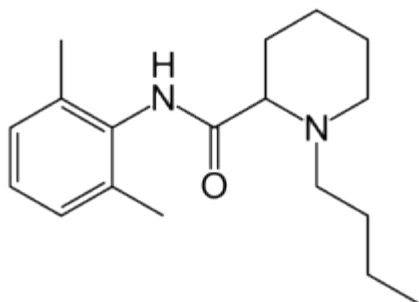
Generic Name: bupivacaine (base) and meloxicam (base)

Code Names: Zynrelef, HTX-011, and HTX-011-056

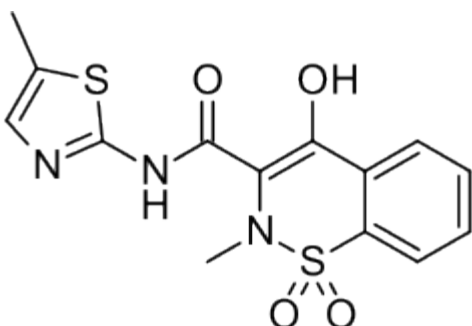
Chemical Name: (RS)-1-butyl-N-(2,6-dimethylphenyl)piperidine-2-carboxamide (bupivacaine) and 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide (meloxicam)

Molecular Formula/Molecular Weight: C₁₈H₂₈N₂O / 288.43 g/mol (bupivacaine); C₁₄H₁₃N₃O₄S₂ / 351.40 g/mol (meloxicam)

Structure or Biochemical Description:



Bupivacaine



Meloxicam

Pharmacologic Class: Amide local anesthetic (bupivacaine); nonsteroidal anti-inflammatory drug (meloxicam)

2.2 Relevant INDs, NDAs, BLAs and DMFs

The development program for this NDA was completed under IND 125927, originally submitted to the Agency on 5/7/2015.

The Applicant is submitting the current New Drug Application under section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act. As such, they are referencing MARCAINE™ (bupivacaine, NDA 016964), Bupivacaine hydrochloride (NDA 18053), Sensorcaine (NDA 18304), MOBIC® (meloxicam, NDA 020938), and Luvox ([fluvoxamine maleate](#), NDA 20243) as the listed drugs.

Table 1: Relevant INDs, NDAs, and DMFs

<i>IND/NDA/DMF</i>	<i>Drug/Compound</i>	<i>Sponsor</i>	<i>Division</i>	<i>Status</i>
IND 125927	HTX-011 (bupivacaine and meloxicam)	Heron Therapeutics Inc	DAAP	Active
NDA 016964	Marcaine (bupivacaine hydrochloride)	Hospira Inc	DAAP	Approved (10/03/1972)
NDA 020938	Mobic (Meloxicam)	Boehringer Ingelheim Pharmaceuticals Inc	DAAP	Approved (04/13/2000)
NDA 18053	Bupivacaine HCl	Hospira Inc	DAAP	Approved (4/17/1978)
NDA 18304	Sensorcaine	Fresenius Kabi USA LLC	DAAP	Approved

				(12/30/1981)
NDA 20243	Luvox (fluvoxamine maleate)	Solvay Pharmaceuticals	DP	Withdrawn (9/3/2003)
DMF	(b) (4)			
DMF				
DMF				
DMF				
DMF				
DMF				
DMF				
DMF				

The Applicant also notes that the vented vial spike has been cleared by the Center for Devices and Radiological Health (CDRH) via the 510(k) process (b) (4). The syringes are also cleared by CDRH via the 510(k) process (b) (4).

2.3 Drug Formulation

HTX-011 is an extended-release solution containing a fixed-dose combination of bupivacaine and meloxicam. HTX-011 uses AP135, a highly viscous polymer to provide extended release of the active ingredients. Dimethyl sulfoxide (DMSO) and triacetin are included as (b) (4).

Table 2: Composition of HTX-011

Component	Concentration (% w/w)	Concentration (mg/g)	Concentration (mg/mL)	Function	Reference to Quality Standard
Bupivacaine base	2.50	25.0	29.25	Active ingredient	Manufacturer
Meloxicam	0.075	0.75	0.88	Active ingredient	USP, Ph Eur
AP135	62.375	623.75	729.788	Release-controlling polymeric excipient	Manufacturer
Dimethyl sulfoxide	10.00	100	117	(b) (4)	USP, Ph Eur
Triacetin	25.00	250	292.5		USP, Ph Eur
Maleic acid	0.05	0.5	0.585		NF, Ph Eur
Totals	100.00	1000.0	1170		

Abbreviations: AP135, tri(ethylene glycol) poly(orthoester); USP, United States Pharmacopeia; Ph Eur, European Pharmacopoeia; NF, National Formulary

Table from page 6 of the Applicant's Drug Product Summary for HTX-011 (2.3.P)

During development the Agency advised the Applicant that the levels of the excipients in the drug product appear to exceed those listed in the CDER Inactive Ingredients Database (IID) and must be adequately qualified for systemic safety.

The Applicant proposed a maximum recommended human dose (MRHD) of 400 mg bupivacaine and 12 mg of meloxicam. This dose would require administration of 13.68 mL of the drug product solution. Based on the total daily dose, AP135, DMSO, triacetin, and maleic acid are all considered new excipients as defined by the FDA guidance document because the dose and/or route of administration exceed that of the any other FDA-approved drug product to date.

2.4 Comments on Novel Excipients

Based on the MRHD and route of administration, there are multiple novel excipients in this drug product that require adequate justification for safety. Each of these will be discussed below.

AP135

AP135 is a polymeric excipient that provides slow diffusional release of the active pharmaceutical ingredients over approximately three days. It is part of the tri(ethylene glycol) poly(orthoester) (TEG-POE) class of polymers (b) (4)

AP135 is not found in the CDER Inactive Ingredients Database (IID). A similar polymeric excipient, APF18A, is used in in the Applicant's FDA-approved and marketed product SUSTOL (granisetron, NDA 022445) for subcutaneous injection.

(b) (4)
 Data from the development of this polymer was submitted as part of the current NDA.

Table 3: Amount of AP135 in Different Formulations

<i>Formulation</i>	<i>Percentage of excipient (w/w)</i>	<i>AP135 concentration</i>	<i>Amount at MRHD¹</i>
HTX-011			(b) (4)
HTX-011 vehicle (HTX-024-002)			
Sustol			
APF18A			

¹ Maximum recommended human dose for HTX-011 is 400 mg/12 mg bupivacaine/meloxicam or 13.68 mL. Maximum recommended human dose for Sustol is 10 mg (0.42 mL) based on product labeling.

The amount of AP135 that a patient would be exposed to by use of SUSTOL at the MRHD from SUSTOL's labeling is about (b) (4) times less than the amount of AP135 at the MRHD of HTX-011. Therefore, the presence of AP135 in SUSTOL does not qualify the levels of this excipient in HTX-011.

The Applicant conducted nonclinical studies with APF18A as part of the development of SUSTOL, including repeat-dose toxicity studies, a genetic toxicity assessment (bacterial forward mutation, mouse lymphoma, and forward mutation assays) and reproductive toxicity studies (fertility and early embryonic development in rat, embryo-fetal development in rat and rabbit, and pre- and post-natal development in rat, see table below) and submitted these to this NDA. The genetic toxicity studies were negative for mutation or clastogenicity. The only treatment-related effects observed in the reproductive studies were local tissue effects at the injection site. Local effects were characterized in more detail in the repeat-dose studies, including histopathological analysis of the injection sites. The repeat-dose studies also characterized the systemic effects of APF18A and the relevance of the local and systemic findings are discussed below. In addition to nonclinical studies with APF18A, the Applicant also characterized the local and systemic effects of AP135 as part of the HTX-011 vehicle used in multiple studies for the current NDA.

Table 4: Reproductive Toxicology Studies with APF18A

<i>Study #</i>	<i>Type</i>	<i>AP135 (mg/mL)</i>	<i>AP135 (mg/kg)</i>	<i>HED (mg)</i>	<i>Systemic Safety margin¹</i>
27-02	Fertility and early embryonic development (rat)				(b) (4)
25-09	Embryo-fetal development (rat)				
25-10	Embryo-fetal development (rabbit)				

27-03	Pre- and post-natal development	(b) (4)

¹ Calculated as a ratio of the non-clinical human equivalent dose divided by the amount of AP135 at the maximum recommended human dose ((b) (4) mg AP135).

Although the rabbit EFD study noted in the table above does not provide complete coverage for the proposed clinical exposure and the high dose tested did not result in clear maternal toxicity, the Applicant concluded that the high dose tested was the maximum feasible dose (MFD) based on the material oozing from the injection sites at larger volumes. Given this feasibility limitation and because the study was able to dose close to the predicted exposure to APF18A via this formulation and the existing adverse effects noted in the labeling for both meloxicam and bupivacaine, no further studies will be required to address the potential embryo-fetal effects of APF135 for the intended clinical use.

Local effects:

The 90-day studies with the APF18A polymer were conducted in rat and dog (Studies 25-07 and 25-08). Dosage was by subcutaneous administration (0.25 mL in rats and 1 mL in dogs) every 24 hours across six dosage sites (16 injections total per site). Local injection site reactions observed consistently in both studies included dermal findings of erythema and edema, macroscopic observations of thickening of the skin, and multiple microscopic findings (chronic inflammation of multiple regions, necrosis/degeneration, fibrosis, and edema). Other findings seen primarily in one species included erosion, ulceration, hyperplasia, and hemorrhage. In general, findings were mostly no longer present following a 6-week recovery period, except for edema and erythema in rats, which was still present, but the severity and incidence was greatly reduced.

Similar injection site findings were seen with the HTX-011 vehicle (HTX-024-002) in 28-day repeat-dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection (0.13-0.19 mL in rats and 5.2-8.9 mL in dogs) every 48 hours across six dosage sites (2-3 injections total per site). In rats, scabbing was seen at the injection sites and microscopic observations included chronic and granulomatous inflammation. In dogs, edema, swelling, and scabs were observed at the injection site and microscopic observations included chronic inflammation, granulomatous inflammation, fibrosis, and hemorrhage. Of note, in both studies chronic inflammation was still present at the end of the study, suggesting that it persists for up to 26-28 days.

When interpreting results from these studies, it must be kept in mind that the studies were conducted under exaggerated conditions; the 90-day study had a total of 16 injections per injection site and the 28-day studies had a total of 2-3 injections per injection site. This exceeds the single dose of HTX-011 that would be administered clinically. However, similar local site reactions were seen following a single subcutaneous dose of the HTX-011 vehicle in rats (Studies 33-48 and 33-55) suggesting that local effects of the polymer seen in repeat-dose studies are likely relevant for the clinical dosing scheme.

The results from both studies together suggest that AP135 contributes to a local tissue reaction. This is not unexpected as the response appears to be consistent with a foreign body reaction. However, some of the findings suggest that the effects may be more adverse than a typical inflammatory reaction, such as the observations of necrosis, erosion, and ulceration. These effects were seen in the APF18A studies, which were of a longer duration and used a higher concentration of AP135 as compared to repeat-dose studies with the HTX-011 vehicle ((b) (4)). It is unlikely that the longer duration plays a role since necrosis was also observed in single-dose rat studies using the HTX-011 vehicle (Studies 33-48 and 33-55). Therefore, it is more likely that the higher dosage of AP135 contributed to the increased severity of findings.

In addition to a higher concentration of AP135, it appears increasing the injection volume may also play a role. The single-dose rat studies (Studies 33-48 and 33-55) where potentially adverse findings were observed used increased injection volumes (0.5 mL and 2 mL) compared to the rat 28-day repeat-dose study with the HTX-011 vehicle (Study 33-85, 0.13-0.19 mL) where only inflammation was observed microscopically. A similar trend of dose volume and severity/incidence of local reactions was seen in the 28-day dog study with HTX-011 (Study 33-86). Dogs treated with the HTX-011 vehicle (5.2-8.9 mL) had increased incidence and severity of injection site findings compared to animals receiving the HTX-011 drug product (2.3-3.9 mL), despite the two formulations having similar levels of AP135. While increased dosage volume appears to result in a more severe local reaction, it is difficult to meaningfully translate the dose volumes to the clinical dose.

The majority of the findings observed in studies with both APF18A and the HTX-011 vehicle either completely recovered, or severity and incidence was greatly reduced by the end of the recovery periods. Based on the 28-day studies with the HTX-011 vehicle, chronic inflammation was the longest lasting effect at the injection site, which was still present to some extent around 28 days later. Chronic inflammation was not observed in recovery animals in the APF18A studies, likely due to the extended recovery period in these studies (6 weeks).

In summary, the local toxicity of AP135 was adequately characterized. Treatment with APF18A and the HTX-011 vehicle, resulted in multiple injection site findings. The injection site findings were, for the most part, consistent with a foreign body reaction to injected material. However, in some cases more severe findings were observed, likely related to the higher concentration of AP135 in APF18A or the use of a large injection volume. In general, the findings recovered with time; the most persistent finding was chronic inflammation, which lasted about 28 days following treatment with the HTX-011 vehicle. It is difficult to put the local findings into context since both adverse (necrosis) and likely tolerable (chronic inflammation) findings were seen with clinically relevant concentrations of AP135. In addition, it is unclear what the clinical significance of chronic inflammation would be as it appears to be a normal, though robust, reaction to foreign material. In that regard, only a minor effect on wound healing was observed in a GLP mini-pig study (Study 33-54). Therefore, we acknowledge that there is clinical experience with HTX-011 from the Phase 2 and 3 studies and defer to the clinical

review team in regard to if the local findings in nonclinical studies are acceptable from a risk-benefit perspective considering the clinical data. However, it appears that more severe findings were seen when either the concentration of AP135 exceeded the clinical formulation or an increased volume of injection was used in the animal studies. Therefore, we would recommend caution regarding increased dosing in humans.

Table 5: Summary of Local Effects Following AP135 Treatment

Study	AP135 (W/W)	Injection site	Injection volume
Rat, 90-day, APF18A, 25-07	(b) (4)	Dermal observations: erythema and edema Macroscopic changes: abrasion and thickening Microscopic observations: hyperplasia, chronic inflammation, necrosis, edema, erosion, ulceration, fibrosis	0.25 mL
Dog, 90-day, APF18A, 25-08	(b) (4)	Dermal observations: edema Macroscopic changes: thickening Microscopic observations: chronic inflammation, granulomatous inflammation, necrosis, edema, hemorrhage, fibrosis	1 mL
Rat, 28-day, HTX-011 vehicle, 33-85	(b) (4)	Dermal observations: scab Macroscopic changes: scab Microscopic observations: chronic inflammation, granulomatous inflammation	0.13-0.19 mL
Dog, 28-day, HTX-011 vehicle, 33-86	(b) (4)	Dermal observations: edema, scab Macroscopic changes: thickening Microscopic observations: chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage	5.2-8.9 mL
Rat, single, HTX-011 vehicle, 33-48	(b) (4)	Dermal observations: edema Macroscopic changes: scab, dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, crust	2 mL
Rat, single, HTX-011 vehicle, 33-55	(b) (4)	Dermal observations: not conducted Macroscopic changes: dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, fibrosis	0.5 mL

Systemic effects:

The 90-day studies with the APF18A polymer were conducted in rat and dog (Studies 25-07 and 25-08). Dosage was by subcutaneous administration (0.25 mL in rats and 1 mL in dogs) every 24 hours across six injection sites (16 injections total per site). Based on body weights at the beginning of the study, rats were exposed to 531.8-

1008.6 mg/kg of AP135 (b) (4) and dogs were exposed to 93.6-170.2 mg/kg (b) (4). While these studies address the direct exposure to the excipient, a few things must be kept in mind. The first is that the studies were multiple-dose studies, which is exaggerated compared to single-dose exposure from the clinical product. The second is that the route of administration was subcutaneous injection as opposed to direct instillation into the surgical area as intended clinically. It is unclear how the different route of administration would affect systemic exposure to the excipient.

In rats, systemic effects observed following APF18A treatment included increases in hematology parameters (platelets, white blood cells, neutrophils, monocytes, reticulocytes (f), and lymphocytes (f)), decreased clinical chemistry parameters (total protein, albumin, albumin/globulin ratio, and calcium (f)), increased organ weights (spleen, adrenal (f), and ovary (f)), and increased incidence of microscopic findings (spleen hematopoiesis, lung alveolus histiocytes, kidney nephropathy (m), kidney corticomedullary junction mineralization (f), myeloid hyperplasia of the sternum and femur marrow, and lymphoid hyperplasia in multiple lymph nodes). A majority of these findings are consistent with a nonspecific immune system response to a foreign material, likely the result of breakdown products of the polymer entering systemic circulation. Findings in dogs were limited to changes in clinical chemistry parameters (decreased albumin (m), increased globulin (m), and decreased albumin/globulin ratio). The more limited number of systemic findings in dogs compared to rats is likely related to the dog receiving a much lower mg/kg dose of APF18A.

Similar systemic findings were seen with the HTX-011 vehicle in 28-day repeat-dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection (0.13-0.19 mL in rats and 5.2-8.9 mL in dogs) every 48 hours across six injection sites (2-3 injections total per site). This resulted in rats being exposed to 646.4 mg/kg of AP135 (b) (4) and dogs being exposed to 760.5 mg/kg (b) (4). When interpreting the results of these studies it is important to keep in mind that the HTX-011 vehicle contained multiple excipients beyond just the AP135 polymer. In addition, the study was a repeat-dose study by a slightly different route of exposure as was discussed for the APF18A studies.

Findings in the rat study were limited to altered hematology parameters in male rats (increased platelets, neutrophils, eosinophils, and reticulocytes). In dogs, multiple systemic effects were observed including: hypoactivity, decreased body weight (f), altered hematology parameters (increased white blood cells, platelets, leukocytes, eosinophils, neutrophils, monocytes, and reticulocytes; decreased red blood cells, HGB, HCT, and lymphocytes), altered clinical chemistry parameters (increased globulin, decreased albumin (f), decreased albumin/globulin ratio, increased LDH, and decreased creatinine clearance), and increased organ weights (liver, thymus (m), adrenal (f), and kidney (f)). The more robust systemic reaction to the HTX-011 vehicle in dogs is likely related to the large dose of vehicle in this study. This is supported by the observation of

very limited systemic findings with the HTX-011 drug product in the same study (limited to increased reticulocytes in males and increased kidney weight in females) which delivered an AP135 dose of 321.1 mg/kg ((b) (4))

Taken together, the potential systemic toxicity of AP135 was adequately characterized across multiple studies. Potentially adverse effects (histopathological findings and/or decreased body weight) were seen in the rat study with APF18A at approximately 0.52-0.98x the MRHD (400 mg/12 mg) and in the dog study with the HTX-011 vehicle at a dose of 2.5x the MRHD (400 mg/12 mg). The adverse effects observed in these studies may not be clinically relevant since they are likely the result of repeat dosing and the drug product is indicated for a single treatment. In that regard, these adverse effects were not observed in single-dose studies with the HTX-011 vehicle by subcutaneous injection at approximately 0.93-7.2x the MRHD (400 mg/12 mg). The most consistent systemic effect was an increase in hematology parameters that were seen in dogs with APF18A at approximately 0.31-0.57x, in rats with the HTX-011 vehicle at approximately 0.63x, and in dogs with HTX-011 at approximately 1.1x the MRHD (400 mg/12 mg). Altered hematology parameters were also seen in one of the two single dose rat studies with the HXT-011 vehicle at approximately 0.93-1.5x the MRHD, suggesting they may be clinically relevant even after a single exposure. Changes in hematology parameters were measured in the clinical studies and we defer to the clinical team in terms of interpreting if the observed effects in nonclinical studies are acceptable from a risk-benefit perspective considering the clinical data.

Table 6: Summary of Systemic Effects Following AP135 Treatment

<i>Study</i>	<i>Dose of AP135 in mg/kg (HED in mg^d)</i>	<i>Effects</i>	<i>Safety Margin³</i>
Rat, 90-day, APF18A, 25-07	531.8-1008.6 (b) (4)	↑ hematology parameters, altered clinical chemistry, ↑ organ weights, microscopic findings	0.52-0.98x ¹
Dog, 90-day, APF18A, 25-08	93.6-170.2 (b) (4)	altered clinical chemistry	0.31-0.57x ¹
Rat, 28-day, HTX-011 vehicle, 33-85	646.4 (b) (4)	↑ hematology parameters	0.63x ²
Dog, 28-day, HTX-011 vehicle, 33-86	760.5 (b) (4)	↓ activity, ↓ BW, altered hematology parameters, altered clinical chemistry, ↑ organ weights	2.5x ²
Dog, 28-day, HTX-011, 33-86	321.1 (b) (4)	↑ reticulocytes	1.1x ²
Rat, single dose, HTX-011 vehicle, 33-55	957.8-1584.4 (b) (4)	↑ hematology parameters	0.93-1.5x ¹
Rat, single dose, HTX-011 vehicle, 33-48	3755.6-7455.6 (b) (4)	none	3.6-7.2x ¹

¹ Safety margins calculated based on initial body weights. The mg/kg dose would decrease over the course of the study as animals weight increased.

² These studies used a constant volume/kg throughout the study.

³ Safety margins are compared to the MRHD of 400 mg/12 mg of bupivacaine/meloxicam.

⁴ Human equivalent dose calculated based on body surface area and a 60 kg individual

Overall Conclusion for AP135:

The local and systemic toxicity of AP135 was adequately characterized across multiple animal studies using different formulations. Locally, the primary finding at clinically relevant concentrations was a local inflammatory reaction at the injection site which could be considered a foreign body reaction. Chronic inflammation persisted for around 28 days. In addition, increased amounts of the excipient (either due to an increased concentration of AP135 or increased dosage volumes) appeared to cause severe local reactions, although it is difficult to translate the increased volumes in a meaningful way to compare to the clinical dose. The only systemic finding after a single exposure was changes in hematology parameters at 0.93-1.5x the level of AP135 at the MRHD (400 mg/12 mg), although no systemic effects were seen in a second single-dose study at 3.6-7.2x the level of AP135 at the MRHD (400 mg/12 mg). In repeat-dose studies, hematology parameters were affected at 0.31-0.57x (dog) and 0.63x (rats) the level of AP135 at the MRHD (400 mg/12 mg). Adverse systemic effects were observed following repeat exposure at 0.52-0.98x the level of AP135 at the MRHD (400 mg/12 mg) in rats and in the dog study with the HTX-011 vehicle at 2.5x the MRHD (400 mg/12 mg); however, these effects are likely the result of exaggerated exposure conditions and not entirely clinically relevant. Taken together, the data suggest the potential for both local and systemic effects at clinically relevant doses and therefore AP135 is not completely qualified for safety in the nonclinical studies. However, we acknowledge that there is clinical experience from the Phase 2 and 3 studies with the formulation and defer to the clinical review team in terms of interpreting if the observed effects in nonclinical studies translate into adverse effects in the clinical setting.

Dimethyl Sulfoxide (DMSO)

Dimethyl sulfoxide (DMSO) was included in the drug product as a (b) (4) w/w or (b) (4) mg/g. The amount of DMSO in the drug product is (b) (4) % This results in a total of (b) (4) mg of DMSO administered at the MRHD ((b) (4) However, the drug product has a specification of (b) (4) w/w DMSO which would result in the maximal potential amount of DMSO being (b) (4) mg/g or (b) (4) mg at the MRHD. DMSO has been used in multiple approved products based on the CDER Inactive Ingredients Database (IID), primarily dosed by the topical route. Based on the levels of DMSO in this drug product, the use of DMSO in this drug product is novel based on total dose via the intended clinical route of administration. The IID provides the following information on FDA-approved drug products that contain DMSO.

Table 7: DMSO FDA-approved Products as Per IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency Per Unit Dose	Comment on Adequacy
Dimethyl Sulfoxide	IV (Infusion)	Powder, For Injection Suspension, Lyophilized	NA	Inadequate information and route
Dimethyl Sulfoxide	Subcutaneous	Implant	104 mg	Inadequate dose
Dimethyl Sulfoxide	Topical	Dressing	NA	Inadequate information and route
Dimethyl Sulfoxide	Topical	Lotion	45.5% W/W	Inadequate information on exposure via this route
Dimethyl Sulfoxide	Topical	Solution	45.5% W/W	Inadequate information on exposure via this route

Based on the information provided above, the proposed dose of DMSO via the SC route of injection clearly exceeds that of any other FDA-approved drug product. In the absence of adequate exposure information, the systemic coverage via the topical drug products is not clear. As such, adequate safety justification is required. The Applicant noted that DMSO is present in the IID as noted in the table above. To support the safety, they reference three publications, summarized below:

Table 8: DMSO Literature Cited by Applicant

Reference	Applicant Conclusion	Doses Tested	Comment on Adequacy
Noel 1975	Based on studies in the dog, pig, rabbit, and rat, the lenticular nucleus of the eye is a target organ of toxicity.	Various doses in multiple studies summarized.	Inadequate but supportive. Review of studies with a focus on lenticular changes. Lacks adequate detail for independent substantive review.
Vogin 1970	Oral NOEL in an 18-month rhesus monkey study was 3 mg/kg/day	HED = 58 mg/60 kg person.	Inadequate dose to inform risk assessment
De la Torre 1981	No significant or long-lasting effects noted following IV administration of 3 g/kg DMSO in a 40% solution for 9 days.	HED = 58064 mg/60 kg person (47 times the MRHD of DMSO).	Inadequate. Supportive at best given study limitations. The study only tested 6 male animals. Battery of tissues examined via histopathology not fully described. May be limited to muscle, lymph nodes, salivary gland and peripheral nerve.

The above published studies were reviewed to determine if these data could be adequate to address the general toxicity of DMSO. Collectively, the data are supportive at best but suggest that DMSO has been tested in numerous species, including rodent

and non-rodent, to inform the potential target organs of toxicity. However, the general toxicity of DMSO was characterized in part via the pivotal toxicology studies testing the drug product formulation.

DMSO was included in HTX-011 and the HTX-011 vehicle that were tested in the 28-day repeat dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection and the rat single dose studies (Studies 33-55 and 33-48) by subcutaneous injection. These studies can be used to help characterize the presence of DMSO in the drug product from a local and general toxicology perspective. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section.

To address the potential reproductive and developmental toxicity of DMSO, the Applicant cited the Organization for Economic Cooperation and Development's (OECD) Screening Information Dataset (SIDS) dossier for DMSO (OECD 2000). The OECD evaluated unpublished studies in this document and a summary of their evaluation is provided here. This included a GLP oral reproduction/developmental toxicity screening study in rats, a GLP oral developmental toxicity study in rats, and a GLP oral developmental toxicity study in rabbits. No reproductive or developmental toxicity was seen in the reproduction/developmental toxicity screening study or the rabbit developmental study (highest dose tested was 1000 mg/kg). Developmental/maternal toxicity (decreased body weight gain in dams manifesting as decreased fetal weight and reduced/delayed ossification of the ribs) was observed in the rat developmental study at 5000 mg/kg but no effects were seen at 1000 mg/kg. Thus, the overall NOAEL for reproductive/developmental toxicity is 1000 mg/kg, which is a human equivalent dose of 9,677.4 mg based on body weight scaling and a 60 kg individual. Thus, the levels of DMSO in the drug product (b) (4) mg) are covered by the available nonclinical reproductive/developmental toxicity data based on body surface area. However, no bridging exposure data were provided to justify why oral administration would provide similar or greater exposure compared to the clinical route of administration and thus the Applicant did not provide adequate data to support their conclusion that these data adequately characterized the safety of the DMSO administered via the clinical route of administration.

Triacetin

(b) (4). The amount of triacetin in the drug product is (b) (4) mg/g. This results in a total of (b) (4) mg of triacetin administered at the MRHD for HTX-011 ((b) (4))

Triacetin has been used in multiple approved products, primarily dosed by the oral route, as identified by the IID. The information from the IID is reproduced in the table below:

Table 9: Triacetin in FDA-approved Products as per CDER IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency Per Unit Dose
Triacetin	Endocervical	Gel	NA
Triacetin	Oral	Capsule	6.42 mg
Triacetin	Oral	Capsule, Coated Pellets	1.21 mg
Triacetin	Oral	Capsule, Enteric Coated Pellets	5.1 mg
Triacetin	Oral	Capsule, Extended Release	46.7 mg
Triacetin	Oral	Capsule, Sustained Action	2.76 mg
Triacetin	Oral	Concentrate	50 mg/1 mL
Triacetin	Oral	Powder, For Oral Suspension	1.02 mg/5 mL
Triacetin	Oral	Suspension	0.32 mg/1 mL
Triacetin	Oral	Suspension, Extended Release	1.18 mg/5 mL
Triacetin	Oral	Tablet	6 mg
Triacetin	Oral	Tablet (Immed./Comp. Release), Film Coated	0.72 mg
Triacetin	Oral	Tablet, Chewable	1.36 mg
Triacetin	Oral	Tablet, Coated	1 mg
Triacetin	Oral	Tablet, Delayed Action, Enteric Coated	6 mg
Triacetin	Oral	Tablet, Delayed Release	1.82 mg
Triacetin	Oral	Tablet, Extended Release	8 mg
Triacetin	Oral	Tablet, Film Coated	15.12 mg
Triacetin	Oral	Tablet, Sustained Action	1.96 mg
Triacetin	Transdermal	Patch	22.1 mg
Triacetin	Transdermal	Patch, Controlled Release	22.1 mg

Based on the MRHD and route of administration, none of the previously approved drug products provide adequate coverage for the proposed dose of triacetin. The Applicant justifies the safety by stating that triacetin is considered generally recognized as safe (GRAS). However, GRAS determinations for foods are in the context of how the material is used in food and oral toxicity data. No data regarding food consumption or metabolism was provided. The Applicant cited a summary of toxicity findings but provided no references other than an LD₅₀ study in rats and mice. From a general toxicology perspective, the NDA lacks reference to adequate published data to support the systemic safety of the proposed levels of triacetin.

Triacetin was included in HTX-011 and the HTX-011 vehicle that were tested in the 28-day repeat-dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection and the rat single-dose studies (Studies 33-55 and 33-48) by subcutaneous injection. These studies can be used to help characterize the presence of triacetin in the drug product. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section.

To address the potential reproductive and developmental toxicity of triacetin, the Applicant cited an unpublished GLP combined oral repeat-dose and

reproduction/developmental toxicity study in rats submitted to the Japanese Ministry of Health and Welfare. A translated copy of the study was provided by the Applicant as part of the second cycle materials. This study was also reviewed by the Organization for Economic Cooperation and Development (OECD 2002a). No findings on reproductive or developmental factors were observed in this study up to a dose of 1000 mg/kg, the highest dose tested. However, no bridging exposure data were provided to justify why oral administration would provide similar or greater exposure compared to the clinical route of administration and thus the Applicant did not provide adequate data to support their conclusion that these data adequately characterized the safety of triacetin administered via the clinical route of administration.

No developmental toxicity data was available in a second species, such as the rabbit. The Applicant cited the argument presented by the Cosmetic Ingredient Review (CIR) that triacetin is quickly metabolized into glycerol and acetic acid, both of which are not developmental toxins (Fiume et al., 2003). The data to support the rapid metabolism of triacetin was not included in the CIR evaluation but this appears to be the case when triacetin is incubated with rat intestine in vitro according to the OECD SIDS for triacetin. The OECD document also describes a study where dogs were given IV triacetin and the majority of the triacetin underwent intravascular hydrolysis. This suggests that rapid metabolism is not limited to the GI tract. However, the study was not provided and the time course for hydrolysis was not described in the summary. As such, data were not submitted to support the hypothesis that there is no systemic exposure to triacetin via this drug product.

To support the lack of developmental toxicity of glycerol and acetic acid, the Applicant cited the OECD SIDS documents for ethyltriacetoxysilane (which contained reproduction and developmental data for acetic acid, OECD 2005) and glycerol (OECD 2002b). The Applicant also provided study reports of the developmental studies for both compounds.

An oral study with sodium acetate was conducted in rats to assess fertility up to a dose of 60 mg/kg with treatment for a week before breeding, during the 9-day breeding period, and throughout pregnancy, lactation, and weaning of the offspring. No effects on fertility were observed. Of note, higher doses could not be tested because of stomach irritation.

An oral developmental study with acetic acid (5% apple cider vinegar) was conducted with doses of up to 1600 mg/kg by gavage in mice, rats, and rabbits. The study is described as 1600 mg/kg acetic acid in the OECD document, but this would be expected to cause severe stomach irritation. The Applicant submitted a copy of the original study report and it appears that the dose is 1600 mg/kg of 5% acetic acid which would correspond to a dose of 80 mg/kg acetic acid. There were no maternal or developmental effects observed in all three species.

Developmental toxicity studies with glycerol were reviewed in the OECD SIDS. No developmental toxicity was seen in the rat (up to 1310 mg/kg), mouse (1280 mg/kg), or rabbit (up to 1180 mg/kg).

The existing data are not adequate because triacetin was only directly tested in one species by the oral route. The weight of the evidence does suggest that there is potentially limited concern for triacetin in the drug product to cause reproductive or developmental toxicity. Although, no effects were seen in rats tested with oral triacetin up to 1000 mg/kg (b) (4), it is not known how exposure to triacetin compare following oral and intra-wound installation of this compound. The major expected metabolites, acetic acid and glycerol, did not show any evidence of developmental or reproductive toxicity in multiple species. However, the Applicant did not provide data to characterize triacetin exposure following administration of HTX-011-56 and therefore, it is not known if the parent compound will be present in the systemic circulation. If triacetin is not present in the systemic circulation, reproductive and developmental effects of the metabolites would be a valid justification for not conducting reproductive and developmental studies for triacetin.

Maleic Acid

(b) (4)
The amount of maleic acid in the drug product is 0.05% w/w or 0.5 mg/g. This results in a total of 8.0 mg of maleic administered at the MRHD for HTX-011 (13.68 mL * 1.17 mg/mL * 0.5 mg/g). Maleic acid has been used in multiple approved drug products by different routes according to the IID, including intramuscular, intravenous, and oral, as noted in the table below.

Table 10: Maleic Acid FDA-approved Products as Per CDER IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency per unit dose
MALEIC ACID	INTRAMUSCULAR	INJECTION	0.01%W/V
MALEIC ACID	INTRAMUSCULAR	POWDER, FOR INJECTION SOLUTION	NA
MALEIC ACID	INTRAVENOUS	INJECTION	0.01%
MALEIC ACID	IV(INFUSION)	INJECTABLE	0.01%
MALEIC ACID	IV(INFUSION)	INJECTION	ADJ PH
MALEIC ACID	ORAL	CAPSULE	2MG
MALEIC ACID	ORAL	SYRUP	1.73MG/5ML
MALEIC ACID	ORAL	TABLET	4MG

ADJPH = adjust PH as necessary

Of the products listed above, the oral syrup product can be dosed up to 12 teaspoons per day (~59 mL), which would correlate with a maximum daily dose (MDD) of 20.41 mg maleic acid. This exceeds the amount of maleic acid that a patient would be exposed to at the MRHD (6.1 mg). However, the oral route is not ideal for justifying the clinical route of exposure due to potential differences in absorption and metabolism and

therefore the approved products do not qualify this excipient for general systemic exposure.

Approved products listed in the IID had a lower concentration of maleic acid than HTX-011 (0.1 mg/mL vs. 0.585 mg/mL in HTX-011). Thus, maleic acid in HTX-011 cannot be qualified for local safety based on the approved products.

Maleic acid was included in HTX-011, but not the HTX-011 vehicle, that was tested in the 28-day repeat-dose rat and dog studies (Studies 33-85 and 33-86) by subcutaneous injection. The studies using HTX-011 can be used to help characterize the presence of maleic acid in the drug product. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section.

The Applicant cited a publicly available Environmental Protection Agency (EPA) action memorandum to address the systemic safety of the proposed dose of maleic acid (EPA, 2006). The EPA reference cited studies with maleic anhydride but that the results could be applied to maleic acid due to rapid hydrolysis of maleic anhydride to maleic acid. The EPA reference contained results of a multi-generation reproduction study and a teratogenic study in rats. The study reports were not provided by the Applicant. According to the action memorandum, in a multigenerational study in rats, maleic anhydride was administered by oral gavage at 0, 20, 55, or 150 mg/kg/day. Renal lesions were noted at all doses. A LOAEL for the study was described as 20 mg/kg/day. The EPA memo states that there were no effects noted on fertility at doses up to 55 mg/kg/day. Further, no adverse effects on litter size and pup survival were noted at doses up to 150 mg/kg/day. In a separate dedicated embryo-fetal development study, the EPA memo noted that there were no external abnormalities, soft tissue abnormalities or skeletal abnormalities when female rats were treated with 0, 30, 90, or 140 mg/kg/day maleic anhydride from Gestation Day 6 to 15. The memo reported that the maternal NOAEL was the high dose of 140 mg/kg.

No reproductive or developmental effects were seen at human equivalent doses of 532 mg (reproductive) and 1354.8 mg (teratogenic), which is 67x and 169x times the levels a patient would be exposed to at the MRHD, respectively. Normally, the Agency does not accept summary information to support the safety of novel excipient use. However, because the EPA is a U.S. regulatory agency, we will accept these summary data based on their conclusions. The Applicant identified oral bioavailability data in the rat which suggests that maleic acid is about 30% orally bioavailable in the Sprague Dawley rat (Wu et al., 2016). The safety margins would still be sufficiently high even if it is assumed that instillation into the wound would result in 100% bioavailability of maleic acid with adjusted safety margins of 20x and 51x.

The Applicant submitted a study testing the developmental toxicity of oral maleic acid in rabbits (Study 34-74). The full review of the study can be found in Section 9 of this review. No effect on developmental toxicity was observed in the study. The highest dose tested was 20 mg/kg, which is a human equivalent dose of 387.1 mg based on body surface area and a 60 kg person, which is 48x the levels a patient would be

exposed to at the MRHD. This dose produced no evidence of maternal toxicity and therefore was not ideally designed as a toxicology study. Further, the study report in the NDA is draft and there are no exposure data to justify why the oral toxicology study resulted in exposures comparable to that which would occur via instillation into the surgical wound. Therefore, although the study likely provides adequate coverage, there is technically an inadequate scientific bridge to support the assumption that these oral data adequately address the reproductive safety of wound instilled maleic acid via this formulation.

The Applicant also states that the safety of maleic acid in terms of embryo-fetal development is supported by the use of maleate salts in FDA-approved prescription products and specifically lists Luvox (fluvoxamine maleate) as a referenced drug. They note that the maximum daily dose of fluvoxamine is 300 mg which is 219.8 mg of fluvoxamine and 80.2 mg of maleic acid. The rabbit EFD study in that referenced label reported no adverse effects at a presumed dose of maleate of 10.9 mg/kg (HED of 3.5 mg/kg person). Compared to the human dose of 8 mg (0.13 mg/kg) via HTX-011, the rabbits would have been exposed to 26-times the levels of maleate based on body surface area. However, the drug product label does not specify that the animals were administered fluvoxamine maleate, only fluvoxamine. Therefore, we cannot assume the animals were treated with the maleate salt. Further, exposure levels for maleic acid via this referenced product were not provided in the referenced label and C_{max} levels via the wound instillation method may be very different than via oral administration. The Applicant did identify oral bioavailability data in the rat but oral bioavailability in the rabbit was not provided.

Taken together with the data reviewed by the EPA, the reproductive and developmental toxicity of maleic acid is most likely of low risk. However, definitive data or justification to support that the levels in the drug product are not expected to result in developmental toxicity in the rabbit via the proposed route of administration are lacking.

Characterization of the excipients by nonclinical data

As noted above, DMSO, triacetin, and maleic acid in the drug product were not characterized for either local or systemic safety based on use in other approved products. However, the Applicant did test these excipients as part of the drug product and vehicle in the nonclinical studies. An important distinction is that the HTX-011 vehicle did not contain maleic acid, but the drug product itself did.

In terms of local toxicity, the HTX-011 vehicle (containing DMSO and triacetin) resulted in an inflammatory reaction that is consistent with a foreign body material. This was characterized as chronic inflammation that lasted for up to 28 days. A similar reaction was seen with the HTX-011 drug product (containing DMSO, triacetin, and maleic acid). Based on studies with APF18A, the inflammatory reaction is very likely driven by the presence of AP135, although a contribution of the other excipients cannot be completely

ruled out. More severe findings, such as necrosis, were seen in the single-dose rat studies but this is possibly due to the increased injection volume used.

In the worst-case scenario, the excipients could have contributed to the observed local inflammatory reaction. It is unclear what the clinical significance of chronic inflammation would be as it appears to be a normal reaction to a foreign material. In that regard, there did not appear to be a significant effect on wound healing in a GLP mini-pig study (Study 33-54). We also acknowledge that there is clinical experience with the excipients from the Phase 2 and 3 studies and defer to the clinical review team regarding if the local findings in non-clinical studies would be of a safety concern. If the clinical team finds the chronic inflammation as tolerable, then the excipients are characterized for local safety at a margin of 1x, as the concentrations of DMSO, triacetin, and maleic acid in the nonclinical studies are the same as those used in the clinical formulation.

In terms of systemic effects, the primary findings with the HTX-011 vehicle (containing DMSO and triacetin) were changes in hematology parameters with similar effects observed with HTX-011 (containing DMSO, triacetin, and maleic acid). Again, it is likely that these changes are the result of AP135 as similar findings were seen with APF18A, although a contribution of the other excipients cannot be completely ruled out. More severe effects (decreased body weights and decreased activity) were seen in the repeat-dose dog study with the HTX-011 vehicle (Study 33-86) but these were not seen in rat studies or when HTX-011 was given at a lower dose. In the worst-case scenario, the excipients contributed to the hematology findings, which could be considered an adverse effect. We acknowledge that there is clinical experience with the excipients from the Phase 2 and 3 studies, which included some monitoring for hematology changes, and defer to the clinical review team regarding if the changes in non-clinical studies would be of a safety concern. If the clinical team finds the changes in hematology parameters tolerable, then the excipients are characterized for general systemic safety at a margin of up to 1.4x (see table below).

While the above studies help characterize the general systemic effects of the excipients, no studies were conducted with any of the individual excipients, HTX-011, or the HTX-011 vehicle to address the potential reproductive toxicology of the excipients. The Applicant did provide data to address the reproductive/developmental toxicity of all of the excipients as described in the previous sections.

Table 11: Summary of Local Effects Potentially Associated with Excipients

<i>Study</i>	<i>Excipients</i>	<i>Injection site</i>	<i>Injection volume</i>
Rat, 28-day, HTX-011 vehicle, 33-85	DMSO: 10%* Triacetin: 25%	Dermal observations: scab Macroscopic changes: scab Microscopic observations: chronic inflammation, granulomatous inflammation	0.13-0.19 mL

Dog, 28-day, HTX-011 vehicle, 33-86	DMSO: 10%* Triacetin: 25%	Dermal observations: edema, scab Macroscopic changes: thickening Microscopic observations: chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage	5.2-8.9 mL
Rat, single, HTX-011 vehicle, 33-48	DMSO: 10%* Triacetin: 25%	Dermal observations: edema Macroscopic changes: scab, dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, crust	2 mL
Rat, single, HTX-011 vehicle, 33-55	DMSO: 10%* Triacetin: 25%	Dermal observations: not conducted Macroscopic changes: dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, fibrosis	0.5 mL

(b) (4)

Table 12: Summary of Systemic Effects Potentially Associated with Excipients

<i>Study</i>	<i>Excipients in mg/kg (HED in mg)</i>	<i>Effects</i>	<i>Safety Margin³</i>
Rat, 28-day, HTX-011 vehicle, 33-85	DMSO: 99.5 (963) Triacetin: 248.6 (2406)	increased hematology parameters	0.60x ²
Rat, 28-day, HTX-011, 33-85	DMSO: 99.5 (963) Triacetin: 248.6 (2406) Maleic acid: 0.50 (4.8)	increased hematology parameters	0.60x ²
Dog, 28-day, HTX-011 vehicle, 33-86	DMSO: 117 (3900) Triacetin: 292.5 (9750)	decreased activity, decreased BW, altered hematology parameters, altered clinical chemistry, increased organ weights	2.4x ²
Dog, 28-day, HTX-011, 33-86	DMSO: 51.5 (1717) Triacetin: 128.7 (4290) Maleic acid: 0.26 (8.7)	Increased reticulocytes	1.1x ²
Rat, single dose, HTX-011 vehicle, 33-55	DMSO: 147-244 (1423-2361) Triacetin: 368-609 (3561-5894)	Increased hematology parameters	0.89-1.4x ¹
Rat, single dose, HTX-011 vehicle, 33-48	DMSO: 578-1147 (5594-11100) Triacetin: 1444-2868 (13974-27755)	none	3.5-6.9x ¹

¹ Safety margins calculated based on initial body weights. The mg/kg dose would decrease over the course of the study as animals weight increased.

² These studies used a constant volume/kg throughout the study.

³ Safety margins are compared to the MRHD of 400 mg/12 mg of bupivacaine/meloxicam.

(b) (4)

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Specifications

Bupivacaine

The drug substance specifications for bupivacaine base are shown in the Applicant's table below. At the MRHD of 400 mg bupivacaine, according to ICH Q3A(R2), the appropriate qualification threshold is 0.15% or 1 mg per day intake (whichever is lower). Based on this the Applicant's specifications for (b) (4) and any unspecified impurity are acceptable.

The drug substance contains one residual solvent, (b) (4) is listed in ICH Q3C as a (b) (4) with a concentration limit of (b) (4) ppm so the Applicant's proposed specification of (b) (4) ppm is acceptable.

The adequacy of the remaining specifications is discussed below.

(b) (4)

(b) (4)

Table 13: Bupivacaine Drug Substance Specifications

Attribute		Acceptance Criteria	Test Method Reference
Appearance		White to off-white crystalline powder or crystals or granules	Visual
Identification	IR	Complies with reference spectrum	USP <197A>, Ph Eur 2.2.24
	Chromatographic	Complies with reference	TLC
Water content (% w/w)		(b) (4)	USP <921>, Ph Eur 2.5.12
Residue on ignition/sulfated ash (% w/w)		(b) (4)	USP <281>, Ph Eur 2.4.14
Loss on drying (% w/w)		(b) (4)	USP <731>, Ph Eur 2.2.32
Assay (% w/w)		(b) (4)	Titration
Chromatographic purity (% w/w)	(b) (4)	(b) (4)	HPLC
	Any unspecified impurity	(b) (4)	
	Total impurities	(b) (4)	
(b) (4)		(b) (4)	GC
(b) (4)		(b) (4)	GC
(b) (4)		(b) (4)	GC
Bacterial endotoxins (EU/g)		(b) (4)	USP <85>, Ph Eur 2.6.14
Microbiological limits (CFU/g)	Total aerobic microbial count ^a	(b) (4)	USP <61>, Ph Eur 2.6.12
	Total combined yeasts/molds count ^a	(b) (4)	

Abbreviations: IR, infrared; USP, United States Pharmacopeia; Ph Eur, European Pharmacopoeia; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; GC, gas chromatography; % w/w, percent weight/weight; ppm, parts per million; EU, endotoxin units; CFU, colony forming units; NMT, not more than

^a Per USP <1111> and Ph Eur 5.1.4; 10² is 200 CFU/g.

Table from page 1 of the Applicant's Specification document (3.2.S.4.1)

Meloxicam

The drug substance specifications for meloxicam are shown below. At the MRHD of 12 mg meloxicam, according to ICH Q3A(R2), the appropriate qualification threshold is 0.15% or 1 mg per day intake (whichever is lower). Based on this, the Applicant's specifications for all impurities are acceptable.

All of the specifications for the residual solvents are within ICH Q3C. ICH Q3C does not specifically delineate (b) (4), but refers to (b) (4) which, as per the guidance, (b) (4) ICH Q3C notes that the PDE for (b) (4) ppm concentration. The proposed specification of NMT (b) (4) ppm (b) (4) would result in (b) (4) mcg of (b) (4) at the MRHD of 12 mg meloxicam (b) (4)) via this product which is below the amount of (b) (4) present at the PDE of (b) (4). The specification is acceptable.

Table 14: Meloxicam Drug Substance Specifications

Attribute		Acceptance Criteria	Test Method Reference
Appearance		Pale yellow powder	Visual
Identification	IR	Complies with reference spectrum	USP <197K>, Ph Eur 2.2.24
	HPLC	The retention time of the major peak of the sample corresponds to that of the reference standard	USP monograph
Loss on drying (% w/w)		NMT 0.5	USP <731>, Ph Eur 2.2.32
Residue on ignition/sulfated ash (% w/w)		NMT 0.1	USP <281>, Ph Eur 2.4.14
Assay, HPLC (% w/w on dried basis)		98.0 – 102.0	USP monograph
Assay, titration (% w/w on dried basis)		99.0 – 101.0	Ph Eur monograph
Related substances (HPLC) (% w/w)	USP related compound A and Ph Eur impurity A ^a	NMT 0.1	HPLC, USP monograph Procedure 1, Ph Eur monograph
	USP related compound B and Ph Eur impurity B ^b	NMT 0.1	
	USP methyl meloxicam and Ph Eur impurity C ^c	NMT 0.05	
	USP ethyl meloxicam and Ph Eur impurity D ^d	NMT 0.05	
	Any unspecified impurity	NMT 0.10	
	Total impurities	NMT 0.3	
Residual solvents (ppm)	(b) (4)		GC
Bacterial endotoxins (EU/g)	(b) (4)		USP <85>, Ph Eur 2.6.14

Table from page 1 the Applicant's Specification document (3.2.S.4.1).

Drug Product

The specifications for the drug product are located in the tables below. At the MRHD of 400 mg bupivacaine, according to ICH Q3B(R2), the appropriate qualification threshold is 0.2% or 3 mg per day intake (whichever is lower). Based on this, the Applicant's specifications for any unspecified impurity are acceptable. The specification for

(b) (4) was NMT (b) (4) % in the original submission, which was higher than ICH Q3B(R2). This was identified as a deficiency. In the resubmission, the Applicant has reduced the specification to (b) (4) %, which is acceptable.

At the MRHD of 12 mg meloxicam, according to ICH Q3B(R2), the appropriate qualification threshold is 0.5% or 200 mcg per day intake (whichever is lower). Based on this, the Applicant's specifications for all meloxicam-related substances are acceptable.

The adequacy of the specifications for other impurities (b) (4) are discussed below.

(b) (4)

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Table 15: HTX-011 Drug Product Specifications (Part 1)

Attribute	Acceptance Criteria	Method Reference
Appearance (Visual)	(b) (4)	
Dynamic Viscosity at 25°C		
Molecular Mass Distribution (GPC)		
Particulate Matter by Light Obscuration		
Identification of Bupivacaine and Meloxicam by HILIC ^a		
Identification of Bupivacaine and Meloxicam by HPLC ^a		
Bupivacaine Assay (HPLC)		
Bupivacaine Related Substances (Area % by HPLC)		
Bupivacaine Dose Uniformity ^a		
Meloxicam Assay (HPLC)		
Meloxicam Related Substances (Area % by HPLC)		

Table from page 38 of the Applicant's Drug Product Summary for HTX-011 document (2.3.P)

Table 16: HTX-011 Drug Product Specifications (Part 2)

Attribute	Acceptance Criteria	Method Reference
Meloxicam Dose Uniformity ^a	(b) (4)	
In Vitro Release (see Table 12 for additional Level 2 and Level 3 acceptance criteria)		
(b) (4)		
Container Closure Integrity (Dye Ingress) ^b		
Bacterial Endotoxins		
Sterility ^a		
Extractable Volume ^a		
	200 mg bupivacaine / 6 mg meloxicam	
	300 mg bupivacaine / 9 mg meloxicam	
	400 mg bupivacaine / 12 mg meloxicam	

Abbreviations: GPC, gel permeation chromatography; GC, gas chromatography; HPLC, high performance liquid chromatography; M_w, weight average molecular weight; M_n, number average molecular weight; LMW, low molecular weight; LC, label claim; NMT, not more than; AV, acceptance value; HILIC, hydrophilic interaction liquid chromatography; NLT, not less than; ppm, parts per million; USP, United States Pharmacopeia; Ph Eur, European Pharmacopoeia

^a Tested on release only

^b Tested on stability only

Table from page 38 of the Applicant's Drug Product Summary for HTX-011 document (2.3.P)

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(b) (4)



Container Closure System

HTX-011 is a solution filled into a 10 or 20 mL (b) (4) clear glass vial with a 20 mm opening ((b) (4) The vials are closed with 20 mm (b) (4) stoppers coated with (b) (4) and capped with 20 mm aluminum overseals ((b) (4) (b) (4)



The preparation and administration components include syringes, a vented vial spike, Luer lock applicators, tip caps, and instructions for use. The sealed glass vial is considered the primary container closure.

Table 18: Primary Container Closure

Description	Manufacturer	DMF	Specification Number
10 mL (b) (4) clear glass vial with a 20 mm opening			(b) (4)
20 mL (b) (4) clear glass vial with a 20 mm opening			
20 mm (b) (4) gray (b) (4) stopper with (b) (4)			
20 mm Aluminum overseal with a matte flip-off cap ^a			

Abbreviations: DMF, Drug Master File; NA, not applicable

^a Different flip-off cap colors are used for each of the product configurations (Table 3).

^b Aluminum overseal with matte flip-off cap is not in direct contact with the product.

Table from page 3 of the Applicant's Container Closure System document (3.2.P.7)

Table 19: Preparation and Administration Components

Description	Manufacturer	Manufacturer Part Number	510(k) Clearance Number	Shelf Life	Storage Condition
VVS					(b) (4)
2 mL (3 mL) ^a Luer lock syringe					
10 mL (12 mL) ^a Luer lock syringe					
Syringe tip cap					
LLA					

Abbreviations: VVS, vented vial spike; LLA, Luer lock applicator; NDA, New Drug Application

^a The first number indicates the nominal volume of the syringe, and the number in parentheses indicates the maximum volume.

^b Refer to Section 3.2.P.8.1.6.

Table from page 4 of the Applicant's Container Closure System document (3.2.P.7)

Extractables/Leachables Evaluation

To justify the safety of leachables arising from the container closure system or from (b) (4) processes that are in direct contact with the drug product, the Applicant performed extractables and leachables studies, which are described in the following documents:

- Module 3.2.P.2 Container Closure System
- Module 3.2.P.2 Manufacturing Process Development

The Applicant conducted extractable and leachable studies with two different contract testing labs, (b) (4). Each contract lab took a different approach to extractable and leachable testing. For example, (b) (4) conducted a single extraction study, while (b) (4) conducted a total of three extraction studies. Each approach is reviewed separately in this section.

Table 20: List of Extractable Studies

(b) (4)



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(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

HTX-011 is indicated for application into the surgical site to reduce pain for 72 hours. The Applicant proposed a limitation of use since safety and efficacy have not been established in highly vascular surgeries. Beyond this limitation of use, the Applicant is proposing a broad indication. However, based on internal discussions with the clinical team, the following surgeries are supported: unilateral bunionectomy, unilateral open inguinal hernia, and unilateral total knee arthroplasty. We defer to the clinical team on which surgeries are adequately supported with the approval of the NDA.

The Applicant proposed a maximum dose of 400 mg/12 mg (13.68 mL).

2.7 Regulatory Background

HTX-011 was originally developed under IND 125927, which was opened on May 7th, 2015. NDA 211988 was submitted on September 21st, 2018 and the NDA was fileable from the nonclinical perspective with no comments for the 74-day letter. Following review of the Applicant's NDA, the Division issued a complete response on April 30th, 2019. The complete response included the following nonclinical deficiencies.

3. You have not provided adequate data to support the safety of the DMSO or triacetin, in your drug product and the levels in the product exceed the maximum potency listed in the CDER Inactive Ingredient Database (IID) and are therefore considered novel. Specifically, your NDA did not include any discussion of the impact of these two excipients on the standard reproductive and developmental battery of studies.

Information needed to address this deficiency:

Submit adequate data to fully characterize the impact of the proposed doses of DMSO and triacetin on all endpoints normally characterized via the standard battery of reproductive and developmental toxicology studies. Should you elect to address this via literature, justify the adequacy of the literature based on current standard study protocols and provide copies of all referenced literature. In the absence of adequate published data, GLP nonclinical toxicology studies should be completed.

4. Your toxicological risk assessment for the excipient maleic acid, which exceeds the maximum potency listing in the CDER Inactive Ingredients Database (IID), does not address the potential impact of maleic acid on embryo-fetal development in a second species (typically rabbit).

Information needed to address this deficiency:

Submit adequate justification for the safety of the proposed maximum daily dose of maleic acid, specifically with respect to the effects of this compound on rabbit embryo-fetal development.

5. You have not provided adequate data to qualify the proposed drug product degradant (b) (4) which exceeds the ICH Q3B(R2) qualification threshold.

Information needed to address this deficiency:

Either tighten the drug product specification for (b) (4) to NMT (b) (4) % or provide adequate qualification in accordance with ICH Q3B(R2) as follows:

- a. Complete a minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
- b. In addition, conduct a repeat-dose toxicology study of appropriate duration to support the proposed indication. In this case, a study of 14 days should be completed.

Alternatively, provide adequate data to support your position that (b) (4)

However, we note that if you elect to pursue this option, the data may not address the local safety concerns via this drug product's proposed dosing regimen.

6. You have not provided adequate data to support the safety of the drug product specification for (b) (4). The proposed specification of NMT (b) (4) %

exceeds the appropriate qualification threshold of NMT (b) (4) % for a drug product with a maximum recommended human dose of greater than 2 grams as outlined in the ICH guidance for industry: *Q3B(R2) Impurities in New Drug Products*. (b) (4)

We also acknowledge that (b) (4) was detected in the clinical HTX-011 lot that was tested in the pivotal 28-day toxicology studies in dogs and rats; however, the level detected in these stability batches do not support the proposed specification.

Information needed to address this deficiency:

Either tighten the specification of (b) (4) to be within the qualification threshold of NMT (b) (4) % or provide adequate qualification in accordance with ICH Q3B(R2) as follows:

- a. Complete a minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
 - b. In addition, conduct a repeat-dose toxicology study of appropriate duration to support the proposed indication. In this case, a study of 14 days should be completed.
7. You have not provided adequate leachable data to permit substantive toxicological evaluation of the safety of the container closure system and the (b) (4) (b) (4) for this drug product. Specifically:
- a) You have not tested at least three drug product stability batches over the entire course of your proposed expiry (early, middle, late) in order to identify trends for leachables over the course of stability.
 - b) You have not tested your drug product for extractable compounds detected above the safety concern threshold (SCT) (b) (4) mcg/day from the (b) (4) in Extractable Study 3 (RPT-694).
 - c) You have not fully identified all of the compounds above the SCT in your extraction studies.
 - d) In your risk assessment for the (b) (4) you have not provided adequate justification for the use of the (b) (4) as a surrogate for the (b) (4).

- e) The variability of (b) (4) present in the existing leachable data do not identify any clear trends and suggest the potential for these compounds to be derived from (b) (4)

Information needed to address this deficiency:

Conduct new leachables studies based on all of the extractables identified in Extractable Study 3 identified above the safety concern threshold (SCT) of (b) (4) mcg/day from the (b) (4) according to USP <1663> and <1664>. In addition, since Extractable Study 3 did not address non-volatile compounds, conduct new leachable studies based on the non-volatile compounds identified in Extractable Studies 1 (RPT-701) and 2 (RPT-693).

Of note, you used a conservative SCT of (b) (4) mcg presumably based on genotoxic concerns. However, for acute products (duration of < 1 month) an SCT of (b) (4) mcg/day can be used for genetic toxicity assessment. However, for general toxicity concerns, employ a SCT of (b) (4) mcg/day in the leachable study.

Include at least three to-be-marketed drug product batches over the entire course of your proposed expiry (early, middle, late) to identify trends for leachables over the course of stability. The three drug batches should be stored in the planned commercial container closure.

The leachable study should use an AET that accurately reflects the potential total daily intake of potential leachables from the drug product and how the product will be dosed. For example, if multiple vials are to be used to deliver a dose, this could affect the AET calculation.

We noted that multiple compounds were not fully identified from your extraction/leachable studies. This included the leachable identified in the final drug product samples from Leachable Study 3 as (b) (4). Provide descriptions of the methods and procedures used to attempt to identify these compounds and justify why these compounds could not be identified or provide further data supporting identification of these chemicals.

Include further justification to support that use of (b) (4) is appropriate to represent the potential toxicity of (b) (4), either by providing literature references or performing adequate QSAR analysis.

Provide a root-cause analysis to identify the source of the (b) (4) or confirm that the (b) (4) present are derived from either (b) (4) or (b) (4). If they are derived from (b) (4), justify how you intend to adequately control (b) (4) to ensure product consistency and quality.

In addition to the comments above, the following general recommendations regarding the design and requirements for adequate extractable leachable safety assessment are provided to guide these studies:

The NDA submission must contain adequate information on potential leachables and extractables from the drug container closure system and/or drug product formulation, unless specifically waived by the Division. The evaluation of extractables and leachables from the drug container closure system or device should include specific assessments [REDACTED] (b) (4) etc. Provide justification for the choice of solvents and conditions for the extraction studies (e.g., time, temperature, agitation). The results of the extraction studies should be used to assure that you are adequately monitoring the drug product stability samples for potential leachables from the primary or secondary container closure systems and from your analysis of data from any [REDACTED] (b) (4) processes that suggest the potential for additional leachable compounds in the final drug product formulation. Your analytical evaluation threshold (AET) must be established to be able to detect, identify, and quantitate levels of compounds based on these thresholds or you must provide adequate justification that these thresholds are not possible to be met by current analytical methodology. If you cannot meet these thresholds, safety evaluations will be based on the limits of quantitation (LOQ). Your submission must include a detailed discussion of how you established your AET as well as justification for the limits of detection (LOD) and LOQ for the analytical methods used.

Evaluate at least three batches of your to-be-marketed drug product for leachables and include assessments at multiple timepoints over the course of your stability studies in order to identify trends in leachable levels over time. The materials tested should include any secondary container closure systems, if present, and be subjected to the same sterilization methods, as appropriate. These data are essential to determine the appropriate shelf life of your product.

For all drug products, establish your AET to be able to detect potentially carcinogenic or genotoxic compounds as per ICH M7 qualification thresholds (e.g., not more than 1.5 mcg/day or up to 120 mcg/day depending on the duration of treatment). However, from a general toxicology perspective, for parenteral products, the AET must be able to detect and identify any leachable that is present in the product at 5 mcg/day and higher, unless justified otherwise, to permit an adequate toxicological risk assessment.

For additional guidance on extractables and leachables testing, refer to the following documents:

- USP <1663>: Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems

- USP <1664>: Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems
- FDA guidance for industry: *Container Closure Systems for Packaging Human Drugs and Biologics*, available at, <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070551.pdf>

In your assessment, include a table listing all compounds, including the concentration in ppm, the experimental conditions, and the maximum daily exposure to these compounds based on the maximum daily dose of the product. The extractable/leachable data must be accompanied by an adequate toxicological risk assessment. Although a toxicological risk assessment based on the results of the extraction studies may be adequate to support the safety assessment during development, for your NDA, the final safety assessment should be based on your evaluation of leachables from at least three batches of your drug product tested at multiple timepoints over the course of your stability studies, as discussed above. The approach for toxicological evaluation of the safety of leachables must be based on good scientific principles and take into account the specific container closure system or patch, drug product formulation, dosage form, route of administration, and dose regimen (chronic or short-term dosing). The safety assessment should be specifically discussed in Module 2.6.6.8 (Toxicology Written Summary/Other Toxicity) of the NDA submission. The risk assessment should be based on the maximum level of each leachable detected in long-term stability samples that include any intended secondary container closure system(s) unless otherwise justified. Include copies of all referenced studies upon which a safety assessment is based.

- If you employ a Permissible Daily Exposure (PDE) assessment as described in ICH Q3C, provide justification for all safety factors employed.
- Published literature to support the safety of any compound rarely provides adequate detail of the study design and study results to permit a thorough independent evaluation of the data. Summary reviews, (e.g., BIBRA, CIR, HERA), although potentially useful to identify original source material, are not acceptable as the source material is not provided and the conclusions cannot be independently verified. Submission of any published study reports must be accompanied by a detailed comparison to modern toxicology study endpoints and any shortcomings of the study must be discussed and justification must be provided to support your assertion that these data are adequate to support the safety of your container closure system.
- Safety justifications based on analogous compounds are also not acceptable unless you can provide adequate data to support your conclusions that a risk assessment based on one compound can be logically interpolated to represent an adequate safety evaluation for your leachable/extractable. This should

include a detailed understanding of the absorption, distribution, metabolism, and elimination of the compounds and an adequate scientific bridge to interpolate a NOAEL for the extractable/leachable compound.

A Type A post-action meeting was held with the Applicant on August 7, 2019. The NDA was resubmitted on September 26th, 2019.

3 Studies Submitted

The Applicant submitted multiple nonclinical studies to support HTX-011 and are summarized in the tables below.

Table 76: Pharmacology Studies Submitted

Study Number	Type of Study	Test System	Method of Administration	Testing Facility
<i>Primary Pharmacodynamics</i>				
33-12	POP ^a	Domestic Piglet	Local Administration into the incision site	(b) (4)
33-14	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-13	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-24	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-61	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-68	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-71	POP ^a	Domestic Piglet	Local Administration into the incision site ^b	
33-87	Incision Model	Domestic Piglet	Local Administration into the incision site	
33-23	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-25	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-29	POP ^a	Domestic Piglet	Local Administration into the incision site	
<i>Secondary Pharmacodynamics</i>				
NA				
<i>Safety Pharmacology</i>				
33-62	In vitro	CHO	In vitro	(b) (4)
<i>Pharmacodynamic Drug Interactions</i>				
NA				

Abbreviation: POP, postoperative pain.

^a Incisional POP employing Von Frey methods for assessment of analgesia.

^b Eloxiject™ (meloxicam) 5 mg/mL Solution for Injection.

Table from Page 2 of the Applicants Pharmacology Tabulated Summary (2.6.3)

Table 77: Pharmacodynamic Studies Submitted-Part 1

Heron Study No. ^{a,b}	Type of Study	Test Article ^c	Composition (w/w%)	MLX:BPV Ratio	BPV Dose/MLX Dose (mg)	Noteworthy Findings
<i>Determination of the optimal fixed-dose ratio and necessity for co-administration of bupivacaine and meloxicam</i>						
33-12	POP	Saline	NA	NA	NA	<i>Cross-comparison between Studies 33-12 and 33-14:</i> Analgesic effect of HTX-002-01 was greater than that of liposomal bupivacaine. The analgesic effect diminished for HTX-002-01 from 48 to 96 hours and returned by 120 hours. The analgesic effect for liposomal bupivacaine diminished from 24 hours but did not return.
		HTX-002-01	BPV 15%	NA	316/0	
33-14	POP	HTX-011-10	BPV 10% + MLX 0.38%	(b) (4)	234/8.9	<i>Cross-comparison among Studies 33-12, 33-13, and 33-14:</i> In contrast to HTX-002-01 and liposomal bupivacaine, HTX-011-03 resulted in a sustained analgesic effect that lasted from the time of administration through 144 hours <i>Cross-comparison among Study 33-12, 33-14, and 33-24:</i> The analgesic effect of HTX-002-01 started to diminish after 24 hours. In contrast, the analgesic effect of HTX-011-10 was at its maximal level at 24 and 48 hours with the benefit maintained through 72 hours. HTX-009-03 did not result in a meaningful analgesic effect.
		Exparel (bupivacaine liposome injectable suspension)	BPV 1.3%	NA	106.4/0	
33-13	POP	HTX-011-03 ^d	BPV 15% + MLX 3.5%	0.233	351/81.9	
33-24	POP	HTX-009-03	MLX 0.15%	NA	0/3	

Table from Page 3 of the Applicants Pharmacology Tabulated Summary (2.6.3)

Table 78: Pharmacodynamic Studies Submitted-Part 2

Heron Study No. ^{a,b}	Type of Study	Test Article ^c	Composition (w/w%)	MLX:BPV Ratio	BPV Dose/MLX Dose (mg)	Noteworthy Findings
33-61	POP	Saline	NA	NA	NA	<i>Cross-comparison among Studies 33-61, 33-68, and 33-71:</i> The lowest effective MLX:BPV ratio in the extended-release combination formulation was determined to be (b) (4) <i>Cross-comparison between Studies 33-61 and 33-71:</i> HTX-011-56 resulted in a greater analgesic effect compared with that for HTX-002-13 with SC administered meloxicam at most evaluation timepoints.
		HTX-011-56 ^e	BPV 2.5% + MLX 0.075%	0.030	100/3	
		HTX-011-62	BPV 2.5% + MLX 0.038%	0.015	100/1.5	
		HTX-011-63	BPV 2.5% + MLX 0.019%	0.008	100/0.75	
33-68	POP	HTX-011-56 ^e	BPV 2.5% + MLX 0.075%	0.030	100/3	
		HTX-011-72	BPV 2.5% + MLX 0.1125%	0.045	100/4.5	
		HTX-011-74	BPV 2.5% + MLX 0.15%	0.060	100/6	
33-71	POP	HTX-011-74	BPV 2.5% + MLX 0.15%	0.060	100/6	
		HTX-002-13 + SC meloxicam ^f	BPV 2.5% + 3 mg MLX	NA	100/3	
<i>Determination of the mechanism by which meloxicam enhances effectiveness of bupivacaine</i>						
33-87	POP incision model	Saline	NA	NA	NA	The pH of the tissues at the incisions treated with HTX-011-56 or left untreated (sham control) dropped after the surgery and was approximately 6.0 at 8 hours. Afterwards, the pH of the tissues at the incisions treated with HTX-011-56 started to increase and was at the baseline level by 48 hours after the surgery. In contrast, the pH of the tissues at the incisions left untreated (sham control) or treated with saline remained approximately 6.0 and further decreased after 30 hours.
		HTX-011-56 ^e	BPV 2.5% + MLX 0.075%	0.030	100/3	
		Sham control	NA	NA	NA	

Table from Page 4 of the Applicants Pharmacology Tabulated Summary (2.6.3)

Table 79: Pharmacodynamic Studies Submitted-Part 3

Heron Study No. ^{a, b}	Type of Study	Test Article ^c	Composition (w/w%)	MLX:BPV Ratio	BPV Dose/MLX Dose (mg)	Noteworthy Findings
<i>Synergistic Effect of Bupivacaine and Meloxicam Co-Formulated in HTX-011</i>						
33-23	POP	HTX-011-19	BPV 5% + MLX 0.15%	0.030	105.3/3.2	<i>Cross-comparison among Studies 33-23, 33-25, 33-29, 33-61, 33-68, and 33-71:</i> Bupivacaine and meloxicam in the extended-release combination formulation are acting synergistically.
33-25	POP	HTX-011-16	BPV 5% + MLX 0.3%	0.060	117/7	
		HTX-011-17	BPV 5% + MLX 0.19%	0.038	117/4.4	
		HTX-011-18	BPV 5% + MLX 0.08%	0.016	117/1.9	
33-29	POP	HTX-011-56 ^e	BPV 2.5% + MLX 0.075%	0.030	100/3	
		HTX-011-19	BPV 5% + MLX 0.15%	0.030	100/3	

Abbreviations: BPV, bupivacaine; MLX, meloxicam; NA, not applicable; No., number; POP, postoperative pain; w/w, weight per weight.

Note: % concentrations for test articles refer to % w/w.

^a Postoperative pain was assessed using the Von Frey method at 1, 3, and 5 hours following the surgery on Study Day 0 and on Study Days 1, 2, 3, 4, 5, and 6. In Studies 33-68 and 33-71, postoperative pain using the Von Frey method was also assessed at 8 hours following surgery on Study Day 0.

^b Test articles, except ELOXIJECT in Study 33-71, were administered by local administration into the incision site. ELOXIJECT in Study 33-71 was administered subcutaneously.

^c Other formulations were studied but only those relevant to HTX-011-56 are presented in this table.

^d In the study report for 33-13, the formulation was identified as HTX-011. The complete name of the formulation is HTX-011-03.

^e HTX-011-056 refers to the HTX-011 formulation.

^f Eloxiject™ (meloxicam) 5 mg/mL Solution for Injection.

Sources: Module 4, Section 4.2.1.1, Study Report 33-12, Study Report 33-14, Study Report 33-13, Study Report 33-24, Study Report 33-61, Study Report 33-68, Study Report 33-71, Study Report 33-87, Study Report 33-23, Study Report 33-25, Study Report 33-29.

Table from Page 5 of the Applicants Pharmacology Tabulated Summary (2.6.3)

Table 80: Safety Pharmacology Studies Submitted

Study	Type of Study	Species	Test Article	Concentration (µM)	Noteworthy Findings
33-62	In vitro	In vitro (CHO cells)	Meloxicam	0.03, 0.3, 3, 30	MLX did not cause a meaningful inhibition of the hERG channel.

Abbreviations: CHO, Chinese hamster ovary; hERG, human ether-a-go-go related gene; MLX, meloxicam.

Source: Module 4, Section 4.2.1.3, Study Report 33-62.

Table from Page 7 of the Applicants Pharmacology Tabulated Summary (2.6.3)

Table 81: Pharmacokinetics Studies Submitted

Overview			Test Article: HTX-011-56		
Type of Study	Species	Method of Administration	Testing Facility	Heron Study No.	
Absorption					
	Rat	Subcutaneous	(b) (4)	33-79	
	Dog	Subcutaneous		33-36	
Excretion					
	Minipig sow and neonate piglet	Subcutaneous		33-77	
Pharmacokinetic Drug Interactions					
	Minipig	Subcutaneous		33-78	
Other					
	Dog	Subcutaneous		33-47	
	Dog	Subcutaneous		33-69	

Table from Page 2 of the Applicants Pharmacokinetics Tabulated Summary (2.6.5)

Table 82: Pharmacokinetic Studies Submitted (Continued)

			Test Article: APF18A (TEG-POE Polymer)	
Distribution				
	Rat	Subcutaneous	(b) (4)	24-21 ^a
Excretion				
	Rat	Subcutaneous	24-21 ^a	

Abbreviations: No., number.

^a Study 24-21 of the vehicle control APF18A was performed as part of the NDA for SUSTOL[®] (araneson) extended-release injection (NDA 022445).

(b) (4) The vehicle control data from the SUSTOL NDA were submitted to the HTX-011-56 IND (IND 125927).

Sources: Module 4, Section 4.2.2.2, [Study Report 33-79](#), [Study Report 33-36](#); Module 4, Section 4.2.2.5, [Study Report 33-77](#); Module 4, Section 4.2.2.6, [Study Report 33-78](#); Module 4, Section 4.2.2.7, [Study Report 33-47](#), [Study Report 33-69](#), [Study Report 24-21](#).

Table from Page 3 of the Applicants Pharmacokinetics Tabulated Summary (2.6.5)

Table 83: Toxicology Studies Submitted-Part 1

Test Article: HTX-011-56 or Supporting Formulations								
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number	
Single Dose Toxicity								
Single Dose Toxicity (HTX-011-56, HTX-002-14)	Rat (SD)	IP	Single Dose	BPV: 0, 15 mg/kg (M) MLX: 0, 0.5 mg/kg (M)	GLP	(b) (4)	33-37	
Single Dose (HTX-002)	Rat (SD)	SC	Single Dose	BPV: 0, 7, 42, 83 mg/kg (M); 0, 11, 63, 126 mg/kg (F) MLX: 0 mg/kg (M/F)	GLP		33-48	
Extended Single Dose (HTX-009-005)	Rat (SD)	SC	Single Dose	BPV: 0 mg/kg (M/F) MLX: 0, 0.2, 0.6, <u>1.2</u> mg/kg (M); 0, 0.3, 0.8, <u>1.6</u> mg/kg (F)	GLP		33-55	
Repeated Dose Toxicity								
28-Day Toxicity Study (HTX-011-56)	Rat (SD)	SC	28 Days (every 48 hours)	BPV: 0, 0, 12.5, <u>25</u> mg/kg MLX: 0, 0, 0.38, <u>0.75</u> mg/kg	GLP		33-85	
28-Day Toxicity Study (HTX-011-56)	Dog (Beagle)	SC	28 Days (every 48 hours)	BPV: 0, 0, 10, <u>13</u> mg/kg MLX: 0, 0, 0.3, <u>0.39</u> mg/kg	GLP	33-86		
Genotoxicity								
Ames Assay (HTX-011-15)	S. typhimurim and E. coli strains	In vitro	Incubated for 48 – 72 hours	Up to 5,000 µg/plate	GLP	33-11		

Table from Page 5 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 84: Toxicology Studies Submitted-Part 2

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Local Tolerance							
Bone Healing Model (HTX-011-19)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 0, 17.80, 44.45, 88.98 mg/kg (M) 0, 0, 21.21, 53.04, 106 mg/kg (F) MLX: 0.0, 0.55, 1.37, 2.74 mg/kg (M) 0, 0, 0.65, 1.63, 3.26 mg/kg (F)	Non-GLP	(b) (4)	33-15
Bone Healing Model (HTX-11-56, HTX-002-14)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 50 mg/kg (F) MLX: 0, 1.5 mg/kg (F)	Non-GLP	(b) (4)	33-39
Bone Healing Model (HTX-011-56)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 14.6 mg/kg (F) MLX: 0, 0.44 mg/kg (F)	GLP	(b) (4)	33-83
Hernia Repair Model (HTX-11-56, HTX-002-14)	Rabbit (NZW)	Test article was administered onto the mesh prior to closure of the SC tissues and skin at the incision site	Single Dose	BPV: 0, 10 (M); 0, 9 mg/kg (F) MLX: 0, 0.3 (M); 0, 0.3 mg/kg (F)	Non-GLP	(b) (4)	33-38
Hernia Repair Model (HTX-011-56)	Rabbit (NZW)	Test article was administered onto the mesh prior to closure of the SC tissues and skin at the incision site	Single Dose	1.0 mL/site BPV: 29.25 mg/mL MLX: 0.88 mg/mL	GLP	(b) (4)	33-80

Table from Page 6 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 85: Toxicology Studies Submitted-Part 3

Test Article: HTX-011-56 or Supporting Formulations								
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number	
Wound Healing Model (HTX-11-56, HTX-002-14)	Minipig (Gottingen)	Dermal	Single Dose	BPV: 0, 2.3, 4.6 mg/kg (M); 0, 2.3, 4.5 mg/kg (F) MLX: 0, 0.1 mg/kg (M); 0, 0.1 mg/kg (F)	GLP	(b) (4)	33-41	
Wound Healing Model (HTX-002-13), HTX-009-05)	Minipig (Gottingen)	Dermal	Single Dose	BPV: 0, 4.6 mg/kg (M/F) MLX: 0, 0.1 mg/kg (M/F)	GLP		33-54	
Brachial Plexus Nerve Block Model (HTX-024-02, HTX-002-14, HTX-011-56)	Dog (Beagle)	Intra-plexus	Single Dose	BPV: 0, 1.4, 8.3 (M); 0, 1.8, 10.5 (F) MLX: 0, 0.2 (M); 0, 0.3 (F)	GLP		33-42	
Perineural Toxicity Study (HTX-011-56)	Dog (Beagle)	Perineural injection to femoral nerve	Single Dose	BPV: 0, 2, 7, 14.1 (M) MLX: 0, 0.2, 0.4 (M)	GLP		33-63	
Other Toxicity Studies Conducted with APF18A Supporting the TEG-POE Polymer								
Repeat Dose Toxicity (APF18A)	Rat/Crl:CD [®] (SD)	SC	Up to 93 days	APF18A: 0, 295 mg/dose	Yes		25-07	
Repeat Dose Toxicity (APF18A)	Dog/Beagle	SC	Up to 93 days	APF18A: 0, 1180 mg/dose	Yes		25-08	

Table from Page 7 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 86: Toxicology Studies Submitted-Part 4

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Genotoxicity (APF18A)	S. typhimurium strains TA98, TA100, TA1535 and TA1537 E. coli strain WP2uvrA	In vitro	52±4 hour incubation	APF18A: ± S9: 0 (DMSO), 100, 333, 1000, 3330 and 5000 µg APF18A/plate	Yes	(b) (4)	24-17
Genotoxicity (APF18A)	Mouse lymphoma L5178Y cells	In vitro	Initial assay: 4 hour incubation Confirmatory assay: 24-hour treatment period	APF18A: ±S9: 0 (DMSO), 78.5, 157, 313, 625, 1250, 2500, 4000 and 5000 µg APF18A/mL	Yes		24-19
Genotoxicity (APF18A)	Rat/Crl:CD (SD)BR	SC	Single dose Animals sacrificed 24 and 48 hours post dose	APF18A: 0 (saline), 750, 1500 and 3000 mg/kg	Yes		24-16
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Males: beginning 28 days before cohabitation through cohabitation Females: beginning 15 days before cohabitation till Gestation Day 7	APF18A: 0 (0.9% NaCl), 0.142 and 0.295 g APF18A/day	Yes		27-02
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Gestation Days 7-17 (0, 0.14 and 0.295 g/day) Gestation Days 7, 10, 13 and 16 (0.295 g/day)	APF18A: 0 (0.9% NaCl), 0.14 and 0.295 g APF18A/day and 0.295 g APF18A/every 72 hours)	Yes		25-09

Table from Page 8 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 87: Toxicology Studies Submitted-Part 5

Test Article: HTX-011-56 or Supporting Formulations								
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number	
Reproductive and Developmental Toxicity (APF18A)	Rabbit/New Zealand white	SC	Gestation Days 7-19 (0.59 and 1.18 g/day) Gestation Days 7, 10, 13, 16 and 19 (1.18 g/day)	APF18A: 0 (0.9% NaCl), 0.59 and 1.18 g APF18A/day and 1.18 g APF18A/every 72 hours)	Yes	(b) (4)	25-10	
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Gestation Day 7 to Lactation Day 20	APF18A: 0 (0.9% NaCl), 0.142 and 0.295 g APF18A/day	Yes		27-03	
(Q)SAR Assessment for HTX-011-56 Drug Product Impurities								
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No			33-98
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No			33-99
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No		34-10	

Table from Page 9 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 88: Toxicology Studies Submitted-Part 6

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Other Studies Conducted with Early HTX-011 Formulations Containing Higher Concentrations of Bupivacaine and Meloxicam than HTX-011-56							
Single Dose Toxicity (HTX-011-12, HTX-011-14, HTX-011-15)	Rat (SD)	SC	Single Dose	BPV: 0, 97.2, 146.7, 195.0 mg/kg (M); 0, 127.7, 193.3, 258.8 mg/kg (F) MLX: 0, 7.4, 14.7, 29.3 (M); 0, 9.7, 19.3, 38.8 mg/kg (F)	Yes	(b) (4)	33-09

Abbreviations: BPV, bupivacaine; DMSO, dimethyl sulfoxide; F, female; GLP, Good Laboratory Practice; IP, intraperitoneal; M, male; MLX, meloxicam; NA, not applicable; NZW, New Zealand White; (Q)SAR, Quantitative Structure Activity Relationship; SC, subcutaneous; SD, Sprague Dawley; TEG-POE, tri(ethylene glycol)-based poly(orthoester) polymer.

^a Unless otherwise specified. For Repeated Dose Toxicity, the highest No Observed Adverse Effect Level (NOAEL) is underlined. Doses listed specify the approximate mg/kg doses of the active pharmaceutical ingredients (APIs), bupivacaine and meloxicam, which incorporates the correction for density of 1.17 g/mL. Studies utilized a variety of vehicles for HTX-011.

Table from Page 10 of the Applicants Toxicology Tabulated Summary (2.6.7)

3.1 Studies Reviewed

The Applicant conducted multiple nonclinical studies, many with previous formulations of the drug product. The following studies were reviewed in the original submission: Studies 33-85 and 33-86 were formally reviewed as these were the pivotal studies for justifying the safety of the clinical HTX-011 formulation, including the levels of bupivacaine and excipients. Special toxicity studies for hernia repair (Study 33-38), bone healing (Study 33-39), and wound healing (Study 33-54) were also reviewed as these studies supported the use of HTX-011 in the various surgeries used clinically. In addition, Studies 33-48, 33-55, 24-17, 24-19, 24-16, 27-02, 25-09, 25-07, 25-08, 25-10, and 25-03 were reviewed to justify the excipients, especially the AP135 polymer. While a formal review of the studies in the previous sentence is not included below, summaries of the results of these studies can be found in the excipients section (Section 2.4) of this review.

The following studies were reviewed as part of the Applicant's resubmission: Study 34-84 was a 14-day subcutaneous injection study in rats to support the safety of exposure to systemic levels of bupivacaine that are equivalent to clinical doses of 400 mg bupivacaine or higher. Study 34-74 was a developmental study in rabbits with maleic acid to support the safety of this excipient in the formulation.

3.2 Studies Not Reviewed

The remaining studies in the Applicant's tables above were not reviewed formally but summaries of relevant studies are provided in some of the sections below.

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

HTX-011 contains bupivacaine, an amide-type local anesthetic, and meloxicam, an NSAID. (b) (4)



4.2 Secondary Pharmacology

Secondary pharmacology studies with HTX-011 were not conducted.

4.3 Safety Pharmacology

The only safety pharmacology study conducted by the Applicant was an in vitro study on cardiovascular targets, including the hERG channel. Meloxicam did not cause a meaningful inhibition of any cardiovascular targets. A 20.5% inhibition of the hERG channel was detected at 30 mcM meloxicam.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Exposure to bupivacaine and meloxicam was assessed following a single subcutaneous administration of HTX-011 in rats and dogs (Study 33-79 and 33-36) and the pharmacokinetic parameters from those studies are included below. In rats, bupivacaine was observed in plasma for 72 to 144 hours with the low dose (25 mg/kg) and for 144 to 168 hours with the high dose (50 mg/kg). In dogs, bupivacaine was observed in the plasma for 96 to 120 hours.

Excretion was measured in minipig sows following a single subcutaneous dose of HTX-011 (Study 33-77). Concentrations of bupivacaine, meloxicam, and the water-soluble end products of AP135 ((b) (4)) were measured in milk, plasma, and urine. The same compounds were measured in the plasma and urine of untreated neonatal piglets. Bupivacaine and meloxicam were found in the plasma, milk, and urine of the sows, while only bupivacaine was found in the urine and plasma of piglets. The water-soluble end products were found in the milk, plasma, and urine of sows, while only (b) (4) was present in piglet plasma. All four products were found in pooled urine from piglets.

Table 89: Highest Concentrations of Bupivacaine and Meloxicam in Plasma, Milk, and/or Urine of Minipig Sows and Neonate Piglets Following a Single SC Administration of HTX-011 to Sows

Matrix	Species	N	Bupivacaine (µg/mL)	Meloxicam (µg/mL)
Milk	Sow	3	0.577	0.0632
Plasma	Sow	3	2.07	0.132
	Piglet	24	0.330	BLQ ^a
Urine	Sow	3	3.70	0.0226
	Piglet ^b	24	0.0552	BLQ ^a

Abbreviation: BLQ, below the limit quantitation.

^a Lower limit of quantitation for meloxicam was 0.010 µg/mL for plasma and urine.

^b Urine samples were pooled for each litter for determination of bupivacaine and meloxicam concentrations.

Source: Module 4, Section 4.2.2.5, Study Report 33-77, Table 6.

Table from page 24 of the Applicant's Pharmacokinetics Written Summary (2.6)

The potential for drug interactions between bupivacaine, meloxicam, and lidocaine were assessed in minipigs (Study 33-78). There was no meaningful impact on the pharmacokinetic profiles of any of the compounds when co-administered.

Table 90: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Rats

Dose of HTX-011-56 (mg/kg)	Analyte	Sex	N	t _{1/2} (h)	T _{max} (h)	C _{max} (ng/mL)	AUC _{inf} (h·ng/mL)
<i>Low dose</i>							
25	Bupivacaine	M	9	20.8	6.00	276	7,260
		F	9	13.6	0.50	321	6,240
0.75	Meloxicam	M	9	26.3	24.00	1,860	175,000
		F	9	NC ^a	72.00	3,770	NC ^a
<i>High dose</i>							
50	Bupivacaine	M	9	18.1	4.00	474	16,400
		F	9	16.2	1.00	510	15,000
1.5	Meloxicam	M	9	36.5	72.00	3,480	340,000
		F	9	40.5	72.00	7,180	962,000

Abbreviations: AUC_{inf}, area under the concentration-time curve from time 0 to infinity; C_{max}, maximum observed concentration; F, female; M, male; NC, not calculated; PK, pharmacokinetic; SC, subcutaneous; t_{1/2}, apparent terminal half-life; T_{max}, time of maximum observed concentration.

^a Not calculated due to insufficient number of samples.

Source: [Module 4, Section 4.2.2.2, Study Report 33-79, Appendix 5, Table 5.](#)

Table from page 30 of the Applicant's Nonclinical Overview (2.4)

Table 91: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Dogs

Dose of HTX-011-56 (mg/kg)	Analyte	Sex	N	t _{1/2} (h)	T _{max} (h)	C _{max} (ng/mL)	AUC _{last} (h·ng/mL)	AUC _{inf} (h·ng/mL)
6.96	Bupivacaine	M	5	35.6	1.80	321	9,527	10,495
6.66		F	4 ^a	36.5	2.50	228	8,230	9,458
0.209	Meloxicam	M	5	154	36.0	301	28,474	69,649
0.200		F	4 ^a	94.1	48.0	257	23,778	44,894

Abbreviations: AUC_{inf}, area under the concentration-time curve from time 0 to infinity; AUC_{last}, area under the concentration-time curve from time 0 to last quantifiable sampling time point; C_{max}, maximum observed concentration; F, female; M, male; PK, pharmacokinetic; SC, subcutaneous; t_{1/2}, apparent terminal half-life; T_{max}, time of maximum observed concentration.

Note: the dose level in mg/kg for the active ingredients was calculated based on the nominal concentration of bupivacaine or meloxicam including the density of 1.17 g/mL, the dose volume administered, and an average body weight for each sex.

^a One female received only half of the targeted dose. The plasma concentration-time data from this female was excluded from the analysis.

Source: [Module 4, Section 4.2.2.2, Study Report 33-36, Table 1.](#)

Table from page 24 of the Applicant's Nonclinical Overview (2.4)

6 General Toxicology

Over the course of development, the toxicity studies tested various formulations of HTX and treatment groups employed code numbers for the formulations. The table below, reproduced from the submission, summarizes many of the compositions of the formulations tested compared to the to-be-marketed formulation coded HTX-011-56.

Table 92: Composition of Test Articles Used in Nonclinical Studies Supporting Development of HTX-011-56

Test Article	Active Ingredient		Excipient			
	Bupivacaine (mg/mL)	Meloxicam (mg/mL)	DMSO (mg/mL)	TEG-POE (mg/mL)	Maleic Acid (mg/mL)	Triacetin (mg/mL)
<i>Extended Release Formulations of Bupivacaine and Meloxicam</i>						
HTX-011-56	29.25	0.88	117.0	729.8	0.59	292.5
HTX-011-12 ^a	58.50	4.45	NA	924.5	7.02	NA
HTX-011-14 ^a	87.75	8.78	NA	887.3	10.6	NA
HTX-011-15 ^a	117.0	17.55	NA	848.1	11.70	NA
HTX-011-19 ^a	58.50	1.76	NA	927.2	7.02	NA
HTX-011-49	29.25	0.88	93.60	635.0	1.76	409.5
<i>Extended Release Formulations of Bupivacaine Only</i>						
HTX-002-13	29.25	NA	117.0	730.7	0.59	292.5
HTX-002-14	29.25	NA	93.60	635.9	1.76	409.5
<i>Extended Release Formulation of Meloxicam Only</i>						
HTX-009-05	NA	0.88	117.0	759.6	NA	292.5
<i>Vehicles for HTX-011-56</i>						
HTX-024-01	NA	NA	117.0	643.5	NA	409.5
HTX-024-02	NA	NA	117.0	760.5	NA	292.5
<i>Vehicle for HTX-011-19</i>						
HTX-018-02 ^a	NA	NA	NA	850	NA	NA
<i>Controls</i>						
Bupivacaine HCl	5	NA	NA	NA	NA	NA
Saline	NA	NA	NA	NA	NA	NA

Abbreviations: DMSO, dimethyl sulfoxide; NA, not applicable; TEG-POE, tri(ethylene glycol) poly(orthoester).

Notes: the mg/mL concentrations of bupivacaine, meloxicam, and excipients, except for HTX-018-02, were calculated based on the density of 1.17 g/mL. In the nonclinical toxicology studies additional formulations were evaluated. They are not listed in this table because they are not relevant for HTX-011-56.

^a Formulation contained (b) (4)

Sources: Module 4, Section 4.2.3.1, Study Report 33-37, Study Report 33-09; Study Report 33-48, Study Report 33-55; Module 4, Section 4.2.3.2, Study Report 33-85, Study Report 33-86; Module 4, Section 4.2.3.3.1, Study Report 33-11; Module 4, Section 4.2.3.6, Study Report 33-15, Study Report 33-39, Study Report 33-83, Study Report 33-38, Study Report 33-38 Amendment 1, Study Report 33-80, Study Report 33-41, Study Report 33-54, Study Report 33-42, Study Report 33-63.

6.1 Single-Dose Toxicity

Single-dose studies with the HTX-011 vehicle were summarized as part of the Comments on Novel Excipients Section (Section 2.4).

A total of four single-dose toxicity studies were conducted, as summarized in the Applicant's table below.

Table 93: Overview of Single-dose Toxicity Studies Conducted by Applicant

Heron Study No./ GLP	Species	Route	Necropsy Day Postdose	Test Articles	TK Assessed
33-37/ Yes	Rat	IP	3, 8, 15	Saline HTX-024-01 (vehicle for HTX-011-56) HTX-002-14 (bupivacaine only comparator) ^a HTX-011-56 ^b	Yes
33-09/ Yes	Rat	SC	3, 14	Saline HTX-011-12 ^c HTX-011-14 ^d HTX-011-15 ^e	Yes
33-48/ Yes	Rat	SC	3, 14	Saline HTX-024-02 (vehicle for HTX-011-56) Bupivacaine HCl HTX-002-14 (bupivacaine only comparator) ^a	Yes
33-55/ Yes	Rat	SC	4, 15	Saline HTX-024-02 (vehicle for HTX-011-56) HTX-009-05 (meloxicam only comparator) ^f	Yes

Abbreviations: GLP, Good Laboratory Practice; IP, intraperitoneal; No., number; SC, subcutaneous; TK, toxicokinetics.

^a HTX-002-14 is an extended-release formulation of bupivacaine only and contains the same amount of bupivacaine as HTX-011-56.

^b HTX-011-56 refers to HTX-011, the Phase 3, Phase 2b, and intended commercial formulation.

^c HTX-011-12 contains 2-times higher concentration of bupivacaine and 5-times higher concentration of meloxicam than HTX-011-56.

^d HTX-011-14 contains 3-times higher concentration of bupivacaine and 10-times higher concentration of meloxicam than HTX-011-56.

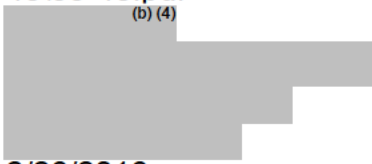
^e HTX-011-15 contains 4-times higher concentration of bupivacaine and 20-times higher concentration of meloxicam than HTX-011-56.

^f HTX-009-05 is an extended-release formulation of meloxicam only and contains the same amount of meloxicam as HTX-011-56.

Sources: [Module 4, Section 4.2.3.1](#), [Study Report 33-37](#), [Study Report 33-09](#), [Study Report 33-48](#), [Study Report 33-55](#).

Only one of the studies actually tested HTX-011-56 and that study tested the drug product via intraperitoneal dosing. As such, these studies are not further reviewed here as relative comparisons to the referenced product cannot be made nor are the formulations directly applicable to the proposed product. The exception was Study number 33-48. This study is briefly reviewed here because it is included treatment with bupivacaine alone as a control, which is useful in evaluating if the local tissue effects are related to the excipients used in the drug product.

Study title: HTX-011: 28-Day Intermittent Subcutaneous Toxicity and Toxicokinetic Study in Sprague-Dawley Rats (with a 2-week Recovery)

Study no.: 33-48
Study report location: SDN 1, Module 4
\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4231-single-dose-tox\33-48\33-48.pdf
Conducting laboratory and location:  (b) (4)
Date of study initiation: 2/26/2016
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Purity values are for bupivacaine
HTX-002-014, Lot 15055, purity 101.6%
HTX-002-013, Lot 16007, purity 101.3%
HTX-024-002, Lot 1273098, purity negative
HTX-024-003, Lot 1273100, purity negative
bupivacaine, Lot 55211DD and 58201DD,
purity not provided

Key Study Findings

- Treatment of animals with saline or bupivacaine resulted in similar local injection site histopathological findings.
- Treatment with HTX (HTX-002-013) caused an increased incidence and/or severity of subcutaneous inflammation, hemorrhage, necrosis (female only), and edema (female only) compared to the saline control 3 days after treatment. An increased incidence and/or severity of subcutaneous inflammation and hemorrhage compared to the saline control was also seen in recovery animals on Day 14.
- Treatment with the HTX vehicle (HTX-024-002) caused an increased incidence and/or severity of subcutaneous inflammation, hemorrhage (females only), necrosis, and edema (female only) compared to the saline control 3 days after treatment. In addition, an increased incidence and/or severity of epidermal crust was observed. An increased incidence and/or severity of subcutaneous inflammation and hemorrhage compared to the saline control was also seen in recovery animals on Day 14.

- In recovery animals only, treatment with HTX (HTX-002-013) caused an increased incidence and/or severity of fibroplasia and epidermal hyperplasia/regeneration (females only).
- In recovery animals only, treatment with the HTX vehicle (HTX-024-002) caused an increased incidence and/or severity of fibroplasia (males only) and epidermal hyperplasia/regeneration (females only).
- The local histopathological findings appear to be related to the excipients (DMSO, AP135, and triacetin) since similar findings were seen with both the HTX vehicle and HTX formulation and the incidence and /or severities were greater than the bupivacaine alone. However, the results were not always consistent making definitive conclusions impossible.

Methods

Doses:	Bupivacaine 2.5 mg, low dose of HTX: 0.585 mg bupivacaine, high dose of HTX: 1.17 mg bupivacaine
Frequency of dosing:	Single injection
Route of administration:	Subcutaneous injection
Dose volume:	See experimental design
Formulation/Vehicle:	See formulation table
Species/Strain:	Sprague-Dawley rat
Number/Sex/Group:	10 for the main experiment, 5 for recovery, and 3 (saline and vehicle) or 27 (low and high dose) for toxicokinetics
Age:	8-9 weeks old
Weight:	193-227 g (male) and 148-193 g (female)
Satellite groups:	Toxicokinetic and recovery groups
Unique study design:	For dose volumes of 2 mL (saline and the vehicle controls), 1 mL was injected into two separate injection sites
Deviation from study protocol:	None that impacted study results

Table 94 Formulations Tested in Study Number 33-48

Test and Control Items	Lot Number	Active Pharmaceutical Ingredients (APIs)	Excipients			
		Bupivacaine w/w% (mg/g)	Maleic Acid w/w% (mg/g)	DMSO w/w% (mg/g)	Triacetin w/w% (mg/g)	AP135 w/w% (mg/g)
HTX-024-002	1273098	NA	NA	10.0 (100.0)	25.0 (250.0)	65.0 (650.0)
HTX-024-003	1273100	NA	NA	8.0	35.0	57.0
HTX-002-013	16007	2.5 (25.0)	0.15 (1.5)	10.0 (100.0)	25.0 (250.0)	62.35 (623.5)
HTX-002-014	15055	2.5 (25.0)	0.15 (1.5)	8.0 (80.0)	35 (350.0)	54.35 (543.5)

Table from page 14 of the Applicant's study report

Experimental design

Group Numbers	Group Designation	Dose Volume (mL)	Toxicology Animals				Toxicokinetic Animals	
			Main		Recovery		Male	Female
			Male	Female	Male	Female		
1	Saline Control	2.0	10	10	5	5	3	3
2	Vehicle Control HTX-024-002	2.0	10	11*	5	4*	3	3
3	Vehicle Control HTX-024-003	2.0	10	10	5	5	3	3
4	Bupivacaine comparator	0.5	10	9*	5	6*	6	6
5	HTX-002-013 Low dose	0.5	10	10	5	5	6	6
6	HTX-002-013 High dose	1.0	10	10	5	5	6	6
7	HTX-002-014 Low dose	0.5	10	10	5	5	6	6
8	HTX-002-014 High dose	1.0	10	10	5	5	6	6

Table from page 14 of the Applicant's study report

Only results from HTX-024-002, referred to as HTX vehicle, and HTX-002-013, referred to as HTX, are presented in this review as these are the closest to the clinical formulation. Furthermore, only the histopathological results are described in detail below as this was the key data for assessing whether the local effects were potentially related to the excipients as opposed to bupivacaine. As the data from this study was not with the clinical formulation and the safety of the clinical formulation was addressed in the pivotal repeat dose studies, detailed review of the remaining data was not deemed critical for this review.

Histological Findings

The incidence and severity of subcutaneous inflammation was increased in males following treatment with the HTX vehicle, the low dose of HTX-002-013, and the high dose of HTX-002-013 compared to both the saline control and bupivacaine. In females, only a small increase in severity was observed with the vehicle, the low dose of HTX-002-013, and the high dose of HTX-002-013 compared to both the saline control and bupivacaine because the saline and bupivacaine groups had a higher incidence and severity of findings. The incidence and severity of this finding was also increased following recovery in both sexes, except in female animals receiving the HTX vehicle.

The incidence and severity of subcutaneous hemorrhage was increased in males following treatment with the low and high dose of HTX-002-013, and the high dose of HTX-002-013 compared to both the saline control and bupivacaine. In females, the incidence and severity of subcutaneous hemorrhage was increased with both doses of HTX and the HTX vehicle. Hemorrhages were still found at a higher incidence/severity following recovery in both sexes with both doses of HTX but not the HTX vehicles.

The incidence and severity of subcutaneous necrosis was increased in males following treatment with the HTX vehicle compared to both the saline control and bupivacaine. In females, the incidence and severity of subcutaneous necrosis was increased in animals receiving HTX or the HTX vehicle compared to both the saline control and bupivacaine. Necrosis was not seen in the recovery animals.

A higher incidence or severity of epidermis crust was observed in both males and females who received the HTX vehicle compared to both the saline control and bupivacaine. An increase was also seen in males receiving the low dose of HTX, but in no other groups receiving HTX. Epidermis crust was seen in some recovery animals but there did not appear to be a pattern to the findings.

The incidence and severity of subcutaneous edema was increased in female animals treated with the HTX vehicle or HTX compared to both the saline control and bupivacaine. Edema was not observed in recovery animals.

Fibroplasia was only observed in recovery animals. There was an increased incidence and severity of this finding in males receiving the HTX vehicle and HTX compared to both the saline control and bupivacaine. In females, the increased incidence and severity were only seen with HTX.

Epidermal hyperplasia/regeneration was only seen in female recovery animals who received HTX or the HTX vehicle.

Table 95 Histopathological Findings on Day 3 at the Primary Injection Site, Males

	Male				
	Saline	Bupivacaine	HTX-024-02 (vehicle)	HTX-002-013 LD	HTX-002-013 HD
Inflammation, Subcutaneous	60% (1.5)	60% (1.2)	90% (1.9)	90% (1.9)	100% (2.0)
Inflammation, epidermal/dermal	50% (1.0)	10% (1.0)	40% (1.0)	50% (1.4)	30% (1.0)
Hemorrhage Subcutaneous	60% (1.2)	70% (1.0)	70% (1.0)	80% (1.4)	90% (1.7)
Necrosis Subcutaneous	0%	10% (1.0)	70% (1.7)	20% (1.0)	10% (1.0)
Crust Epidermis	30% (1.0)	10% (1.0)	40% (1.0)	50% (1.6)	20% (1.0)
Edema Subcutaneous	20% (1.0)	0%	30% (1.3)	0%	20% (1.5)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe
Table prepared by Reviewer based on data from study report

Table 96 Histopathological Findings on Day 3 at the Secondary Injection Site, Males

	Male	
	Saline	HTX-024-02 (vehicle)
Inflammation, Subcutaneous	70% (1.3)	100% (1.7)
Inflammation, epidermal/dermal	20% (1.0)	40% (1.3)
Hemorrhage Subcutaneous	40% (1.0)	80% (1.1)
Necrosis Subcutaneous	0%	60% (1.3)
Crust Epidermis	10% (1.0)	70% (1.1)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe
Table prepared by Reviewer based on data from study report

Table 97 Histopathological Findings on Day 14 (Recovery) at the Primary Injection Site, Males

	Male				
	Saline	Bupivacaine	HTX-024-02 (vehicle)	HTX-002-013 LD	HTX-0020013 HD
Inflammation, Subcutaneous	40% (1.0)	20% (1.0)	60% (1.3)	100% (1.6)	80% (1.5)
Inflammation, epidermal/dermal	0%	20% (2.0)	0%	0%	20% (3.0)
Hemorrhage Subcutaneous	0%	0%	0%	40% (1.5)	40% (2.0)
Crust Epidermis	0%	20% (2.0)	0%	0%	0%
Fibroplasia	0%	0%	20% (2.0)	40% (1.5)	60% (2.3)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 98 Histopathological Findings on Day 14 (Recovery) at the Secondary Injection Site, Males

	Male	
	Saline	HTX-024-02 (vehicle)
Inflammation, Subcutaneous	0%	20% (1.0)
Hemorrhage Subcutaneous	0%	0%
Fibroplasia	0%	0%

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 99 Histopathological Findings on Day 3 at the Primary Injection Site, Females

	Female				
	Saline	Bupivacaine	HTX-024-02 (vehicle)	HTX-002-013 LD	HTX-0020013 HD
Inflammation, Subcutaneous	100% (1.9)	78% (1.9)	91% (2.5)	100% (2.1)	90% (2.7)
Inflammation, epidermal/dermal	70% (1.3)	89% (1.3)	45% (1.6)	60% (1.3)	70% (1.1)
Hemorrhage Subcutaneous	90% (1.0)	67% (1.2)	91% (1.8)	100% (1.3)	90% (1.7)
Necrosis Subcutaneous	10% (1.0)	0%	54% (1.7)	20% (1.0)	60% (1.3)
Crust Epidermis	60% (1.0)	100% (1.3)	54% (1.7)	70% (1.0)	50% (1.4)
Edema Subcutaneous	0%	0%	40% (1.8)	40% (1.0)	70% (1.4)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 100 Histopathological Findings on Day 3 at the Secondary Injection Site, Females

	Female	
	Saline	HTX-024-02 (vehicle)
Inflammation, Subcutaneous	80% (2.3)	100% (2.3)
Inflammation, epidermal/dermal	30% (1.3)	64% (1.6)
Hemorrhage Subcutaneous	80% (1.1)	100% (1.5)
Necrosis Subcutaneous	20% (1.0)	54% (1.2)
Crust Epidermis	40% (1.0)	72% (1.6)
Edema, Subcutaneous	10% (1.0)	9% (1.0)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 101 Histopathological Findings on Day 14 (Recovery) at the Primary Injection Site, Females

	Female				
	Saline	Bupivacaine	HTX-024-02 (vehicle)	HTX-002-013 LD	HTX-0020013 HD
Inflammation, Subcutaneous	0%	50% (1.0)	25% (1.0)	80% (1.5)	80% (1.5)
Inflammation, epidermal/dermal	0%	0%	25% (2.0)	0%	0%
Hemorrhage Subcutaneous	0%	0%	0%	40% (1.0)	40% (1.5)
Crust Epidermis	0%	17% (3.0)	25% (3.0)	20% (2.0)	0%
Fibroplasia	0%	17% (1.0)	0%	40% (1.5)	40% (2.0)
Hyperplasia/rege neration Epidermal	0%	0%	25% (3.0)	40% (1.5)	20% (2.0)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

Table 102 Histopathological Findings on Day 14 (Recovery) at the Secondary Injection Site, Females

	Female	
	Saline	HTX-024-02 (vehicle)
Inflammation, Subcutaneous	20% (1.0)	100% (1.5)
Inflammation, epidermal/dermal	0%	25% (1.0)
Hemorrhage Subcutaneous	0%	0%
Crust Epidermis	0%	50% (2.5)
Fibroplasia	0%	50% (1.5)
Hyperplasia/rege neration Epidermal	0%	25% (1.0)

Values in parentheses = average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table prepared by Reviewer based on data from study report

6.2 Repeat-Dose Toxicity

Study title: HTX-011: 28-Day Intermittent Subcutaneous Toxicity and Toxicokinetic Study in Sprague-Dawley Rats (with a 2-week Recovery)

Study no.: 33-85
Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\33-85\33-85.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/17/2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: HTX-011-056, Lot 026517, 99.9% bupivacaine and 97.3% meloxicam

Key Study Findings

- All animals survived until necropsy.
- No treatment-related effects were observed on body weight, food consumption, ophthalmic parameters, clinical chemistry parameters, organ weights, or urinalysis parameters.
- An increased incidence of scabbing was seen at the injection site of some animals receiving HTX-011 or the vehicle at some injection sites. The incidence of scabbing was reduced following recovery.
- In males, increased platelets, neutrophils, eosinophils, and reticulocytes were observed in all treated groups (including the vehicle) compared to saline. Increases in hematology parameters were no longer present following recovery.
- Histopathological findings that appeared to be related to systemic exposure were limited to minimal mononuclear cell infiltrates in the liver of high dose males and minimal accumulation of erythrocytes in the thymus of low and high dose males.
- Injection site histopathological findings included chronic inflammation, hemorrhage, and presence of test material following treatment with the vehicle and HTX-011. Treatment with HTX-011 resulted in an increase in the severity or incidence of some findings. Chronic inflammation was still present in recovery animals, although the overall incidence was reduced in animals that had a longer recovery time.

- Bupivacaine and meloxicam toxicokinetics were measured on Days 1, 5, and 27. For both compounds, AUC increased linearly with dose. AUCs also appeared to increase with time suggesting some accumulation. Bupivacaine T_{max} values were generally short (0.1-1 hours), while meloxicam T_{max} values were longer (0.5-48 hours) but with more variance. C_{max} values for bupivacaine were highly variable and no trends could be deciphered. In contrast, meloxicam C_{max} values followed a linear trend with dose and increased with time.
- Polymer breakdown products were evaluated in urine on Day 20. (b) (4)
[REDACTED]
were all detected at levels above the calibration range.
- In terms of systemic effects, findings were limited to males and included effects on hematology parameters in all treated animals (vehicle and both doses of HTX-011) and potentially associated histopathological findings of infiltration of mononuclear cells into the liver (high dose of HTX-011) and accumulation of erythrocytes in the thymus (low and high dose of HTX-011). Changes in hematology parameters were small and did not follow a dose response. The observed histopathological findings in the liver and thymus were of minimal severity and had a small incidence (30%). They were not considered toxicologically relevant.
- A local inflammatory reaction was observed following treatment with the vehicle and HTX-011. The effects are consistent with a biological response to injection of foreign material. The majority of the effects resolved as indicated by the lack of findings in recovery animals. However, chronic inflammation was still present in recovery animals up to 26 days following the last injection. Effects were at times worse (increased incidence and/or severity) with HTX-011 compared to the vehicle suggesting that bupivacaine or meloxicam may have exacerbated the effects of the vehicle. There was no dose response, except for the presence of test material, which is expected since the dose was controlled by injecting different volumes of the clinical formulation (same concentration of drug substance and excipients). The Applicant claims that the effects are non-adverse and therefore the NOAEL is the highest dose tested. While the effects observed are not unexpected, it is difficult to ascertain the adversity and clinical relevance of the observed persisting chronic inflammation. Therefore, in terms of local effects, this study demonstrates that the clinical formulation causes chronic inflammation up to 26 days that is likely primarily the result of the vehicle.

Methods

Doses: Saline, vehicle control, 12.5/0.38 mg/kg bupivacaine/meloxicam, and 25/0.75 bupivacaine/meloxicam (see table below)

Frequency of dosing: Once every 48 hours (see injection site rotation below)

Route of administration: Subcutaneous injection

Dose volume: 0.85 mL/kg (saline, vehicle, and 25/0.75 mg/kg) or 0.43 mL/kg (12.5/0.38 mg/kg)

Formulation/Vehicle: **HTX-011-056 (HTX-011)**: 25% triacetin, 10% DMSO, 62.375% AP135 polymer, 0.05% maleic acid, 2.5% bupivacaine, and 0.075% meloxicam
HTX-024-002 (vehicle): 25% triacetin, 10% DMSO, and 65% AP135 polymer

Species/Strain: Sprague-Dawley rat

Number/Sex/Group: 10 for the main experiment, 5 for recovery, and 3 (saline and vehicle) or 27 (low and high dose) for toxicokinetics

Age: 8-9 weeks old

Weight: 193-227 g (male) and 148-193 g (female)

Satellite groups: Toxicokinetic and recovery groups

Unique study design: None

Deviation from study protocol: None that impacted study results

Experimental Design

Group ^a	Description	Number of Rats/Sex/Group			Dose Level		Dose Volume (mL/kg)	Dose Concentration ^d	
		Core	Recovery	TK	bupivacaine (mg/kg/day)	meloxicam (mg/kg/day)		bupivacaine (mg/mL)	meloxicam (mg/mL)
1 ^b	Saline Control	10	5	3	0	0	0.85	0	0
2 ^c	Vehicle Control	10	5	3	0	0	0.85	0	0
3	Low Dose	10	5	27	12.5	0.38	0.43	29.25	0.88
4	High Dose	10	5	27	25	0.75	0.85	29.25	0.88

^aAnimals were dosed once every 48 hours ($\pm$ 1 hour) for a total of 14 doses. Animals were dosed on Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27.

^bGroup 1 was administered saline control (sterile saline) only.

^cGroup 2 was administered vehicle control (HTX-024-002) only.

^dConcentrations of bupivacaine and meloxicam were 2.5% and 0.075% w/w (or 25 mg/g and 0.75 mg/g), respectively. When corrected for density (1.17 g/mL) the final concentrations of bupivacaine and meloxicam were 29.25 mg/mL and 0.88 mg/mL, respectively.

Injection Site Rotations

Dose Site	Location	Injection Day	Dose Free Period to Terminal Necropsy
A	Left Cranial Back	1, 13, and 25	4
B	Right Cranial Back	3, 15, and 27	2
C	Left Mid Back	5 and 17	12
D	Right Mid Back	7 and 19	10
E	Left Caudal Back	9 and 21	8
F	Right Caudal Back	11 and 23	6

Observations and Results

Mortality

All animals survived until scheduled necropsies.

Clinical Signs

Dosing sites were scored using a modified Draize scoring system prior to dosing and 2-4 hours post dose. Detailed clinical examinations were performed weekly during the study and cage-side observations were performed at the time of dose site observations.

No evidence of edema or erythema was observed during the study. The most remarkable finding was scabbing at the injection sites. Findings were seen with both the vehicle and HTX-011 in males and females, with an increasing incidence of findings in the low- and high-dose groups in some cases. Scabs were most prevalent at the injection sites that were injected three times (A and B). However, Site C also had a large amount of scabbing and it was only injected a total of two times. Furthermore, Site A and B both had scabbing occur prior to the third injection and in some cases prior to the second injection. The incidence of scabbing was greatly reduced in recovery animals with only a few sporadic findings.

Table 103: Incidence of Clinical Observations in Males

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Scab, A, small, 1-10 mm	1	2	5	8
Scab, B, small, 1-10 mm	-	6	6	6
Scab, C, small, 1-10 mm	-	-	4	6
Sore, C, small 1-10 mm	-	-	-	3

N = 15

Table 104: Incidence of Clinical Observations in Females

	Female			
	Saline	Vehicle	12.5/0.38	25/0.75
Scab, A, small, 1-10 mm, multiple	-	1	-	3
Scab, A, small, 1-10 mm	-	9	7	9
Scab, B, small, 1-10 mm	-	5	5	10
Scab, B, medium, >10-20 mm	-	1	1	3
Scab, C, small, 1-10 mm	-	1	5	5
Scab, D, small, 1-10 mm, multiple	-	1	4	5
Scab, D, small, 1-10 mm	-	1	5	8

N = 15

Body Weights

Body weights were recorded prior to randomization, prior to treatment, and weekly for the remainder of the study.

There were no differences in body weights between groups.

Feed Consumption

Food consumption was measured in conjunction with body weight.

Rats of all groups consumed a similar amount of food.

Ophthalmoscopy

Indirect fundusoscopic examinations were performed pretest and on the main experiment animals one week prior to the end of treatment.

There were no treatment-related ophthalmic changes observed.

Hematology

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of hematology and coagulation parameters was evaluated.

Changes in hematology parameters compared to the saline control were limited to male animals and included increased absolute platelets (all treated groups), increased neutrophils as a percentage of red blood cells (high dose), increased absolute neutrophils (all treated groups), increased absolute eosinophils (all treated groups), increased absolute and as a percentage of red blood cells reticulocytes (all treated groups), and increased fibrinogen (all treated groups). Following recovery, all hematology parameters were similar between the saline control and treated groups. Overall, the changes were relatively small and the fact that the changes were seen in all treated groups indicated that the effects are likely secondary to the local inflammatory reaction.

Table 105: Notable Changes in Hematology Parameters in Male Rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Platelets (x 10 ³ cells/mcL)	1170 ± 107	1312 ± 135* (12)	1285 ± 112 (10)	1331 ± 131* (14)
Neutrophils (%)	13.4 ± 4.4	14.0 ± 5.8 (4)	14.2 ± 4.9 (6)	16.5 ± 4.4 (23)
Neutrophils (x 10 ³ cells/mcL)	1.11 ± 0.34	1.33 ± 0.56 (20)	1.52 ± 0.60 (37)	1.45 ± 0.47 (31)
Eosinophils (x 10 ³ cells/mcL)	0.10 ± 0.02	0.12 ± 0.04 (20)	0.14 ± 0.04* (40)	0.11 ± 0.04 (10)
Reticulocytes (x 10 ⁹ cells/L)	196.5 ± 28.3	228.8 ± 29.0 (16)	223.5 ± 33.5 (14)	257.8 ± 47.0* (31)
Reticulocytes (%)	2.37 ± 0.41	2.72 ± 0.43 (15)	2.81 ± 0.51 (19)	3.18 ± 0.55* (34)
Fibrinogen (mg/dL)	327 ± 31	390 ± 28* (19)	391 ± 31* (20)	394 ± 19* (20)

* Significantly different from saline control, p ≤ 0.05

Values in () represent % change compared to saline.

N = 10

Clinical Chemistry

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of clinical chemistry parameters was evaluated.

A statistically significant decrease in albumin was reported in males receiving the vehicle and high dose of HTX-011. The magnitude of change was small and there was no difference following the recovery period, therefore the change is not likely toxicologically relevant.

A large increase in aspartate aminotransferase (AST) was noted in one male receiving the low dose of HTX-011, which caused the average AST level in this group to be elevated. Since the increase in AST was only seen in one animal across the entire study, this was considered an incidental finding not related to treatment.

Table 106: Notable Changes in Clinical Chemistry Parameters in Rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Albumin (g/dL)	2.8 ± 0.1	2.6 ± 0.1* (7)	2.7 ± 0.1 (4)	2.6 ± 0.1* (7)
Aspartate aminotransferase (%)	119 ± 20	133 ± 25 (12)	168 ± 110 (41)	130 ± 13 (9)

* Significantly different from saline control, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10

Urinalysis

Urine was collected from fasted main study and recovery rats the day prior to terminal sacrifice. A complete battery of urinalysis parameters was evaluated.

There were no treatment-related changes in urinalysis parameters.

Gross Pathology

Following sacrifice, all main study and recovery animals received a complete necropsy.

Gross observations were primarily at the injection site and consisted of scabs and accumulations in animals receiving HTX-011 or the vehicle. In males, findings were primarily at Injection Site A and B, which were the injection sites with the least time between the last injection and sacrifice (4 and 2 days, respectively). In females, findings were also seen at Injection Site C, which had the longest time between the last injection and sacrifice (12 days). There was no clear dose-response to the findings, although the findings were overall more prevalent in animals who received HTX-011 than the vehicle. The lack of a dose response between the two HTX-011 doses is not unexpected since the gross observations represent a local effect and dose was controlled by injecting different volumes of the same concentration of HTX-011. Scabbing and accumulation were not observed in recovery animals.

Table 107: Gross Observations in Male Rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Accumulation (A)	-	-	-	1
Accumulation (B)	-	1	3	1
Scab (A)	-	-	2	-
Scab (B)	-	1	2	-

Table 108: Gross Observations in Female Rats

	Female			
	Saline	Vehicle	12.5/0.38	25/0.75
Accumulation (A)	-	-	-	2
Accumulation (B)	-	-	3	2
Accumulation (C)	-	1	2	-
Scab (A)	-	2	-	1
Scab (B)	-	-	1	3
Scab (C)	-	-	3	-

N = 10

Organ Weights

The following tissues were weighed:

Adrenal Glands	Liver	Testes
Brain	Ovaries	Thymus
Epididymides	Pituitary gland	Thyroid
Heart	Prostate gland	Uterus
Kidneys	Spleen	

There were no treatment-related changes in organ weights.

Histopathology**Adequate Battery: Yes****Peer Review: Yes**

Tissue Collection Table

Adrenals*	Pancreas
Aorta	Pituitary*
Brain* (entire)	Prostate* (with seminal vesicles/ coagulating gland/empty bladder)*
Epididymides*	Salivary glands (mandibular)
Esophagus	Sciatic nerve
Eyes with optic nerve	Skeletal muscle
Femur with marrow	Skin (with mammary gland for females)
Harderian gland	Spinal cord (cervical, thoracic, lumbar)
Heart*	Spleen*
Intestines (gut-associated lymphoid tissue, examined only if present in plane of section)	Sternum (bone with marrow)
Cecum	Stomach
Colon	Testes*
Duodenum	Thymus*
Ileum	Thyroid* (parathyroids, examined if present in plane of section)
Jejunum	Tongue
Rectum	Trachea
Kidneys*	Uterus* (with cervix/empty bladder)
Larynx	Vagina
Liver*	Gross lesions/masses
Lungs with bronchi	Identification (ear tag)
Lymph nodes (mandibular and mesenteric)	Injection sites A, B, C, D, E, F (full thickness at each site; including underlying muscle)
Nasal Cavity	
Ovaries* (with oviducts)	

Histological Findings

Histopathological findings related to systemic exposure were only seen in male animals and included minimal mononuclear cell infiltrates in the liver at the high dose and minimal accumulation of erythrocytes in the thymus at the low and high doses. These effects were not seen in recovery animals. These findings were not considered adverse due to the minimal severity and low incidence.

The remaining histopathological findings were seen at the injection sites with the vehicle and HTX-011. While not consistent, treatment with HTX-011 did result in a greater incidence and or severity of some findings compared to the vehicle control. Observations included chronic inflammation, granulomatous inflammation, hemorrhage, and the presence of test material. Granulomatous inflammation was seen at all injection sites with an average severity between minimal and mild but was no longer

seen in the recovery groups indicating that it resolved by 14 days post treatment. Findings of hemorrhage were more prevalent at the injection sites that were more recently injected although there were some exceptions. Hemorrhage was not seen in the recovery animals suggesting it resolves after 16 days.

Chronic inflammation was described in the report as either "active" or having no additional description. Active chronic inflammation appeared to be most prevalent at Injection Site B, where there was only 2 days following injection before necropsy. In general, the incidence of active chronic inflammation decreased with time after injection, although there were some exceptions. Active chronic inflammation was not observed in recovery animals. In contrast, chronic inflammation was most prevalent at Injection Site C, where there was 12 days following injection before necropsy. Based on the incidence data for both types of chronic inflammation, it appears that it is active following injection and eventually transitions over to chronic inflammation, although it is difficult to determine the precise time course based on the available data. What is clear is that for up to 12 days post injection there is some form of chronic inflammation at the injection site. Chronic inflammation, but not active chronic inflammation, was observed in recovery animals. While still present, the incidence was reduced in the animals which had the longest recovery time (26 days).

There did not appear to be a clear difference between the low and high dose of HTX-011 in terms of incidence or severity for histopathological findings, except for the presence of test material. This is not unexpected since the histopathological observations represent a local effect and the dose was controlled by injecting a different volume of the same concentration of HTX-011. However, the presence of test material would be expected to be related to the volume injected as a higher volume would result in a larger absolute amount of test material.

Table 109: Systemic Histopathological Observations

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Liver- mononuclear cell infiltrate	1 (1)	1 (1)	1 (1)	3 (1)
Thymus- accumulation of erythrocyte	0	0 (1)	2 (1)	3 (1)

N = 10

Table 110: Chronic Inflammation (Incidence)

Inj Site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	2 (2)	1 (1)	2 (1.5)	2 (1)	1 (1)	2 (1)	1 (1)	3 (1.3)
A [4]	2 (1)	1 (2)	3 (2)	4 (1.3)	1 (1)	4 (1)	6 (1.7)	6 (1.5)
F [6]	0	2 (1)	2 (2)	6 (2)	0	4 (1)	4 (1)	2 (2.5)
E [8]	0	4 (1.5)	4 (2)	2 (2.5)	1 (1)	4 (1.3)	4 (1.5)	2 (2.5)
D [10]	0	2 (1.5)	6 (2)	4 (1.5)	0	4 (1.8)	6 (1.7)	4 (2.3)
C [12]	0	8 (1.1)	7 (1.7)	7 (1.4)	0	4 (1)	4 (1.3)	9 (1.4)

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 111: Chronic Inflammation, Active (Incidence)

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	3 (1)	7 (1.7)	8 (2.1)	8 (2)	1 (1)	5 (1.6)	9 (1.7)	7 (1.9)
A [4]	0	5 (2.2)	5 (2.4)	3 (1.7)	0	3 (2)	2 (1.5)	4 (1.5)
F [6]	0	3 (2.7)	5 (2.6)	3 (2.3)	0	0	1 (3)	3 (3)
E [8]	0	2 (1.5)	4 (2.5)	6 (2)	0	2 (1.5)	1 (1)	5 (2.2)
D [10]	0	2 (2)	1 (2)	5 (2.4)	0	0	3 (2)	2 (1)
C [12]	0	0	1 (3)	2 (1.5)	0	1 (2)	0	1 (1)

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 112: Granulomatous Inflammation (Incidence)

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	2 (1)	1 (2)	0	0	0	0	1 (1)
A [4]	0	3 (1)	2 (1.5)	1 (1)	0	2 (1)	0	1 (1)
F [6]	0	2 (1.5)	2 (1.5)	2 (1)	0	2 (1)	1 (1)	3 (1.7)
E [8]	1 (2)	1 (1)	4 (1)	5 (1)	0	1 (1)	1 (1)	5 (1.4)
D [10]	0	3 (1)	2 (1.5)	4 (1)	0	1 (1)	2 (1)	3 (1.7)
C [12]	0	0	4 (1)	4 (1)	0	2 (1.5)	0	3 (1)

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 113: Hemorrhage (Incidence)

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	1 (2)	8 (2.9)	6 (1.8)	0	2 (1)	3 (2)	4 (1.8)
A [4]	0	2 (1.5)	2 (3)	2 (1)	0	2 (1.5)	5 (1.2)	2 (1)
F [6]	0	1 (4)	1 (2)	5 (1.8)	0	0	1 (3)	1 (2)
E [8]	1 (2)	0	2 (2)	4 (1)	0	0	0	2 (1)
D [10]	0	1 (3)	1 (1)	1 (1)	0	1 (1)	1 (1)	1 (2)
C [12]	1 (1)	1 (3)	0	2 (3)	0	1 (2)	0	0

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 114: Presence of Test Material (Incidence)

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	0	4 (2.8)	5 (3)	0	2 (1)	3 (1.7)	3 (1.3)
A [4]	0	1 (1)	1 (3)	3 (2.7)	0	2 (2)	2 (1.5)	1 (1)
F [6]	0	1 (1)	0	2 (2.5)	0	0	0	3 (3)
E [8]	0	0	2 (2)	3 (2.7)	0	0	1 (1)	2 (3.5)
D [10]	0	0	0	3 (2)	0	0	2 (1)	0
C [12]	0	0	0	1 (3)	0	1 (2)	0	0

N = 10/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 115: Chronic inflammation, Recovery Animals

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [16]	0	1 (1)	3 (1.3)	2 (1.5)	0	1 (1)	1 (1)	2 (1.5)
A [18]	0	0	2 (1)	5 (1.4)	0	2 (1)	2 (2)	0
F [20]	0	0	0	0	0	1 (1)	1 (1)	1 (1)
E [22]	0	4 (1)	4 (1.5)	4 (1.3)	0	3 (1.3)	0	3 (1.7)
D [24]	0	2 (1.5)	2 (1)	1 (1)	0	2 (1)	3 (1)	1 (1)
C [26]	0	2 (2)	1 (1)	1 (2)	0	2 (1)	1 (1)	0

N = 5/group

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Toxicokinetics

Toxicokinetic animals (3 rats/sex/timepoint) were bled via the sublingual vein on Days 1, 5, and 27. Urine was collected from vehicle and high-dose toxicokinetic animals in Week 3 for analysis of polymer breakdown products.

Bupivacaine

Bupivacaine AUC followed a nearly linear dose response on Day 1 and Day 5, but not on Day 27. It appeared that there was some accumulation of bupivacaine over time as AUC increased with time in males, but not females, with both doses. By Day 27, the AUC was 1.8-2.3 times higher in males compared to AUC on Day 1. In addition, there were still small, but detectable, amounts of bupivacaine in plasma at 48 hours after dosing in both males and females. Bupivacaine appeared to release quickly with T_{max} s ranging from 0.5 – 1 hour. Bupivacaine C_{max} values were highly variable and it is difficult to interpret any patterns. In general, C_{max} , T_{max} , and AUC were comparable between males and females.

Table 116: Bupivacaine Toxicokinetic Parameters

Dose (mg/kg/dose)	Day	Sex		C_{max} (ng/mL)	t_{max} (h)	AUC _{0-48h} (ng·h/mL)	R ^a	$C_{max}/Dose$	AUC _{0-48h}/Dose}
12.5	1	Male	Mean	348	0.5	2570	NA	27.8	206
			SE	235		278			
		Female	Mean	303	0.5	3220	NA	24.3	258
			SE	45.2		420			
	5	Male	Mean	1250	0.5	3640	1.42	100	291
			SE	852		498			
		Female	Mean	326	1	3000	0.930	26.1	240
			SE	94.4		302			
	27	Male	Mean	470	0.5	5860	2.28	37.6	469
			SE	149		938			
		Female	Mean	487	1	4650	1.44	38.9	372
			SE	188		605			
25	1	Male	Mean	254	6	4580	NA	10.1	183
			SE	86.5		478			
		Female	Mean	718	0.5	6600	NA	28.7	264
			SE	184		574			
	5	Male	Mean	328	0.5	6360	1.39	13.1	254
			SE	104		487			
		Female	Mean	558	1	7290	1.10	22.3	292
			SE	80.1		1250			
	27	Male	Mean	948	1	8120	1.77	37.9	325
			SE	367		753			
		Female	Mean	566	1	6490	0.980	22.6	260
			SE	211		465			

NA: Not Applicable; SE: standard error (C_{max} and AUC_{0-48h} only)

$C_{max}/Dose$ units: [(ng/mL)/(mg/kg/dose)]

AUC_{0-48h}/Dose units: [(ng·h/mL)/(mg/kg/dose)]}

^a R (Accumulation Ratio) = Day 5 or Day 27 AUC_{0-48h}/Day 1 AUC_{0-48h}}}

Taken from page 552 of Study report 33-85

Meloxicam

Meloxicam AUC followed a linear dose response on all days for both sexes. Meloxicam AUC increased with time; by Day 27 AUC was 2.7-3.9 times higher than on Day 1. There were still high levels of meloxicam in plasma 48 hours after dosing, which is reflected by the high T_{max} values ranging from 0.5-48 hours. C_{max} values also followed a

linear dose response and increased with time. For both C_{max} and AUC, the values were higher in females compared to males. The T_{max} values were highly variable in both sexes but generally seemed comparable.

Table 117: Meloxicam Toxicokinetic Parameters

Dose (mg/kg/dose)	Day	Sex		C_{max} (ng/mL)	t_{max} (h)	AUC _{0-48h} (ng•h/mL)	R ^a	$C_{max}/Dose$	AUC _{0-48h}/Dose}
0.38	1	Male	Mean	597	48	25600	NA	1570	67400
			SE	75.8		989			
		Female	Mean	1200	48	42300	NA	3170	111000
			SE	119		4540			
	5	Male	Mean	1340	6	50100	1.96	3510	132000
			SE	473		3240			
		Female	Mean	3080	12	112000	2.65	8110	295000
			SE	457		11200			
	27	Male	Mean	1810	12	70200	2.74	4770	185000
			SE	219		4040			
		Female	Mean	4190	12	163000	3.85	11000	429000
			SE	280		22400			
0.75	1	Male	Mean	1280	24	49000	NA	1710	65300
			SE	159		2520			
		Female	Mean	2740	48	79500	NA	3660	106000
			SE	198		8840			
	5	Male	Mean	2770	6	109000	2.22	3700	145000
			SE	318		6370			
		Female	Mean	5200	36	209000	2.63	6930	279000
			SE	1520		28800			
	27	Male	Mean	3680	6	142000	2.90	4900	189000
			SE	89.9		8190			
		Female	Mean	7710	0.5	289000	3.64	10300	385000
			SE	647		39900			

NA: not applicable; SE: standard error (C_{max} and AUC_{0-48h} only)

$C_{max}/Dose$ units: [(ng/mL)/(mg/kg/dose)]

AUC_{0-48h}/Dose} units: [(ng•h/mL)/(mg/kg/dose)]

^a R (Accumulation Ratio) = Day 5 or Day 27 AUC_{0-48h}/Day 1AUC_{0-48h}}

Taken from page 556 of Study report 33-85

Polymer breakdown products

Urine collected on Day 20 was analyzed for the presence of breakdown products of the polymer (AP135) using a non-validated method for exploratory purposes. The breakdown products measured were (b) (4)

For all compounds, the levels were over the calibration range and extrapolated values were reported when available. For (b) (4) the values were above the calibration range (b) (4) mcg/mL) but estimated values were not reported. Excluding (b) (4) the most prevalent breakdown product was (b) (4) followed by (b) (4) and then (b) (4). Levels of (b) (4) were similar between males and females while levels of (b) (4) and (b) (4) were higher in males.

Table 118: Levels of Polymer Breakdown Products in the Urine

Dose Group	Animal ID	Sex
Group 2 Vehicle Control	507	Male
	508	Male
	509	Male
	510	Female
	511	Female
	512	Female
Group 4 High Dose	567	Male
	568	Male
	569	Male
	594	Female
	595	Female
	596	Female

(b) (4)

^a The assays used to measure the concentrations were not validated, and all samples were above the calibration range. The extrapolated values are shown for informational purposes only, when available.

Taken from page 28 of Study 33-85

Dosing Solution Analysis

The vehicle was analyzed at the end of the study (March 6, 2018) to compare to the results from the Certificate of Analysis provided by the Applicant dated March 19th, 2018. There were no differences between the CoA and the analysis. Meloxicam and bupivacaine were not detected in the vehicle.

HTX-011 was not analyzed. The Applicant provided characterization of the test substance including stability data. The CoA for Lot 026517 indicated that the test substance had 99.9% bupivacaine and 97.3% meloxicam with a retest date of November 2018 (24 months after manufacture), that is later than the end of Study 33-85.

Study title: HTX-002: 14-Day Intermittent Subcutaneous Toxicity and Toxicokinetic Study in Sprague-Dawley Rats (with a 2-week Recovery)

Study no.: 34-84
Study report location: SDN 48, Module 4
<\\cdsesub1\levsprod\nda211988\0048\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\34-84\34-84.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: 9/17/2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: **HTX-002-14**, Lot 082719, 100.9%
bupivacaine
HTX-002-19, Lot 090519, 99.8%
bupivacaine
HTX-024-03, Lot 083019, 0% bupivacaine

Key Study Findings

- No treatment related mortalities were observed.
- No toxicologically significant effect was seen on body weight, ophthalmoscopy, clinical chemistry parameters, urinalysis parameters, or organ weights.
- An increased incidence of clinical signs compared to the vehicle were observed at the injection site. These included raised areas, scabbing, and sores (females only). These effects were consistent with other repeat dose studies with HTX formulations.
- Hematology parameters were altered compared to the control group in animals receiving bupivacaine formulations. These effects were consistent with other repeat dose studies with HTX formulations.
- Gross observations were limited to the injection site in animals receiving the bupivacaine formulations and included accumulations, crust (females only), and scabbing (females only). These effects were consistent with other repeat-dose studies with HTX formulations.
- Histopathological findings were limited to the injection site. Increased incidences of mononuclear cell infiltration, fibrosis, edema, erythrocyte accumulation, ulcer (females at Injection Site A), and acute inflammatory exudate (females at Injection Site A) were seen in animals who received the bupivacaine

formulations. These effects were consistent with other repeat-dose studies with HTX formulations.

- Bupivacaine AUC was nearly dose proportional on Days 1 and 13 with an expected fold difference of 1.5 (100 vs. 150 mg/kg) but with an actual dose ratio of 1.6-1.8 fold. It appeared that there was some accumulation of bupivacaine over time as AUC increased with time in both sexes and doses. Bupivacaine AUC and C_{max} was similar between males and females.
- The goal of the study was to assess the systemic safety of high-dose bupivacaine delivered via depot formulations. In that regard, no systemic toxicity was seen up to a dose of 150 mg/kg (HED of 1451.6 mg bupivacaine for a 60 kg individual). The corresponding AUC_{inf} at this dose was 52,900 ng*h/mL using the combined data from males and females. This value is greater than the highest human AUC_{inf} for bupivacaine in the Applicant's clinical trials (Study 209, total knee arthroplasty, 31,173 h*ng/mL) by a factor of ~1.7.
- A local inflammatory reaction was seen in the animals who received the bupivacaine in vehicle formulation which was more pronounced compared to the vehicle alone which showed a very mild response. This is interesting because a similar vehicle was used in the pivotal 28-day studies in rat and dog (Study 33-85 and 33-86) and caused a rather strong local immune response similar to the HTX formulation containing bupivacaine. Since the vehicle in this study has a different excipient profile compared to the bupivacaine formulations it is unclear whether the increased local response was due to the presence of bupivacaine or other excipients.

Methods

Doses: Vehicle control (HTX-024-03, 0 mg/kg bupivacaine), HTX-002-14 (100 mg/kg bupivacaine), and HTX-002-19 (150 mg/kg bupivacaine). See table below.

Frequency of dosing: Once every 48 hours

Route of administration: Subcutaneous injection

Dose volume: 1 mL/kg (vehicle), 3.4 mL/kg (HTX-002-14), and 2.6 mL/kg (HTX-002-19)

Formulation/Vehicle: **HTX-002-14**: 29.25 mg/mL bupivacaine, 93.6 mg/mL DMSO, 635.9 mg/mL AP135, 1.755 mg/mL maleic acid, and 409.5 mg/mL triacetin)
HTX-024-003: 93.6 mg/mL DMSO, 666.9 mg/mL AP135, 409.5 mg/mL triacetin).
 The chemical composition of **HTX-002-19** is unknown. The toxicology written summary states that HTX-002-19 contains bupivacaine in only the TEG-POE polymer.

Species/Strain: Sprague-Dawley rat

Number/Sex/Group: 10 for the main experiment, 5 for recovery, and 3 (vehicle) or 9 (bupivacaine) for toxicokinetics

Age: 8-9 weeks old

Weight: 218-355 g (male) and 164-261 g (female)

Satellite groups: Toxicokinetic and recovery groups

Unique study design: None

Deviation from study protocol: None that impacted study results

Experimental Design (From page 14 of the Applicant's Study Report 34-84)

Group ^a	Description	Number of			Dose Level	Dose	Dose
		Core	Recovery	TK	bupivacaine	Volume	Concentration ^c
					(mg/kg/day)	(mL/kg)	(mg/mL)
1 ^b	HTX-024-03 Vehicle Control	10	5	3	0	1	0
2	HTX-002-14	10	5	9	100	3.4	29.25
3	HTX-002-19	10	5	9	150	2.6	58.5

^aAnimals were dosed once every 48 hours ($\pm$ 2 hours), except on TK collection days when it was 48 hours ($\pm$ 1 hour), for a total of 7 doses. Animals will be dosed on Days 1, 3, 5, 7, 9, 11 and 13.

^bGroup 1 was administered vehicle control (HTX-024-03) only.

^cConcentrations of bupivacaine were 2.5% w/w for HTX-002-14 and 5.0% w/w for HTX-002-19. When corrected for density (1.17 g/mL) the final concentration of bupivacaine were 29.25 and 58.5 mg/mL, respectively.

Injection Site Rotations (from page 15 of the Applicant's Study Report 34-84)

Dose Site	Locations	Days
A	Left cranial back	1 and 13
B	Right cranial back	3
C	Left mid back	5
D	Right mid back	7
E	Left caudal back	9
F	Right caudal back	11

Observations and Results

Mortality

One high-dose male was found dead following TK blood collection on Day 1. No test-article related lesions were observed during gross necropsy.

One high-dose female had declining health status and was sacrificed on Day 15. Clinical signs included body weight loss, discolored paws and mouth, discolored inguinal fur, and red material around the eyes/nose. Necropsy revealed red, mottled, and fluid filled lungs. The cause of death was labeled as acute pneumonia.

Clinical Signs

Dosing sites were scored using a modified Draize scoring system prior to dosing and at least once following dosing. Detailed clinical examinations were performed weekly during the study and cage-side observations were performed at the time of dose site observations.

An increased incidence of clinical signs at the injection site compared to the vehicle control was observed. In both sexes, a raised area and scabbing was seen at the injection site. Sores at the injection site were only seen in female animals. Clinical signs at the injection sites were still present following the recovery period.

Table 119: Incidence of Clinical Observations in Males

Males			
	Vehicle	100 mg/kg	150 mg/kg
Raised area, left mid back	0	5	9
Raised area, right mid back	0	5	8
Raised area, left caudal back	0	3	11
Scab, left cranial back	0	7	6
Scab, right cranial back	2	7	4

N = 15

Table 120: Incidence of Clinical Observations in Females

Females			
	Vehicle	100 mg/kg	150 mg/kg
Raised area, left mid back	0	1	3
Raised area, left caudal back	0	2	4
Scab, left cranial back	4	11	14
Scab, right cranial back	5	9	14
Scab, left mid back	1	3	11
Scab, right mid back	1	6	7
Scab, left caudal back	0	3	3
Scab, right caudal back	0	1	3
Sore, left cranial back	1	5	2
Sore, right cranial back	1	3	2

N = 15

Body Weights

Body weights were recorded prior to randomization, prior to treatment, and weekly for the remainder of the study.

There were no differences in body weights between groups based on the mean body weight measured during the treatment period. However, there was a statistically significant 7% decrease in fasted body weight compared to the vehicle control in males

of the 150 mg/kg group on the final day of sacrifice. Male body weights in the 150 mg/kg group were higher (11%) than the vehicle control following recovery. The decrease in body weight is not considered toxicologically relevant by the reviewer since it was only seen at the end of the study, was less than 10%, and recovered.

Food Consumption

Food consumption was measured in conjunction with body weight.

Rats of all groups consumed a similar amount of food except males receiving 150 mg/kg during the recovery period. This increase in food consumption corresponds to the observed increase in body weight during the recovery period.

Ophthalmoscopy

Indirect fundoscopic examinations were performed pretest and near the end of the treatment period.

There were no treatment-related ophthalmic changes observed.

Hematology

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of hematology and coagulation parameters was evaluated.

Hematology parameters were altered in both males and females receiving bupivacaine compared to the vehicle controls. Some changes were small in magnitude but statistically significant. There did not appear to be a dose response for many parameters suggesting these effects could be secondary to the local inflammation reaction at the injection site, although it could also be related to the narrow dose spacing in the study.

The following changes were observed at one or both doses: increased white blood cells (M/F, no recovery), decreased red blood cells (M/F, recovery except males at 100 mg/kg), decreased hemoglobin (M/F, males did not recover), decreased hematocrit (M/F, males did not recover), increased platelets (M/F, recovered), increased neutrophils (absolute and %, M/F, recovery except females at 100 mg/kg), decreased eosinophils (absolute and %, M only, recovered), increased reticulocytes (M only, recovered), increased fibrinogen (M/F, recovered), and decreased MCHC (F only, recovered).

Table 121: Notable Changes in Hematology Parameters in Male Rats

Males				
	Vehicle	100 mg/kg	150 mg/kg	Recovery
WBC (x10 ³ cells/mcL)	7.86	10.40* (32%)	9.62 (22%)	Still elevated:
RBC (x10 ⁶ cells/mcL)	7.65	7.25* (-5%)	7.53 (-2%)	Still decreased:
HGB (g/dL)	14.6	14.1* (-3%)	14.2 (-3%)	Still decreased:
HCT (%)	43.7	42.6 (-3%)	42.5 (-3%)	Still decreased:
MCHC (g/dL)	33.4	33.1 (-1%)	33.4 (0%)	Similar to control
PLAT (x10 ³ cells/mcL)	1364	1493 (9%)	1564* (15%)	Similar to control
NEUT (%)	20.0	22.2 (11%)	25.9* (30%)	Decreased
NEUT (x10 ³ cells/mcL)	1.58	2.27* (44%)	2.47* (56%)	Similar to control
EOS (%)	1.3	0.8* (-38%)	0.7* (-46%)	Similar to control
EOS (x10 ³ cells/mcL)	0.11	0.08 (-28%)	0.07 (-36%)	Similar to control
Retic (x10 ⁹ cells/L)	290.1	381.2* (31%)	323.0 (11%)	Similar to control
Retic (%)	3.80	5.27* (39%)	4.32 (14%)	Similar to control
FIB (mg/dL)	390	455* (17%)	461* (18%)	Similar to control

* Significantly different from vehicle control, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10

Table 122: Notable Changes in Hematology Parameters in Female Rats

Females				
	Vehicle	100 mg/kg	150 mg/kg	Recovery
WBC (x10 ³ cells/mcL)	5.18	6.62 (28%)	7.14 (38%)	Still elevated
RBC (x10 ⁶ cells/mcL)	7.97	7.40* (-7%)	7.44* (-7%)	Similar to control
HGB (g/dL)	15.0	13.9* (-7%)	13.7* (-9%)	Similar to control
HCT (%)	43.4	41.1* (-5%)	40.3* (-7%)	Similar to control
MCHC (g/dL)	34.6	33.7* (-3%)	33.9* (-2%)	Similar to control
PLAT (x10 ³ cells/mcL)	1349	1495 (11%)	1471 (9%)	Similar to control
NEUT (%)	15.4	19.9 (29%)	21.9 (42%)	Similar to control
NEUT (x10 ³ cells/mcL)	1.20	1.72 (43%)	1.44 (20%)	100 mg/kg: still elevated 150 mg/kg: similar to control
EOS (%)	1.1	1.1 (0%)	1.1 (0%)	Similar to control
EOS (x10 ³ cells/mcL)	0.07	0.08 (14%)	0.08 (14%)	Similar to control
Retic (x10 ⁹ cells/L)	202.3	190.0 (-6%)	219.7 (9%)	100 mg/kg: elevated but large SD 150 mg/kg: similar to control
Retic (%)	2.51	2.41 (-4%)	2.87 (14%)	100 mg/kg: elevated but large SD 150 mg/kg: similar to control
FIB (mg/dL)	255	260 (2%)	279 (9%)	Similar to control

* Significantly different from vehicle, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10, except for the 150 mg/kg females where N = 9

Clinical Chemistry

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of clinical chemistry parameters was evaluated.

Clinical chemistry parameters were altered in both males and females receiving bupivacaine compared to the vehicle controls. Changes were small in magnitude but statistically significant. Based on the small magnitude of change and lack of a clear dose response the findings are not likely toxicologically relevant. In addition, most parameters were similar to the vehicle following the recovery phase.

Table 123: Notable Changes in Clinical Chemistry Parameters in Male Rats

Males				
	Vehicle	100 mg/kg	150 mg/kg	Recovery groups
ALB (g/dL)	2.8	2.6*	2.6*	No difference
GLU (mg/dL)	99	84*	88	No difference
T. PRO (g/dL)	5.2	4.9*	4.9*	No difference
K (mmol/L)	4.9	5.0	5.2*	Slight decrease

* Significantly different from vehicle, $p \leq 0.05$

N = 10

Table 124: Notable Changes in Clinical Chemistry Parameters in Female Rats

Females				
	Vehicle	100 mg/kg	150 mg/kg	Recovery groups
ALB (g/dL)	3.2	2.9*	2.8*	No difference
GLU (mg/dL)	99	105	100	100 mg/kg: slight elevation 150 mg/kg: No difference
T. PRO (g/dL)	5.4	5.1*	4.9*	No difference
Na (mmol/L)	141	143*	142*	No difference
K (mmol/L)	4.9	5.0	5.2*	100 mg/kg: no difference 150 mg/kg: Elevated
Cl (mmol/L)	105	106	107*	No difference

* Significantly different from vehicle, $p \leq 0.05$

N = 10, except for the 150 mg/kg females where N = 9

Urinalysis

Urine was collected the day prior to terminal sacrifice. A complete battery of urinalysis parameters was evaluated.

The only treatment related finding was a small but statistically significant decrease in urine pH of males receiving bupivacaine. Due to the small magnitude of the change, the fact it was seen in one sex and that values were similar to the control following recovery suggests the finding is likely not toxicologically relevant.

Table 125: Urine pH in Male and Female Rats

	Vehicle	100 mg/kg	150 mg/kg	Recovery groups
pH (males)	6.7	6.2*	6.4*	No difference
pH (females)	6.3	6.4	5.9	No difference

* Significantly different from vehicle, $p \leq 0.05$

N = 10, except for the 150 mg/kg females where N = 9

Gross Pathology

Following sacrifice, all main study and recovery animals received a complete necropsy.

Gross observations were limited to the injection sites. In males, accumulations were observed at the injection site while, in females, accumulations, crust, and scabbing were present. Findings were similar to the vehicle-treated group following a recovery period.

Table 126: Gross Observations in Male Rats (Incidence)

Males			
	Vehicle	100 mg/kg	150 mg/kg
Injection site-subcutaneous fluid filled accumulation	0	3	1
Injection site-subcutaneous yellow accumulation	0	1	3
Injection site-subcutaneous accumulation	0	4	1

N = 10

Table 127: Gross Observations in Female Rats (Incidence)

Females			
	Vehicle	100 mg/kg	150 mg/kg
Injection site-crust	1	3	2
Injection site-scab	0	1	3
Injection site-subcutaneous accumulation	0	4	1

N = 10, except for the 150 mg/kg females where N = 9

Organ Weights

The following tissues were weighed:

Adrenal Glands	Ovary
Brain	Spleen
Heart	Testes

Kidneys
Liver

Thymus

Statistically significant changes in organ weights were observed in males but were generally small in magnitude. This included decreased heart weight (abs), increased liver weight relative to body weight, increased brain weight (relative to body weight), and increased testes weight (relative to body weight). Effects on the brain and testis seemed to follow somewhat of a dose response. The effects were no longer present following the recovery phase. Due to the small magnitude of change and lack of histopathological correlates the effects seem to not be toxicologically relevant.

In females the only organ weight change was increased spleen weight relative to body weight at 150 mg/kg compared to the vehicle treated animals. Spleen weight was still slightly elevated (7%) in the recovery animals but this small difference is likely not toxicologically relevant.

Table 128: Organ Weights in Male Rats

	Males			
	Vehicle	100 mg/kg	150 mg/kg	Recovery
Heart (g)	1.182	1.082* (-8%)	1.073* (-9%)	Similar to control
Liver (rel)	3.15	3.38* (7%)	3.36 (7%)	Similar to control
Brain (rel)	0.612	0.632 (3%)	0.660* (8%)	Similar to control
Testis (rel)	0.924	0.986 (7%)	1.040* (13%)	Similar to control

* Significantly different from vehicle, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10

Table 129: Organ Weights in Female Rats

	Females			
	Vehicle	100 mg/kg	150 mg/kg	Recovery
Spleen (rel)	0.235	0.248 (6%)	0.270* (15%)	Slight elevation

* Significantly different from vehicle, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10, except for the 150 mg/kg females where N = 9

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Tissue Collection Table

Adrenals*	Pancreas
Aorta	Pituitary
Brain* (entire)	Prostate (with seminal vesicles and coagulating gland)
Epididymides	Salivary glands (mandibular)
Esophagus	Sciatic nerve
Eyes with optic nerve	Skeletal muscle
Femur with marrow	Skin (untreated; with mammary gland for females)
Harderian gland	Spinal cord (cervical, thoracic, lumbar)
Heart*	Spleen*
Intestines (gut-associated lymphoid tissue, examined only if present in plane of section)	Sternum (bone with marrow)
Cecum	Stomach
Colon	Testes*
Duodenum	Thymus*
Ileum	Thyroid (parathyroids, examined if present in plane of section)
Jejunum	Tongue
Rectum	Trachea
Kidneys*	Urinary bladder
Larynx	
Liver*	Uterus (with cervix)
Lungs with bronchi	Vagina
Lymph nodes (mandibular and mesenteric)	Gross lesions/masses
Ovaries* (with oviducts)	Identification (ear tag)
Regional Lymph Nodes (axillary)	Injection sites A, B, C, D, E, F (full thickness at each site; including underlying muscle)

While all tissues were collected, only the brain, heart, liver, lungs, kidneys, and injection sites underwent histopathological evaluation. This was considered adequate because the largest concern with bupivacaine is cardiotoxicity.

Histological Findings

There were no systemic histopathological findings identified following treatment with bupivacaine. An increased incidence of lesions was observed at the injection site of animals who received bupivacaine including mononuclear cell infiltration, fibrosis, edema, erythrocyte accumulation, ulcer (females at Injection Site A), and acute inflammatory exudate (females at Injection Site A). Findings at the injection site were mostly resolved following the recovery period.

Table 130: Infiltrate of Mononuclear Cells (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	3 (1.3)	7 (1.4)	8 (1.3)	3 (1.7)	3 (1.3)	6 (1.3)
F [3]	2 (2)	8 (1.5)	7 (1.1)	2 (1.0)	8 (1.5)	6 (2)
E [5]	5 (1.2)	9 (1.6)	9 (1.9)	5 (1.4)	10 (1.9)	8 (1.5)
D [7]	4 (2)	10 (1.6)	7 (1.3)	7 (1.6)	9 (1.7)	7 (1.4)
C [9]	7 (1)	9 (1.6)	9 (1.2)	1 (2)	4 (1.3)	7 (1.6)
B [11]	3 (1)	3 (1.3)	5 (1.2)	5 (1.2)	8 (1.4)	7 (1.1)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Table 131: Fibrosis (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	1 (2.0)	5 (2.0)	4 (2.3)	0	0	1 (1.0)
F [3]	2 (2.5)	2 (2.5)	2 (2.5)	0	2 (1.5)	2 (2.5)
E [5]	3 (2.7)	7 (2.3)	6 (1.7)	1 (2.0)	7 (2.4)	4 (1.8)
D [7]	3 (2.7)	8 (3.0)	5 (2.0)	3 (1.0)	5 (2.2)	6 (2.0)
C [9]	4 (2.3)	9 (2.6)	7 (2.6)	0	3 (2.3)	5 (2.4)
B [11]	1 (2.0)	3 (1.7)	4 (1.8)	0	2 (3.0)	3 (1.0)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Table 132: Edema (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	2 (2.5)	4 (3.0)	6 (2.5)	0	1 (3.0)	1 (1.0)
F [3]	0	5 (4.2)	3 (4.7)	0	2 (2.0)	3 (4.7)
E [5]	0	3 (3.3)	5 (3.8)	0	5 (3.2)	2 (3.0)
D [7]	0	6 (3.7)	6 (3.5)	0	1 (3.0)	3 (4.0)
C [9]	0	4 (3.0)	2 (4.0)	0	1 (3.0)	2 (2.5)
B [11]	0	0	1 (3.0)	0	5 (3.0)	5 (3.4)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Table 133: Erythrocyte Accumulation (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	2 (1.5)	6 (3.2)	9 (3.7)	0	2 (4.5)	2 (4.5)
F [3]	2 (2.0)	6 (3.5)	4 (2.8)	0	2 (2.0)	3 (2.3)
E [5]	1 (3.0)	7 (3.0)	6 (3.8)	1 (3.0)	5 (3.2)	3 (3.0)
D [7]	2 (1.0)	7 (3.6)	2 (3.0)	0	4 (4.0)	2 (2.0)
C [9]	1 (1.0)	3 (3.0)	5 (1.8)	0	4 (3.3)	4 (3.5)
B [11]	0	2 (3.5)	1 (3.0)	4 (2.0)	6 (3.0)	6 (3.7)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Table 134: Ulcer (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	0	1 (3.0)	0	1 (4.0)	2 (3.0)	6 (2.7)
F [3]	0	1 (4.0)	0	0	1 (3.0)	2 (4.5)
E [5]	0	0	0	0	1 (3.0)	0
D [7]	0	0	0	0	0	1 (3.0)
C [9]	0	0	0	0	0	2 (4.0)
B [11]	1 (3.0)	0	0	1 (1.0)	3 (3.0)	2 (3.0)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 135: Acute Inflammatory Exudate (Incidence)

Inj Site	Male			Female		
	Vehicle	100	150	Vehicle	100	150
A [1, 2x inj]	0	1 (4.0)	1 (3.0)	1 (3.0)	5 (3.2)	8 (3.9)
F [3]	0	1 (4.0)	0	0	1 (3.0)	2 (4.5)
E [5]	0	0	0	0	1 (4.0)	1 (1.0)
D [7]	0	0	0	0	0	2 (4.0)
C [9]	0	0	0	0	0	2 (4.0)
B [11]	1 (3.0)	0	0	1 (3.0)	4 (4.0)	2 (3.5)

N = 10, except for 150 mg/kg females (9)

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe.

Values in [] represent number of days since last injection

Toxicokinetics

Toxicokinetic animals (3 rats/sex/timepoint) were bled via the sublingual vein on Days 1 and 13. Vehicle control animals had blood collected at 6 hours post dose on both days. Bupivacaine treated animals had blood collection at the following timepoints: pre dose, and 0.5, 1, 4, 6, 12, 24, 36, 48, 60, and 72 hours post dose on Day 13; pre dose, and 0.5, 1, 4, 6, 12, 24, 36, and 48 hours post dose on Day 1.

Bupivacaine

Bupivacaine AUC was nearly dose proportional on Days 1 and 13 with an expected fold difference of 1.5 (100 vs. 150 mg/kg) but with an actual dose ratio of 1.6-1.8 fold. It appeared that there was some accumulation of bupivacaine over time as AUC increased with time in both sexes and doses. Bupivacaine AUC and C_{max} were similar between males and females.

Table 136: Bupivacaine Toxicokinetic Parameters

Treatment	BPV Dose (mg/kg)	Day	Sex	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC ₀₋₂₄ ^a (ng•h/mL)	AUC ₀₋₄₈ ^b (ng•h/mL)	AUC _{last} (ng•h/mL)	AUC _{inf} (ng•h/mL)	R _c ^c
HTX-002-14	100	1	Male	684	4	15.8	10200	14300	14300	16100	NA
			Female	686	6	15.3	11400	15500	15500	17400	NA
			Combined	655	4	15.2	10800	14900	14900	16700	NA
		13	Male	1350	4	28.8	17300	26000	28400	31200	1.8
			Female	1180	4	16.8	19900	26600	29500	30800	1.7
			Combined	1260	4	17.2	18600	26300	28900	30500	1.8
HTX-002-19	150	1	Male	1120	6	14.6	18000	23700	23700	26500	NA
			Female	1050	12	16.1	18900	24400	24400	27000	NA
			Combined	1010	6	13.2	18400	24000	24000	26400	NA
		13	Male	2060	12	ND	34100	43600	48300	ND	1.8
			Female	3030	1	16.3	36200	48600	53800	55700	2.0
			Combined	2180	1	16.4	35200	46100	51000	52900	1.9

BPV = bupivacaine; NA = Not Applicable; ND = Not Determined.

^a AUC₀₋₂₄ is area under the plasma concentration-time from time zero to 24 h post-dose.

^b AUC₀₋₄₈ is area under the plasma concentration-time from time zero to 48 h post-dose.

^c R_c is accumulation ratio Day 13 AUC₀₋₄₈/Day 1 AUC₀₋₄₈.

Taken from page 469 of Study report 34-84

Dosing Solution Analysis

Dosing solution analysis was not performed. End-of-use stability analysis of the test article formulations was supposed to be conducted and appended to the final study report, but it does not appear to have been completed yet at the time of review.

Study title: HTX-011: 28-day intermittent subcutaneous toxicity and toxicokinetic study in Beagle dogs (with a 2-week recovery)

Study no.: 33-86
Study report location: SDN 1, Module 4
<\\cdsesub1\levsprod\nda211988\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\33-86\33-86.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/22/2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: HTX-011, Lot 026517, 99.9% bupivacaine and 97.3% meloxicam

Key Study Findings

- All animals survived until necropsy.
- No treatment-related effects were observed on ophthalmic parameters, electrocardiography parameters, or urinalysis parameters.
- There was an increased incidence of edema at the injection site following vehicle and HTX-011 treatment. Incidence and severity were highest with the vehicle, while both doses of HTX-011 caused similar incidence and severity scores that were on average lower than the vehicle. Edema was not seen in recovery animals.
- Treatment with the vehicle also resulted in hypoactivity, dermal scabs, and swollen sites. These were not observed in recovery animals.
- Decreased body weight was observed following treatment with the vehicle. This did not appear to be toxicologically relevant in males due to lower weight prior to treatment and similar body weight gain to saline control. However, the effect was toxicologically relevant in females where body weight gain was greatly reduced over the course of the study.
- Treatment with the vehicle affected multiple hematology parameters while treatment with HTX-011 only resulted in increased reticulocytes. There was no difference in any hematology parameters in recovery animals.
- Treatment with the vehicle affected multiple clinical chemistry parameters while treatment with HTX-011 only resulted in increased glucose at the interim sacrifice. There was no difference in any clinical chemistry parameters in recovery animals.

- Gross pathologic inspection revealed thickening at the injection site in vehicle-treated animals and accumulation in HTX-011 and vehicle-treated animals. No treatment-related macroscopic findings were present in recovery animals.
- Changes in organ weights were observed in vehicle-treated animals compared to saline control. This included increased absolute and relative liver weights, increased absolute and relative thymus weights (males only), increased absolute and relative adrenal weights (females only), and increased absolute and relative kidney weights (females only). Most of these changes were also observed in recovery animals.
- Injection site histopathological findings included chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage, and presence of test material following treatment with the vehicle and HTX-011. In general, findings were more prevalent or more severe in female animals and vehicle-treated animals. Multifocal chronic inflammation, multifocal granulomatous inflammation, and multifocal fibrosis were still present in recovery animals at reduced severity and/or incidence up to around 28 days.
- Bupivacaine and meloxicam toxicokinetics were measured on Days 1, 9, and 27. Bupivacaine and meloxicam C_{max} and AUC increased with time and were similar between the two dose groups. T_{max} values for both compounds were highly variable.
- Polymer breakdown products were detected in urine on Day 22. (b) (4)
were all detected at levels above the calibration range.
- Effects that could be potentially related to systemic exposure included hypoactivity, decreased body weight, altered hematology parameters, altered clinical chemistry parameters, and increased organ weights. All of these effects were observed following treatment with the vehicle and not saline or HTX-011. The majority of findings were similar in both males and females (except for effects on body weight). No histopathological effects on any organs were observed and most effects (except organ weight changes) were reversible. Overall, the data support that the excipients in the vehicle cause a systemic response.
- The injection volume used for the vehicle was larger than that for HTX-011; therefore, vehicle animals received higher exposure to certain excipients which may have caused the systemic effects. The vehicle was not a perfect match for the drug product as it does not contain maleic acid and contains more AP135 (650 mg/g vs. 623.75 mg/g) than HTX-011. Compared to HTX-011, the vehicle resulted in about twice as much exposure to DMSO (117 vs. 51.5 mg/kg), triacetin (292.5 vs. 128.7 mg/kg), and AP135 (760.5 vs. 321.1 mg/kg).

Compared to human exposure at the MRHD of 400 mg/12 mg, HTX-011 showed limited systemic effects (increased reticulocytes in females) with an exposure margin of ~1.1x for DMSO, triacetin, and AP135, while the vehicle showed systemic effects with an exposure margin of ~2.5x for DMSO, triacetin, and AP135 based on BSA and a 60 kg individual.

- A local inflammatory reaction was observed following treatment with the vehicle and HTX-011. The effects are consistent with a biological response to injection of foreign material. The majority of the effects resolved following a recovery period as indicated by the lack of those findings in recovery animals. However, multifocal chronic inflammation, granulomatous inflammation, and fibrosis were still present in recovery animals up to 28 days following the last injection. Local effects were predominantly in females and were worse with vehicle compared to HTX-011, likely due to the increased injection volume used for the vehicle. In addition, there appeared to be more effects with the high dose of HTX-011 compared to the low dose. The increased local findings with the vehicle and HTX-011 high dose is slightly unusual as generally the concentration of the formulation is considered the dose metric for local effects. However, the results in dogs support that the absolute amount of the excipients delivered to the local tissue seems to be the driving force to the local effects.
- The Applicant claims that the effects are non-adverse and therefore the NOAEL is the highest dose tested. While the effects observed are not unexpected, it is difficult to ascertain the adversity and clinical relevance of the observed persisting chronic inflammation, granulomatous inflammation, and fibrosis. Therefore, in terms of local effects, this study demonstrates that the clinical formulation causes a local inflammatory response up to 28 days that is likely the result of the vehicle. The total amount of excipients seems to be a driving force in the local reaction. However, if that is the case, it is difficult to compare the local effects in animals to humans since the dosing in the animal study is different from the clinical dosing regimen and it is unclear how to translate the volume of injection into a HED dose.

Methods

Doses: Saline, vehicle control, 10/0.30 mg/kg bupivacaine/meloxicam, and 13/0.39 bupivacaine/meloxicam

Frequency of dosing: Once every 48 hours

Route of administration: Subcutaneous injection

Dose volume: 0.44 mL/kg (saline and 13/0.39 mg/kg), 1 mL/kg for vehicle (start of study), 0.5 mL/kg for vehicle (after seeing decreased body weight and injection site findings), or 0.34 mL/kg (10/0.30 mg/kg)

Formulation/Vehicle: HTX-011-056 (aka HTX-011): 25% triacetin, 10% DMSO, 62.375% AP135 polymer, 0.05% maleic acid, 2.5% bupivacaine, and 0.075% meloxicam
HTX-024-002 (vehicle): 25% triacetin, 10% DMSO, and 65% AP135 polymer

Species/Strain: Beagle dog

Number/Sex/Group: 4 for the main experiment, and 2 for recovery

Age: 5-6 months old

Weight: 6.4-8.9 kg (male) and 5.2-7.2 kg (female)

Satellite groups: Recovery groups

Unique study design: None

Deviation from study protocol: None that impacted study results

Experimental Design

Group ^a	Description	Bupivacaine Dose Level (mg/kg/day)	Meloxicam Dose Levels (mg/kg/day)	Dose Volume (mL/kg)	Bupivacaine Conc. ^d (mg/mL)	Meloxicam Conc. ^d (mg/mL)	Number of Dogs/Group	
							Core	Recovery
1 ^b	Saline Control	0	0	0.44	0	0	4/sex	2/sex
2 ^c	Vehicle Control	0	0	1/0.5 ^e	0	0	4/sex	2/sex
3	Low Dose	10	0.30	0.34	29.25	0.88	4/sex	2/sex
4	High Dose	13	0.39	0.44	29.25	0.88	4/sex	2/sex

^aAnimals will be dosed once every other day for a total of 14 doses, on Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27.

^bGroup 1 will be administered saline control (sterile saline) only.

^cGroup 2 will be administered vehicle control (HTX-024) only.

^dConcentrations of bupivacaine and meloxicam will be 2.5 and 0.075% w/w (or 25 mg/g and 0.75 mg/g), respectively. When corrected for density (1.17 g/mL) the final concentrations of bupivacaine and meloxicam will be 29.25 mg/mL and 0.88 mg/mL, respectively.

^eDue to decreased body weights and adverse injection site findings, the dose volume for the Vehicle Control group was decreased to 0.5 mL/kg for Days 21, 23, 25, and 27.

Injection Site Rotations

Dose Site	Location	Injection Day	Dose Free Days to Terminal Necropsy
A	Left Cranial Back	1 and 13	16
B	Right Cranial Back	3 and 15	14
C	Left Mid Back	5 and 17	12
D	Right Mid Back	7 and 19	10
E	Left Caudal Back	9, 21, and 25	4
F	Right Caudal Back	11, 23, and 27	2

Observations and Results

Mortality

All animals survived until scheduled necropsies.

Clinical Signs

Dosing sites were scored using a modified Draize scoring system prior to dosing and two hours post dose. Detailed clinical examinations were performed weekly during the study in conjunction with body weight. Cage-side observations were performed at the time of dose site/dermal observations.

The primary finding at the dermal site was increased edema. The largest effect was seen with the vehicle treatment resulting in edema scores of four (severe) at most time points. Treatment with either HTX-011 dose resulted in less frequent and less severe observations of edema than the vehicle, but they were still increased compared to the saline control. Edema scores were highest two days after dosing. There was no dose response to the incidence or severity; however, this is not unexpected since the doses are very similar and similar volumes were injected. At the end of the recovery period, the incidence and severity of edema was greatly reduced and no difference between treatment groups was observed.

The dose volume of the vehicle control was reduced due to the observation of injection site findings. After reducing the volume from 1 mL/kg to 0.5 mL/kg (similar to the high-dose group), the edema scores were reduced as well.

Some of the animals receiving the vehicle control exhibited serosanguinous fluid with expulsion from the injection site. The fluid was tested for bacteria and did not contain any. Additional findings only seen in the vehicle control animals were hypoactivity, dermal scabs, and swollen sites.

Table 137: Incidence of Edema at the Injection Site

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
A	0	6 (4)	6 (2)	5 (2)	0	6 (4)	6 (2)	6 (2)
B	0	6 (4)	6 (1)	6 (1)	0	6 (4)	6 (1)	6 (1)
C	0	6 (4)	3 (1)	3 (1)	0	6 (4)	2 (1)	6 (1)
D	0	6 (4)	5 (1)	6 (2)	0	6 (4)	5 (1)	5 (1)
E	0	6 (4)	6 (2)	6 (2)	0	6 (4)	4 (2)	6 (2)
F	0	6 (4)	6 (2)	6 (2)	0	6 (4)	6 (3)	6 (2)

N = 6

Values in () represent average severity: 1 = very slight, 2 = well-defined, 3 = moderate, 4 = severe.

Table 138: Average Severity of Edema in Vehicle Control Group

Inj site	Day 11 (1 ml/kg) M/F	Day 13 (1 ml/kg) M/F	Day 23 (0.5 ml/kg) M/F	Day 25 (0.5 ml/kg) M/F
E	3/3.8	-	2.8/2.3	-
F	-	3.7/3.7	-	2.7/2.7

Body Weights

Body weights were recorded prior to randomization, prior to treatment, and weekly for the remainder of the study.

Body weights in male vehicle treated animals were less than saline control. However, it appears that this group of animals weighed less than the saline control prior to treatment (Day 0). Furthermore, the group as a whole had a similar level of body weight gain over the first 19 days of treatment (0.28 kg for both the saline and vehicle control group) compared to the saline control group.

Body weight was also decreased in female vehicle treated animals compared to the saline control. Similar to males, it appears that this group of animals weighed less than the saline control prior to treatment (Day 0). In contrast to males, there did appear to be an effect on body weight by the vehicle treatment as the % difference between the two groups increased over the first 19 days of treatment. This can also be seen by comparing body weight gain over the 19 days between the saline control (0.31 kg) and the vehicle control (-0.15 kg).

No differences between the HTX-011 groups and the saline control were observed.

Table 139: Body Weights

Day	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
0	7.78	91.9%	98.2%	96.5%	6.32	96.2%	98.6%	101.3%
1	7.69	91.7%	98.5%	97.1%	6.18	96.4%	97.6%	100.8%
7	7.90	89.4%	98.7%	99.0%	6.32	94.3%	102.5%	106.3%
13	7.81	92.2%	102.2%	100.5%	6.50	90.0%	99.2%	103.2%
19	8.06	92.2%	102.7%	100.4%	6.62	89.6%	99.2%	104.8%
25	8.34	91.7%	100.2%	99.4%	6.74	93.6%	100.3%	103.0%
27	8.00	95.0%	103.4%	100.8%	6.67	95.8%	100.9%	105.4%

N = 6

Ophthalmoscopy

Indirect fundoscopic examinations were performed pretest, at the end of the treatment period, and during recovery.

There were no treatment-related ophthalmic changes observed.

Electrocardiography

A six-lead electrocardiogram was performed on each dog prior to treatment, on Day 27, and during the second week of recovery. ECGs were evaluated by a qualified veterinary cardiologist.

There were no treatment-related electrocardiographic effects.

Hematology

Blood was collected from the jugular vein prior to treatment, at about 3 weeks, at end of the treatment period, and the end of recovery. A complete battery of hematology and coagulation parameters was evaluated.

A number of hematology parameters were affected by treatment with the vehicle in both males and females. Consistently effected parameters were increased white blood cells, decreased red blood cells, decreased hemoglobin, decreased hematocrit, increased platelets, increased absolute and relative neutrophils, decreased relative lymphocytes, decreased relative basophils, increased absolute monocytes, increased absolute leukocytes, increased red blood cell distribution width, increased absolute eosinophils, and increased absolute and relative reticulocytes. All parameters were similar to the saline control after a two-week recovery period.

In contrast, there were very few effects on hematology with the drug product. In males an absolute and relative increase in reticulocytes were seen at the interim blood collection with both doses but values were similar at the terminal blood collection. In females, absolute and relative reticulocytes were increased at both the interim and terminal blood collections. All parameters were similar to the saline control after a two-week recovery period.

Table 140: Hematology Parameters in Male Dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
WBC (x10 ³ cells/mcL)	11.05	433.9%*	107.9%	111.9%
	7.99	301.1%*	110.4%	115.5%
RBC (x10 ³ cells/mcL)	6.71	75.3%*	100.4%	99.3%
	6.24	78.5%*	101.0%	96.6%
HGB (g/dL)	15.2	73.7%*	98.0%	95.4%
	13.9	79.1%*	100.7%	94.2%
HCT (%)	46.2	76.6%*	98.7%	95.7%
	43.2	80.0%*	99.1%	93.5%
RDW (%)	14.3	103.5%	104.9%	105.6%
	14.0	111.4%*	104.3%	105.0%
PLAT (x10 ³ cells/mcL)	321	144.2%	101.9%	114.6%
	269	208.9%*	105.9%	110.0%
NEUT (%)	56.5	151.2%*	111.7%	110.3%
	54.9	135.2%*	107.5%	104.6%
LYMP (%)	34.5	20.6%*	84.6%	87.5%
	34.5	47.8%*	93.6%	93.0%
EOS (%)	1.4	28.6%*	114.3%	114.3%
	2.1	104.8%	95.2%	133.3%
MONO (%)	6.7	97.0%	80.6%	79.1%
	7.4	86.5%	77.0%	93.2%
LUC (%)	0.5	100.0%	80.0%	80.0%
	0.7	85.7%	85.7%	85.7%
Retic (%)	0.71	129.6%	163.4%	171.8%
	0.78	239.7%*	101.3%	125.6%
NEUT (x10 ³ cells/mcL)	6.25	657.3%*	120.6%	125.8%
	4.47	410.5%*	116.8%	119.9%
LYMPH (x10 ³ cells/mcL)	3.80	85.8%	91.1%	95.0%
	2.69	133.1%*	105.6%	107.4%
EOS (x10 ³ cells/mcL)	0.15	113.3%	120.0%	126.7%
	0.16	281.3%*	112.5%	168.8%
MONO (x10 ³ cells/mcL)	0.76	415.8%*	84.2%	85.5%
	0.58	256.9%*	86.2%	108.6%
LUC x10 ³ cells/mcL	0.05	460.0%	100.0%	80.0%
	0.05	280.0%*	100.0%	100.0%
Retic (x10 ⁹ cells/L)	47.4	96.6%	165.0%	171.5%*
	48.0	188.1%*	104.6%	123.1%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Table 141: Hematology Parameters in Female Dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
WBC (x10 ³ cells/mcL)	12.82	256.9%*	83.5%	83.5%
	9.47	276.2%*	107.5%	92.8%
RBC (x10 ³ cells/mcL)	7.04	78.6%*	99.3%	98.7%
	6.65	84.1%*	99.7%	98.8%
HGB (g/dL)	15.7	75.8%*	94.9%	100.0%
	14.9	81.9%*	95.3%	98.7%
HCT (%)	48.5	77.5%*	94.8%	98.8%
	45.7	83.6%*	95.6%	99.3%
RDW (%)	14.7	103.4%	104.1%	101.4%
	14.2	109.2%*	107.7%*	102.8%
PLAT (x10 ³ cells/mcL)	392	114.3%	91.8%	91.8%
	333	148.0%*	109.9%	103.0%
NEUT (%)	55.9	143.1%*	106.1%	107.5%
	51.5	146.0%*	121.7%*	112.0%
LYMP (%)	36.1	33.5%*	91.1%	89.2%
	39.5	38.2%*	74.9%*	87.3%
EOS (%)	1.4	71.4%	135.7%	157.1%
	2.2	104.5%	83.4%	127.3%
MONO (%)	5.8	110.3%	87.9%	82.8%
	5.8	117.2%	86.2%	72.4%
LUC (%)	0.5	100.0%	80.0%	80.0%
	0.5	80.0%	80.0%	80.0%
Retic (%)	0.96	94.8%	124.0%	157.3%*
	0.70	198.6%	171.4%	162.9%
NEUT (x10 ³ cells/mcL)	7.24	369.8%*	87.8%	89.1%
	4.92	404.1%*	134.4%	103.9%
LYMPH (x10 ³ cells/mcL)	4.56	78.9%*	77.2%*	75.0%*
	3.71	103.0%	75.2%*	81.1%
EOS (x10 ³ cells/mcL)	0.17	152.9%	117.6%	141.2%
	0.21	261.9%*	85.7%	109.5%
MONO (x10 ³ cells/mcL)	0.73	287.7%*	75.3%	69.9%
	0.54	325.9%*	100.0%	68.5%
LUC x10 ³ cells/mcL	0.06	233.3%*	66.7%	66.7%
	0.05	200.0%*	80.0%	60.0%
Retic (x10 ⁹ cells/L)	67.5	75.6%	123.6%	155.3%*
	46.5	164.7%	173.3%	163.9%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Clinical Chemistry

Blood was collected from the jugular vein prior to treatment, at about 3 weeks, at end of the treatment period, and at the end of recovery. A complete battery of hematology and coagulation parameters was evaluated.

Treatment with the vehicle caused multiple changes in clinical chemistry parameters in male and female animals. Consistent changes in both sexes at both blood collection time points were decreased albumin, decreased ALT, decreased creatinine, decreased glucose, increased LDH, increased globulin, and decreased A/G ratio. Other less

consistent changes included decreased total bilirubin (males at both time points; females at interim only), decreased calcium (males at interim only; females at both time points), increased cholesterol (both sexes at interim only), decreased blood urea nitrogen (males at interim only; females at both time points), decreased potassium (both sexes at interim only), increased CPK (males at terminal only), increased triglycerides (males at terminal only), decreased chloride (males at terminal only), and decreased GGT (females at terminal only). All parameters were similar to the saline control animals following a 2-week recovery period.

The only change observed in animals treated with the drug product was an increase in glucose. This change was seen in both sexes and only at the interim time point and similar to saline treated animals after the 2-week recovery period.

Table 142: Clinical Chemistry Parameters in Male Dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Alb (g/dL)	2.9	72.4%	96.6%	96.6%
	2.8	82.1%	100.0%	96.4%
Alt (U/L)	29	34.5%	93.1%	93.1%
	27	48.1%	111.1%	103.7%
Crea (mg/dL)	0.83	43.4%	90.4%	101.2%
	0.59	54.2%	91.5%	98.3%
Glu (mg/dL)	80	55.0%	120.0%	120.0%
	88	71.6%	102.3%	98.9%
LDH (U/L)	72	370.8%	90.3%	102.8%
	112	313.4%	100.9%	127.7%
GLB (g/dL)	2.5	128.0%	96.0%	92.0%
	2.5	124.0%	92.0%	92.0%
A/G	1.2	58.3%	100.0%	100.0%
	1.2	66.7%	100.0%	100.0%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Table 143: Clinical Chemistry Parameters in Female Dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Alb (g/dL)	3.1	74.2%	93.5%	96.8%
	3.0	80.0%	96.7%	96.7%
Alt (U/L)	25	48.0%	112.0%	112.0%
	24	54.2%	112.5%	104.2%
Crea (mg/dL)	0.87	51.7%	80.5%	90.8%
	0.59	54.2%	89.8%	84.7%
Glu (mg/dL)	84	79.8%	111.9%	119.0%
	92	80.4%	97.8%	106.5%
LDH (U/L)	78	174.4%	85.9%	91.0%
	100	194.0%	131.0%	93.0%
GLB (g/dL)	2.4	137.5%	95.8%	91.7%
	2.3	130.4%	100.0%	95.7%
A/G	1.3	53.8%	100.0%	107.7%
	1.3	61.5%	100.0%	107.7%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Urinalysis

Urine was collected directly from the bladder of animals at scheduled necropsy. A complete battery of urinalysis parameters was evaluated.

There were no treatment-related changes in urinalysis parameters.

Gross Pathology

Following sacrifice, all main study and recovery animals received a complete necropsy.

Macroscopic observations were limited to the injection sites. Accumulations were observed in animals treated with the vehicle and HTX-011. The incidence of accumulations appeared to be higher in HTX-011 dosed animals but a clear dose response was not apparent. Thickening of the injection site was primarily observed in vehicle treated animals with the highest incidences occurring at Injection Sites E and F, the injection sites treated closest to the end of the study. No macroscopic findings were present following the 2-week recovery period.

Table 144: Gross Observations in Male Dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Accumulation (A)	-	1	-	2
Accumulation (B)	-	-	-	1
Accumulation (D)	-	2	-	1
Accumulation (E)	-	1	2	2
Accumulation (F)	-	1	1	3
Thickened (E)	-	3	1	1
Thickened (F)	-	3	-	-

N = 4

Table 145: Gross Observations in Female Dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Accumulation (A)	-	1	-	2
Accumulation (B)	-	1	-	1
Accumulation (D)	-	2	-	-
Accumulation (E)	-	1	3	3
Accumulation (F)	-	-	1	2
Thickened (E)	-	3	1	-
Thickened (F)	-	4	2	2

N = 4

Organ Weights

The following tissues were weighed:

Adrenal Glands	Liver	Testes
Brain	Ovaries	Thymus
Epididymides	Pituitary gland	Thyroid
Heart	Prostate gland	Uterus
Kidneys	Spleen	

Changes in organ weights were primarily seen in the vehicle control group. In males, absolute and relative liver weights were increased. Following recovery, absolute liver weights were still increased but relative liver weights were similar to the saline control. Absolute and relative thymus weights were increased at terminal sacrifice and following recovery.

In females, altered organ weights were observed in the adrenal, liver, and kidney. Absolute and relative adrenal weights were increased but only absolute adrenal weight

was increased following recovery. In the main study animals, only relative liver weight was increased but following recovery an increase in absolute liver weight was also observed. Increased absolute and relative kidney weights were observed in both main study and recovery animals.

Table 146: Organ Weights in Male Dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Liver	225	278 (23%)	246 (9%)	237 (5%)
	2.94	3.88 (32%)*	3.29 (11%)	3.12 (9%)
Thymus	8.7	10.28 (18%)	8.94 (3%)	9.41 (8%)
	0.116	0.143 (23%)	0.118 (2%)	0.125 (8%)
Liver- recovery	204	254 (24%)	228 (12%)	242 (19%)
	2.88	3.00 (4%)	2.91 (1%)	3.08 (7%)
Thymus- recovery	9.63	14.60 (52%)	11.64 (21%)	6.88 (-29%)
	0.131	0.173 (32%)	0.151 (15%)	0.089 (-32%)

For each row, the top value is for absolute weight and the bottom value is for organ weight relative to body weight

N = 4 for main study and N = 2 for recovery

Table 147: Organ Weights for Female Dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Adrenal	1.12	1.48 (32%)*	1.02 (-9%)	1.16 (4%)
	0.017	0.026 (52%)*	0.016 (-6%)	0.018 (6%)
Liver	190	203 (7%)	184 (-3%)	207 (9%)
	2.95	3.54 (20%)*	2.92 (-1%)	3.24 (10%)
Kidney	31.8	37.9 (19%)	33.7 (6%)	38.6 (21%)
	0.494	0.660 (34%)*	0.533 (8%)	0.605 (22%)
Adrenal- recovery	1.08	1.24 (15%)	0.98 (-9%)	0.95 (-12%)
	0.017	0.018 (6%)	0.015 (-12%)	0.013 (-24%)
Liver-recovery	180	224 (24%)	190 (6%)	194 (8%)
	2.85	3.17 (11%)	2.88 (1%)	2.67 (-6%)
Kidney-recovery	28.8	46.0 (60%)	34.9 (21%)	36.2 (26%)
	0.454	0.651 (43%)	0.528 (16%)	0.495 (9%)

For each row, the top value is for absolute weight and the bottom value is for organ weight relative to body weight

N = 4 for main study and N = 2 for recovery

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Tissue Collection Table

Adrenals*	Pancreas
Aorta	Pituitary*
Brain* (entire)	Prostate*
Epididymides*	Salivary glands (mandibular and parotid)
Esophagus	Sciatic nerve
Eyes with optic nerve	Skeletal muscle (biceps femoris)
Femur with marrow	Skin (with mammary gland for females)
Gallbladder	Spinal cord (cervical, thoracic, lumbar)
Harderian gland	Spleen*
Heart*	Sternum (bone with marrow)
Intestines	Stomach
Cecum	Testes*
Colon	Thymus*
Duodenum	Thyroid* (parathyroids if present in the section)
Ileum (with Peyer's patches)	Tongue
Jejunum	Trachea
Rectum	Urinary bladder
Kidneys*	Uterus* (with cervix)
Larynx	Vagina
Liver*	Gross lesions
Lungs with bronchi	Identification (ear tattoo)
Lymph nodes (mandibular and mesenteric)	Injection sites A, B, C, D, E, and F (full thickness at each site, including underlying muscle)
Ovaries* (with oviducts)	

A number of histopathological findings were observed at the injection sites with treatment of either the vehicle or HTX-011 as compared to saline control where few or no findings were observed. These included chronic inflammation, granulomatous inflammation, fibrosis, presence of test material, and hemorrhage. Recovery animals were included who were treated for the same amount of time as the main study animals except they were sacrificed 14 days after treatment was stopped. It is important to note that the decreased number of animals used for recovery (two versus four) made it difficult to compare incidence back to the main experiment animals since a single finding represented 50% of the animals studied.

Chronic inflammation was described as focal, multifocal, or active/multifocal with multifocal and multifocal/active being the more prevalent types of chronic inflammation observed. For active multifocal chronic inflammation, the highest incidences and most severe findings were seen at Injection Sites E and F, the injection sites treated closest to the end of the study, with both the vehicle and HTX-011. Overall, the highest incidences and average severity was observed with treatment of the vehicle and overall incidence for all treatments was higher in females. The overall severity and incidence were lower at injection sites that had a longer time between treatment and necropsy for all treatments. Active chronic multifocal inflammation was not observed at a significant level in recovery animals.

In contrast, multifocal chronic inflammation incidence and severity seemed to be higher at injection sites where there was a longer time between the last injection and necropsy with both the vehicle and HTX-011. As with active chronic multifocal inflammation a higher incidence of findings were observed in females but in this case it appeared that the greatest incidence and severity was seen with the high dose of HTX-011. Chronic multifocal inflammation was also observed in recovery animals. Again, findings were primarily in females and with HTX-011 (both doses). The incidence and severity seemed to decrease at injection sites that had longer time since last injection such that findings were not really seen in either sex around 28-30 days.

Taken together, it appears that active chronic multifocal inflammation is seen early following treatment, which transitions to a chronic multifocal inflammation. Based on the female data, where findings were more prevalent, this occurs around 12 days following injection and the chronic active multifocal inflammation lasts until 28 days following injection.

Granulomatous inflammation was primarily seen in female animals treated with the vehicle (all injection sites) and the high dose of HTX-011 (Injection Sites A, B, E, and F). It was also observed in males receiving the high dose at Sites A, B, C, and D. Overall, the average severity was minimal to mild. It was also observed in recovery females at all injection sites except A, although the overall severity was decreased. Taken together, the data suggests that the incidence and severity of granulomatous inflammation peaks around Day 16, decreases with time since injection, and was completely resolved by Day 30.

Fibrosis was described as either focal or multifocal with multifocal being the more prevalent form. Multifocal fibrosis was observed in both sexes with both the vehicle and HTX-011. The overall severity of fibrosis was high at multiple injection sites with average severity values of moderate to marked. Fibrosis was observed at all injection sites in main study animals, suggesting it lasts at least 16 days following injection. Findings of fibrosis were still observed in some recovery animals. Based on the observed injection sites, fibrosis with an average severity of mild to marked was observed in female animals up to 26 days after the last injection. No observations of fibrosis were seen at Injection Sites A or B indicating that fibrosis was completely resolved 28-30 days post injection.

Hemorrhage was observed in both males and females receiving the vehicle and HTX-011. In both sexes, findings were more prevalent with the vehicle and the high dose. The majority of the findings were seen at Injection Sites E and F suggesting that hemorrhage resolved by Day 10-12. Hemorrhage was not observed at a significant level in recovery animals.

The presence of test material at the injection site was seen in some animals and defined as either focal or multifocal, with multifocal being more prevalent. In males, the presence of multifocal test material was seen with an incidence of 50% or more with the vehicle at Injection Site F, and the high dose at Injection Site F and D. In females, the presence of multifocal test material was seen with an incidence of 50% or more with the vehicle at Injection Site E and F, the low dose at Injection Site E, and the high dose at Injection Site E and F. This suggests that the test material was only present at the injection site for up to 10 days. However, test material was seen in individual animals with severity scores ranging from minimal to marked up to 16 days after injection. The test material was not observed in recovery animals.

In summary, treatment with the vehicle lead to an increase in incidence/severity of chronic active inflammation, granulomatous inflammation, hemorrhage, and fibrosis compared to the saline control. These findings were primarily in female animals and were mostly not present in recovery animals, with some exceptions. In some cases, findings with the vehicle were worse than with HTX-011 (active chronic inflammation, and fibrosis). This is interesting since the vehicle had a similar concentration of excipients as HTX-011 (the vehicle did not contain maleic acid and has slightly less AP135). The major difference was a much larger injection volume of the vehicle at the beginning of the study (1 vs. 0.44 mL/kg). Thus, despite being local effects, the increased absolute amount of the excipients could have played a role in these findings.

Treatment with HTX-011 lead to an increase in incidence/severity of chronic active inflammation, granulomatous inflammation, hemorrhage, and fibrosis compared to the saline control. In general, females were more affected than males but it is unclear why there would be a sex difference for these local effects. For many of the observed findings there was a slight increase in the severity and incidence with the high dose of HTX-011 compared to the low dose of HTX-011. It is unclear why this would be the case since the dose was controlled by injecting different volumes of the same concentration solution. It appears that the local effects are somewhat related to the absolute amount of the solution present which is consistent with the increased findings with the vehicle which had a much larger injection volume. Taken together, it appears that the majority of the findings are likely related to the presence of one or more excipients in the drug product.

Table 148: Percentage of Main Study Animals with Chronic Multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	25 (1)	0	0	25 (2)	25 (1)	0	25 (1)	25 (1)
E (4)	0	0	0	75 (1.3)	25 (1)	0	50 (1)	50 (1)
D (10)	0	25 (1)	25 (1)	25 (1)	0	25 (1)	0	50 (1)
C (12)	0	25 (1)	25 (1)	25 (2)	25 (1)	50 (1)	50 (1)	75 (1.3)
B (14)	0	0	50 (1.5)	0	0	0	25 (1)	75 (1.7)
A (16)	0	0	75 (1)	0	0	50 (1.5)	25 (1)	100 (2)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 149: Percentage of Recovery Animals with Chronic Multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	0	50 (1)	50 (2)	0	50 (1)	50 (2)	50 (1)
E (18)	0	50 (1)	0	50 (1)	0	0	50 (2)	50 (2)
D (24)	0	50 (1)	0	0	0	0	50 (1)	50 (2)
C (26)	0	0	0	50 (1)	0	0	50 (1)	50 (1)
B (28)	0	0	0	0	0	0	50 (1)	0
A (30)	0	0	50 (1)	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 150: Percentage of Main Study Animals with Active Chronic Multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	75 (3.7)	75 (1.3)	50 (2)	25 (1)	75 (3.3)	25 (3)	75 (3.3)
E (4)	0	75 (1.7)	25 (1)	25 (2)	0	100 (3.8)	50 (2)	50 (2.7)
D (10)	0	25 (2)	0	50 (2.5)	0	50 (2.5)	50 (2)	0
C (12)	0	0	50 (2)	25 (1)	0	25 (3)	0	25 (1)
B (14)	0	0	0	50 (1.5)	0	75 (2.3)	25 (1)	0
A (16)	0	0	0	50 (1.5)	0	75 (2.3)	25 (1)	0

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 151: Percentage of Main Study Animals with Multifocal Granulomatous Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	0	25 (1)	0	0	50 (1)	0	50 (1.5)
E (4)	0	0	0	0	0	50 (1)	25 (1)	50 (1.5)
D (10)	0	0	0	25 (1)	0	75 (1.7)	25 (1)	0
C (12)	0	0	50 (1)	50 (1)	0	50 (1.5)	0	0
B (14)	0	0	0	25 (1)	0	75 (2)	25 (1)	75 (1.3)
A (16)	0	0	0	25 (2)	0	50 (1)	0	75 (1.7)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 152: Percentage of Recovery Animals with Multifocal Granulomatous Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	0	0	0	0	100 (1.5)	50 (1)	0
E (18)	0	0	0	0	0	0	50 (1)	0
D (24)	0	0	0	0	0	50 (1)	50 (1)	0
C (26)	0	0	0	0	0	50 (1)	50 (1)	50 (1)
B (28)	0	0	0	0	0	0	50 (1)	50 (1)
A (30)	0	0	0	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 153: Percentage of Main Study Animals with Multifocal Fibrosis

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	100 (3.8)	0	0	0	75 (4.3)	50 (3.5)	25 (4)
E (4)	0	100 (4.5)	25 (1)	0	0	100 (3)	25 (2)	75 (2.7)
D (10)	0	100 (1.8)	0	75 (1.3)	0	75 (2.7)	25 (2)	25 (1)
C (12)	0	50 (3)	50 (1.5)	50 (2)	0	75 (1.7)	50 (1.5)	50 (1.5)
B (14)	0	25 (3)	25 (2)	0	0	50 (3.5)	0	75 (2.7)
A (16)	0	0	25 (2)	25 (2)	0	75 (2.3)	0	25 (3)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 154: Percentage of Recovery Animals with Multifocal Fibrosis

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	50 (1)	0	0	0	0	50 (1)	0
E (18)	0	0	0	0	0	50 (4)	0	50 (2)
D (24)	0	50 (1)	0	0	0	100 (2.5)	0	0
C (26)	0	0	0	0	0	0	0	50 (2)
B (28)	0	0	0	0	0	0	0	0
A (30)	0	0	0	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 155: Percentage of Main Study Animals with Multifocal Hemorrhage

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	25 (3)	0	50 (3)	0	75 (1.7)	50 (1.5)	75 (2)
E (4)	0	75 (1.7)	0	0	0	50 (2.5)	0	50 (1.5)
D (10)	0	25 (1)	0	50 (1.5)	0	0	0	0
C (12)	0	0	25 (1)	25 (1)	0	0	0	25 (1)
B (14)	0	0	0	25 (2)	0	0	0	0
A (16)	0	0	0	0	0	0	0	0

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 156: Percentage of Main Study Animals with Multifocal Presence of Test Material

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	50 (3.5)	25 (2)	75 (4.3)	0	50 (4)	25 (3)	75 (4)
E (4)	0	0	25 (4)	25 (4)	0	50 (4)	50 (3)	50 (3.5)
D (10)	0	25 (2)	0	75 (1.7)	0	25 (4)	25 (1)	0
C (12)	0	0	25 (2)	0	0	0	0	25 (4)
B (14)	0	0	0	0	0	0	0	0
A (16)	0	0	25 (2)	0	0	0	0	25 (4)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Toxicokinetics

All animals were bled via the jugular vein on Days 1, 9, and 27. Urine was collected from vehicle and high-dose animals in Week 3 for analysis of polymer breakdown products.

Bupivacaine

Bupivacaine C_{max} increased with time; a large increase was seen between Day 1 and Day 9 and a smaller increase between Day 9 and Day 27. C_{max} values were similar between the low- and high-dose groups, which isn't surprising considering how closely spaced the doses were. There was a good deal of variability to the T_{max} with values ranging from 1-8 hours across the study. There did not appear to be any time or dose trend to the T_{max} values. Over the course of the experiment, females generally had higher T_{max} values. The AUC values increased with time; again a larger increase was seen between Day 1 and Day 9 compared to Day 9 and Day 27. AUC values were generally comparable between the low- and high-dose groups in females. In males, AUC in the high-dose group tended to be higher (27-61%) and was generally in line with the increased amount of bupivacaine in the high dose (30%).

Table 157: Bupivacaine PK Parameters

Dose (mg/kg/dose)	Day	Sex		C _{max} (ng/mL)	t _{max} (h) ^a	AUC _{0-48h} (ng•h/mL)
10	1	Male	Mean	289	1	7460
			SD	160	1-4	2010
			n	6	6	6
		Female	Mean	315	5	11100
			SD	68.7	1-12	3010
			n	6	6	6
	9	Male	Mean	514	1	13900
			SD	152	1-4	3120
			n	6	6	6
		Female	Mean	567	1	15100
			SD	126	0.5-6	3700
			n	6	6	6
27	Male	Mean	424	2.5	11600	
		SD	152	1-4	4050	
		n	6	6	6	
	Female	Mean	736	1	18400	
		SD	693	0.5-4	13000	
		n	6	6	6	
13	1	Male	Mean	280	4	10500
			SD	80.1	1-24	2910
			n	6	6	6
		Female	Mean	271	8	9890
			SD	53.5	1-48	1760
			n	6	6	6
	9	Male	Mean	581	1	17600
			SD	142	1-1	4330
			n	6	6	6
		Female	Mean	498	4	15600
			SD	106	1-24	2710
			n	6	6	6
	27	Male	Mean	639	1	18700
			SD	183	1-4	6500
			n	6	6	6
		Female	Mean	622	2.5	17500
			SD	312	1-4	8230
			n	6	6	6

^a t_{max} is reported as median and minimum-maximum for group summary statistics instead of mean and standard deviation (SD).

Meloxicam

Meloxicam C_{max} increased with time; a large increase was seen between Day 1 and Day 9 and there was essentially no difference between Day 9 and Day 27. C_{max} values were slightly higher with the high dose, which is in line with the small difference between the two dose groups. There was a good deal of variability to the T_{max} with values ranging

from 0.75-48 hours across the study. The majority of the T_{max} values were on the higher end (18-48 hours). There did not appear to be any time, sex, or dose trend to the T_{max} values. The AUC values increased with time; again a larger increase was seen between Day 1 and Day 9 compared to Day 9 and Day 27. AUC values in the high dose group tended to be higher (15-44%) and were generally in line with the increased amount of meloxicam in the high dose (30%).

Table 158: Meloxicam PK Parameters

Dose (mg/kg/dose)	Day	Sex		C _{max} (ng/mL)	t _{max} (h) ^a	AUC _{0-48h} (ng•h/mL)
0.30	1	Male	Mean	359	36	13100
			SD	57.0	24-48	2200
			n	6	6	6
		Female	Mean	370	48	12700
			SD	78.1	24-48	2750
			n	6	6	6
	9	Male	Mean	760	18	30900
			SD	146	4-24	6010
			n	6	6	6
		Female	Mean	749	0.75	28700
			SD	212	0.5-24	8100
			n	6	6	6
27	Male	Mean	876	8	35500	
		SD	118	4-24	5480	
		n	6	6	6	
	Female	Mean	728	3.5	30400	
		SD	228	0.5-48	9300	
		n	6	6	6	
0.39	1	Male	Mean	412	36	15200
			SD	33.8	24-48	1630
			n	6	6	6
		Female	Mean	470	48	15500
			SD	95.0	24-48	3480
			n	6	6	6
	9	Male	Mean	1050	24	43100
			SD	342	4-24	15500
			n	6	6	6
		Female	Mean	895	18	33700
			SD	343	0-24	11700
			n	6	6	6
27	Male	Mean	1150	4	45500	
		SD	272	4-24	13000	
		n	6	6	6	
	Female	Mean	1050	36	45400	
		SD	207	0.5-48	8790	
		n	6	6	6	

^a t_{max} is reported as median and minimum-maximum for group summary statistics instead of mean and standard deviation (SD).

Polymer breakdown products

Urine collected on Day 22 was analyzed for the presence of breakdown products of the polymer (AP135) using a non-validated method for exploratory purposes. The breakdown products measured were (b) (4)

(b) (4) For all compounds, the levels were over the calibration range and extrapolated values were reported when available. For (b) (4) the values were above the calibration range (b) (4) mcg/mL) but estimated values were not reported. Excluding (b) (4) the most prevalent breakdown product was (b) (4) followed by (b) (4) and then (b) (4)

Dosing Solution Analysis

The vehicle was analyzed at the end of the study (March 6, 2018) to compare to the results from the Certificate of Analysis provided by the Applicant dated March 19th 2018. There were no differences between the CoA and the analysis. Meloxicam and bupivacaine were not detected in the vehicle.

HTX-011 was not analyzed. The Applicant provided characterization of the test substance including stability data. The CoA for Lot 026517 indicated that the test substance had 99.9% bupivacaine and 97.3% meloxicam with a retest date of November 2018 (24 months after manufacture) that is later than the end of Study 33-86.

7 Genetic Toxicology

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

Study title:

Study no.: 33-11
 Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\33-11\33-11.pdf>

Conducting laboratory and location:

(b) (4)

Date of study initiation: July 18, 2014
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: HTX-011-15 (10.0% bupivacaine, 1.5% meloxicam, 72.29% APF135, 1.21% maleic acid, (b) (4) Lot 1257021, not applicable since it is a mixture

Key Study Findings

The study was considered invalid due to a very low response from the positive controls that was well below the laboratories historical control values.

Methods

Strains: TA1535, TA1537, TA98, TA100, and WP2 *uvrA*

Concentrations in definitive study: 50, 158, 500, 1581, and 5000 mcg/plate

Basis of concentration selection: Based on maximum recommended dose in ICH S2 (R1)

Negative control: DMSO

Positive control: Sodium azide (TA1535 and TA100, without metabolic activation), 9-aminoacrididine hemihydrate (TA1537 without metabolic activation), 2-nitrofluorene (TA98 without metabolic activation), 4-nitroquinoline N-oxide (WP2 *uvrA* without metabolic activation), 2-aminoanthracene (TA 1535 and WP2 *uvrA*, with metabolic activation), and benzo[a]pyrene (TA1537, TA98, and TA100, with metabolic activation)

Formulation/Vehicle: HTX-011-15 was dissolved in DMSO

Incubation & sampling time: An aliquot of 0.5 mL of S9 mix (+S9) or phosphate buffer 0.1 M pH 7.4 (0S9) was combined with 0.1 mL bacterial culture in a sterile container. Appropriate volume, as indicated in the study design, of the test/negative and positive control items was added. Molten top agar (approximately 2 mL) supplemented with 0.05 mM biotin and minimal (0.05 mM) histidine and minimal (0.05 mM) tryptophan was added to the sample. The solution was mixed and overlaid on a minimal glucose plate (1.5% agar, Vogel-Bonner medium E, 2% glucose). After the overlay solidified, the plates were inverted and placed in an incubator set to maintain 37°C for 48 to 72 hours. The sterile containers and the plates were labeled with the ^{(b) (4)} study number and plate identification number.

Study Validity

The following criteria were determined to be valid:

- Selection of bacterial tester strains was adequate based upon Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (ICH-S2(R1); June 2012).
- Historical data were provided.
- Cytotoxicity was properly evaluated.
- The appropriate positive and negative controls were employed according to OECD guideline no. 471
- All strains contain the appropriate genetic markers.
- Dose selection for the plate incorporation were adequate based upon use of the limit dose (i.e., 5000 mcg/plate).
- The S9 concentration was within acceptable limits.

However, the assay was determined to be invalid because the responses from the positive controls were well below the historical averages for the laboratory (see tables below).

Results

Overall there was not a positive response with the HTX-011-15 formulation in the presence and absence of S9. The positive controls did cause a small increase in the number of revertant colonies but in many cases they were barely 2-fold above the vehicle control. Generally a positive response is a doubling or tripling over the negative control. The laboratory included historical control data. The positive controls were far below the historical control values for the laboratory. Therefore, the assay is considered invalid.

Table 159: Plate Incorporation in Absence of S9

Strains	Dose level (µg/plate of RYR)	Number of revertants			Mean	SD	Background observations			Fold response†
		x1	x2	x3			x1	x2	x3	
TA1535	DMSO	1	1	1	1.0	0.0				
	50	2	1	0	1.0	1.0				1.0
	158	0	2	4	2.0	2.0				2.0 ^b
	500	2	3	1	2.0	1.0				2.0 ^b
	1581	1	2	1	1.3	0.6				1.3
	5000	1	1	1	1.0	0.0				1.0
TA1537	DMSO	12	8	8	9.3	2.3				
	50	10	8	8	8.7	1.2				0.9
	158	7	6	11	8.0	2.7				0.9
	500	9	13	15	12.3	3.1				1.3
	1581	5	5	9	6.3	2.3				0.7
	5000	22	12	12	15.3	5.8				1.6
TA98	DMSO	9	12	6	9.0	3.0				
	50	8	7	10	8.3	1.5				0.9
	158	1	6	7	4.7	3.2				0.5
	500	5	6	10	7.0	2.7				0.8
	1581	7	5	1	4.3	3.1				0.5 ^a
	5000	5	9	6	6.7	2.1				0.7
TA100	DMSO	44	50	32	42.0	9.2				
	50	41	46	66	51.0	13.2				1.2
	158	36	27	54	39.0	13.8				0.9
	500	36	30	41	35.7	5.5				0.9
	1581	20	24	42	28.7	11.7				0.7
	5000	31	21	34	28.7	6.8				0.7
WP2 uvrA	DMSO	4	4	3	3.7	0.6				
	50	3	8	4	5.0	2.7				1.4
	158	5	5	5	5.0	0.0				1.4
	500	4	5	4	4.3	0.6				1.2
	1581	3	6	7	5.3	2.1				1.5
	5000	2	3	2	2.3	0.6				0.6

Comments on plate and/or background lawn (as appropriate) : Normal (no comment), MC: Methylcellulose

†: Fold response in mean revertants compared to concurrent vehicle; SD: Sample standard deviation

^a: Not biologically relevant decrease; ^b: Not biologically relevant positive response

The table above is from page 18 of the study report.

Table 160: Plate Incorporation in Presence of S9

Strains	Dose level (µg/plate of RZR)	Number of revertants			Mean	SD	Background observations			Fold response†
		<i>x1</i>	<i>x2</i>	<i>x3</i>			<i>x1</i>	<i>x2</i>	<i>x3</i>	
TA1535	DMSO	4	8	2	4.7	3.1				
	50	3	4	3	3.3	0.6				0.7
	158	1	1	3	1.7	1.2				0.4
	500	3	1	7	3.7	3.1				0.8
	1581	3	5	6	4.7	1.5				1.0
	5000	2	3	3	2.7	0.6				0.6
TA1537	DMSO	7	15	13	11.7	4.2				
	50	13	10	9	10.7	2.1				0.9
	158	5	10	10	8.3	2.9				0.7
	500	9	8	7	8.0	1.0				0.7
	1581	13	11	6	10.0	3.6				0.9
	5000	6	5	5	5.3	0.6				0.5T
TA98	DMSO	16	27	23	22.0	5.6				
	50	12	23	16	17.0	5.6				0.8
	158	14	14	18	15.3	2.3				0.7
	500	18	16	18	17.3	1.2				0.8
	1581	12	11	9	10.7	1.5				0.5 ^a
	5000	8	15	13	12.0	3.6				0.6
TA100	DMSO	35	48	51	44.7	8.5				
	50	55	36	54	48.3	10.7				1.1
	158	51	62	43	52.0	9.5				1.2
	500	44	42	66	50.7	13.3				1.1
	1581	39	54	46	46.3	7.5				1.0
	5000	44	38	35	39.0	4.6				0.9
WP2 <i>uvrA</i>	DMSO	10	7	8	8.3	1.5				
	50	6	9	10	8.3	2.1				1.0
	158	5	5	9	6.3	2.3				0.8
	500	6	4	10	6.7	3.1				0.8
	1581	8	6	1	5.0	3.6				0.6
	5000	2	2	4	2.7	1.2				0.3T

Comments on plate and/or background lawn (as appropriate) : Normal (no comment)

†: Fold response in mean revertants compared to concurrent vehicle; SD: Sample standard deviation

T: Toxic; ^a: Non biologically relevant decrease

The table above is from page 19 of the study report.

Table 161: Positive Control Data

Strains	S9	Compound	Dose level (µg/plate)	Number of revertants			Mean	SD	Fold response†
				x1	x2	x3			
TA1535	-	SAZ	0.5	71	78	91	80.0	10.1	80.0
TA1537	-	9AC	20	7	22	26	18.3	10.0	2.0
TA98	-	2NF	1.25	11	29	30	23.3	10.7	2.6
TA100	-	SAZ	0.5	111	117	72	100.0	24.4	2.4
WP2 <i>uvrA</i>	-	NQO	0.5	9	5	11	8.3	3.1	2.3
TA1535	+	2AA	5	35	46	52	44.3	8.6	9.4
TA1537	+	B[a]P	5	34	29	36	33.0	3.6	2.8
TA98	+	B[a]P	2	167	165	174	168.7	4.7	7.7
TA100	+	B[a]P	5	568	586	518	557.3	35.2	12.5
TA100	+	B[a]P	10	497	481	521	499.7	20.1	11.2
WP2 <i>uvrA</i>	+	2AA	15	28	16	27	23.7	6.7	2.9

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

The table above is from page 20 of the study report

Table 162: Historical Control Data

Vehicle controls, without S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	18.0	11.7	25.0	141.6	26.8
Standard deviation	12.9	3.0	6.4	24.9	7.2
Minimum	6	7	14	105	16
Maximum	64	19	38	185	40
Number of experiments	6	6	6	6	6
Vehicle controls, with S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	15.7	13.5	34.0	141.4	29.2
Standard deviation	5.3	5.7	9.0	25.1	9.2
Minimum	6	4	22	90	14
Maximum	25	28	56	196	52
Number of experiments	6	6	6	6	6
Positive controls, without S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	489.2	327.7	182.6	611.9	140.2
Standard deviation	88.9	166.5	38.5	189.5	51.4
Minimum	400	152	137	400	84
Maximum	626	697	237	875	222
Number of experiments	3	3	3	3	3
Positive controls, with S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	229.3	-	134.0	-	107.9
Standard deviation	98.7	-	28.2	-	28.8
Minimum	91	-	100	-	69
Maximum	347	-	191	-	163
Number of experiments	3	-	3	-	3

Positive controls without S9 Mix

TA1535 SAZ (0.5 µg/plate)
 TA1537 9AC (20 µg/plate)
 TA98 2NF (1.25 µg/plate)
 TA100 SAZ (0.5 µg/plate)
 WP2 *uvrA* NQO (0.5 µg/plate)

Positive controls with S9 Mix

TA1535 2AA (5 µg/plate)
 TA98 B[a]P (2 µg/plate)
 WP2 *uvrA* 2AA (5 µg/plate)

Table above is from page 22 of the study report.

The Applicant is also relying upon the data for the two drug substances as described in the referenced drug product labels.

The referenced Marcaine label (NDA 16964) contains the following information:

The mutagenic potential and the effect on fertility of bupivacaine hydrochloride have not been determined.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

Meloxicam was not mutagenic in an Ames assay, or clastogenic in a chromosome aberration assay with human lymphocytes and an in vivo micronucleus test in mouse bone marrow.

Although the exposure to bupivacaine via this drug product technically exceeds that of the referenced drug product, the increase in exposure is not considered to be of the magnitude that would warrant requiring an Applicant to conduct these studies for an older drug product.

8 Carcinogenicity

Carcinogenicity studies with HTX-011 are not required for an acute-use drug.

The referenced Marcaine label (NDA 16964) contains the following information:

Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine hydrochloride have not been conducted.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

No carcinogenic effect of meloxicam was observed in rats given oral doses up to 0.8 mg/kg/day (approximately 0.4-fold the human dose at 15 mg/day for a 50 kg adult based on body surface area conversion) for 104 weeks or in mice given oral doses up to 8.0 mg/kg/day (approximately 2.2-fold the human dose, as noted above) for 99 weeks.

Exposure margins will be adjusted for this drug product maximum daily dose of meloxicam.

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicity studies were conducted with HTX-011. The Applicant is relying upon the Agency previous finding of safety of bupivacaine and

meloxicam via the 505(b)(2) pathway. A combination embryo-fetal development study was not required because the existing data on meloxicam and bupivacaine, as described in the referenced product labeling, already report significant adverse effects at doses below the clinical dose and the pharmacological and systemic toxicological profile of the two drugs do not suggest the potential for additive toxicity.

As part of the requirement under the Pregnancy Lactation and Labeling Rule (PLLR), the Applicant conducted a review of the nonclinical literature for bupivacaine (1972 to 2018) and meloxicam (1990 to 2018). Many of the articles referenced by the Applicant were already previously reviewed during recent label updates for meloxicam and bupivacaine containing products. As such, these articles were already taken into consideration as part of the labeling of other meloxicam and bupivacaine products and the labeling for HTX-011 will reflect the existing data in the referenced labels adjusted for dose plus any relevant information identified in the PLLR updates for meloxicam and bupivacaine products. The following section describes the literature data submitted by the Applicant that was not evaluated for other products and the relevance of these studies for labeling.

In addition, the Applicant submitted an embryo-fetal developmental study with maleic acid to justify the safety of this excipient and is reviewed below in Section 9.2.

The referenced Marcaine label (NDA 16964) contains the following information:

The mutagenic potential and the effect on fertility of bupivacaine hydrochloride have not been determined.

Pregnancy

Bupivacaine hydrochloride produced developmental toxicity when administered subcutaneously to pregnant rats and rabbits at clinically relevant doses.

Bupivacaine hydrochloride was administered subcutaneously to rats at doses of 4.4, 13.3, & 40 mg/kg and to rabbits at doses of 1.3, 5.8, & 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). The high doses are comparable to the daily maximum recommended human dose (MRHD) of 400 mg/day on a mg/m² body surface area (BSA) basis. No embryo-fetal effects were observed in rats at the high dose which caused increased maternal lethality. An increase in embryo-fetal deaths was observed in rabbits at the high dose in the absence of maternal toxicity with the fetal No Observed Adverse Effect Level representing approximately 1/5th the MRHD on a BSA basis.

In a rat pre- and post-natal development study (dosing from implantation through weaning) conducted at subcutaneous doses of 4.4, 13.3, & 40 mg/kg, decreased pup survival was observed at the high dose. The high dose is comparable to the daily MRHD of 400 mg/day on a BSA basis.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

Meloxicam did not impair male and female fertility in rats at oral doses up to 9 and 5 mg/kg/day, respectively (4.9-fold and 2.5-fold the human dose, as noted above). However, an increased incidence of embryoletality at oral doses ≥ 1 mg/kg/day (0.5-fold the human dose, as noted above) was observed in rats when dams were given meloxicam 2 weeks prior to mating and during early embryonic development.

Exposure margins will be adjusted for this drug product based on the maximum daily dose of meloxicam.

Bupivacaine and Pregnancy/Lactation – Animals literature review of studies not previously reviewed for other bupivacaine and meloxicam products

Intravenous injection of bupivacaine in the pregnant ewe. (Craft et al., 1979)

Objective: To evaluate the effect of bolus intravenous injections of bupivacaine on maternal and fetal cardiovascular dynamics, catecholamines, uterine blood flow and fetal acid-base status

Method: Bupivacaine (10, 20, 30, or 40 mg) was injected *intravenously* into eight pregnant ewes 24 hours following hysterotomy under anesthesia. Maternal, uterine and fetal vascular dynamics were monitored continuously. Maternal and fetal arterial blood samples were obtained at 1, 3, 5, 15, 30, 45, and 60 minutes after injection for acid-base, local anesthetic, and catecholamine determination.

Key Findings:

- 10 mg of bupivacaine increased maternal cardiac output but did not affect maternal or fetal acid-base status.
- 40 mg of bupivacaine resulted in sustained fetal hypoxemia resulting from decreased uterine blood flow, increased uterine tone, and decreased maternal PaO₂.

Reviewer's note: The study data are from a meeting abstract with limited information. The results are mechanistic in nature. While they describe an effect on the fetus at 40 mg in terms of decreased PaO₂, it is unclear what the toxicological significance of this finding would be. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Transfer of bupivacaine, lignocaine, and pethidine across the rabbit placenta: influence of maternal protein binding and fetal flow. (Hamshaw-Thomas et al., 1984)

Objective: To evaluate the effect of umbilical flow on the placental transfer of compounds (including bupivacaine) with differing lipid solubility and protein binding

Method: Bupivacaine, lignocaine, pethidine (1.25 mg/mL each), and antipyrine (4.0 mg/mL) were infused intravenously into a neck vein of 17 anesthetized pregnant New Zealand white rabbits (3.44 to 4.95 kg) within three days of term (30 days) at a rate of 12.0 mL/h for 20 minutes and 6.0 mL/h thereafter. The rabbit placentas were perfused in situ under different flow rates. Placental perfusions and maternal blood samples were analyzed for bupivacaine, lignocaine, pethidine, and antipyrine. Maternal and fetal plasma were analyzed for protein binding.

Key Findings:

- Transfer rates across the placenta at flow rates of 0.5 to 2.0 mL/min reflected the compounds protein binding not their lipid solubilities.
- Bupivacaine, which had the highest % drug bound (76.5%), had the lowest transfer rates of the compounds studied.

Reviewer's note: The study is mechanistic in nature and only describes the ability of bupivacaine to cross the placenta. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Placental transfer of bupivacaine, pethidine and lignocaine in the rabbit. Effect of umbilical flow rate and protein content. (Hamshaw-Thomas et al., 1985)

Objective: To evaluate the effects of protein binding, fetal perfusate glycoprotein content, and umbilical flow on placental transfer of bupivacaine, lignocaine and pethidine with antipyrine

Method: Bupivacaine, lignocaine, pethidine (1.25 mg/mL each), and antipyrine (4.0 mg/mL) were infused intravenously into 11 anesthetized pregnant New Zealand white rabbits (4.4 to 5.5 kg) within three days of term (30 days) at a rate of 12.0 mL/h for 20 minutes and 6.0 mL/h thereafter. The rabbit placentas were perfused in situ under different flow rates. Placental perfusions and maternal blood samples were analyzed for bupivacaine, lignocaine, pethidine, and antipyrine. Maternal and fetal plasma were analyzed for protein binding.

Key Findings:

- Protein on the fetal side enhanced transfer of highly bound drugs, such as bupivacaine.
- Clearance of the drugs was flow-dependent.

Reviewer's note: The study is mechanistic in nature and only describes the ability of bupivacaine to cross the placenta. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal

development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

The placental transfer of bupivacaine and lidocaine and their myocardial and cerebral uptake in the rabbit. (Hollmen et al., 1973)

Objective: To compare the extent of binding of lidocaine and bupivacaine to fetal heart and brain in the rabbit when injected intravenously

Method: Tritiated bupivacaine (0.3 mg/kg) or lidocaine (0.6 mg/kg) were administered IV to 40 near-term anesthetized pregnant rabbits. Caesarian section was performed at 2, 5, 8, 20, and 45 minutes after injection and fetuses were sacrificed. Maternal heart and arterial blood were also collected. Maternal heart, maternal blood, fetal heart, fetal brain, and fetal ventricular blood were all analyzed for bupivacaine and lidocaine.

Key Findings:

- Fetal heart/blood and brain/blood ratios were lower for bupivacaine as compared to lidocaine.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine can cross the placenta and reach the fetal heart and brain to some extent. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

In vivo comparison of the effects of bupivacaine and levobupivacaine on the pregnant rat myometrium using electrohysterogram. (Kaynak et al., 2012)

Objective: To compare the effects of bupivacaine and levobupivacaine on rat uterine contractility in an in vivo setting

Method: 20 rats (23 days pregnant) weighing 150-200 g where anesthetized and underwent laparotomy. Electrical activity of the uterus was measured every five minutes for a total of 35 minutes. Bupivacaine or levobupivacaine were administered intramyometrially at a dose of 2 mg/kg after the first recording (at five minutes).

Key Findings:

- Both compounds led to lower frequencies of uterine contractions compared to the controls.
- Levobupivacaine led to less muscle relaxation as compared to bupivacaine.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine can decrease uterine contractions. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal

development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Uptake and distribution of bupivacaine in fetal lambs. (Kennedy et al., 1986)

Objective: To determine the uptake and distribution of bupivacaine in fetal lambs

Method: Bupivacaine (3 mg/kg) was infused into a jugular vein at a constant rate over 1 hour into five pregnant ewes. It is unclear at what time of pregnancy the ewes were, but single pregnancy was confirmed by x-ray. Following injection of bupivacaine, blood was sampled from the maternal femoral artery, the umbilical vein, and the fetal distal aorta. After five hours the fetuses were killed, removed, and dissected.

Key Findings:

- Fetal blood concentrations were less than maternal blood concentrations.
- PPX was not found in urine or tissue samples suggesting that neither the mother nor fetus was metabolizing bupivacaine.
- Bupivacaine diffused back from the fetus to the mother as evidenced by a lower umbilical vein concentration compared to the fetal aorta concentration.
- Fetal uptake was linear over the course of the infusion.
- The high correlation of mean fetal bupivacaine uptake to the maternal dose suggests a flow-independent fetal uptake.
- The highest levels of fetal bupivacaine were in the lung (~0.09 nmoles/g tissue). Bupivacaine was also detected in the brain (~0.06 nmoles/g tissue), kidney (~0.08 nmoles/g tissue), heart (~0.06 nmoles/g tissue), and liver (~0.06 nmoles/g tissue).

Reviewer's note: The study is mechanistic in nature and describes placental transfer of bupivacaine in sheep, including distribution data in the fetus. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Effects of spinal blockade with bupivacaine on parturition in rats. (Li et al., 1985)

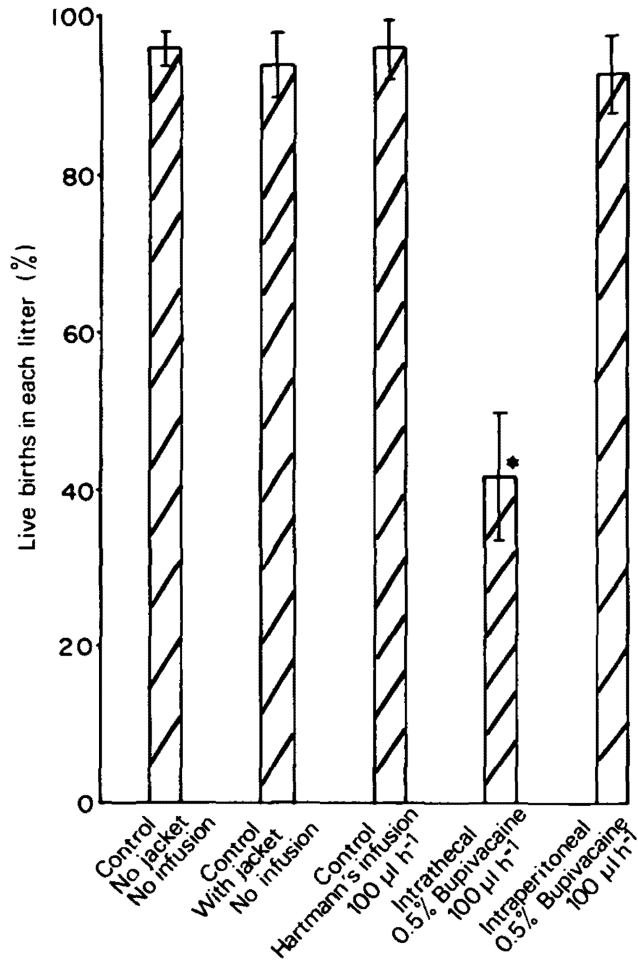
Objective: To observe the effects of differential degrees of motor and sensory blockade on the parturition process

Method: Pregnant Wistar rats (180-220 g prior to pregnancy) were anesthetized on the 16-18th day of gestation and fitted with a catheter in the intrathecal space. The animals were divided into five groups: 1) control with no jacket or infusion, 2) control with jacket

but no infusion, 3) 100 mcL/h infusion of Hartmann's solution, 4) 100 mcL/h infusion of 0.5% bupivacaine intrathecal, 5) 100 mcL/h infusion of 0.5% bupivacaine intraperitoneal. The rats were then left until delivery was complete monitored by video.

Key Findings:

- There was no difference in the number of pups per litter.
- Delivery was ~3 times longer in the group receiving intrathecal blockade with 0.5% bupivacaine. This appeared to be due to a lack of maternal effort.
- The mean percentage of live births per litter surviving the first 6 hours was reduced in the group receiving intrathecal blockade with 0.5% bupivacaine. (42% vs. 94-96% in the control groups). There was no reduction in the group receiving IP bupivacaine (93%).



Reviewer's note: The study describes an effect on fetal mortality following intrathecal administration of bupivacaine. A similar effect was not observed following IP administration. The authors attributed the effects on perinatal mortality to the prolonged

labor and not to a direct effect on the pups, which is supported by the lack of an effect following IP dosing. The information in this report is supportive of the embryo-fetal mortality but does not add any additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Effects of levobupivacaine and bupivacaine on rat myometrium. (Li et al., 2006)

Objective: To study the effect of levobupivacaine and bupivacaine on the contractility of isolated muscle strips from pregnant and non-pregnant rats.

Method: Pregnant rats (18-21 days) and non-pregnant female rats (~200 g) were treated with 40 mcg diethylstilbestrol by SC injection for two days to induce estrus. Rats were killed and myometrial strips were prepared. Once contractions of the strips became regular, baseline frequency and amplitude were measured followed by addition of either bupivacaine or levobupivacaine.

Key Findings:

- In non-pregnant rat myometrial strips, both bupivacaine and levobupivacaine (at cumulative concentrations ranging from 10^{-8} to 10^{-4} mol/L) decreased contractile activity. Levobupivacaine was stronger than bupivacaine.
- In pregnant rat myometrial strips, bupivacaine and levobupivacaine did not have an effect at lower cumulative concentrations but did reduce contractile activity at higher cumulative concentrations.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine can reduce muscle contractility. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Comparative effects of sodium channel blockers in short term rat whole embryo culture. (Nilsson et al., 2013)

Objective: To examine the effect on the rat embryonic heart of two experimental drugs (AZA and AZB)

Method: Pregnant rats were euthanized on Gestational Day 13. The uterus was removed and opened so that the decidual swellings could be removed. From the swellings, the embryo was removed and cultured in Dulbecco's Modified Eagle Medium. After 1 hour, embryos were examined for a normal heart rate of 140 beats per minute. Then 20 mcL of test compound was added. Embryos were inspected 1, 2, and 3 hours after addition of compound. Bupivacaine was tested as a control in this experiment at a concentration of 10 mcM.

Key Findings:

- Bupivacaine caused severe bradycardia that increased in severity with time. This effect was reversed by a washout.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine can adversely affect the fetal heart in vitro. Due to the in vitro nature of the experiment it is unclear the clinical significance of the findings. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

The effect of foetal acidosis on bupivacaine levels in utero. (Pickering et al., 1981)

Objective: To determine if the highly protein bound local anesthetic bupivacaine demonstrates ion-trapping in the acidotic fetus

Method: Six near term (mean gestational age of 135 days) underwent hysterectomy to insert catheters for blood collection. Animals were given 24 hours to recover before dosing. The experiment consisted of three phases: 1) maternal bupivacaine infusion (120 minutes; 0.08 mg/kg/min bupivacaine), 2) maternal bupivacaine infusion plus fetal acid infusion (45 minutes; 2.5 M lactic acid), and 3) maternal bupivacaine infusion and fetal bicarbonate infusion (45 minutes; 0.1M sodium bicarbonate). Measurements included maternal and fetal arterial blood pressure, pulse, blood gases, pH, and bupivacaine levels.

Key Findings:

- Infusion of acid into the fetus caused an increase in the fetal arterial concentration of bupivacaine and the fetal/maternal ratio.
- Infusion of bicarbonate to correct the pH reduced the fetal arterial concentration of bupivacaine and the fetal/maternal ratio back to control values.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine concentration in the fetus is pH dependent. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

The electrophysiologic effects of bupivacaine on adult, neonatal, and fetal guinea pig papillary muscles. (Sun et al., 1991)

Objective: To study developmental changes in the effects of bupivacaine on the transmembrane potentials of adult, neonatal, and fetal guinea pig papillary muscles using standard microelectrode techniques

Method: Neonatal (postnatal age of 1-10 days), nonpregnant adult, and pregnant adult guinea pigs were used in this experiment. Hysterotomies were performed on pregnant

animals and fetuses (gestational age of 62-66 days) were removed. Sternotomies were performed on all animals and the hearts were collected. Papillary muscles from either ventricle were dissected for measurement of transmembrane potentials. Tonic block was achieved using bupivacaine concentrations of 0.2, 1.0, and 1.5 mcg/mL for 30 mins each.

Key Findings:

- 1.5 mcg/mL bupivacaine hyperpolarized maximum diastolic potential in adult and neonatal guinea pig muscles, but not fetal muscles.
- Bupivacaine did not change the action potential duration (APD) in adult or neonatal tissue. In the fetal tissue, 1.0 and 1.5 mcg/mL bupivacaine shortened APD₅₀ and APD₁₀₀.

Reviewer's note: The study is mechanistic in nature and indicates that bupivacaine may affect heart muscle APD to a greater extent than in adults or neonates. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Epidural anesthesia worsens uterine blood flow and fetal oxygenation during hemorrhage in gravid ewes. (Vincent et al., 1992)

Objective: To determine whether epidural anesthesia alters maternal and fetal hemodynamic and acid-base responses to hemorrhage in gravid ewes

Method: Pregnant ewes of 120 days of gestation underwent laparotomy and hysterotomy for inserting catheters and allowed to recover for at least three days. On the day of the experiment, each animal received an intravenous bolus of 500 mL saline. 15 minutes later 6-20 mL of 0.5% bupivacaine or saline was delivered through the epidural catheter. At 30 mins after the first injection, an additional 0.5% of bupivacaine was delivered if the sensory level of analgesia was lower than T10. Also at 30 mins, each animal received 15,000 units of heparin intravenously. At 45 minutes after the first injection, each animal was bled for 20 ml/kg via the femoral arterial catheter. At 110 minutes after the first injection, the collected maternal arterial blood was reinfused over 55 minutes. Hemodynamic measurements were continued until the end of the experiment (170 minutes after the first injection). Parameters measured included maternal arterial blood pressure, maternal central venous pressure, maternal pulmonary artery pressure, fetal arterial blood pressure, maternal heart rate, fetal heart rate, uterine artery blood flow, maternal/fetal arterial blood gas, maternal/fetal arterial pH, and plasma norepinephrine and epinephrine concentrations.

Key Findings:

- Two fetal deaths occurred in the epidural group.

- Maternal heart rate was lower during hemorrhage in the epidural group.
- Maternal mean arterial blood pressure was lower in the epidural group before and during hemorrhage.
- Maternal cardiac output was lower in the epidural group before and during hemorrhage.
- Maternal systemic vascular resistance in the epidural group increased during hemorrhage.
- Uterine blood flow was lower during hemorrhage in the epidural group.
- Fetal PO₂ and pH were lower during hemorrhage in the epidural group.
- Plasma epinephrine concentrations dropped below baseline in the epidural group.

Reviewer's note: The study indicates that bupivacaine may adversely affect the compensatory response to untreated hemorrhage. Given the long clinical history of epidural anesthesia, the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Meloxicam

Effect of meloxicam on pregnancy rate of recipient heifers following transfer of in vitro produced embryos (Aguilar et al., 2013)

Objective: To determine if administration of meloxicam is beneficial in heifers in which embryo transfer is more difficult

Method: In vitro embryo production blastocysts were transferred non-surgically. Recipients were divided into two groups depending on the degree of difficulty in passing the catheter through the cervix; easy (< 60 seconds) or difficult (> 80 seconds). Immediately after embryo transfer the recipients received an IM injection of 200 mg meloxicam.

Key Findings:

- Meloxicam increased the overall pregnancy rate (66.67% vs. 49.02% in control).
- When stratified by difficulty, meloxicam did not increase the pregnancy rate of easy heifers but did increase the pregnancy rate of difficult heifers (66.13% vs. 23.91% in controls).

Reviewer's note: The study indicates that meloxicam improves the pregnancy rate of difficult heifers receiving embryo transfer. This is not a toxicological finding and the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Combined administration of gonadotropin-releasing hormone, progesterone, and meloxicam is an effective treatment for the repeat-breeder cow. (Amiridis et al., 2009)

Objective: To test the hypothesis that a combined treatment would benefit the conception rate of repeat-breeder cows

Method: 402 cows in their second to fifth lactation, with a body condition score of 2.75 to 3.5 (scale of 1 to 5), producing more than 25 kg milk per day, with at least four unsuccessful inseminations, and were 128 to 285 days in milk were used in this study. Animals received either 0.012 mg IM buserelin 4 to 6 hours before AI, 100 mg P4 gelatin capsules administered intravaginally every 12 hours on Days 4, 5, and 6, 0.5 mg/kg meloxicam subcutaneously on Days 16, 17, and 18, or the combination of all three.

Key Findings:

- Pregnancy rate was increased by each individual treatment, but the highest effect was from the combination of all three treatments.
- Conception rate was increased by P4 and meloxicam but a larger effect was observed with the combination of all three treatments.

Reviewer's note: The study indicates that meloxicam slightly improves the pregnancy and conception rates in the cows treated. This is not a toxicological finding and the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Analysis of the nonsteroidal anti-inflammatory drug literature for potential developmental toxicity in rats and rabbits. (Cook et al., 2003)

Objective: To determine whether NSAIDs other than aspirin induce developmental anomalies similar to those elicited by aspirin.

Method: Developmental toxicity studies were identified through literature searches of PubMed and TOXNET, and pregnancy outcome data were extracted and tabulated. By using a set of defined criteria, each study was evaluated for quality and assigned to one of five tiers. The relation between certain malformations and NSAID treatment was analyzed for the best studies (tiers 1–4) by using concurrent control data (Mantel–

Haenszel and permutation tests) and by combining the concurrent control data with historical control data (w2 test and permutation tests).

Key Findings:

- In rats, the incidence of ventricular septal defects and midline defects in non-aspirin treated NSAID treated rats was about 1.5 times that of controls.
- In rabbits, the incidence of diaphragmatic hernia, ventricular septal defects and midline defects in non-aspirin treated NSAID treated rabbits were greater than the controls.

Reviewer's note: The study indicates that non-aspirin NSAIDs are possibly associated with developmental anomalies based on a literature review. The data are not specific to meloxicam and only suggests that there is a potential risk for all non-aspirin NSAIDs. In that regard, the developmental toxicity of meloxicam has already been characterized by nonclinical studies in rats and rabbits and is described in the labeling. This includes the finding of septal heart defects in rabbits treated with high doses of meloxicam. As such, this study does not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Hot topic: Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield. (Carpenter et al., 2016)

Objective: To replicate the results of a previous study showing that postpartum administration of sodium salicylate can increase 305-day milk yield in older dairy cattle by using meloxicam in place of sodium salicylate

Method: Multiparous cows (12 to 36 hours after calving) received either an oral drench of 125 g/d of sodium salicylate (~185 mg/kg) for three consecutive days or 675 mg of meloxicam (~1 mg/kg) as a bolus treatment on Day 1.

Key Findings:

- Both treatments increased daily and 305-day milk production compared to control.
- Both treatments increased the protein yield of the milk.

Reviewer's note: The study indicates that meloxicam can increase milk yield in dairy cows. This is not a toxicological finding and the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Prostaglandins regulate conceptus elongation and mediate effects of interferon tau on the ovine uterine endometrium. (Dorniak et al., 2011)

Objective: To test the hypothesis that prostaglandin (PG)s from the conceptus and perhaps the endometrium itself have autocrine, paracrine, and perhaps intracrine effects on conceptus development, endometrial function, and endometrial responses to progesterone and interferon tau (IFNT) in ruminants

Method: Ewes used in this study only if they exhibited at least two estrous cycles of normal duration (16-18 days). Mated ewes underwent a mid-ventral laparotomy on day 8 post-mating for insertion of a catheter and osmotic pump. The osmotic pumps contained either 2% ethanol saline or meloxicam (612.5 ng/mL). The pumps delivered 10 mcL/hour for seven days. Ewes were euthanized on Day 14 post-mating for evaluation of the blastocyst/conceptus development, uterine flushing, and processing of the uteri.

A second experiment was conducted with unmated ewes on day 10 postestrous. Ewes underwent a mid-ventral laparotomy for insertion of a catheter and osmotic pump. The osmotic pumps contained either 2% ethanol saline with 101 mcg ovine serum proteins, 101 mcg of recombinant ovine IFNT, meloxicam with 101 mcg ovine serum proteins, or recombinant ovine IFNT plus meloxicam. Ewes were euthanized on Dday 14 post-mating for uterine flushing, and processing of the uteri.

Key Findings:

- Meloxicam inhibits conceptus elongation, reduces endometrial PG synthase activity, and reduces PGs in the uterine lumen.
- IFNT increases endometrial production of PGs and PGG/H synthase and cyclooxygenase 2 activity in cyclic ewes.
- Meloxicam impaired the ability of IFNT to modulate expression of genes in the endometrium.

Reviewer's note: The study is mechanistic in nature. It indicates that when meloxicam is given by intrauterine infusion, it has an inhibitory effect on prostaglandin synthase activity resulting in reduced prostaglandins and this results in elongation of the conceptus. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Comparison of milk and plasma pharmacokinetics of meloxicam in postpartum versus mid-lactation Holstein cows. (Gorden et al., 2018)

Objective: To determine whether differences occurred in meloxicam pharmacokinetics between postpartum cows and mid-lactation cows

Method: Cows were divided into two groups of either < 26 hours postpartum or in mid-lactation (>90 days in milk). Both groups received meloxicam. Cows were milked three times daily. Treatment with meloxicam was given following milking as a gelatin bolus dose of 1 mg/kg. Blood samples were collected from the jugular or coccygeal vein prior to dosing and at 2, 4, 6, 8, 12, 16, and 24 hours. Blood samples were collected every 8 hours past the 24-hour sample until the drug was cleared from the system. Milk samples were collected prior to dosing at every 8 hours after until meloxicam was below the detection limit.

Key Findings:

- Plasma levels of meloxicam were lower in mid-lactation cows as compared to postpartum cows, starting at around 8 hours post dosing.
- The milk concentration of meloxicam was lower in mid-lactation cows as compared to postpartum cows, starting at around 8 hours post dosing.
- Postpartum cows had a higher mean plasma C_{max} , T_{max} , AUC_{inf} , AUC_{last} and mean residence time compared to mid-lactation cows.
- The relative bioavailability in postpartum cows was 210% that of mid-lactation cows.

Reviewer's note: The study is a PK study as opposed to a toxicity study. It indicates that in cows, meloxicam stays in the milk longer at postpartum as opposed to mid-lactation. The clinical significance of this finding is unknown. In terms of the label, there is already animal data suggesting that meloxicam can be found in milk of rats and pigs. The addition of data that it is also in the milk of cows does not really enhance the label in a meaningful way. This reviewer does not consider the study data necessary to be included in the label.

Effects of meloxicam on reproduction parameters in dairy cattle. (Hirsch and Philipp 2009)

Objective: To investigate possible drug effects on different reproductive parameters as well as on the progeny

Method: 38 healthy lactating cows were used in the study (18 meloxicam and 20 control). Day 0 was defined as the day of artificial insemination. Cows received a single IV injection of 1.5 mg/kg meloxicam or placebo into the jugular vein on Days -12, 9, 35, 105, 203, and 259. The following indices were calculated: insemination index, pregnancy rate, embryo-fetal death rate, live birth rate, and rate of obstetrics. The calves were examined at birth and at 28 days.

Key Findings:

- There was no difference between meloxicam treated and control cows for the parameters listed in the methods.
- There were no differences observed in terms of the calves at either birth or at Day 28 between the two groups, including live birth rate and survival rate.

Reviewer's note: The study reports that there were no effects on fertility, pregnancy, or other reproductive parameters in cows who received meloxicam. The current labeling for meloxicam products indicates that there are potential reproductive and developmental effects identified in nonclinical studies. As such, this reviewer does not consider the lack of findings in this study need to be included in the label.

Effects of Meloxicam on Implantation and Parturition of Rat. (Jaffal et al., 2006)

Objective: To study the anti-implantation and parturition effects of meloxicam on pregnant rats

Method: For effects on early implantation, 0, 7.5, or 10 mg/kg meloxicam was administered orally to rats with a positive detection of a vaginal plug on Days 1, 2, and 3 of gestation. For effects on late implantation, 0, 7.5, or 10 mg/kg meloxicam was administered orally to rats with a positive detection of a vaginal plug on Days 3, 4, and 5 of gestation. For effects on parturition, 0, 7.5, or 10 mg/kg meloxicam was administered orally to rats with a positive detection of a vaginal plug on Days 20, 21, and 22 of gestation. One group was laparotomized on Day 23 while the second group went through natural delivery. For the implantation experiments, uteri were examined visually and implants were counted and scored as resorptions, dead, or live fetuses. Fresh uteri tissue was stored for histopathological evaluation. For parturition experiments, the number of live and dead pups were counted, body weights of pups were measured, and pups underwent macrophotography. Rats that were sacrificed on Day 23 were inspected for the number of live, dead, or resorbed fetuses.

Key Findings:

- All doses of meloxicam caused an increase in resorptions, a decrease in viable implanted sites, and a decrease in the number of rats with viable implantation sites. The effects appeared to be worse when the animals were dosed during late implantation (Days 3-5) as opposed to early implantation (Days 1-3).
- All doses of meloxicam caused a decrease in the number of viable fetuses and an increase in the number of dead fetuses.
- The high dose (10 mg/kg) of meloxicam caused an increase in maternal plasma levels of oxytocin.

Reviewer's note: The study reports that there were adverse developmental effects of meloxicam in rats, including embryo-fetal lethality. The lowest dose used in this study

was 7.5 mg/kg. The current labeling for meloxicam products includes nonclinical data in rats and rabbits which describe the developmental effects of meloxicam. These studies indicated similar findings as seen in the Jaffal et al., 2006 study. The rat study in the label for meloxicam reported these effects at a lower dose (1 mg/kg) than tested in the Jaffal et al., 2006 study. As such, the findings from Jaffal et al., 2006 study are already covered by the information in the label and this reviewer does not consider the study data necessary to be included in the label.

Pregnancy rates and serum 13,14-dihydro-15-keto-PGF₂alpha concentrations in recipient Nelore heifers treated with meloxicam after the transfer of in vitro-produced embryos. (Lopes et al., 2018)

Objective: To determine if the administration of meloxicam to Nelore heifers would improve the pregnancy rates by decreasing serum 13,14-dihydro-15-keto-PGF₂alpha (PGFM) concentrations

Method: Cycling Nelore heifers of 33 months of age with a body score of 6 or 7 (1-9 scale) were used as recipients for embryo transfer of in vitro-produced blastocysts. Recipients were classified one month before the experiment based on the degree of difficulty in passing a catheter through the cervix (easy, < 80 seconds or difficult, >80 seconds). One hour before embryo transfer, animals received either a 200 mg meloxicam IM injection or an equal amount of saline. Blood samples were collected from all animals 1 hour before embryo transfer and at 4 and 8 hours after transfer to determine PGFM serum concentrations. Pregnancy was confirmed by ultrasound 30 days after embryo transfer.

Key Findings:

- Meloxicam increased the pregnancy rates of Grade I (easy) Nelore heifers but had no effect on Grade II (difficult) Nelore heifers.
- Grade II (difficult) animals receiving meloxicam had lower serum PGFM concentrations 4 and 8 hours after treatment compared to control and Grade I (easy) meloxicam animals.

Reviewer's note: The study indicates that meloxicam can increase the pregnancy rate of Nelore heifers graded as easy based on the difficulty to pass a catheter through the cervix. This is not a toxicological finding and the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. (Malreddy et al., 2013)

Objective: To determine the depletion of gabapentin and meloxicam into milk

Method: Twelve Holstein-Friesian cows (34-62 months old and 543-891 kg) in their 1st-3rd lactation were used. Six cows received 10 mg/kg gabapentin and 1 mg/kg meloxicam in a gelatin capsule. The remaining six cows received 20 mg/kg gabapentin and 1 mg/kg meloxicam in a gelatin capsule. Milk was collected prior to drug administration and then every 8 hours for 7 days. At each milk sampling time, 10 mL of blood was collected by venipuncture of the jugular vein.

Key Findings:

- The concentration of meloxicam in plasma was higher than in milk.
- Milk concentrations of meloxicam depleted below detectable levels within 80 hours.

Reviewer's note: The study is a PK study as opposed to a toxicity study. It indicates that in cows, meloxicam can be found in the milk of lactating cows. In terms of the label, there is already animal data suggesting that meloxicam can be found in milk of rats and pigs. The addition of data that it is also in the milk of cows does not really enhance the label in a meaningful way. This reviewer does not consider the study data necessary to be included in the label.

Altered fetal pituitary-adrenal function in the ovine fetus treated with RU486 and meloxicam, an inhibitor of prostaglandin synthase-II. (McKeown et al., 2000)

Objective: To examine the effects of specific PGHS-II inhibition in sheep during induced labor.

Method: Catheters and electromyogram electrodes were placed into pregnant ewes of known gestational age. A 5-day period of recovery was allowed after the operation. All animals received a SC injection of 10 mg/kg RU486 to induce labor on Day 127 of gestation. Once in labor, animals received either 1) IV saline (only 18-hour infusion due to animals delivering), 2) IV infusion of 2 mcg/kg meloxicam per minute for 2 hours then 4 mcg/kg per minute for 2 hours, then increased by 4 mcg/kg per minute until the uterine activity pattern returned to baseline. Meloxicam was continued at this dose until termination., 3) meloxicam infusion as in 2 except that once contractions returned to baseline, the dose was decreased by 4 mcg/kg per minute until 2 mcg/kg per minute. Meloxicam was continued at this dose until termination. A second injection of RU486 was given within 12 hours of starting the saline or meloxicam infusions. Meloxicam infusions were terminated at 48 hours and the animals were killed. Maternal and fetal arterial blood samples were collected at 8-hour intervals starting 48 hours prior to the first injection of RU486.

Key Findings:

- Both infusions of meloxicam significantly decreased uterine contraction frequency.

- Both infusions of meloxicam significantly decreased maternal PGFM concentrations and fetal PGE₂ concentrations.
- Both infusions of meloxicam significantly decreased fetal ACTH and cortisol concentrations.

Reviewer's note: The study is mechanistic in nature. It indicates that meloxicam can attenuate the effects of RU486, a progesterone receptor agonist, in sheep. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Effects of meloxicam on milk production, behavior, and feed intake in dairy cows following assisted calving. (Newby et al., 2013)

Objective: To evaluate the effect of meloxicam on behavior, health, and production in cows with assisted calving

Method: Holstein cows (42) and heifers (61) that experienced assisted parturition were enrolled in the study. Calving difficulty was scored as either: 1) easy pull by one person, or 2) a difficult pull with more than one person and/or with mechanical assistance. Animals were blocked into primiparous and multiparous groups and into calving difficulty and randomly assigned within each block to receive either meloxicam (0.5 mg/kg) subcutaneously 25.4 hours following calving or a similar volume of placebo. Dry matter intake, milk yield, body weight, rectal temperatures, and clinical health events were measured. In addition, blood samples were obtained by coccygeal venipuncture after calving and at 1, 3, 6, 9, and 12 days in milk. Feeding and lying behaviors were also measured.

Key Findings:

- Animals who received meloxicam visited the feed bunk more often than the placebo group and spent a longer time feeding.
- No other differences (including in dry matter intake, milk production, or metabolic indicators) were seen between the meloxicam and placebo groups.

Reviewer's note: The study did not indicate any toxicological effect in cows or heifers undergoing assisted calving. There was a change in feeding behavior, but the clinical significance of this finding is unknown. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

The potential danger of COX-2 inhibitors. (Norman and Wu, 2004)

Objective: None. Article is an editorial.

Method: None

Key Findings:

- None

Reviewer's note: The study is an editorial opinion. The Authors believe that "normal women of childbearing age attempting to have a baby need to pay more attention to COX inhibitors generally and COX-2 inhibitors specifically. They should avoid taking these drugs or reduce their dose while seeking pregnancy. Women who require these drugs for chronic inflammatory diseases should consider the benefit–risk profile before trying to become pregnant. Fertility experts should ensure that they take an adequate drug history and give appropriate advice."

The author's present some nonclinical data for meloxicam, but they do not cite a reference. The label for meloxicam products already includes nonclinical data to characterize the potential developmental and reproductive risks of meloxicam. This editorial does not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Evaluation of the contraceptive effects of carprofen, flunixin meglumine and meloxicam in rats. (Paksoy and Kirbas, 2017)

Objective: To determine the suitability of carprofen, flunixin meglumine, and meloxicam for use in emergency contraception

Method: Mated female Sprague-Dawley rats were divided into six groups: 1) carprofen 10 mg/kg SC, 2) carprofen 10 mg/kg SC given twice 12 hours apart, 3) flunixin meglumine 5 mg/kg IM, 4) flunixin meglumine 5 mg/kg IM given twice 12 hours apart, 5) meloxicam 2 mg/kg SC, and 6) saline. Administrations were given on the third day after mating. Rats were euthanized on the 7th day post mating to determine pre-implantation loss.

Key Findings:

- Meloxicam resulted in a significant decrease in implantation sites (3.00 vs. 11.38 in the controls).
- Only 3 of 8 animals treated with meloxicam became pregnant. All 8 control animals became pregnant.

Reviewer's note: The study indicated that meloxicam can result in a decrease in implantation sites leading to pre-implantation loss and decreased pregnancy in rats following a single dose on the 3rd day after mating. The labels for meloxicam products already contain information from animal studies describing pre- and post-implantation

loss. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Dose-dependent effects of meloxicam administration on cyclooxygenase-1 and cyclooxygenase-2 protein expression in intrauterine tissues and fetal tissues of a sheep model of preterm labor. (Rac et al., 2007)

Objective: To determine the effect of different dosage regimens of meloxicam on COX-1 and COX-2 protein expression in ovine endometrium, myometrium, amnion, placenta, and fetal tissues in a model of RU486-induced preterm labor.

Method: Polled Dorset time-pregnant ewes (12 for Study 1 and 6 for Study 2) were surgically prepared under anesthesia on Day 121 of gestation to insert catheters for blood monitoring and drug infusion and electrodes to measure uterine contractions. Animals were allowed to recover 5 days before the experiment began.

In Study 1, labor was introduced by maternal SC injection of 10 mg/kg RU486 on Day 127. The animals were divided into three groups: 1) IV infusion of saline (control), 2) IV infusion of meloxicam at 2 mcg/kg/min for 2 hours doubled every 2 hours until uterine contractile activity reversed back to baseline (~17 mcg/kg/min) and maintained until the end of the protocol, and 3) same as 2) except when contractions returned to baseline, meloxicam dose was reduced by 4 mcg/kg/min until 2 mcg/kg/min was achieved and this dose was maintained until the end of the protocol.

In Study 2, labor was introduced by maternal SC injection of 10 mg/kg RU486 on Day 127. The animals were divided into two groups: 1) IV infusion of saline (control), 2) IV infusion of meloxicam at 16 mcg/kg/min increased by 4 mcg/kg/min every 2 hours until contractile activity was inhibited (average dose of 16-20 mcg/kg/min) and maintained for 10 hours. After that the dose was dropped to 4 mcg/kg/min until the end of the protocol.

For both studies, the duration was 48 hours. Also, a second injection of RU486 was given within 12 hours of starting the infusions. Maternal and fetal blood were collected starting 48 hours prior to the first RU486 injection. At the end of the studies, animals were euthanized and tissue samples were collected.

Key Findings:

- Meloxicam treatment (Study 1) did not affect maternal or fetal arterial: pH, pCO₂, pO₂, or hemoglobin.
- Meloxicam (Study 1) reduced COX-2 protein expression in the endometrium, myometrium, and amnion. No change was observed for COX-2 in the placenta or in COX-1 in any of the tissues. No effect was observed on either COX-1 or COX-2 in any tissue with treatment of meloxicam in Study 2.

- Meloxicam treatment (Study 1) did not affect fetal tissue expression of COX-1 or COX-2 protein.

Reviewer's note: The study is mechanistic in nature. The study did indicate that meloxicam can stop preterm labor contractions and prolong pregnancy. However, the study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Effects of non-steroidal anti-inflammatory drugs on blood pressure in dogs. (Montoya et al., 2012)

Objective: To evaluate the potential effects on blood pressure of a 2-month course of orally administered meloxicam or firocoxib in healthy dogs.

Method: Six cross-bred dogs (Age 2-6 years, weighing 17-24 kg) were used in the study. Each dog received 5 mg/kg firocoxib and 1 mg/kg orally for 60 days with a 90-day washout between treatments.

Key Findings:

- Treatment with meloxicam had no effect on mean, diastolic, or systolic blood pressure was observed.

Reviewer's note: The study is only a meeting poster, did not evaluate any developmental or reproduction parameters and did not find a toxicological effect of meloxicam. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Risk factors, microbiological findings and outcome of necrotizing fasciitis in New Zealand; a retrospective chart review. (Das et al., 2012)

Objective: To investigate the relationship between the incidence of necrotizing fasciitis (NF) in New Zealand and traditional Samoan tattooing and other risk factors, and to document the microbiology, complications, treatment and outcomes of NF.

Method: 209 hospital charts of patients discharged with a NF diagnosis in eight hospitals in New Zealand from 2000 to 2006 were retrospectively reviewed.

Key Findings:

- ~25% of the patients were taking NSAIDs but the role of NSAIDs was unclear.

Reviewer's note: This study did not specify what NSAIDs were used and it was unclear if usage was a risk factor for NF. Furthermore, it is unclear what such an association

would have with developmental or reproductive parameters. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

Diverse roles of prostaglandins in blastocyst implantation. (Salleh, 2014)

Reviewer's note: This article is a review article which summaries the effects of prostaglandins on blastocyst implantation. Meloxicam or NSAIDs are not mentioned at all. The label already includes a brief description of the potential effects of prostaglandin inhibition and its potential developmental effects. The study data do not appear to provide additional safety information to the existing embryo-fetal development and pre-/post-natal development animal data in the label; this reviewer does not consider the study data necessary to be included in the label.

9.2 Embryonic and Fetal Development*

9.2.1 Study Title: Maleic Acid: An Oral Developmental Toxicity Study in Pregnant New Zealand White Rabbit

Study no.: 34-74
Study report location: Module 4:
<\\cdsesub1\evsprod\nda211988\0053\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\34-74\34-74-audited-draft.pdf>
Conducting laboratory and location: (b) (4)
GLP compliance: Yes
Drug, lot #, and % purity: Maleic acid, 2HH0135, 100.5%

Key Study Findings

- New Zealand White rabbits were administered 0, 2, 5, and 20 mg/kg/day maleic acid by oral gavage once daily from Gestational Day 6 to 18.
- No evidence of test article-related maternal toxicity was observed in the study.
- There was no treatment related effect on litter viability based on the following parameters: pre- and post-implantation loss, number of corpora lutea, number of

live fetuses, total number of implants, early/late resorption, total resorptions, early/late death, total deaths, and total non-live resorptions and deaths

- There was a small decrease in the male to female pup ratio in treated groups compared to the vehicle. The magnitude of the effect was small and did not follow a dose response so it was not considered significant.
- There was no treatment-related effect on fetal malformations/ variations of any kind. All observed effects did not follow a dose response, were similar to control, or within historical control ranges.
- The Applicant identified 20 mg/kg/day as the NOAEL for maternal and embryo-fetal development. The reviewer agrees. The NOAEL of 20 mg/kg is a human equivalent dose of 387.1 mg based on body surface area and assuming a 60 kg person. The amount of maleic acid at the MRHD is 8.0 mg resulting in a margin of exposure of 48.4x.

Methods

Doses:	2, 5, and 20 mg/kg/day
Frequency of dosing:	Once daily
Number/Sex/Group:	23 females/group
Dose volume:	10 mL/kg
Formulation/Vehicle:	Maleic acid was dissolved in deionized water.
Route of administration:	ORAL
Species:	Rabbit
Strain:	New Zealand White
Comment on Study Design and Conduct:	
Dosing Solution Analysis:	Dose solution analysis was conducted on the first day of preparation and at the end of the dosing period. All maleic acid formulations were between -0.5 and + 0.5% of the target concentration. The vehicle control did not have any detectable maleic acid (below LOQ of 50 mcg/mL).

Observations and Results

F₀ Dams

Mortality

All does were observed twice daily on weekdays and once daily on weekends/holidays.

All animals survived until scheduled C-section on GD 29.

Clinical Signs

Each doe was evaluated during dosing for overt signs of toxicity. A detailed clinical examination was performed pre-test and daily during the study. Clinical observations were performed ~1-hour post dosing.

No treatment related clinical signs were observed.

Body Weight

Does were weighed upon arrival and on GD 6, 9, 12, 15, 17, 19, 21, 23, 25, 27, and 29.

Treatment with maleic acid did not appear to affect the body weight or body weight gain of the rabbits.

Food Consumption

Food consumption was measured on the same days as body weight measurements.

Food consumption was generally similar across the groups except for between GD 12-15 where all treated groups had a lower food consumption compared to the vehicle treated group. There was no dose response relationship to the observation and decreased food consumption during this time period was not considered treatment related or adverse.

Necropsy/ Histopathology

All surviving rabbits were euthanized with a euthanasia solution on GD 29. A gross necropsy was performed and the uterus of each doe was weighed with the ovaries. Corpora lutea were counted for both ovaries. Each uterine horn was inspected for tissue resorptions and fetal death. Uteri of does that appeared to be non-gravid were stained with 10% ammonium sulfide. Tissue masses and suspected lesions were dissected and fixed in 10% neutral buffered formalin.

No gross abnormalities were observed during the necropsy in maternal animals. All animals were found to be pregnant except two control animals who were confirmed to be non-gravid by ammonium sulfide staining and one 5 mg/kg animal which was gravid with one implantation site.

Corpora Lutea/Preimplantation Loss

Litter viability was assessed by the following parameters: pre- and post-implantation loss, number of corpora lutea, number of live fetuses, total number of implants,

early/late resorption, total resorptions, early/late death, total deaths, and total non-live resorptions and deaths.

There were no treatment related effects on any of the parameters measured. An increase in the % of preimplantation loss was observed in animals receiving 2 mg/kg maleic acid compared to the control. This effect was not observed at higher doses and appeared to be driven by an increased average number of corpora lutea in this group as opposed to a decrease in the number of implantations.

Table 163: Litter Viability

	Vehicle	2 mg/kg	5 mg/kg	20 mg/kg
% preimplantation loss	5.9	15.4	3.2	4.9
% post implantation loss	10.5	6.7	11.5	7.2
# of corpora lutea	9.6	10.2	9.0	9.1
Total # of implants	9.0	8.7	8.7	8.7
Live fetuses	8.0	8.0	8.0	8.0
Resorptions, early	0.9	0.4	0.5	0.5
Resorptions, late	0	0.1	0.1	0.1
Resorption, total	0.9	0.5	0.6	0.6
Deaths, early	0	0.1	0.1	0
Deaths, late	0	0.1	0.1	0
Deaths, total	0	0.1	0.1	0.1
Resorptions and death, total	1.0	0.6	0.7	0.7

N = 21 for the vehicle control group and 23 for all other groups.

Values presented as means

F₁ Offspring

Litter body weights were similar amongst all groups. There was a slight shift in the male:female pup ratio in treated groups compared to the control group with an increase in the number of females born and a decrease in the number of males born. There did not appear to be a dose response as all maleic acid treated groups had a similar male:female ratio. This effect was not considered treatment related or meaningful.

Table 164: Sex and Sex Ratio of Litter

	Vehicle N = 20	2 mg/kg N = 21	5 mg/kg N = 22	20 mg/kg N = 23
# of Males (mean)	4.6	4.1	4.0	3.5
# of females (mean)	3.7	4.3	4.4	4.4
M/F ratio	1.2	0.9	0.9	0.8

Terminal Observations

Fetal Malformations/ Variations (external, visceral, skeletal)

Removed fetuses were given a gross morphological examination. Fetuses were euthanized by induction of hypothermia. Visceral, cephalic, and skeletal examinations were performed on the fetuses.

During the external examinations, one fetus from the 2 mg/kg group was found with multiple malformations: arthrogryposis of both forelimbs, cleft palate, club foot, protruding tongue, short snout, and small upper jaw. In addition, one fetus from the 5 mg/kg group was identified with a malformation of arthrogryposis. The malformations were considered sporadic due to the low incidence and lack of findings at the highest dose tested.

No test-article related visceral or cephalic findings were noted. Overall there was a low number of variations and malformations observed across all groups. Any variations and malformations observed were either also seen in the vehicle treated animals or did not follow a dose response.

Only the vehicle control group and high-dose group were evaluated for skeletal variations/malformations. The overall rate of skeletal variations in vertebrae, skull, sternbrae, and ribs were similar across all groups. Skeletal malformations were limited to a single fetus in the 20 mg/kg group (fused rib). The fetal and litter incidence of this finding was within the historical control range reported by the (b) (4)

10 Special Toxicology Studies

10.1 Wound Healing and Bone Studies

Wound and bone healing studies were conducted with HTX-011 and were reviewed during the IND. In summary, there did not appear to be any effect on bone healing or adverse interaction with the Prolene mesh with HTX-011-56. From the nonclinical perspective, no additional testing to assess these parameters are necessary. There was some evidence of a potential effect on wound healing in the rabbit study. However, the minipig study (33-54), which used a higher dose, supported that there was only a very slight difference in wound healing with HTX-011-56 compared to control treatments. However, it must be kept in mind that when the dose volume was normalized to the size of the incision site (estimated incision sizes for the surgeries proposed in the clinical studies were provided by the clinical team) the minipig study (1 mL/cm) was lower than the proposed clinical surgeries (herniorrhaphy, 3-4 cm, 2.5-3.4 mL/cm; (b) (4)

Study title: HTX-011: A Hernia Repair Model with Mesh in New Zealand White Rabbit

Study no.: 33-38
Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-to\33-38\33-38.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: September 17, 2015
GLP compliance: No
QA statement: No (Study Director Statement only stating the study was conducted with (b) (4) SOPs)
Drug, lot #, and % purity: HTX-011-019, Lot 14072, 97.7% Meloxicam and 101.0% Bupivacaine
HTX-011-49, Lot 15011, 97.9% Meloxicam and 101.5% Bupivacaine
HTX-011-056, Lot 025774, 101.4% Meloxicam and 103.6% Bupivacaine

Key Study Findings

- New Zealand White Rabbits were administered a single application of 25 mg bupivacaine formulation or 25 mg bupivacaine and 0.75 mg meloxicam formulations onto Prolene mesh in a hernia repair model. There was no bupivacaine alone control arm to permit comparison to the approved drug products.
- No treatment-related mortalities were observed.
- There were no treatment-related effects on body weight or food consumption.
- Fecal output was reduced in males receiving the bupivacaine formulation comparator and all three HTX formulations. Fecal output was primarily reduced in females receiving HTX-011-19. This finding was not considered adverse.
- Treatment with the vehicle, bupivacaine comparator formulation, and all three HTX formulations resulted in increased incidence/severity of erythema and edema at the injection site in males and females on Days 3 and 15 although there did not appear to be a consistent pattern across formulation or sex. Overall, there were more findings on the last day of the study in animals treated with the bupivacaine comparator and the HTX formulations than in the saline and

vehicle treated groups.

- Treatment with the vehicle, bupivacaine comparator formulation, and all three HTX formulations resulted in increased incidence/severity of wound observations at the injection site in males and females on Days 3 and 15 although there did not appear to be a consistent pattern across formulation or sex. Overall, the most commonly observed effect, hemorrhage, was no longer present in most animals by Day 15 regardless of treatment.
- On Day 3, various effects on hematology parameters were seen following treatment with the various formulations used compared to the saline control, including increased white blood cells, increased neutrophils, decreased monocytes, decreased reticulocytes, and decreased platelets. These changes were less pronounced by Day 15, suggesting recovery.
- Changes in clinical chemistry parameters noted were decreased triglycerides (20-30%) in male animals and decreased phosphorous (13-22%) in female animals. The toxicological significance of both findings is unknown.
- Occasionally, elevations in the number of gross findings at the injection site were increased in some treated groups compared to the saline control.
- On the Day 3 sacrifice, injection site histopathology findings were of similar incidence and severity across the saline control and all treatments.
- On Day 15, there appeared to be some differences compared to the saline control animals in histopathological lesions of the injection site. This included increased incidences/severity of cell debris, cystic change, hemorrhage, epidermal hyperplasia, dermal granulation, necrosis, epidermal ulcer, and subcutaneous/muscular/intermuscular granulation.
- Taken together, the various formulations, including the vehicle control, bupivacaine formulation comparator, and the three HTX formulations, caused increased incidences/severities in dermal, wound site, gross, and histopathological findings throughout the study. The observations at the wound site appeared to be similar to saline control by the end of the study and are likely not adverse. In contrast, the presence of erythema and edema at the end of the study was only seen with the bupivacaine formulation comparator and HTX formulations. Similarly, increased incidence and severity of various histopathological findings were seen at the end of the study with the majority of them occurring with the HTX formulations. This suggests that some shared component of the different formulations may be interfering with wound healing in this study.
- It is unclear if the treatments caused an interaction with the Prolene mesh as no

clear evidence of the mesh was seen in any sections, including on Day 3. The Applicant attributed the lack of mesh to loss of the fibers during tissue processing. The similar incidence and severity of histopathological effects on Day 3, especially in cystic change, suggest that the mesh performed similarly in all groups.

Methods

Doses: 25 mg bupivacaine and 0.75 mg meloxicam
 Frequency of dosing: Once prior to closing the subcutaneous tissues and the skin.
 Route of administration: Injection
 Dose volume: 0.5 (HTX-011-19) – 1.0 mL (HTX-024-001, HTX-002-014, HTX-011-49, and HTX-011-56)
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-011-19, HTX-011-49, and HTX-011-56). The vehicle control was HTX-024-001. See table below for further details.
 Species/Strain: Rabbit/New Zealand White, Crl:KBL (NZW) BR
 Number/Sex/Group: 4/sex/group (Main)
 Age: ~4 months
 Weight: 2.5-3.4 kg (m) and 2.6-3.9 kg (f)
 Satellite groups: none
 Unique study design: Hernia repair model using Prolene mesh
 Deviation from study protocol: None that impacted the results of the study.

Table 165: Treatment Groups Hernia Repair Study

Group	Test and Control Items	Active Pharmaceutical Ingredients (APIs)		Excipients				
		Bupivacaine w/w% ¹ (mg/g)	Meloxicam w/w% (mg/g)	Maleic Acid w/w% (mg/g)	^{(b) (4)} w/w% (mg/g)	DMSO w/w% (mg/g)	Triacetin w/w% (mg/g)	API35 w/w% (mg/g)
1	Saline Control	NA	NA	NA	NA	NA	NA	NA
2	HTX-024-001 Vehicle Control	NA	NA	NA	NA	10.00 (100.0)	35.00 (350.0)	55.00 (550.0)
3	HTX-002-014 Comparator	2.50 (25.0)	NA	0.15 (1.5)	NA	8.00 (80.0)	35.00 (350.0)	54.35 (543.5)
4	Test Item HTX-011-19	5.0 (50.0)	0.15 (1.5)	0.60 (6.0)	^{(b) (4)}	NA	NA	79.25 (792.5)
5	Test Item HTX-011-49	2.50 (25.0)	0.075 (0.75)	0.15 (1.5)	NA	8.00 (80.0)	35.00 (350.0)	54.275 (572.75)
6	Test Item HTX-011-56	2.50 (25.0)	0.075 (0.75)	0.05 (0.5)	NA	10.00 (100.0)	25.00 (250.0)	62.375 (623.75)

Data from page 8 of the study report.

Observations and Results

Mortality

One female was found dead during the surgery procedures prior to being dosed and was replaced by a spare animal. Since the animal wasn't dosed this death was not considered treatment related.

All other animals survived until scheduled termination of the study.

Clinical Signs

Cage side observations were conducted once daily throughout the study except on days with detailed clinical observations. Detailed clinical observations were conducted once before treatment, weekly throughout the study period, and before necropsy.

In males, the number of animals with and the total number of occurrences of reduced fecal output were increased slightly in animals who received the bupivacaine comparator, HTX-011-19, HTX-011-49, and HTX-011-56 compared to the vehicle and saline controls. In females, reduced fecal output was seen in all groups except the saline control. The incidence of this finding was higher in the bupivacaine comparator and HTX-011-19 formulation. This finding was not considered adverse due to the sporadic nature, observation in the control animals, and unclear toxicological significance.

All other clinical signs observed were not considered treatment related.

Table 166: Clinical Signs Rabbit Hernia Repair Study

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Reduced fecal output (total # of observations)	3	3	6	7	6	6
Reduced fecal output (# of animals)	3/8	2/8	5/8	4/8	5/8	3/8
Females						

Reduced fecal output (total # of observations)	0	2	3	6	1	1
Reduced fecal output (# of animals)	0/8	1/8	2/8	4/8	1/8	1/8

Dermal Observations

Dermal observations were made daily for the first seven days post-surgery (except Day 1), weekly thereafter, and prior to necropsy. Dermal observations were recorded using a modified Draize scoring system.

In animals sacrificed on Day 3, there were minor increases in the incidence and severity of erythema in males and females compared to the saline control. In males, increased severity was seen with the vehicle, HTX-011-19 and HTX-011-56 formulations while in females increases in severity were seen with the bupivacaine formulation comparator and all three HTX formulations. In animals sacrificed on Day 3, there were also increases in the incidence and severity of edema compared to the saline control in males and females. In males, all treated groups showed an increased incidence compared to saline with the highest incidence and severity in animals who received the vehicle, bupivacaine formulation comparator, and HTX-011-56. In females, all treated groups showed an increased incidence compared to saline, with the highest severity seen following treatment with the vehicle and HTX-011-56.

In animals sacrificed on Day 15, all groups in males showed an increased severity in erythema compared to the saline treated animals with HTX-011-49 and HTX-011-56 showing the highest increases in severity. In females, an increased incidence of erythema was seen in all treated groups compared to the saline control with the highest severity seen with HTX-011-19. The incidence and severity of edema in males was similar across all treatment groups compared to the saline control, while in females an increase in severity was seen in all groups compared to the saline control. In the saline control groups, findings of erythema (females only) and edema resolved by the middle of the study (Day 6). This was also true for the vehicle treated groups except for one case of edema in a female animal with edema from Day 8-15. For the groups treated with the bupivacaine formulation comparator and the HTX formulations, erythema and edema were more frequently seen on the last day of treatment suggesting a lack of recovery or a prolonged effect.

Overall, the vehicle resulted in more skin observations than the saline control. The bupivacaine formulation comparator displayed a pattern of skin findings very similar to the vehicle control. The three formulations of HTX appeared similar to both the comparator and the vehicle control, although there were some instances where the formulations appeared slightly worse. Taken together, the formulations appeared slightly worse than the saline control, although there did not appear to be a consistent pattern across formulation or sex. In addition, there were more findings at the end of the study with the bupivacaine formulation comparator and the HTX formulations.

Table 167: Dermal Observations of Day 3 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	2 (1)	3 (1)	2 (1)	3 (1.3)	1 (1)	4 (1.5)
Edema	0	3 (1.7)	3 (1.3)	2 (1)	1 (1)	3 (1.7)
Females						
Erythema	2 (1)	1 (1)	3 (1)	2 (1.5)	2 (1.5)	3 (1.3)
Edema	1 (1)	4 (1.5)	3 (1)	3 (1.3)	3 (1.3)	3 (1.7)

N=4

Bold = increased incidence/severity over saline control

Table 168: Dermal Observations of Day 15 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	3 (1.3)	4 (1.3)	4 (1.3)	2 (1.5)	4 (1.8)	3 (2)
Edema	4 (1.8)	4 (2)	4 (2)	3 (2.3)	4 (2)	4 (1.5)
Females						
Erythema	0	3 (1.3)	4 (1.5)	4 (1.8)	4 (1.5)	2 (1.5)
Edema	4 (1.3)	4 (1.8)	4 (2)	3 (1.7)	4 (1.5)	4 (1.5)

N=4

Bold = increased incidence/severity over saline control

Table 169: Dermal Observations on Day 15

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	0	0	0	0	1	1
Edema	0	0	1	3	3	2
Females						
Erythema	0	0	2	3	2	0
Edema	0	1	2	1	1	1

N=4

Wound Observations

Wound observations were made daily for the first seven days post-treatment, weekly thereafter, and prior to necropsy.

In animals sacrificed on Day 3, there were no clear differences between any treatments in males. In females, a minor increase in severity of hemorrhage was observed in animals receiving the bupivacaine comparator with larger increases in severity in animals receiving the vehicle and HTX-011-56. In addition, females receiving the bupivacaine comparator and HTX-011-49 had increased incidence and severity of exudate.

In animals sacrificed on Day 15, there were increases in the severity of hemorrhage at the wound site in females receiving all treatments compared to the saline control. In males, the severity of hemorrhage was similar across all groups. In females, there was increased severity in exudate in animals treated with the vehicle, HTX-011-19, and HTX-011-49 compared to controls. The severities for the presence of exudate were similar across all groups in males. Males did have an increased incidence of granulation in animals who received the bupivacaine formulation comparator, HTX-011-19 and HTX-011-49.

Since there were several findings in the saline control animals, only the severity of the findings was increased with some treatments and a lack of clear effects across sex and time, it is difficult to make a clear conclusion from the wound observations. The primary finding at the wound site was hemorrhage which was no longer present by Day 15 in most animals, although it was still present in half the female animals who received HTX-011-56. Therefore, while there were slight differences in wound healing during the study, wound healing was on average similar to saline treated animals by the end of the study.

Table 170: Wound Observations of Day 3 Sacrifice Group (Incidence)

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
hemorrhage	4 (2.5)	4 (2.8)	4 (2)	4 (1.3)	3 (1.7)	3 (3)
exudate	1 (1)	1 (1)	0	1 (1)	2 (1)	2 (1)
granulation	0	0	0	0	0	0
Females						
hemorrhage	4 (2)	3 (3)	4 (2.5)	4 (1.3)	3 (2)	4 (2.8)
exudate	1 (1)	1 (1)	2 (2)	2 (1)	3 (1.7)	3 (1)
granulation	0	0	0	0	0	0

N=4

Values in () represent average area affected: 1 = 1 mm, 2 = 2 mm, 3 = 1/2 of wound, 4 = whole wound
 Bold = increased incidence/severity over saline control

Table 171: Wound Observations of Day 15 Sacrifice Group (Incidence)

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
hemorrhage	4 (2.8)	4 (1.8)	4 (3)	3 (3)	4 (3.3)	4 (2.5)
exudate	3 (1.3)	2 (1.5)	4 (1.5)	3 (1)	3 (1.3)	2 (1)
granulation	0	0	1 (2)	2 (2)	1 (3)	0
Females						

hemorrhage	4 (2.3)	4 (3.5)	4 (2.8)	4 (3.3)	4 (3)	4 (2.8)
exudate	3 (1.3)	4 (2)	4 (1)	4 (1.8)	3 (2)	3 (1)
granulation	0	0	1 (1)	1 (1)	0	0

N=4

Values in () represent average area affected: 1 = 1 mm, 2 = 2 mm, 3 = 1/2 of wound, 4 = whole wound

Bold = increased incidence/severity over saline control

Table 172: Last Day of Wound Hemorrhage

Group	Saline	Vehicle	Bup comparator	HTX-011- 19	HTX-011- 49	HTX-011- 56
Males						
Hemorrhage- last day of finding	8, 6, 6, 7	8, 8, 7, 6	15, 8, 8, 7	6, 5, 6	8, 6, 6, 7	7, 4, 6, 6
Females						
Hemorrhage- last day of finding	6, 6, 8, 8	8, 8, 7, 8	8, 8, 8, 7	8, 8, 8, 7	15, 8, 5, 8	15, 15, 8, 7

Body Weights

Body weights were recorded once prior to group assignment, one week prior to treatment, and on Days -1, 4, 8, and 15.

No treatment-related changes in absolute body weight or body weight gain were observed.

Food Consumption

Food consumption was measured daily during the last week of the pre-treatment period and throughout the treatment and observation periods.

No treatment-related changes in food consumption were observed.

Hematology

Blood samples were collected from all animals (fasted) on prior to the start of treatment and on necropsy days via the central ear artery. A complete battery of hematology and coagulation parameters was evaluated.

In animals sacrificed on Day 3, there were several effects on hematology parameters with different treatments. In males, treatment with the vehicle lead to decreased platelets, monocytes, and reticulocytes, as well as, increased white blood cells and neutrophils. Similar changes were seen in females, except there was no difference in reticulocyte counts. The bupivacaine formulation comparator caused similar changes

as the vehicle control, except that white blood cell counts were only elevated in females. Overall, the three HTX formulations seemed to cause the same effects as the vehicle and the bupivacaine formulation comparator, except that the effects were less consistently observed in both sexes.

Of note, differences of similar magnitude for some parameters were also seen prior to treatment. For example, decreased platelets were seen in males receiving the vehicle, the bupivacaine formulation comparator, HTX-011-19, and HTX-011-56, decreased reticulocytes were seen in males receiving vehicle and the HTX formulations and increased white blood cells were seen in males receiving HTX-011-19. The pretreatment differences could not explain all the changes observed with treatment of the various formulations. Taking the pretreatment changes into consideration, the following effects were still observed:

Vehicle: Increased WBC (male/female), increased neutrophils (male/female), decreased monocytes (males)

Bupivacaine formulation comparator: Decreased reticulocytes (male), increased neutrophils (male), decreased monocytes (male/female), decreased platelets (female), increased WBC (female)

HTX-011-19: Increased neutrophils (male/female), decreased reticulocytes (female), increased WBC (female), decreased monocytes (female)

HTX-011-49: Decreased platelets (male), increased neutrophils (male), increased WBC (female)

HTX-011-56: Increased WBC (male/female), decreased monocytes (male/female), decreased platelets (female)

Across all formulations and the vehicle, the most consistent findings were increased WBCs, increased neutrophils, and decreased monocytes suggesting these are likely related to the shared excipients.

In animals sacrificed on Day 15, the effects on hematology seen on Day 3 were not as apparent, possibly indicating these effects are recoverable. Still, there were isolated changes in hematological parameters that were consistent with those on Day 3, such as decreased platelets in males receiving HTX-011-56 and females receiving HTX-011-19. Overall, on Day 15, hematology parameters were similar between treated and saline control animals.

Table 173: Hematology Findings on Day 3, Percentage of Saline Control

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						

Platelets	100	76.7	87.5	90.6	77.5	75.1
Retic (%)	100	76.5	83.8	70.6	75.0	80.9
WBC	100	123.3	102.4	117.6	104.6	112.8
Neutrophil	100	149.9	121.2	127.2	115.4	84.6
Monocytes	100	50.0	61.1	111.1	0	33.3
Females						
Platelets	100	89.4	80.0	97.6	99.5	76.9
Retic (%)	100	102.3	88.6	70.5	100.0	127.3
WBC	100	135.9	115.4	132.3	115.1	113.2
Neutrophil	100	150.0	124.3	141.4	101.7	94.9
Monocytes	100	70.0	55.0	65.0	80.0	45.0

Table 174: Hematology Findings on Day 15, Percentage of Saline Control

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Platelets	100	94.5	105.0	95.0	106.5	88.4
Retic (%)	100	113.3	100.0	100.0	82.2	75.6
WBC	100	92.3	86.6	89.2	105.1	100.8
Neutrophil	100	108.7	104.8	111.9	130.9	130.2
Monocytes	100	58.8	111.8	88.2	123.5	129.4
Females						
Platelets	100	90.9	102.8	84.4	106.7	101.2
Retic (%)	100	77.3	122.7	79.5	93.2	90.9
WBC	100	92.5	91.0	88.7	73.3	85.6
Neutrophil	100	115.5	109.2	119.1	75.3	92.4
Monocytes	100	125.0	216.7	158.3	91.7	216.7

Clinical Chemistry

Blood samples were collected from all animals (fasted) prior to the start of treatment and on necropsy days via the central ear artery. A complete battery of clinical chemistry parameters was evaluated.

The only changes in clinical chemistry parameters noted were decreased triglycerides (20-30%) in male animals treated with the HTX formulations compared to the saline control and decreased phosphorous (13-22%) in females of all groups compared to the saline controls. The toxicological significance of both findings is unknown.

Gross Pathology

A complete necropsy was performed on all animals.

Numerous lesions were observed at the incision site consisting of adhesion, dark area, firm, gelatinous material, mass, soft, pale area, raised area, and thickening. Occasionally, these findings were elevated compared to the saline control in the treated

groups as indicated in the tables below.

Table 175: Gross Findings of Day 3 Sacrifice Group

Groups	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f
Number of animals INCISION SITE	Males						Females					
	4	4	4	4	4	4	4	4	4	4	4	4
Adhesion	0	0	0	0	0	1	0	0	0	0	0	0
Dark area	4	3	3	4	3	2	3	4	4	4	4	3
Firm	0	1	3	0	0	0	1	1	0	1	0	0
Gelatinous material	0	1	0	0	0	0	0	0	0	0	0	0
Mass	0	1	0	0	0	0	1	0	0	0	0	0
Pale area	0	1	0	2	1	1	1	1	2	2	1	1
Raised area	1	0	0	0	0	0	0	0	0	0	0	0
Soft	3	0	0	0	0	0	0	2	1	0	0	0
Thickening	3	2	1	3	3	2	2	1	3	2	4	4

^a – Saline Control; ^b – Vehicle Control; ^c – Comparator (HTX-002-014); ^d – HTX-011-19;
^e – HTX-011-49; ^f – HTX-011-56

Table 176: Gross Findings of Day 15 Sacrifice Group

Groups	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f
Number of animals INCISION SITE	Males						Females					
	4	4	4	4	4	4	4	4	4	4	4	4
Adhesion	1	0	0	0	0	0	0	0	0	0	0	0
Dark area	0	0	1	0	2	1	0	1	0	1	0	1
Firm	0	1	1	0	0	1	0	2	2	0	0	1
Gelatinous material	0	0	0	0	1	0	0	0	0	0	0	0
Mass	0	0	1	1	0	0	0	0	0	0	0	0
Pale area	0	1	1	0	1	0	1	0	2	1	1	0
Raised area	1	0	0	0	0	0	0	0	0	0	1	0
Scab	0	0	0	0	0	0	0	0	0	0	1	2
Soft	0	0	0	0	1	0	1	0	1	1	0	0
Thickening	3	4	1	3	3	2	4	4	2	4	1	3

^a – Saline Control; ^b – Vehicle Control; ^c – Comparator (HTX-002-014); ^d – HTX-011-19;
^e – HTX-011-49; ^f – HTX-011-56

Histopathology

Adequate Battery: Yes**Peer Review: No.**

Histopathological examinations were only performed on incision sites and gross lesions identified during necropsy.

Histological Findings

On the Day 3 sacrifice, injection site findings consisted of minimal to mild cell debris, minimal to moderate cystic change, minimal to mild epidermal erosion/ulcer, minimal to moderate hemorrhage, minimal epidermal hyperplasia, minimal to moderate subcutaneous acute inflammation, and minimal to mild necrosis. These findings were of similar incidence and severity across the saline control and all treatments. No clear evidence of the mesh material was seen in the sections. However, cystic change was related to the space created by the mesh and since this finding was similar amongst all treatment groups it is unlikely that the mesh performed differently amongst the groups.

On Day 15, similar findings were present at the injection site except both dermal granulation and subcutaneous/muscular/intermuscular granulation were also present. At this time point, there appeared to be some differences compared to the saline control animals. In males, there was an increased incidence of cell debris in animals receiving the HTX formulations, increased incidences of cystic change and hemorrhage in animals of all treatment groups, increased incidence/severity of epidermal hyperplasia with HTX-011-19 and HTX-011-49, and an increased incidence of dermal granulation in animals receiving HTX-011-19. In females, an increased incidence of epidermal ulcer was seen in animals receiving all treatments except the vehicle control, an increased severity of subcutaneous/muscular/intermuscular granulation was observed in animals receiving all treatments except HTX-011-49, an increased incidence of hemorrhage in animals receiving the vehicle, the bupivacaine formulation comparator, and HTX-011-56, and an increased incidence of necrosis in the bupivacaine formulation comparator. Taken together, it appears there was some impact on wound healing on Day 15 following treatment with the various formulations, primarily in males who received the HTX formulations.

Table 177: Histological Findings of Day 15 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						

Cell debris	0	0	0	1 (2)	1 (2)	1 (2)
Cystic change	1 (2)	3 (2)	4 (2)	4 (2)	4 (2)	4 (2)
Epidermal ulcer	0	0	0	2 (1)	0	0
Hemorrhage	2 (1.5)	3 (1.7)	3 (1.7)	3 (1.7)	3 (1.3)	4 (1.5)
Epidermal hyperplasia	1 (1)	2 (1)	2 (1)	2 (1.5)	1 (2)	2 (1)
Dermal granulation	2 (1.5)	4 (1)	3 (1.3)	4 (1.3)	2 (1.5)	3 (1)
Granulation subcutaneous/muscular/intermuscular	4 (2.8)	4 (3)	4 (3)	4 (2.3)	4 (2.8)	4 (2.5)
Necrosis	1 (2)	1 (2)	3 (1.1)	2 (1.5)	2 (1)	2 (1)
Females						
Cell debris	2 (2)	0	1 (1)	2 (1.5)	1 (3)	1 (2)
Cystic change	3 (2.3)	4 (1.8)	4 (2.3)	4 (2)	3 (2)	4 (1.8)
Epidermal ulcer	0	0	1 (1)	3 (1.3)	1 (3)	1 (2)
hemorrhage	0	4 (1.5)	2 (1.5)	1 (1)	1 (1)	2 (1.5)
Epidermal hyperplasia	4 (2.3)	3 (1)	1 (2)	2 (2)	2 (1)	3 (1.7)
Dermal granulation	4 (2)	3 (1.7)	2 (1)	3 (1.3)	3 (2)	4 (2)
Granulation subcutaneous/muscular/intermuscular	4 (1)	4 (1.5)	4 (2)	4 (1.8)	3 (1.3)	4 (1.5)
necrosis	2 (1.5)	3 (1)	4 (1.5)	3 (1.3)	3 (1.7)	3 (1.3)

Bold = increased incidence/severity over saline control

Dosing Solution Analysis

Dosing solution analysis was not conducted by the testing laboratory. Instead, data on the achieved concentrations of the test and control items was provided by the Applicant in the form of certificates of analysis and stability data to cover the period of dosing.

Study title: Single Subcutaneous Dose Effect of HTX-002-014, HTX-001-49 and HTX-011-56 on Bone Healing in the Cortical Defect Model in Female Sprague Dawley Rats

Study no.: 33-39
Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-to\33-39\33-39.pdf>
Conducting laboratory and location:  (b) (4)
Date of study initiation: September 26, 2015
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: HTX-024-001, Lot 1237047
HTX-002-14, Lot 1273049
HTX-011-49, Lot 15011
HTX-011-056, Lot 025774
Purities were not provided

Key Study Findings

- SD rats were administered a single application of 12.5 mg bupivacaine or 12.5 mg bupivacaine and 0.375 mg meloxicam by subcutaneous injection following surgery to induce cortical defect.
- At least six animals were found to have lameness following surgery. Of these, six animals were sacrificed early due to associated fractures. Since none of these animals were from the control group, the treatment-related nature of this finding could not be ruled out.
- Skin lesions (scabs and ulceration) were seen in animals receiving the bupivacaine comparator and HTX formulations.
- There were no treatment-related effects on body weight.
- No treatment-related effects on bone mineral content, bone mineral density, or the healing stage of the bone were identified throughout the study.
- The study should have been conducted in accordance with Good Laboratory Practices (GLPs). Although this study was not conducted according to GLPs, according to the Applicant the only deviation from GLP was that the study was not quality assured. The results were reviewed, and the study was designed reasonably well. The Division concluded that based on the results obtained, repeating the study in accordance with full GLPs was not likely to impact the clinical assessment of this critical endpoint and would not be required in this unusual circumstance.

Methods

Doses: 12.5 mg bupivacaine and 0.375 mg meloxicam
 Frequency of dosing: Once
 Route of administration: Subcutaneous Injection
 Dose volume: 0.5 mL
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-002-014, HTX-011-49, and HTX-011-56). The vehicle control was HTX-024-001. See table below for further details.
 Species/Strain: Rat/Sprague-Dawley
 Number/Sex/Group: 8/females/group
 Age: 15-16 weeks
 Weight: 293.59 ± 10.93 g
 Satellite groups: none
 Unique study design: Cortical defect surgery and CT scans.
 Deviation from study protocol: Part way through the study, animals began being housed individually to minimize the occurrence of fractures.
 Husbandry checks were only performed once daily instead of twice daily.
 Macroscopic observations were not collected automatically in a way that allowed cross referencing with histological observations.

Group	Test and Control Items	Active Pharmaceutical Ingredients			Excipients		
		Bup	Mel	Maleic Acid	DMSO	Triacetin	AP135
		w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)
1	Saline Control	NA	NA	NA	NA	NA	NA
2	Vehicle Control HTX-024-001	NA	NA	NA	10.00 (100.0)	35.00 (350.0)	55.00 (550.0)
3	Comparator HTX-002-014	2.5 (25.0)	NA	0.15 (1.5)	8.00 (80.0)	35.00 (350.0)	54.35 (543.5)
4	Test Item HTX-011-49	2.5 (25.0)	0.075 (0.75)	0.15 (1.5)	8.00 (80.0)	35.00 (350.0)	54.275 (572.75)
5	Test Item HTX-011-56	2.5 (25.0)	0.075 (0.75)	0.05 (0.5)	10.00 (100.0)	25.00 (250.0)	62.375 (623.75)

Abbreviations: Bup = Bupivacaine; Mel = Meloxicam

Data from page 7 of the study report.

Observations and Results

Mortality

Six animals were identified by CT scan as having fractures. These animals were sacrificed early. All other animals survived to scheduled sacrifice.

Clinical Signs

Animals were monitored daily for lameness, death, and morbidity. Animals that showed signs of lameness were x-rayed and/or CT scanned and sacrificed if a fracture was detected.

As described above, at least six animals were found to have lameness and underwent a subsequent CT scan. A total of six animals were identified with a fracture and they consisted of two animals from the vehicle control group, two animals from the bupivacaine comparator group, one animal from the HTX-011-49 group, and one animal from the HTX-011-56 group. Fractures were primarily identified on the right femur (5/6) which is the leg that underwent injection. Since the fractures only occurred in animals receiving one of the formulations and not the saline control, the treatment-related nature of this finding could not be ruled out.

Skin lesions at the injection site, consisting of scabs and ulceration, were observed in six animals receiving the bupivacaine comparator, four animals receiving HTX-011-49, and four animals receiving HTX-011-56. These lesions were found during cage side observations on the day of necropsy.

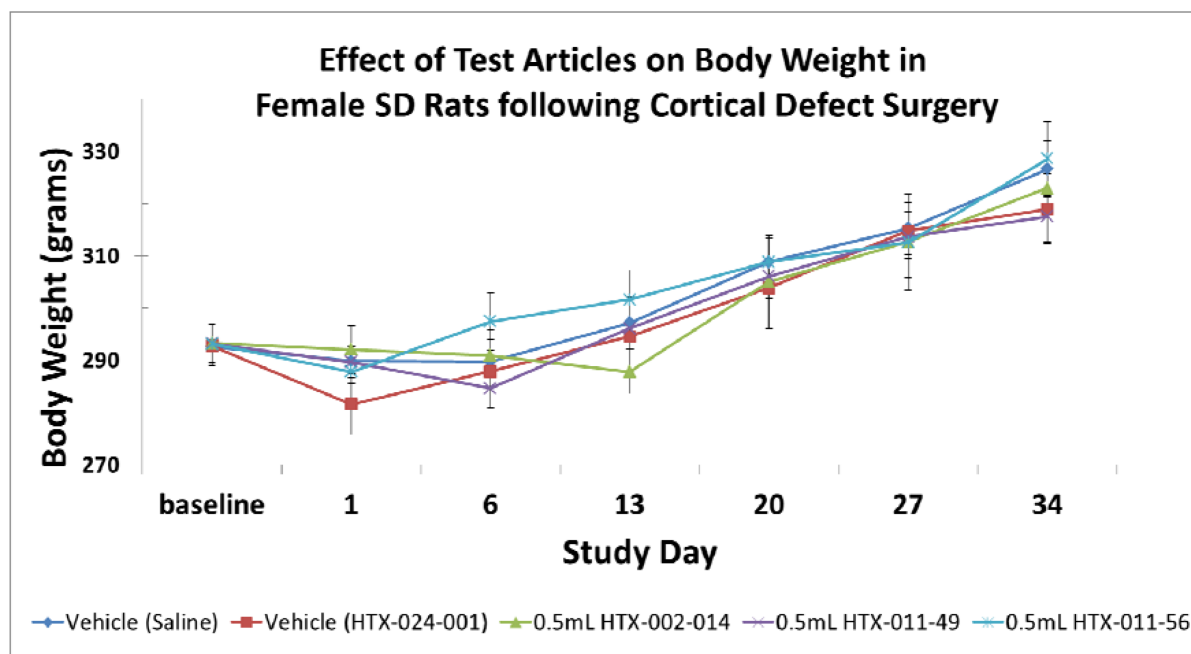
Table 178: Clinical Signs

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Fracture	0/8	2/8 (right, both)	2/8 (right, both)	1/8 (left)	1/8 (right)
Skin lesions	0/8	0/8	6/8	4/8	4/8

Body Weights

Body weights were recorded on Day 5 of the predose phase and on Days 1, 6, 13, 20, and 34 of the dosing phase.

No treatment-related differences in absolute body weight were observed.

Figure 5: Body Weight in Rat Bone Defect Study

Data from page 12 of the study report.

CT Imaging

Animals underwent CT imaging on Days 1, 6, 13, 20, and 34 of the dosing phase. Total bone mineral density (BMD) and bone mineral content (BMC) of the callus were measured for both the left and right femur.

No significant differences were noted in total BMD for the left and right femurs and total BMC in the left femurs from Days 1-34. No significant change was observed in total BMC of the right femurs from Days 6 to 34. A significant increase in total BMC was observed in the right femurs of animals receiving HTX-011-56 on Day 1 only. The toxicological significance of this finding is unknown.

Table 179: Bone Mineral Content of Left Femur (Percent of Saline Control)

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	87.6	84.8	113.8	117.1
Day 6	100	97.7	107.1	95.2	111.7
Day 13	100	112.7	117.8	112.3	147.7
Day 20	100	113.0	123.0	115.6	131.9
Day 34	100	105.6	116.8	108.5	117.5

Table 180: Bone Mineral Content of Right Femur (Percent of Saline Control)

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	104.5	97.4	116.5	128.8
Day 6	100	104.7	96.6	107.9	119.2
Day 13	100	113.2	83.5	95.3	125.9
Day 20	100	106.1	86.2	91.8	108.9
Day 34	100	99.9	96.8	96.2	103.9

Table 181: Bone Mineral Density of Left Femur (Percent of Saline Control)

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	89.6	86.4	111.3	110.2
Day 6	100	100.2	108.7	93.0	106.0
Day 13	100	115.6	119.8	110.1	140.5
Day 20	100	116.1	125.7	113.4	125.9
Day 34	100	108.6	119.4	106.5	112.2

Table 182: Bone Mineral Density of Right Femur (Percent of Saline Control)

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	103.7	96.3	109.5	117.4
Day 6	100	103.9	95.7	101.5	109.2
Day 13	100	114.3	82.1	90.8	116.8
Day 20	100	106.3	85.0	86.6	100.6
Day 34	100	100.1	96.0	90.8	96.0

Gross Pathology

A complete necropsy was performed on all animals.

Due to an error, no macroscopic findings were recorded.

Histopathology

Adequate Battery: Not for a full toxicology study, because only left and right femur were analyzed. As this study was intended to characterize the effects on bone, this is acceptable.

Peer Review: No.

Histopathological examinations were only performed on the left and right femur. The stage of the healing process was scored by the pathologist according to a scoring system described by Xu et al., 2011.

Histological Findings

There was no significant difference in the mean healing score for any group compared to the saline control. All animals had a healing score of 4-5, except one animal receiving the vehicle control which had a healing score of 2.

Table 183: Healing Score of Femur

Group	Saline	Vehicle	Bup comp	HTX-011-49	HTX-011-56
Healing score-left	4.00	4.50	4.00	4.00	4.00
Healing score-right	4.13	3.67	4.00	4.14	4.14

2.1 Scoring System: Healing Stages

- Stage 1: Predominance of soft tissue reaction (mixture of hematoma and granulation tissue) with a small amount of intramedullary woven bone located adjacent to the bone defect.
- Stage 2: Intramedullary woven bone predominates, mixed with smaller amounts of soft tissue reaction composed of immature mesenchymal tissue. Bone defect is not completely bridged by the new bone formation.
- Stage 3: Complete bridging of the bone defect on the endosteal surface and moderate amount of remodeled intramedullary trabecular bone remaining in the medullary cavity with inter-trabecular spaces containing varying amounts of immature connective tissue and/or re-established marrow population.
- Stage 4: Complete bridging and partial filling of bone defect with remodeled woven bone, reduced amount of intramedullary woven bone as compared with Stage 3, and reestablishment of marrow population.
- Stage 5: All cortical surfaces bridged by the remodeled bone with the absence of the intramedullary bone and complete resolution of soft tissue reaction and bone defect.

From page 74 of Study 33-39

Dosing Solution Analysis

Dosing solution analysis was not conducted.

Study title: HTX-002, HTX-009 and HTX-011: A Single Dose 15-Day Wound Healing Dermal Toxicity Study in the Gottingen Minipig

Study no.: 33-54
 Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-to\33-54\33-54.pdf>

Conducting laboratory and location:  (b) (4)

Date of study initiation: June 20, 2016
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: HTX-002-013, Lot 16007, 2.5% meloxicam, >99.9% purity
 HTX-009-005, Lot 15011, 0.075% meloxicam, purity not reported
 HTX-011-056, Lot 026126, 0.075% meloxicam and 2.5% bupivacaine, purity not reported

Key Study Findings

- HTX-011 caused slightly worse Draize scoring and wound observations compared to saline and sham control.
- Wounds were not completely healed by Day 15 in any group. A slight increase in the severity of histopathological findings was observed at incision sites treated with HTX-011 compared to saline and sham controls.
- Overall the impact on wound healing appeared to be minimal since only small increases in severity were observed by histopathology.
- The doses used in this study are lower than those used in the clinical studies based on normalization to the incision size. The volume per incision size used in this study was 1 mL/cm. Clinical studies are expected to be herniorrhaphy; 2.5-3.4 mL/cm,  (b) (4) based on information provided by the clinical team. Because clinical studies were permitted to proceed, the reader is referred to the clinical review for definitive information on the impact of the drug product on soft tissue wound healing in humans.

Methods

Doses: 87.75 mg bupivacaine and 2.63 mg meloxicam
 Frequency of dosing: Once by wound surface application into a surgical incision.
 Route of administration: Wound surface application
 Dose volume: 3 mL
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-002-013, HTX-009-005, and HTX-011-56). The vehicle control was HTX-024-002. See table below for further details.
 Species/Strain: Gottingen Minipig, *Sus scrofa domestica*
 Number/Sex/Group: 3/sex/group (Main)
 Age: 9-10 months
 Weight: 17.8-20.5 kg (m) and 17.9-21.5 kg (f)
 Satellite groups: None
 Unique study design: Wound healing following surgical incision. Each animal received six incisions and received all treatments: saline, HTX-011-056, vehicle, HTX-009-005, HTX-002-013, and sham; one treatment per incision.

Deviation from study protocol: None that impacted the results of the study.

Incision	Test and Control Items	Active Pharmaceutical Ingredients (APIs)		Excipients			
		Bupivacaine w/w% ¹ (mg/g)	Meloxicam w/w% (mg/g)	Maleic Acid w/w% (mg/g)	DMSO w/w% (mg/g)	Triacetin w/w% (mg/g)	AP135 w/w% (mg/g)
1	Saline Control	NA	NA	NA	NA	NA	NA
2	Vehicle Control HTX-024-002	NA	NA	NA	10.00 (100.00)	25.00 (250.00)	65.00 (650.00)
3	HTX-002-013	2.50 (25.00)	NA	0.05 (0.50)	10.00 (100.00)	25.00 (250.00)	62.45 (624.5)
4	HTX-011-056	2.50 (25.00)	0.075 (0.75)	0.05 (0.50)	10.00 (100.00)	25.00 (250.00)	62.375 (623.75)
5	HTX-009-005	NA	0.075 (0.75)	NA	10.00 (100.00)	25.00 (250.00)	64.925 (649.25)
6	Sham Control	NA	NA	NA	NA	NA	NA

Data from page 10 of the study report.

Observations and Results

Mortality

No mortalities were observed.

Clinical Signs

Cage side observations were conducted once daily throughout the study except on days with detailed clinical observations. Detailed clinical observations were conducted once before treatment, weekly throughout the study period, and before necropsy.

Bruising, crust, and decreased activity were seen sporadically throughout the study. Since each animal received all treatments, these findings could not be ascribed to any of the test articles.

Dermal Observations

Dermal observations were assessed post-surgery on Days 2-8 and Day 15 for animals being sacrificed on Day 15. Dermal observations were assessed post-surgery on Days 2-4 for animals being sacrificed on Day 4.

In animals sacrificed on Day 4, increased number of days with erythema was observed with treatment of HTX-011 compared to the saline and sham controls in both males and females. Increased number of days with erythema were also observed with treatment of the bupivacaine formulation comparator (male and female) and vehicle (female). The average severity was similar between all groups. For edema, there did not appear to be a major difference in the number of days observed. There was a small increase in the average severity of edema for females at incision sites treated with HTX-011.

In animals sacrificed on Day 15, there did not appear to be a difference in the number of days edema was observed between the different treatments. However, there did appear to be an increase in the incidence of observations on Day 15 compared to the saline and vehicle control with HTX-011.

Taken together, HTX-011 appeared to result in a slightly worse Draize scoring across the study.

Table 184: Dermal Observations in Day 4 Sacrifice Animals, Male

Group	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
Erythema 3 animals	-	1 (1)	3 (1)	3 (1)	2 (1)	5 (1)
Edema 3 animals	8 (1.2)	8 (1)	8 (1.3)	9 (1)	9 (1)	8 (1.2)

Total # of days observed (average severity); n=3 animals/group

Table 185: Dermal Observations in Day 4 Sacrifice Animals, Female

Group	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
Erythema 3 animals	2 (1)	6 (1)	4 (1)	2 (1)	2 (1)	4 (1)
Edema 3 animals	9 (1.1)	9 (1.3)	9 (1.1)	9 (1.1)	9 (1.2)	9 (1.5)

Total # of days observed (average severity); n=3 animals/group

Table 186: Dermal Observations in Day 15 Sacrifice Animals, Male

Animal	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
M1	5 (1.2)	6 (1)	7 (1.2)	5 (1.2)	6 (2)	6 (2) Day 15 (2)
M2	6 (1.2) Day 15 (1)	5 (1)	5 (1)	6 (1)	5 (1)	6 (2) Day 15 (1)
M3	6 (2)	1 (2)	1 (1)	5 (1)	5 (1)	6 (1.3) Day 15 (1)

Total # of days observed (average severity); n=3 animals/group

Table 187: Dermal Observations in Day 15 Sacrifice Animals, Female

Animal	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
F1	6 (1.8)	6 (1.8)	6 (2)	6 (1.8)	5 (2)	6 (2.2) Day 15 (3)
F2	6 (1.5)	6 (1.8) Day 15 (1)	6 (1.8) Day 15 (1)	6 (1.8)	7 (1.5)	6 (2) Day 15 (1)
F3	6 (2)	6 (2)	6 (1.8)	6 (1.8)	6 (1.8)	6 (2) Day 15 (1)

Total # of days observed (average severity); n=3 animals/group

Wound Observations

Wound observations were made on Day 4 (all animals), and on Days 8 and 15 for animals in the Day 15 sacrifice group.

On Day 4, there was a slight increase in the appearance of the wound (erythema), condition of the skin surrounding the wound (erythema), and the presence of hemorrhage in males at incision sites treated with HTX-011 compared to saline and sham controls. In females, this was only true for the condition of skin surrounding the wound.

In animals sacrificed on Day 15, increased severity was observed in males on Day 8 for presence of hemorrhage only. No difference in severity for any findings was observed on Day 15.

Table 188: Wound Observations on Day 4

Group	Saline	Vehicle	Bup comp	Meloxicam	Sham	HTX- 011-56
Males						
Appearance of Wound and Wound Edge n = 6	6 (1.5)	6 (1.3)	6 (1.8)	6 (1.5)	5 (1.6)	6 (1.8)
Condition of skin surrounding the wound n = 6	5 (1)	5 (1)	5 (1.6)	4 (1)	4 (2)	5 (1.8)
Presence of hemorrhage n = 6	5 (1.8)	5 (1.6)	6 (2.0)	3 (2.3)	4 (1.8)	6 (2.3)
Presence of exudates n = 6	0	1 (2)	1 (3)	0	0	2 (1)
Females						
Appearance of Wound and Wound Edge n = 6	6 (2.2)	6 (2.3)	6 (1.7)	6 (2.0)	6 (2.3)	5 (2.2)
Condition of skin surrounding the wound n = 6	6 (1.5)	6 (1.2)	4 (1.5)	4 (1.5)	5 (1.6)	6 (1.8)
Presence of hemorrhage n = 6	6 (2.8)	6 (2.3)	6 (2.7)	6 (2.5)	6 (2.7)	6 (2.5)

Presence of exudate n = 6	4 (2.5)	1 (2)	4 (2.8)	3 (2)	1 (3)	4 (2.3)

of animals with finding (average severity); n = 6/sex/group

Table 189: Wound Observations on Day 8

Group	Saline	Vehicle	Bup comp	Meloxicam	Sham	HTX-011-56
Males						
Appearance of Wound and Wound Edge (1/3)	3 (1)	3 (1)	3 (1.3)	2 (1.5)	3 (1.3)	2 (1.5)
Condition of skin surrounding the wound (1/3)	3 (1.3)	2 (1)	2 (2.5)	1 (1)	3 (2)	3 (2)
Presence of hemorrhage (3/3)	3 (1.3)	2 (1.5)	3 (2.3)	2 (2)	3 (1)	3 (2.7)

of animals with finding (average severity); n = 6/group

Gross Pathology

Only the dosing sites were evaluated for gross lesions. There did not appear to be any difference between groups in terms of gross pathological findings at the surgical site.

Table 190: Gross Pathology of the Incision Site, Day 4

Sex	Male						Female					
Incisions *	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Clear fluid	0	1	0	0	1	0	2	2	3	2	0	0
Creptus	0	0	0	0	0	2	0	0	0	0	0	2
Dark area	2	3	2	3	2	3	3	3	3	3	3	3
Dark material	0	0	0	0	0	0	0	0	1	1	0	0
Gelatinous material	1	1	2	3	3	1	1	0	0	1	0	1
Pale material	0	0	1	1	0	0	0	2	1	0	0	0
Scar	3	2	2	3	3	2	1	1	1	1	1	2
Wound	0	1	1	0	0	1	2	2	2	2	2	1

* Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 29 of the study report

Table 191: Gross Pathology of the Incision Site, Day 15

Sex	Male						Female					
Incisions *	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Creptus	0	0	0	0	0	0	0	0	0	0	0	1
Dark area	0	0	3	0	0	0	1	2	2	2	1	1
Mass	0	0	0	1	0	0	0	0	0	1	0	0
Pale material	0	0	0	0	0	0	0	0	0	0	1	0
Scar	3	3	3	3	3	3	3	3	3	3	3	3
Thickening	0	0	0	0	0	0	0	0	1	1	0	0

* Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 30 of the study report

Histopathology

Adequate Battery: Yes**Peer Review: No.**

Histopathological examinations were only performed on incision sites and gross lesions identified during necropsy.

Histological Findings

Overall, there was a similar incidence of histopathological findings across the different treatments. The exception was that one male in HTX-011-56 (Group 4) on Day 15 had a mild abscess that was not seen with any other treatment group. There were some minor increases in the severity of findings with HTX-011 for a couple of findings, including serro-cellular crust on Day 4, inflammatory cell infiltrates on Day 15, and granulomatous inflammation of the subcutis on Day 15.

Table 192: Summary of Histopathological Findings, Day 4

Sex	Male						Female					
Incisions*	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Cell infiltrate, inflammatory	3	3	3	3	3	3	3	3	3	3	3	3
minimal	0	0	0	0	0	0	2	1	0	1	2	1
mild	3	1	3	2	3	2	1	2	3	2	1	2
moderate	0	2	0	1	0	1	0	0	0	0	0	0
Eosinophilic material	3	3	3	3	3	3	3	3	3	3	3	3
minimal	3	2	2	1	2	2	1	1	0	0	2	1
mild	0	1	1	2	1	1	2	2	3	2	1	1
moderate	0	0	0	0	0	0	0	0	0	1	0	1
Sero-cellular crust	3	3	3	3	2	3	3	3	3	3	3	3
minimal	2	1	2	1	2	1	1	1	1	1	3	2
mild	1	2	0	2	0	2	2	1	1	1	0	1
moderate	0	0	1	0	0	0	0	1	1	1	0	0
Ulcer	3	3	3	2	2	3	3	3	3	3	2	2
minimal	2	2	2	1	2	2	2	3	2	2	2	2
mild	1	1	1	1	0	1	1	0	1	1	0	0

*Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 31 of the study report

Table 193: Summary of Histopathological Findings, Day 15

Sex	Male						Female					
	1	2	3	4	5	6	1	2	3	4	5	6
Incisions*	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Abscess	0	0	0	1	0	0	0	0	0	0	0	0
mild	0	0	0	1	0	0	0	0	0	0	0	0
Cell infiltrate, inflammatory	1	0	0	1	0	0	1	0	0	1	0	0
minimal	1	0	0	0	0	0	1	0	0	0	0	0
mild	0	0	0	1	0	0	0	0	0	1	0	0
Contraction dermal (present)	3	1	1	2	1	3	3	3	3	2	3	2
Inflammation, granulomatous, dermis	3	3	3	3	3	3	3	3	3	3	3	3
minimal	2	2	0	3	2	2	0	1	0	0	0	0
mild	1	1	3	0	1	1	3	0	2	3	2	3
moderate	0	0	0	0	0	0	0	2	1	0	1	0
Inflammation, granulomatous subcutis	3	3	3	3	3	3	3	3	3	3	3	3
minimal	1	2	0	0	1	3	0	0	1	0	0	3
mild	2	1	3	1	2	0	3	2	1	1	3	0
moderate	0	0	0	2	0	0	0	1	1	2	0	0
Sero-cellular crust	3	2	2	3	3	3	3	3	1	1	3	2
minimal	1	1	2	2	2	1	1	3	1	1	3	2
mild	2	1	0	1	1	2	2	0	0	0	0	0

*Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 32 of the study report

10.2 Juvenile Animal Studies

As noted in the agreed iPSP, the Applicant will be completing two juvenile animal toxicology studies. The first is a study in the rat model to evaluate the impact of DMSO on brain development. This study is being completed because the Division raised concerns with the potential impact of DMSO via this product on brain development based on published report describing widespread neuronal apoptosis in young animals (Hanslick et al., 2009). (b) (4)

(b) (4). Likewise, because kidney, liver, lung, and testes are known target organs of toxicity for NSAIDs, a juvenile animal study will be completed (b) (4). The (b) (4). Feedback on the protocols has already been provided by the Division. According to the currently agreed amended IPSP, these studies are to be completed in accordance with the following timelines.

Milestone	Agreed iPSP Date	Amended Agreed iPSP
(b) (4)		

11 Integrated Summary and Safety Evaluation

The Applicant's original NDA submission was issued a Complete Response with multiple nonclinical deficiencies which are summarized as follows: the Applicant did not provide adequate justification for the safety of excipients (DMSO, triacetin, and maleic acid) in terms of reproductive and developmental toxicity, the Applicant did not provide adequate justification for the specification of the impurities (b) (4) or (b) (4) and the Applicant did not provide adequate extractable/leachable information for toxicological evaluation of the safety of the container closure system. In addition, to the deficiencies, the Applicant was reminded that they did not have nonclinical support for their proposed dose of 400 mg bupivacaine and that the safety of this dose was not adequately supported in their submission. The following sections describe the adequacy of the Applicants attempts to address the deficiencies and the overall pharm/tox conclusions regarding the drug product.

Excipients

In the original submission, the Applicant did not provide adequate justification for the safety of excipients (dimethyl sulfoxide, triacetin, and maleic acid) in terms of reproductive and developmental toxicity. The resubmission included new data to address these deficiencies.

For dimethyl sulfoxide (DMSO), the Applicant referenced the Organization for Economic Cooperation and Development's (OECD) Screening Information Dataset (SIDS) dossier for DMSO, which contained information regarding GLP reproductive and developmental toxicity studies. No evidence of reproductive or developmental toxicity was reported in an oral reproduction/developmental toxicity screening study in rats, an oral developmental toxicity study in rats, or an oral developmental toxicity study in rabbits up to 1000 mg/kg. Developmental toxicity associated with maternal toxicity (decreased body weight gain in dams manifesting as decreased fetal weight and reduced/delayed ossification of the ribs) was observed in the rat developmental study at 5000 mg/kg. The overall NOAEL for reproductive and developmental toxicity was 1000 mg/kg, which is a human equivalent dose of 9,677.4 mg based on body weight scaling and a 60 kg individual. However, no bridging exposure data were provided to justify why oral administration would provide similar or greater exposure compared to the clinical route of administration and thus the Applicant did not provide adequate data to support their conclusion that these data adequately characterized the safety of the DMSO administered via the clinical route of administration.

For triacetin, the Applicant cited an unpublished GLP oral combined reproduction/developmental toxicity study in rats in Japanese and provided a translated copy of the study report. This study was also evaluated by the Organization for Economic Cooperation and Development (OECD 2002a). No findings of reproductive or developmental toxicity were observed in the study, which tested up to 1000 mg/kg (human equivalent dose of 9,677.4 mg based on body weight scaling and a 60 kg individual). However, no bridging exposure data were provided to justify why oral administration would provide similar or greater exposure compared to the clinical route of administration and thus the Applicant did not provide adequate data to support their conclusion that these data adequately characterized the safety of triacetin administered via the clinical route of administration.

Developmental data in a second species was not available. To address this data gap, the Applicant cited developmental data located in OECD SIDS documents for the major expected metabolites of triacetin, glycerol and acetic acid. Glycerol showed no evidence of developmental toxicity when tested in the rat (up to 1310 mg/kg), mouse (1280 mg/kg), or rabbit (up to 1180 mg/kg). Acetic acid showed no evidence of developmental toxicity when tested in the rat, mouse, and rabbit (all up to 80 mg/kg). Higher doses of acetic acid could not be tested due to stomach irritation. While the major expected metabolites, acetic acid and glycerol, did not show any evidence of developmental or reproductive toxicity in multiple species, the Applicant did not provide data to characterize triacetin exposure following administration of HTX-011-56 and therefore, it is not known if the parent compound will be present in the systemic circulation. If triacetin is not present in the systemic circulation, reproductive and

developmental effects of the metabolites would be a valid justification for not conducting reproductive and developmental studies for triacetin.

For maleic acid, the Applicant provided rat oral reproduction and developmental toxicity data from an EPA action memorandum in the original submission. No reproductive or developmental effects were seen at human equivalent doses of 532 mg (reproductive) and 1354.8 mg (teratogenic), which is 67x and 169x times the levels a patient would be exposed to at the MRHD, respectively, based on body surface area and a 60 kg individual. The oral bioavailability in rats was about 30% based on a reference provided by the Applicant. The safety margins would still be sufficiently high even if it is assumed that instillation into the wound would result in 100% bioavailability of maleic acid with adjusted safety margins of 20x and 51x.

In the resubmission, the Applicant submitted an oral developmental study in rabbits with maleic acid (Study 34-74). No evidence of developmental toxicity was observed in the study up to an oral dose of 20 mg/kg, which is a human equivalent dose of 387.1 mg based on body surface area and a 60 kg person. Higher doses could not be tested due to the potential for stomach irritation. This dose produced no evidence of maternal toxicity and therefore was not ideally designed as a toxicology study. Further, the study report in the NDA is draft and there are no exposure data to justify why the oral toxicology study resulted in exposures comparable to that which would occur via instillation into the surgical wound. The Applicant did identify oral bioavailability data in the rat but oral bioavailability in the rabbit was not provided.

In terms of local toxicity, treatment with the HTX-011 vehicle (containing DMSO and triacetin) resulted in an inflammatory reaction that is consistent with a reaction to foreign material. This was characterized as chronic inflammation that lasted for up to 28 days. A similar reaction was seen with the HTX-011 drug product (containing DMSO, triacetin, and maleic acid). Based on studies with APF18A (a similar polymer (b) (4)), the inflammatory reaction is very likely driven by the presence of AP135, although a contribution of the other excipients cannot be completely ruled out. In addition to the local inflammatory reaction, more severe findings, such as necrosis, were seen in the single-dose rat studies but this is possibly due to the increased injection volume used in these studies (0.5-2 mL in single dose studies as compared to 0.13-0.19 mL in repeat dose studies).

In terms of systemic effects, the primary findings with the HTX-011 vehicle (containing DMSO and triacetin) were changes in hematology parameters with similar effects observed with HTX-011 (containing DMSO, triacetin, and maleic acid). Again, it is likely that these changes are the result of AP135 as similar findings were seen with APF18A, although a contribution of the other excipients cannot be completely ruled out. More severe effects (decreased body weights and decreased activity) were seen in the repeat-dose dog study with the HTX-011 vehicle (Study number 33-86). The more robust systemic reaction to the HTX-011 vehicle in dogs is likely related to the large dose of vehicle in this study as the effects were not observed with the clinical formulation in the same study, which delivered about ½ the level of excipients.

In conclusion, not all of the deficiencies were fully addressed by the Applicant. Although the existing data suggest low concern for reproductive or developmental toxicity at the MRHD based on body surface area, the safety margins may not be accurate given that this drug is instilled directly into wounds rather than via the oral route of administration.

Based on the local tissue assessment conducted with various formulations, the data clearly suggest that the excipients contribute to the observed local inflammatory reaction and the systemic hematology findings. However, we acknowledge that there is clinical experience with the excipients from the Phase 2 and 3 studies and defer to the clinical review team regarding if the effects observed in non-clinical studies translate into clinical adverse effects. If the clinical team concludes that the nonclinical findings do not translate into clinical adverse effects, then the excipients are characterized for at a margin of 1.5x for AP135, 1.4x for DMSO and triacetin, and 1.1x for maleic acid and locally at a margin of ~1x (clinical concentration used in experiments) at the MRHD of 400 mg bupivacaine/ 12 mg meloxicam.

Impurities

In the original submission, the Applicant did not provide adequate justification for two impurities that were above the ICH Q3B(R2) qualification threshold, (b) (4). These were addressed by the Applicant in the resubmission.

The Applicant has reduced the specification of (b) (4) in the drug product to NMT (b) (4) %, which is acceptable based on the qualification thresholds in ICH Q3B(R2).

For (b) (4) the Applicant justified the levels using a combination of unpublished nonclinical studies with (b) (4) to address systemic toxicity and clinical/nonclinical experience with HTX-011 to address local toxicity. (b) (4) has been reported to be negative in an Ames bacterial reverse mutation assay, a chromosomal aberration assay, and a sister chromatid exchange assay. In an unpublished GLP 13-week dietary repeat-dose toxicity study in rats, decreased body weight was seen at a dose of (b) (4) mg/kg with a NOAEL of (b) (4) mg/kg. This would correspond to an HED of (b) (4) mg based on body weight scaling and 60 kg individual. The Applicant also cited a report from the Cosmetic Ingredient Review (CIR, 2006) which included data in rats indicating that (b) (4) is well absorbed by males following oral administration, with (b) (4) % of the administered dose found in urine after 5 days. Thus, based on the high oral bioavailability of (b) (4) the dietary study is appropriate for use to support the clinical route of administration.

The maximum amount of (b) (4) in the drug product based on the Applicant's proposed specification of NMT (b) (4) % would be (b) (4) mg at the MRHD ((b) (4)), which is covered by the dosing in the above study. (b) (4)

(b) (4)
The maximum amount of (b) (4) from the drug product would be less than (b) (4)

The results of the repeat-dose study support that even the overestimate of (b) (4) g of (b) (4) would not be expected to result in any systemic toxicological effects and since the levels of (b) (4) in the drug product will be <13 g, it is not of a toxicological concern.

(b) (4)
As such, their hypothesis is not supported by data. Of note, the HTX-011 drug product did cause local effects including an inflammatory reaction that is consistent with a reaction to foreign material that lasted for up to 28 days. In the worst-case scenario, (b) (4) could have contributed to the observed local inflammatory reaction.

It is unclear what the clinical significance of chronic inflammation would be as it appears to be a normal reaction to a foreign material. In that regard, there did not appear to be a significant effect on wound healing in a GLP mini-pig study (Study number 33-54). We also acknowledge that there is clinical experience with (b) (4) exposure from the drug product in the Phase 2 and 3 studies; however, product with (b) (4) at levels up to (b) (4) % was not tested.

The specifications of all other impurities were either within ICH guidelines or adequate justification was available. Other than (b) (4) there are no nonclinical deficiencies regarding drug substance impurity or drug substance/product degradant specifications.

Extractables/Leachables

In the original submission, the Applicant did not provide adequate leachable data to permit substantive toxicological evaluation of the safety of the container closure system and the (b) (4) of the drug product. Specifically, the

Applicant did not test at least three drug product stability batches over the entire course of proposed expiry, did not perform leachable studies for all compounds identified above the safety concern threshold (b) (4) mcg/day), did not adequately identify the compounds they detected, and did not provide adequate justification for the use of a surrogate approach for (b) (4) found in the drug product.

The Applicant addressed these deficiencies in their resubmission. This included conducting new more comprehensive extractable and leachable studies, updating the results of their previous studies, and providing a detailed risk assessment for the leachables identified. In their new leachable studies ((b) (4) Leachable Study 3 and 4), the Applicant evaluated 24 or more lots of drug product aged between 2 and 38 months, which allowed evaluation of trends over time. The range of samples tested covers the entire proposed expiry of the drug product (36 months) and includes samples at early, mid, and late timepoints. In total, the Applicant conducted five leachable studies and when considered together they cover all of the extractables identified over (b) (4) mcg/day. A number of previously unidentified compounds had updated designations in the resubmission including further identification of (b) (4) identifying some compounds as (b) (4) and the identification of (b) (4). Finally, the Applicant provided detailed risk assessments for each compound to support the PDEs they chose and the data they relied on. This included an updated justification for the use of (b) (4) for (b) (4).

The Applicant conducted four extractable studies (b) (4). All four extractable studies resulted in very similar extractable profiles. However, there was less agreement in terms of the leachables identified above the AET and the quantitative levels of the leachables across the five leachable studies. For example, the leachables identified by the (b) (4) leachable study above the AET ((b) (4) and (b) (4) were not found above the AET in any of the (b) (4) leachable studies. It is not clear why the studies gave different results, but possible reasons could include differential targeting of leachables, differences in the samples evaluated, and differences in analytical methods. Due to the discrepancies, the highest level of any leachable identified above the AET from any of the leachable studies was considered to require safety justification.

In total, eight individual leachables were identified above the AET across the five leachable studies: (b) (4)

(b) (4) In addition, the combined level of (b) (4) was reported above the AET in one leachable study.

The safety of the (b) (4) was addressed with a surrogate approach. The Applicant chose (b) (4) as a surrogate compound and the Division of Applied Regulatory Science Computational Toxicology Consultation Service (CTCS) was consulted to assist in evaluating the adequacy of this approach. The CTCS agreed with the use of (b) (4) but also suggested (b) (4) as a surrogate based on its

structure, more comparable molecular weight, and availability of data (see Appendix). The combined maximum detected levels of the two (b) (4) were below the PDEs derived from both (b) (4) and (b) (4).

The (b) (4) were identified as potential leachables in the original submission (b) (4). The Applicant performed root cause analysis to determine the source of the (b) (4) as part of the resubmission. Their analysis supported that the primary source of the (b) (4) was the drug substances and the excipients. This is in good agreement with their extraction studies; the (b) (4) were only identified as a potential leachable from the (b) (4) in (b) (4) Extractable Study 2 where the (b) (4) used were in contact with the drug product prior to analysis. Regardless of the source, (b) (4) Leachable Study 2 and 4 identified (b) (4) in the drug product above the AET and the safety of the levels of the (b) (4) needed to be justified.

In the original submission, (b) (4) were identified above the AET in (b) (4) Leachable Study 2 based on a conservative correction factor for quantitation. For the resubmission, the Applicant recalculated the values for the (b) (4) using response factors that were specific to the (b) (4) and a more accurate maximum dose volume (b) (4). While (b) (4) mL is technically below the MRHD of (b) (4) mL, it was determined that the difference would not significantly alter the Applicant's results. Following the recalculation, the levels of (b) (4) were lower than originally determined and only three (b) (4) (b) (4) and (b) (4) were above the AET. The results are consistent with the Applicant's new leachable study where only (b) (4) but not the other (b) (4) was observed above the AET.

The Applicant used a surrogate approach to address the potential for toxicity of the (b) (4) because toxicological data was not available for (b) (4) and the toxicity profiles of (b) (4) and (b) (4) were similar. The available data for both (b) (4) and (b) (4) suggested that the liver was the major target organ and the compounds caused similar irritation effects following inhalation exposure. PDEs calculated by the FDA reviewer were also similar (b) (4) and using the more conservative value for both (b) (4) and (b) (4) is reasonable. Since no toxicological data are available for (b) (4) the Division of Applied Regulatory Science Computational Toxicology Consultation Service (CTCS) was consulted to assist in evaluating the adequacy of the Applicant's approach to use (b) (4) and (b) (4) as surrogates. The CTCS concluded that the Applicant's approach was acceptable. Since all of the (b) (4) were predicted to result in similar toxicological effects, the highest values of (b) (4) (b) (4) and (b) (4) reported in the leachables studies were combined and compared to the most conservative PDE, 1 mg/day. The highest level reported for each (b) (4) when combined was below the PDE by a factor of 15x. Therefore, the levels of (b) (4) detected in the drug product are not of a toxicological concern.

The Applicant did not directly measure the levels of (b) (4) in the leachable studies despite detecting it in extractable studies above the SCT because they did not consider it a leachable from the container closure (b) (4).

Since (b) (4) could end up in the final drug product (b) (4) (based on it being identified in the extraction studies), the Division felt that the total amount in the final drug product needed to be justified for safety, which was communicated to the Applicant as an information request during the second cycle. To address the safety of (b) (4) in the drug product, the Applicant provided a worst-case estimate of the level of (b) (4) and performed a risk assessment based on their estimated worst-case levels.

The Applicant's worst-case levels were based on the specification for (b) (4) at the MRHD and the maximal level extracted from (b) (4) with (b) (4) in the (b) (4) Extractable study. The level of (b) (4) at the MRHD was estimated as (b) (4) mg and the level reported in the extraction study was (b) (4) (b) (4) resulting in a total worst-case estimate of (b) (4) mg. The amount of (b) (4) reported in the (b) (4) extractable study was less than the levels reported in other extraction studies, such as (b) (4) Extractable Study 2 where (b) (4) mcg was extracted from (b) (4) with bulk drug product. This suggests that (b) (4) mcg may be an underestimate of the amount of (b) (4) from (b) (4). The FDA reviewer believed that since no (b) (4) was detected above the minimum quantifiable limit of (b) (4) mcg/mL (or (b) (4) mcg based on the MRHD) in (b) (4) leachable study after subtracting for background (b) (4) in the drug product that (b) (4) mcg would be a more reasonable estimate of the worst-case level of (b) (4) in the drug product from (b) (4). This would make the final worst-case estimate of (b) (4) in the drug product as (b) (4) mg. The Applicant provided publicly available toxicity data for (b) (4) which was deemed to be adequate to assess its toxicity. The worst-case estimate of the levels of (b) (4) were below the PDE calculated from the data.

(b) (4) and (b) (4) were addressed by published toxicology studies and deriving PDEs. The data for both compounds were very limited with only two studies identified for (b) (4) and one study identified for (b) (4) is a reactive metabolite of (b) (4) and was found to cause hemorrhaging and reduce coagulation factors more potently than (b) (4) (b) (4) is also a metabolite of (b) (4) but it was much less potent in causing hemorrhage and effects on blood coagulation as compared to (b) (4). The highest levels of each compound detected in the drug product were below the calculated PDEs and are likely not of a toxicological concern.

The (b) (4) were addressed by referencing published risk assessments from the Environmental Protection Agency, and the Massachusetts Department of Environmental Protection. These regulatory agencies have taken a fraction approach to (b) (4) dividing them into categories (b) (4). The highest level of the summed (b) (4) in the drug product were below the calculated PDEs for (b) (4) by a factor of (b) (4) and are likely not of a toxicological concern.

In summary, the Applicant has adequately addressed the safety of all the identified leachables.

400 mg Bupivacaine dose

In the original submission, it was determined that the exposure (AUC) to bupivacaine via the drug product at 400 mg bupivacaine exceeded that of the referenced product. The pivotal 28-day nonclinical studies in rats and dogs included exposure data at 24 and 48 hours but not AUC_{inf}. Compared to human exposures in clinical study 209 (total knee arthroplasty), the rat exposures were ~ 0.5x and 0.3x for AUC₀₋₂₄ and AUC₀₋₄₈, respectively, and the dog exposures were ~ 1.0x and 0.8 x for AUC₀₋₂₄ and AUC₀₋₄₈, respectively. It was communicated to the Applicant in the CR letter as a non-hold comment that the nonclinical data did not fully support the safety of bupivacaine above a dose of 300 mg and that further data would be needed to support the 400 mg dose of bupivacaine.

	28-day rat (33-85)	28-day dog (33-86)	Total knee arthroplasty (209) ¹	Exposure margin
AUC ₀₋₂₄ (h * ng/mL)	5710 (M, 25 mg/kg)	11700 (F, 10 mg/kg)	11223 (n = 50)	0.5x, rat 1.0x, dog
AUC ₀₋₄₈ (h * ng/mL)	8120 (M, 25 mg/kg)	18700 (M, 13 mg/kg)	23580 (n = 50)	0.3x, rat 0.8x, dog

¹Value calculated by the FDA clinical pharmacology team and differs from the values reported by the Applicant. See clinical pharmacology review for more information.

To address the lack of coverage in the rat, the Applicant conducted a 14-day study in rats by subcutaneous injection (dosing every 48 hours) of HTX formulations containing only bupivacaine (not the to be marketed formulation). The highest dose of bupivacaine used was 150 mg/kg, which is equivalent to a human dose of ~1450 mg based on body surface area and a 60 kg individual. Results from the study were similar to those in other nonclinical studies with HTX formulations or the APF18A polymer; the only effects that could potentially be due to systemic exposure were changes in hematology parameters. The most prominent effects were increased WBC, increased platelets, increased neutrophils, increased reticulocytes and increased fibrinogen. It is difficult to assess if the magnitudes of the changes are more severe in this study compared to previous studies which used lower doses of bupivacaine due to differences in dosing volumes, formulations, and durations of treatment. No other significant adverse effects, beyond local site reactions, were observed suggesting that the overall impact on the health of the animals was minor. Since the findings appear similar to those observed with lower doses of bupivacaine and are likely caused by the excipients since multiple HTX vehicle formulations also showed effects on hematology parameters, we defer to the clinical team to determine if the effects observed are acceptable from a risk-benefit perspective. If so, then the 14-day rat study supports the 400 mg dose of bupivacaine as the highest systemic exposure (AUC_{inf}) of bupivacaine achieved in the study (55,700 ng*h/mL for females) was higher than the highest reported bupivacaine exposure reported in the Applicants clinical trials (209, total knee arthroplasty, 31,173 h*ng/mL) by a factor of ~1.8x.

It was communicated to the Applicant during a Type A meeting on August 7th, 2019 that if they planned to rely on the nonclinical data to support the 400 mg bupivacaine dose that they would need adequate data in two species. The Applicant did not submit any additional studies in dogs in the resubmission. While the data from the 28-day dog study (Study 33-86) included in the original submission is not ideal (AUC_{inf} was not calculated) it does provide data in a second species that had similar exposure to bupivacaine over 24 hours as humans exposed to 400 mg bupivacaine (~1x). The only systemic finding in dogs at this exposure level was an increase in reticulocytes. Taken together with the results from the 14-day rat study which achieved a higher exposure multiple, the safety of the 400 mg dose of bupivacaine is supported by the nonclinical data as long as the clinical team does not believe that changes in hematology parameters are of a clinical concern considering the human experience.

Wound and bone healing

The rat bone healing study and the rabbit hernia repair studies were not conducted in accordance to good laboratory practices (GLP). The Applicant claims that the only deviations from GLP were the lack of quality assurance statements and involvement of the Quality Assurance department during study conduct. In addition, the Applicant provided the standard operating procedures of the conducting laboratories, which have been quality assured. Based on the data provided by the Applicant and the study reports, it is this reviewer's opinion that while the studies were not conducted under ideal GLP conditions, the results of the studies appear to be reliable and repeating the study would be an inappropriate use of animals based on the findings to date. The Applicant was instructed to complete any future studies intended to support human safety in accordance with GLPs and was required to include enhanced monitoring in the clinical setting to fully evaluate the potential impact on bone healing.

The main goal of the rat bone healing study was to determine if the clinical formulation would have an adverse impact on bone healing to support the proposed bunionectomy clinical study. In that regard, bone mineral density, bone mineral content, and histopathological evaluation of the bone healing stage were evaluated. No treatment related effects on bone mineral content, bone mineral density, or the healing stage of the bone were identified in this study. Based on the conditions used in this study, there was no difference in bone healing with the clinical formulation compared to the control.

The main goal of the rabbit hernia repair model was to determine the effects of the clinical formulation on Prolene mesh to support its use in the herniorrhaphy clinical study, which also included the use of mesh as part of the surgery. The interaction with the mesh was evaluated by histopathological evaluation. It was difficult to determine how the formulation interacted with the Prolene mesh as no clear evidence of the mesh was seen in any of the sections, including on Day 3, the earliest sacrifice time point. As noted above, the Applicant attributed the lack of evidence of the mesh in the histopathology slides as likely due to loss of the fibers from the mesh during tissue processing. They note that the open spaces in the tissue where the mesh was present

were noted, suggesting recent loss. However, the similar incidence of histopathological findings, including in cystic change, on Day 3 suggests that there was no adverse interaction with the mesh.

Across the two studies there was limited evidence of systemic effects following treatment with the HTX-formulations. In the rat bone healing study, no effects related to systemic exposure were observed, although the number of parameters assessed was limited. In the rabbit hernia repair study, the primary systemic effect observed was changes in hematology parameters on Day 3 following treatment with the vehicle, the bupivacaine comparator (HTX-011 without meloxicam), and the different HTX formulations. The most consistent finding across all treatments were increased white blood cells, increased neutrophils, and decreased monocytes, which is consistent with other studies with HTX-011. Additionally, decreased platelets and reticulocytes were seen with some treatments. Most of these changes were not seen on Day 15 suggesting partial to complete recovery. Since effects were seen in the vehicle treated group and across multiple formulations, this suggests that effects on hematology parameters could be related to one or more shared excipients. In this study, the proposed clinical formulation was used at a dose of ~8.3 mg/kg bupivacaine and 0.25 mg/kg meloxicam which is a human equivalent dose of 160.6 mg and 4.8 mg, respectively. Therefore, the effects were observed at a human equivalent dose that is ~2.7 times higher than lowest proposed human dose (60 mg/1.8 mg) and ~2.5 times lower than the highest proposed human dose (400 mg/12 mg).

Dermal and wound site effects were the prominent findings in the two studies. In the rat bone healing study, skin lesions (scab and ulceration) were seen at the injection site on Day 33 in the groups receiving the bupivacaine comparator and both HTX formulations. These were the only local findings reported in this study.

A more detailed analysis of local effects was conducted in the rabbit hernia repair model. In this study, dermal observations, wound observations and histopathological effects were reported. Dermal observations were reported according to the Draize scoring system with the severity and incidence of edema and erythema reported. Increased incidences and or severity of both findings compared to saline control were seen across the vehicle, bupivacaine comparator, and HTX formulations, although there did not appear to be a consistent pattern across formulation or sex.

At the wound site, the most common observation was hemorrhage and was primarily observed in the middle of the study in animals who were sacrificed on Day 15. The severity of hemorrhage was increased in females receiving the vehicle, bupivacaine comparator, and all three HTX formulations. Hemorrhage was not present at the end of the study in most animals, except for 1 female receiving HTX-011-49 and 2 females receiving HTX-011.

Finally, histopathological analysis of the injection sites also revealed increased incidences and severity of findings in treated groups compared to the saline control for animals undergoing necropsy on Day 15. These included the findings of cell debris,

cystic change, epidermal ulcer, hemorrhage, epidermal hyperplasia, dermal granulation, and subcutaneous/muscular/intermuscular granulation. There did not appear to be a consistent pattern across formulation or sex making it difficult to interpret if any formulation adversely affected wound healing.

Taken together, it did appear that the vehicle, bupivacaine formulation comparator, and the HTX formulations had an impact at the local injection site compared to the saline control. The effect seemed to be most pronounced at the end of the study (Day 15). Based on the observation of findings with multiple formulations, it suggests that the effects were potentially related to one or more shared excipients. The dose of the proposed clinical formulation used in this study was 25 mg bupivacaine and 0.75 mg meloxicam, which is less than the lowest proposed human dose of 60 mg bupivacaine and 1.8 mg meloxicam. The size of the incisions in the rat and rabbit studies were not reported, so a comparison in terms of volume per cm of incision between the nonclinical and the clinical studies could not be made.

The clinical formulation was also tested for wound healing in minipigs (Study 33-54). In this study, male and female minipigs were given a single 3 mL dose of saline, vehicle, bupivacaine alone, meloxicam alone, and HTX-011. In addition, a sham control was included where an incision was made but no treatment was given. Minipigs were sacrificed on Day 15, the same duration as the rabbit study. There were limited increases in the severity of wound site observations on Day 4 compared to the sham and vehicle control. Observations included increased redness around the wound site, increased severity of hemorrhage, and increased severity of exudate. These findings were no longer elevated in severity compared to the vehicle or sham control on Day 15. In terms of histopathological findings, there was increased severity of inflammatory cell infiltrate and granulomatous inflammation of the sub cutis on Day 15. Taken together, there was a very slight difference in wound healing in this study. The dose used in this study was 3 mL (75/2.25 mg bupivacaine/meloxicam) with a 3 cm incision or 1 mL/cm. Based on estimated incision sizes provided by the clinical team, for herniorrhaphy (3-4 cm), (b) (4) the dose used in this study is lower than those proposed in the clinical studies (herniorrhaphy; 2.5-3.4 mL/cm (b) (4)).

In summary, there did not appear to be any effect on bone healing or overt adverse interaction with the Prolene mesh with HTX-011. From the nonclinical perspective, no additional testing to assess these parameters are necessary. There was some evidence of a potential effect on wound healing in the rabbit study. However, the minipig study, which used a higher dose, supported that there was only a very slight difference in wound healing with HTX-011 compared to control treatments. Even the minipig study did not test local doses as high as those in the Phase 3 studies and therefore the nonclinical data does not fully support these studies. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in terms if further evaluation of the potential effect on wound healing at clinically relevant doses.

Local and systemic toxicity of the drug product

Nonclinical studies were submitted to justify the safety of the drug product formulation which contains novel excipients, and two previously approved drug products. These studies were designed to address the local safety of meloxicam as this is a new route of administration and the systemic safety of bupivacaine since exposure of bupivacaine from the drug product exceeded the reference product both in terms of AUC_{0-24h} and overall duration of exposure.

The local tissue effects of this drug product when dosed in accordance with the proposed indication are not well characterized from a nonclinical perspective. The development program included many changes to the formulation which precluded a clear delineation of the local tissue response of the to-be-marketed drug product except under exaggerated conditions. However, these studies were considered acceptable to inform the toxicological effects of the materials in the drug product formulations to be tested during development. Specifically, the Division leveraged these data to inform the clinical monitoring program and the informed consent for the early clinical studies. However, pivotal repeat-dose studies testing the to-be-marketed drug product formulation were required to support the NDA. The pivotal studies were two 28-day repeat-dose studies (rat and dog by subcutaneous injection, Studies 33-85 and 33-86) conducted with the final clinical formulation, HTX-011. These two studies provided the only clear product-specific information characterizing the local tissue effects of the to-be-marketed formulation. Local effects observed throughout development consisted of a prolonged inflammatory response, including areas of tissue necrosis. The findings appear to be related primarily to the excipients as similar findings were often observed with the vehicles used in the studies. The local tissue findings were discussed with the clinical review team during development and the clinical team concluded that clinical studies could proceed with adequate monitoring and informed consent to determine if the adverse effects translated into adverse clinical findings.

In terms of local toxicity, the primary finding following treatment with HTX-011 and the HTX-011 vehicles (similar levels of excipients but no active drug products) in both rats and dogs in the pivotal studies was an inflammatory response that is consistent with a foreign body reaction. This was primarily characterized by persistent chronic inflammation that was still present up to 28 days following injection. The clinical significance of prolonged chronic inflammation is unclear. Based on a minipig study (33-54) by wound instillation, HTX-011 appears to only have a very minor impact on soft tissue wound healing consistent with herniorrhaphy procedures. However, it must be kept in mind that when the dose volume was normalized to the size of the incision site (estimated incision sizes for the surgeries proposed in the clinical studies were provided by the clinical team) the minipig study (1 mL/cm) was lower than the proposed clinical surgeries (herniorrhaphy, 3-4 cm, 2.5-3.4 mL/cm; (b) (4)

The local effects appear to be primarily driven by the presence of the tri(ethylene glycol)-based poly(orthoester) polymer (AP135) since similar local effects were seen with both HTX-

011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4))
 Since the clinical formulation was used in the repeat-dose studies, the studies adequately characterize the local toxicity of the drug product, including meloxicam and the excipients, at a margin of 1x. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in determining if the chronic inflammation noted in the animals translates to clinically meaningful adverse effects.

While the 28-day studies were conducted under exaggerated conditions (each injection site received 2-3 injections) similar dermal effects were observed after single injections with the HTX-011 vehicle in rats (Studies 33-48 and 33-55) suggesting the findings in the repeat-dose studies are relevant for a single administration. The findings in the single-dose studies with the HTX-011 vehicle were more severe (e.g., necrosis) than in the repeat-dose studies. This is likely due to the increased volume of injection used in these studies. Increased volume of injection was also linked to increased severity in the 28-day dog study where the HTX-011 vehicle (5.2-8.9 mL) showed increased severity and incidence of local findings compared to the HTX-011 clinical formulation (2.3-3.9 mL). It is difficult to translate the increased volumes into a clinically meaningful dose except to say that increased amounts of locally applied HTX-011 increases the risk for local toxicity.

In terms of systemic safety, the primary finding following treatment with HTX-011 and the HTX-011 vehicle in the pivotal studies was a change in hematology parameters, primarily an increase in reticulocytes, although increases in white blood cells, platelets, leukocytes, eosinophils, neutrophils, monocytes and lymphocytes were also observed. The effects seem to be derived from the AP135 polymer since similar local effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4)). This is likely due to breakdown of the polymer into smaller fragments that are then systemically distributed.

More severe effects were seen in the 28-day dog study where a larger volume of injection was used for the HTX-011 vehicle which resulted in higher exposure to the excipients (2.4-2.5x at the MRHD based on BSA scaling). These effects included decreased activity, decreased body weight, and increased organ weights (liver, thymus, adrenal, and kidney). It is likely that these effects are not relevant for a single-dose exposure since no effects were seen in single-dose studies using the HTX-011 vehicle in rats at exposure levels of the excipients of ~7.2x those of the MRHD based on BSA scaling. Increased hematology parameters were seen in one single-dose rat study using the HTX-011 vehicle at excipient exposures of 0.9-1.5x those of the MRHD based on BSA scaling. Based on all of the studies, increased hematology parameters occur at excipient exposures of ~0.5-1.5x those of the MRHD based on BSA scaling. We acknowledge that there is clinical experience with the drug product that included monitoring for changes in hematology parameters and defer to the clinical review team in determining if these increases also occur in the clinical setting and are clinically relevant.

12 Appendix/Attachments

12.1 Consults from DARS

The Division of Applied Regulatory Science Computational Toxicology Consultation Service consultation review of surrogates for (b) (4)



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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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TABLE OF CONTENTS

1 EXECUTIVE SUMMARY.....	8
1.1 INTRODUCTION	8
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS	8
1.3 RECOMMENDATIONS	12
2 DRUG INFORMATION.....	18
2.1 DRUG	18
2.2 RELEVANT INDS, NDAs, BLAs AND DMFs.....	19
2.3 DRUG FORMULATION	20
2.4 COMMENTS ON NOVEL EXCIPIENTS	21
2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	34
2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN.....	85
2.7 REGULATORY BACKGROUND	85
3 STUDIES SUBMITTED.....	85
3.3 PREVIOUS REVIEWS REFERENCED.....	91
4 PHARMACOLOGY	92
4.1 PRIMARY PHARMACOLOGY	92
4.2 SECONDARY PHARMACOLOGY	92
4.3 SAFETY PHARMACOLOGY	92
5 PHARMACOKINETICS/ADME/TOXICOKINETICS	92
5.1 PK/ADME	92
6 GENERAL TOXICOLOGY.....	94
6.1 SINGLE-DOSE TOXICITY	94
6.2 REPEAT-DOSE TOXICITY	94
7 GENETIC TOXICOLOGY.....	132
7.1 <i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES)	132
8 CARCINOGENICITY.....	139
9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	139
10 SPECIAL TOXICOLOGY STUDIES	140
11 INTEGRATED SUMMARY AND SAFETY EVALUATION	170
12 APPENDIX/ATTACHMENTS.....	173

Table of Tables

Table 1: Relevant NDAs 19

Table 2: Composition of HTX-001 20

Table 3: Amount of AP135 in Different Formulations 21

Table 4: Reproductive Toxicology Studies with APF18A 22

Table 5: Summary of Local Effects Following AP135 Treatment 24

Table 6: Summary of Systemic Effects Following AP135 Treatment 27

Table 7: DMSO FDA-approved products as per IID 28

Table 8: DMSO Literature Cited by Applicant..... 28

Table 9: Triacetin in FDA-approved products as per CDER IID 30

Table 10: Maleic Acid FDA-approved products as per CDER IID 31

Table 11: Summary of Local Effects Potentially Associated with Excipients..... 33

Table 12: Summary of Systemic Effects Potentially Associated with Excipients..... 34

Table 13: Bupivacaine Drug Substance Specifications 36

Table 14: Meloxicam Drug Substance Specifications 37

Table 15: HTX-011 Drug Product Specifications (Part 1)..... 40

Table 16: HTX-011 Drug Product Specifications (Part 2)..... 41

Table 17: Specifications for AP135 43

Table 18: Primary Container Closure 44

Table 19: Preparation and Administration Components..... 45

Table 20: List of Extractable Leachable Studies 46

Table 21: Components Tested in Extraction Study 1 47

Table 22: Analytical Methods Used in Extraction Study 1 48

Table 23: Elemental Impurities from (b) (4) 50

Table 24: Elemental Impurities from (b) (4) 50

Table 25: Extractables from the (b) (4) 50

Table 26: Extractables from the (b) (4) 51

Table 27: Compounds Targeted for Leachable Testing 52

Table 28: Samples Tested in Leachable Study 1 53

Table 29: Extractables from the (b) (4) by GC/MS 56

Table 30: Extractables from the (b) (4) by LC/MS 56

Table 31: Samples Tested in Leachable Study 2 57

Table 32: Components Tested in Extractable Study 3 59

Table 33: Rationale for the Extraction Media in Extractable Study 3 60

Table 34: Analytical Methods Used in Extraction Study 3 61

Table 35: Elemental Impurities from the (b) (4) (Part 1) 62

Table 36: Elemental Impurities from the (b) (4) (Part 2) 63

Table 37: Extractables Identified from the (b) (4) by GC/MS ((b) (4) Extract - Part 1)..... 65

Table 38: Extractables Identified from the (b) (4) by GC/MS ((b) (4) - Part 2) ... 66

Table 39: Extractables Identified from the (b) (4) by GC/MS ((b) (4) - Part 1). 67

Table 40: Extractables Identified from the (b) (4) by GC/MS ((b) (4) - Part 2). 68

Table 41: Extractables Identified from the (b) (4) by GC/MS ((b) (4) - Part 3). 69

Table 42: Extractables Identified from the (b) (4) GC/MS ((b) (4) (b) (4) - Part 1)..... 70

Table 43: Extractables Identified from the (b) (4) by GC/MS
 ((b) (4) - Part 2) 71

Table 44: Extractables Identified from the (b) (4) by GC/MS Bulk Drug Product.....71

Table 45: Extractables Identified from the (b) (4) by GC/MS
 ((b) (4) 72

Table 46: Extractables Identified from the (b) (4) by LC/MS (b) (4) 72

Table 47: Extractables Identified from the (b) (4) by LC/MS ((b) (4) 73

Table 48: Extractables Identified from the (b) (4) by LC/MS (b) (4) 73

Table 49: Extractables Identified from the (b) (4) by LC/MS
 ((b) (4) 74

Table 50: Extractables Identified from the (b) (4) by LC/MS (b) (4) 74

Table 51: Extractables Identified from the (b) (4) by LC/MS (b) (4) 75

Table 52: Extractables Identified from the (b) (4) by LC/MS (b) (4) 75

Table 53: Extractables Identified from the (b) (4) by LC/MS
 ((b) (4) 76

Table 54: Extractables Identified from the (b) (4) by GC/MS ((b) (4) 77

Table 55: Extractables Identified from the (b) (4) by GC/MS (b) (4) 78

Table 56: Extractables Identified by GC/MS that were Targeted for Leachable Analysis 78

Table 57: Drug Products Tested in Leachable Study 3 80

Table 58: Leachables Detected in the Drug Product in Leachable Study 3 82

Table 59: Elemental Impurities in the Drug Product from Leachable Study 3 (Part 1) ..83

Table 60: Elemental Impurities in Drug Product from Leachable Study 3 (Part 2) 84

Table 61: Pharmacology Studies Submitted 86

Table 62: Pharmacokinetics Studies Submitted 86

Table 63: Pharmacokinetic Studies Submitted (Continued) 87

Table 64: Toxicology Studies Submitted-Part 1 87

Table 65: Toxicology Studies Submitted-Part 2 88

Table 66: Toxicology Studies Submitted-Part 3 89

Table 67: Toxicology Studies Submitted-Part 4 90

Table 68: Toxicology Studies Submitted-Part 5 91

Table 69: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Rats 93

Table 70: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Dogs 94

Table 71: Incidence of clinical observations in males.....98

Table 72: Incidence of clinical observations in females.....98

Table 73: Notable changes in hematology parameters in male rats 99

Table 74: Notable changes in clinical chemistry parameters in rats.....100

Table 75: Gross observations in male rats 101

Table 76: Gross observations in female rats 101

Table 77: Systemic histopathological observations 103

Table 78: Chronic Inflammation.....104

Table 79: Chronic Inflammation, active 104

Table 80: Granulomatous inflammation.....	104
Table 81: Hemorrhage.....	105
Table 82: Presence of Test Material.....	105
Table 83: Chronic inflammation, recovery animals.....	105
Table 84: Bupivacaine toxicokinetic parameters	106
Table 85: Meloxicam toxicokinetic parameters.....	107
Table 86: Levels of polymer breakdown products in the urine	108
Table 87: Incidence of edema at the injection site	114
Table 88: Average severity of edema in vehicle control group.....	114
Table 89: Body weights	115
Table 90: Hematology parameters in male dogs.....	116
Table 91: Hematology parameters in female dogs.....	117
Table 92: Clinical chemistry parameters in male dogs	118
Table 93: Clinical chemistry parameters in female dogs	119
Table 94: Gross observations in male dogs	120
Table 95: Gross observations in female dogs	120
Table 96: Organ weights in male dogs	121
Table 97: Organ weights for female dogs.....	121
Table 98: Percentage of main study animals with chronic multifocal Inflammation.....	125
Table 99: Percentage of recovery animals with chronic multifocal Inflammation	125
Table 100: Percentage of main study animals with active chronic multifocal Inflammation	125
Table 101: Percentage of main study animals with multifocal granulomatous Inflammation	126
Table 102: Percentage of recovery animals with multifocal granulomatous Inflammation	126
Table 103: Percentage of main study animals with multifocal fibrosis	126
Table 104: Percentage of recovery animals with multifocal fibrosis	127
Table 105: Percentage of main study animals with multifocal hemorrhage	127
Table 106: Percentage of main study animals with multifocal presence of test material	127
Table 107: Bupivacaine PK parameters	129
Table 108: Meloxicam PK parameters.....	131
Table 109: Plate incorporation in absence of S9.....	135
Table 110: Plate incorporation in presence of S9.....	136
Table 111: Positive control data	137
Table 112: Historical control data	138
Table 113: Treatment Groups Hernia Repair Study	144
Table 114: Clinical Signs Rabbit Hernia Repair Study	145
Table 115: Dermal Observations of Day 3 Sacrifice Group.....	146
Table 116: Dermal Observations of Day 15 Sacrifice Group.....	146
Table 117: Dermal Observations on Day 15	147
Table 118: Wound Observations of Day 3 Sacrifice Group.....	148
Table 119: Wound Observations of Day 15 Sacrifice Group.....	148
Table 120: Last Day of Wound Hemorrhage	148
Table 121: Hematology Findings on Day 3, Percentage of Saline Control	150

Table 122: Hematology Findings on Day 15, Percentage of Saline Control	151
Table 123: Gross Findings of Day 3 Sacrifice Group	152
Table 124: Gross Findings of Day 15 Sacrifice Group	152
Table 125: Histological Findings of Day 15 Sacrifice Group	153
Table 126: Clinical Signs	157
Table 127: Bone Mineral Content of Left Femur	159
Table 128: Bone Mineral Content of Right Femur	159
Table 129: Bone Mineral Density of Left Femur	159
Table 130: Bone Mineral Density of Right Femur.....	159
Table 131: Healing Score of Femur.....	160
Table 132: Dermal Observations in Day 4 Sacrifice Animals, Male	164
Table 133: Dermal Observations in Day 4 Sacrifice Animals, Female	164
Table 134: Dermal Observations in Day 15 Sacrifice Animals, Male	164
Table 135: Dermal Observations in Day 15 Sacrifice Animals, Female	165
Table 136: Wound Observations on Day 4.....	166
Table 137: Wound Observations on Day 8.....	167
Table 138: Gross Pathology of the Incision Site, Day 4	168
Table 139: Gross Pathology of the Incision Site, Day 15	168
Table 140: Summary of Histopathological Findings, Day 4	169
Table 141: Summary of Histopathological Findings, Day 15	170

Table of Figures

Figure 1: Body Weight in Rat Bone Defect Study.....	158

1 Executive Summary

1.1 Introduction

The Applicant, Heron Therapeutics Inc, has developed an extended-release fixed-dose combination product containing bupivacaine and meloxicam, which they referred to as HTX-011 during development. HTX-011 is a viscous solution that is formulated in a proprietary tri(ethylene glycol)-based poly(orthoester) polymer. The Applicant developed HTX-011 for application into a surgical site to reduce postoperative pain for 72 hours and reduce the need for opioid analgesics. The Applicant has submitted a 505(b)(2) application relying on the Agency's previous finding of safety for MARCAINE® (bupivacaine HCl injection; NDA 16964) and MOBIC® (meloxicam; NDA 20938) with additional support from published literature. However, the latest 356h form indicates that the Applicant is also relying on two other bupivacaine NDAs (18053 and 18304) and has patent certified to these products. From a nonclinical perspective, these three FDA-approved bupivacaine labels contain the same data.

The Applicant has proposed a maximum dose of 400 mg/12 mg (bupivacaine/meloxicam). Following internal discussions among the review teams, the review team determined that the data available would only support an approval of a maximum dose of 300 mg/9 mg (see clinical review for further details). Therefore, throughout this document, 300 mg/9 mg is referred to as the maximum recommended human dose (MRHD), as this is the highest approvable dose at this time.

1.2 Brief Discussion of Nonclinical Findings

The drug product formulation contains several "new" excipients as defined by the FDA guidance document as excipients used at higher doses, concentrations or routes of administration than previously approved drug products. Specifically, the proposed doses of AP135, dimethyl sulfoxide (DMSO), triacetin, and maleic acid exceeded levels present in FDA-approved drug products as per the CDER Inactive Ingredients Database (IID). The Applicant was informed of the need to adequately justify the safety of these new excipients in their NDA early in development. Adequate data were provided to characterize the safety profile of AP135 at the MRHD. However, the Applicant failed to fully justify the safety of DMSO, maleic acid, and triacetin with respect to the standard reproductive and developmental toxicology battery endpoints. As such, these excipients are not adequately qualified for safety at the proposed dosing regimen.

The Applicant has proposed adequate drug substance specifications for both bupivacaine and meloxicam. However, the drug product specification for (b) (4) exceeds the ICH Q3B(R2) qualification threshold (QT). This must either be tightened or adequately qualified for safety. The Applicant also proposed a drug product specification for (b) (4) that exceeds the ICH Q3B(R2) QT. The proposed specification may be able to be tightened given that the actual levels detected in the clinical drug product lots on stability (e.g., (b) (4) %

(w/w) with a mean of (b) (4) % were much lower than the proposed specification of NMT (b) (4) %. Although these levels still exceed the ICH QT, a level higher than the QT may potentially be supported based on the amount of (b) (4) that may be present in the local environment via this drug product, the levels present in drug product tested in the pivotal toxicology studies, or the levels tested in clinical studies provided that the Applicant also address the genotoxic potential of (b) (4) per ICH Q3B(R2). Nevertheless, the data available do not adequately justify the proposed specification. This should be tightened or adequately justified for safety.

Nonclinical studies were submitted to justify the safety of the drug product formulation which contains novel excipients. These studies were also specifically designed to address the local safety of meloxicam as this is a new route of administration and the systemic safety of bupivacaine since exposure of bupivacaine from the drug product exceeded the reference product both in terms of AUC_{0-24h} and overall duration of exposure.

The local tissue effects of this drug product when dosed in accordance with the proposed indication are not well characterized from a nonclinical perspective. The development program included many changes to the formulation which precluded a clear delineation of the local tissue response of the to-be-marketed drug product except under exaggerated conditions. However, these studies were considered acceptable to inform the toxicological effects of the materials in the drug product formulations to be tested during development. Specifically, the Division leveraged these data to inform the clinical monitoring program and the informed consent for the early clinical studies. However, pivotal repeat-dose studies testing the to-be-marketed drug product formulation were required to support the NDA. The pivotal studies were two 28-day repeat dose studies (rat and dog by subcutaneous injection, Studies 33-85 and 33-86) conducted with the final clinical formulation, HTX-011. These two studies provided the only clear product-specific information characterizing the local tissue effects of the to-be-marketed formulation. Local effects observed throughout development consisted of a prolonged inflammatory response, including areas of tissue necrosis. The findings appear to be related primarily to the vehicle. The local tissue findings were discussed with the clinical review team during development and the clinical team concluded that clinical studies could proceed with adequate monitoring and informed consent to determine if the adverse effects translated into adverse clinical findings.

In terms of local toxicity, the primary finding following treatment with HTX-011 and the HTX-011 vehicle (similar levels of excipients but no active drug products) in both rats and dogs in the pivotal studies was an inflammatory response that is typical following injection of foreign material. This was primarily characterized by persistent chronic inflammation that was still present up to 28 days following injection. The clinical significance of prolonged chronic inflammation is unclear. Based on a minipig study (33-54) by wound instillation, HTX-011 appears to only have a very minor impact on soft tissue wound healing consistent with herniorrhaphy procedures. However, it must be kept in mind that when the dose volume was normalized to the size of the incision site (estimated incision sizes for the surgeries proposed in the clinical studies were provided

by the clinical team) the minipig study (1 mL/cm) was lower than the proposed clinical surgeries (herniorrhaphy, 3-4 cm, 2.5-3.4 mL/cm; (b) (4)

The local effects appear to be primarily driven by the presence of the tri(ethylene glycol)-based poly(orthoester) polymer (AP135) since similar local effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4) Since the clinical formulation was used in the repeat-dose studies, the studies adequately characterize the local toxicity of the drug product, including meloxicam and the excipients, at a margin of 1x. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in determining if the chronic inflammation noted in the animals translates to clinically meaningful adverse effects.

Given the prolonged and rather robust local tissue response, the potential impact of the drug product on a variety of surgical meshes was tested in nonclinical models. The results suggest that the drug product did not appear to impact the integrity of the surgical meshes tested nor translate into clinically meaningful changes in wound healing. In addition, to support the proposed bunionectomy study and indication, the impact of the drug product on bone healing and integrity was studied in an animal model. The study did not suggest a clear clinically meaningful delay in bone healing, a finding that was further and carefully monitored in clinical studies.

While the 28-day studies were conducted under exaggerated conditions (each injection site received 2-3 injections) similar dermal effects were observed after single injections with the HTX-011 vehicle in rats (33-48 and 33-55) suggesting the findings in the repeat-dose studies are relevant for a single administration. The findings in the single-dose studies with the HTX-011 vehicle were more severe (e.g., necrosis) than in the repeat-dose studies. This is likely due to the increased volume of injection used in these studies. Increased volume of injection was also linked to increased severity in the 28-day dog study where the HTX-011 vehicle (5.2-8.9 mL) showed increased severity and incidence of local findings compared to the HTX-011 clinical formulation (2.3-3.9 mL). It is difficult to translate the increased volumes into a clinically meaningful dose except to say that increased amounts of locally applied HTX-011 increases the risk for local toxicity.

In terms of systemic safety, the primary finding following treatment with HTX-011 and the HTX-011 vehicle in the pivotal studies was a change in hematology parameters, primarily an increase in reticulocytes, although increases in white blood cells, platelets, leukocytes, eosinophils, neutrophils, monocytes and lymphocytes were also observed. The effects seem to be derived from the AP135 polymer since similar local effects were seen with both HTX-011 and the HTX-011 vehicle, as well as in studies using an APF18A polymer ((b) (4) This is likely due to breakdown of the polymer into smaller fragments that are then systemically distributed.

More severe effects were seen in the 28-day dog study where a larger volume of injection was used for the HTX-011 vehicle which resulted in higher exposure to the excipients (3.3x at the MRHD based on BSA scaling). These effects included

decreased activity, decreased body weight, and increased organ weights (liver, thymus, adrenal, and kidney). It is likely that these effects are not relevant for a single-dose exposure since no effects were seen in single-dose studies using the HTX-011 vehicle in rats at exposure levels of the excipients of ~9x those of the MRHD (300 mg/9 mg) based on BSA scaling. Increased hematology parameters were seen in one single-dose rat study using the HTX-011 vehicle at excipient exposures of 1.2-1.9x those of the MRHD (300 mg/9 mg) based on BSA scaling. Based on all of the studies, increased hematology parameters occur at excipient exposures of ~0.78-1.9x those of the MRHD (300 mg/9 mg) based on BSA scaling. We acknowledge that there is clinical experience with the drug product that included monitoring for changes in hematology parameters and defer to the clinical review team in determining if these increases also occur in the clinical setting and are clinically relevant.

The exposure (AUC) to bupivacaine via the drug product formulation at the proposed maximum daily dose of 400 mg bupivacaine exceed that of the referenced drug products (e.g., Marcaine). Exposure data from the pivotal 28-day rat and dog studies with HTX-011 indicate that on Day 27, exposure to bupivacaine (AUC_{0-24h}) is 1.2x (5,710 h*ng/mL in male rat) and 2.5x (11,700 h*ng/mL in female dog) higher than exposure ($AUC_{0-24h}=4712$ h*ng/mL; data provided by the clinical pharmacology team, please see their review for further details) at the MRHD in the herniorrhaphy study (Study 202, 300 mg/9 mg). AUC_{0-24h} was determined to be the most relevant comparison as the majority of the bupivacaine is released from the drug product on Day 1 based on in vivo and in vitro release data. There was a great deal of variability in the human pharmacokinetic data and the nonclinical data likely cannot cover the exposure of all individuals who received the drug product based on the relatively small margins of exposure. However, the only systemic findings in the 28-day nonclinical studies at these exposures were increased hematology parameters, which were likely related to the AP135 polymer and not bupivacaine exposure. Furthermore, the toxicity of bupivacaine is well characterized from the literature and clinical experience. Taken together, the nonclinical data covers the systemic exposure of bupivacaine at the MRHD and defer to the clinical team to evaluate if these safety margins translate into adverse effects in humans. Because the clinical team has recommended that the maximum human daily dose be reduced to 300 mg of bupivacaine, which was tested in the clinical studies, no further toxicology studies are necessary if the labeling restricts dosing to not more than 300 mg bupivacaine. However, to support the proposed exposure after 400 mg bupivacaine, the Applicant should conduct toxicology studies in two species, at least one nonrodent, to adequately characterize the systemic safety of bupivacaine.

To justify the safety of the container closure system, the Applicant conducted multiple extraction studies to identify potential leachables from the (b) (4) and the container closure system. Review by the chemistry, manufacturing, and controls (CMC) team has concluded that these studies are inadequate to fully characterize the leachable profile across the proposed expiry. As such, a final toxicology risk assessment cannot be completed. Of the three studies, Extraction Study 3 (RPT-694) used the most sensitive methods and identified the most compounds from the container

closure system. From the nonclinical perspective, Extraction Study 3 appeared to be the most robust extractable study and the data from this study should have been used to inform leachable assessment of the final drug product. However, the Applicant never performed leachable testing on the final drug product for the compounds identified (b) (4) in Extraction Study 3. Leachable testing on the final drug product was conducted based on potential leachables from the (b) (4) based on Extraction Studies 1 (RPT-701) and 2 (RPT-693), but this is considered inadequate since the targeting strategies employed were not sufficient and the studies do not address all the potential leachables identified in Extractable Study 3. Therefore, adequate leachable data for potential leachables from the container closure are still needed to support an approval recommendation.

Leachable testing was conducted based on the extractables identified in Extraction Study 3 (b) (4). However, this was only conducted on six samples, each representing a single lot at a single time point. The timepoints ranged from (b) (4) months. The results from these studies identified six leachables above the AET, five of which were (b) (4). The levels of the (b) (4) varied by up to 10-fold across the different samples. There did not appear to be a clear trend of the levels with the time points assessed. It is unclear from the data why the different samples had such a large difference in the levels of (b) (4). Based on Extraction Study 3, it appears that the (b) (4) are from the (b) (4). In that regard, the highest levels of (b) (4) would be expected to be (b) (4). Therefore, it is unclear what the highest levels of (b) (4) or other potential leachables, could be in the final drug product based on the Applicant's current data.

Taken together, from the nonclinical perspective, the Applicant has not submitted adequate extractable/leachable testing in order to assure the safety of potential leachables from the container closure or (b) (4). Technically the design of the extractable leachable testing is a CMC deficiency. However, lack of adequate studies prevents a definitive toxicological risk assessment, thus the CMC deficiency has pharmacology toxicology implications.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, there are inadequate data to support an approval recommendation at this time and a complete response is recommended. Specific deficiencies are listed below:

Nonclinical Deficiencies:

1. You have not provided adequate data to support the safety of the DMSO or triacetin, in your drug product and the levels in the product exceed the maximum potency listed in the CDER Inactive Ingredient Database (IID) and are therefore considered

novel. Specifically, your NDA did not include any discussion of the impact of these two excipients on the standard reproductive and developmental battery of studies.

Information needed to address this deficiency:

Submit adequate data to fully characterize the impact of the proposed doses of DMSO and triacetin on all endpoints normally characterized via the standard battery of reproductive and developmental toxicology studies. Should you elect to address this via literature, justify the adequacy of the literature based on current standard study protocols and provide copies of all referenced literature. In the absence of adequate published data, GLP nonclinical toxicology studies should be completed.

2. Your toxicological risk assessment for the excipient maleic acid, which exceeds the maximum potency listing in the CDER Inactive Ingredients Database (IID), does not address the potential impact of maleic acid on embryo-fetal development in a second species (typically rabbit).

Information needed to address this deficiency:

Submit adequate justification for the safety of the proposed maximum daily dose of maleic acid, specifically with respect to the effects of this compound on rabbit embryo-fetal development.

3. You have not provided adequate data to qualify the proposed drug product degradant (b) (4) which exceeds the ICH Q3B(R2) qualification threshold.

Information needed to address this deficiency:

Either tighten the drug product specification for (b) (4) to NMT (b) (4) % or provide adequate qualification in accordance with ICH Q3B(R2) as follows:

- a. Complete a minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
- b. In addition, conduct a repeat-dose toxicology study of appropriate duration to support the proposed indication. In this case, a study of 14 days should be completed.

Alternatively, provide adequate data to support your position that (b) (4)

However, we note that if you elect to pursue this option, the data may not

address the local safety concerns via this drug product's proposed dosing regimen.

4. You have not provided adequate data to support the safety of the drug product specification for (b) (4). The proposed specification of NMT (b) (4) % exceeds the appropriate qualification threshold of NMT (b) (4) % for a drug product with a maximum recommended human dose of greater than 2 grams as outlined in the ICH guidance for industry: *Q3B(R2) Impurities in New Drug Products*. (b) (4)

(b) (4)

We also acknowledge that (b) (4) was detected in the clinical HTX-011 lot that was tested in the pivotal 28-day toxicology studies in dogs and rats; however, the level detected in these stability batches do not support the proposed specification.

Information needed to address this deficiency:

Either tighten the specification of (b) (4) to be within the qualification threshold of NMT (b) (4) % or provide adequate qualification in accordance with ICH Q3B(R2) as follows:

- a. Complete a minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
 - b. In addition, conduct a repeat-dose toxicology study of appropriate duration to support the proposed indication. In this case, a study of 14 days should be completed.
5. You have not provided adequate leachable data to permit substantive toxicological evaluation of the safety of the container closure system and the (b) (4) (b) (4) for this drug product. Specifically:
- a) You have not tested at least three drug product stability batches over the entire course of your proposed expiry (early, middle, late) in order to identify trends for leachables over the course of stability.
 - b) You have not tested your drug product for extractable compounds detected above the safety concern threshold (SCT) of (b) (4) mcg/day from the (b) (4) in Extractable Study 3 (RPT-694).
 - c) You have not fully identified all of the compounds above the SCT in your extraction studies.

- d) In your risk assessment for the (b) (4) you have not provided adequate justification for the use of the (b) (4) as a surrogate for the (b) (4)
- e) The variability of (b) (4) present in the existing leachable data do not identify any clear trends and suggest the potential for these compounds to be derived from (b) (4)

Information needed to address this deficiency:

Conduct new leachables studies based on all of the extractables identified in Extractable Study 3 identified above the safety concern threshold (SCT) of (b) (4) mcg/day from the (b) (4) according to USP <1663> and <1664>. In addition, since Extractable Study 3 did not address non-volatile compounds, conduct new leachable studies based on the non-volatile compounds identified in Extractable Studies 1 (RPT-701) and 2 (RPT-693).

Of note, you used a conservative SCT of (b) (4) mcg presumably based on genotoxic concerns. However, for acute products (duration of < 1 month) an SCT of (b) (4) mcg/day can be used for genetic toxicity assessment. However, for general toxicity concerns, employ a SCT of (b) (4) mcg/day in the leachable study.

Include at least three to-be-marketed drug product batches over the entire course of your proposed expiry (early, middle, late) to identify trends for leachables over the course of stability. The three drug batches should be stored in the planned commercial container closure.

The leachable study should use an AET that accurately reflects the potential total daily intake of potential leachables from the drug product and how the product will be dosed. For example, if multiple vials are to be used to deliver a dose, this could affect the AET calculation.

We noted that multiple compounds were not fully identified from your extraction/leachable studies. This included the leachable identified in the final drug product samples from Leachable Study 3 as (b) (4). Provide descriptions of the methods and procedures used to attempt to identify these compounds and justify why these compounds could not be identified or provide further data supporting identification of these chemicals.

Include further justification to support that use of (b) (4) is appropriate to represent the potential toxicity of (b) (4) either by providing literature references or performing adequate QSAR analysis.

Provide a root-cause analysis to identify the source of the (b) (4) or confirm that the (b) (4) present are derived from either the (b) (4)

(b) (4) If they are derived from (b) (4) (b) (4), justify how you intend to adequately control (b) (4) to ensure product consistency and quality.

In addition to the comments above, the following general recommendations regarding the design and requirements for adequate extractable leachable safety assessment are provided to guide these studies:

The NDA submission must contain adequate information on potential leachables and extractables from the drug container closure system and/or drug product formulation, unless specifically waived by the Division. The evaluation of extractables and leachables from the drug container closure system or device should include specific assessments (b) (4) etc. Provide justification for the choice of solvents and conditions for the extraction studies (e.g., time, temperature, agitation). The results of the extraction studies should be used to assure that you are adequately monitoring the drug product stability samples for potential leachables from the primary or secondary container closure systems and from your analysis of data from any (b) (4) processes that suggest the potential for additional leachable compounds in the final drug product formulation. Your analytical evaluation threshold (AET) must be established to be able to detect, identify, and quantitate levels of compounds based on these thresholds or you must provide adequate justification that these thresholds are not possible to be met by current analytical methodology. If you cannot meet these thresholds, safety evaluations will be based on the limits of quantitation (LOQ). Your submission must include a detailed discussion of how you established your AET as well as justification for the limits of detection (LOD) and LOQ for the analytical methods used.

Evaluate at least three batches of your to-be-marketed drug product for leachables and include assessments at multiple timepoints over the course of your stability studies in order to identify trends in leachable levels over time. The materials tested should include any secondary container closure systems, if present, and be subjected to the same sterilization methods, as appropriate. These data are essential to determine the appropriate shelf life of your product.

For all drug products, establish your AET to be able to detect potentially carcinogenic or genotoxic compounds as per ICH M7 qualification thresholds (e.g., not more than 1.5 mcg/day or up to 120 mcg/day depending on the duration of treatment). However, from a general toxicology perspective, for parenteral products, the AET must be able to detect and identify any leachable that is present in the product at 5 mcg/day and higher, unless justified otherwise, to permit an adequate toxicological risk assessment.

For additional guidance on extractables and leachables testing, refer to the following documents:

- USP <1663>: Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems
- USP <1664>: Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems
- FDA guidance for industry: *Container Closure Systems for Packaging Human Drugs and Biologics*, available at, <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070551.pdf>

In your assessment, include a table listing all compounds, including the concentration in ppm, the experimental conditions, and the maximum daily exposure to these compounds based on the maximum daily dose of the product. The extractable/leachable data must be accompanied by an adequate toxicological risk assessment. Although a toxicological risk assessment based on the results of the extraction studies may be adequate to support the safety assessment during development, for your NDA, the final safety assessment should be based on your evaluation of leachables from at least three batches of your drug product tested at multiple timepoints over the course of your stability studies, as discussed above. The approach for toxicological evaluation of the safety of leachables must be based on good scientific principles and take into account the specific container closure system or patch, drug product formulation, dosage form, route of administration, and dose regimen (chronic or short-term dosing). The safety assessment should be specifically discussed in Module 2.6.6.8 (Toxicology Written Summary/Other Toxicity) of the NDA submission. The risk assessment should be based on the maximum level of each leachable detected in long-term stability samples that include any intended secondary container closure system(s) unless otherwise justified. Include copies of all referenced studies upon which a safety assessment is based.

- If you employ a Permissible Daily Exposure (PDE) assessment as described in ICH Q3C, provide justification for all safety factors employed.
- Published literature to support the safety of any compound rarely provides adequate detail of the study design and study results to permit a thorough independent evaluation of the data. Summary reviews, (e.g., BIBRA, CIR, HERA), although potentially useful to identify original source material, are not acceptable as the source material is not provided and the conclusions cannot be independently verified. Submission of any published study reports must be accompanied by a detailed comparison to modern toxicology study endpoints and any shortcomings of the study must be discussed and justification must be provided to support your assertion that these data are adequate to support the safety of your container closure system.

- Safety justifications based on analogous compounds are also not acceptable unless you can provide adequate data to support your conclusions that a risk assessment based on one compound can be logically interpolated to represent an adequate safety evaluation for your leachable/extractable. This should include a detailed understanding of the absorption, distribution, metabolism, and elimination of the compounds and an adequate scientific bridge to interpolate a NOAEL for the extractable/leachable compound.

1.3.2 Additional Nonclinical Recommendations

Although the current nonclinical data appear to support the safety of bupivacaine when the product is dosed up to 300 mg of bupivacaine, the data do not appear to provide adequate coverage for the maximum AUC_{0-24h} via this drug product at your proposed maximum dose of 400 mg bupivacaine. Because your drug product exposures above 300 mg of bupivacaine via this drug product will exceed that of the referenced drug product, additional data will be required to support any proposed dose above 300 mg bupivacaine.

1.3.3 Labeling

Labeling was not discussed during this review cycle.

2 Drug Information

2.1 Drug

CAS Registry Number: 38396-39-3 (bupivacaine); 71125-38-7 (meloxicam)

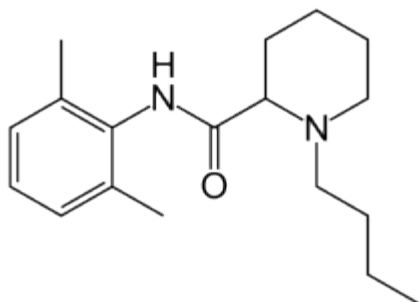
Generic Name: bupivacaine (base) and meloxicam (base)

Code Name: Zynrelef or HTX-011 or HTX-011-056

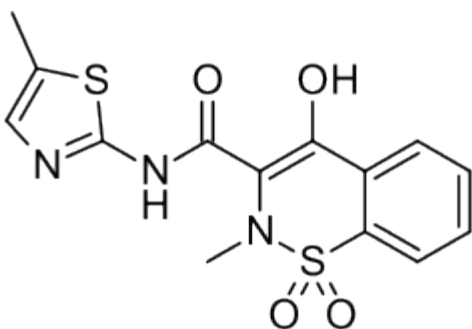
Chemical Name: (RS)-1-butyl-N-(2,6-dimethylphenyl)piperidine-2-carboxamide (bupivacaine) and 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide (meloxicam)

Molecular Formula/Molecular Weight: $C_{18}H_{28}N_2O$ / 288.43 g/mol (bupivacaine); $C_{14}H_{13}N_3O_4S_2$ / 351.40 g/mol (meloxicam)

Structure or Biochemical Description



Bupivacaine



Meloxicam

Pharmacologic Class: Amide local anesthetic (bupivacaine); nonsteroidal anti-inflammatory drug (meloxicam)

2.2 Relevant INDs, NDAs, BLAs and DMFs

The development program for this NDA was completed under IND 125927, originally submitted to the Agency on 5/7/2015.

The Applicant is submitting the current New Drug Application under section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act. As such, they are referencing MARCAINE™ (bupivacaine, NDA 016964), Bupivacaine hydrochloride (NDA 18053), Sensorcaine (NDA 18304), and MOBIC® (meloxicam, NDA 020938) as the listed drugs.

Table 1: Relevant NDAs

<i>IND/NDA/DMF</i>	<i>Drug/Compound</i>	<i>Sponsor</i>	<i>Division</i>	<i>Status</i>
IND 125927	HTX-011 (bupivacaine and meloxicam)	Heron Therapeutics Inc	DAAAP	Active
NDA 016964	Marcaine (bupivacaine hydrochloride)	Hospira Inc	DAAAP	Approved (10/03/1972)
NDA 020938	Mobic (Meloxicam)	Boehringer Ingelheim Pharmaceuticals Inc	DAAAP	Approved (04/13/2000)
NDA 18053	Bupivacaine HCl	Hospira Inc	DAAAP	Approved (4/17/1978)
NDA 18304	Sensorcaine	Fresenius Kabi USA LLC	DAAAP	Approved (12/30/1981)

DMF	(b) (4)
DMF	
DMF	
DMF	
DMF	
DMF	
DMF	

The Applicant also notes that the vented vial spike has been cleared by CDRH via the 510(k) process ((b) (4)). The syringes are also cleared by CDRH via the 510(k) process ((b) (4)).

2.3 Drug Formulation

HTX-011 is an extended-release solution containing a fixed-dose combination of bupivacaine and meloxicam. HTX-011 uses AP135, a highly viscous polymer to provide extended release of the active ingredients. DMSO and triacetin are included as

(b) (4)

Table 2: Composition of HTX-001

Component	Concentration (% w/w)	Concentration (mg/g)	Concentration (mg/mL)	Function	Reference to Quality Standard
Bupivacaine base	2.50	25.0	29.25	Active ingredient	Manufacturer
Meloxicam	0.075	0.75	0.88	Active ingredient	USP, Ph Eur
AP135	62.375	623.75	729.788	Release-controlling polymeric excipient	Manufacturer
Dimethyl sulfoxide	10.00	100	117	(b) (4)	USP, Ph Eur
Triacetin	25.00	250	292.5		USP, Ph Eur
Maleic acid	0.05	0.5	0.585		NF, Ph Eur
Totals	100.00	1000.0	1170		

Abbreviations: AP135, tri(ethylene glycol) poly(orthoester); USP, United States Pharmacopeia; Ph Eur, European Pharmacopoeia; NF, National Formulary

Table from page 6 of the Applicant's Drug Product Summary for HTX-011 (2.3.P)

During development the Agency advised the Applicant that the levels of the excipients in the drug product appear to exceed those listed in the CDER Inactive Ingredients Database and must be adequately qualified for systemic safety.

The Applicant proposed a maximum daily dose of 400 mg bupivacaine and 12 mg of meloxicam. This dose would require administration of 13.67 mL of the drug product solution. However, during review of this NDA the clinical review team noted that they only have adequate data to support a maximum recommended human dose of up to 300 mg of bupivacaine. This dose would be obtained by administration of 10.5 mL of the drug product. Further discussion of the excipient safety qualification below will be based on the 10.5 mL maximum volume referred to as the MRHD.

Based on the total daily dose, AP135, DMSO, triacetin, and maleic acid are all considered new excipients as defined by the FDA guidance document because the dose and/or route of administration exceed that of the any other FDA-approved drug product to date.

2.4 Comments on Novel Excipients

Based on the MRHD and route of administration, there are multiple novel excipients in this drug product that require adequate justification for safety. Each of these will be discussed below.

AP135

AP135 is a polymeric excipient that provides slow diffusional release of the active pharmaceutical ingredients over approximately three days. It is part of the tri(ethylene glycol) poly(orthoester) (TEG-POE) class of polymers (b) (4)

AP135 is not found in the CDER Inactive Ingredients Database (IID). A similar polymeric excipient, APF18A, is used in in the Applicant's FDA-approved marketed product SUSTOL (granisetron, NDA 022445) for subcutaneous injection. (b) (4)

and data from the development of this polymer was submitted as part of the current NDA.

Table 3: Amount of AP135 in Different Formulations

<i>Formulation</i>	<i>Percentage of excipient</i>	<i>AP135 concentration</i>	<i>Amount at MRHD¹</i>
HTX-011	(b) (4)		
HTX-011 vehicle (HTX-024-002)			
Sustol			

APF18A	80 w/w%	936.0 mg/mL	N/A

¹ Maximum recommended human dose for HTX-011 is 300 mg/9 mg bupivacaine/meloxicam or 10.5 mL based on the clinical review team's recommendations. Maximum recommended human dose for Sustol is 10 mg (0.42 mL) based on product labeling.

The amount of AP135 that a patient would be exposed to by use of SUSTOL at the maximum recommended human dose (MRHD) from SUSTOL's labeling is about (b) (4) times less than the MRHD of HTX-011 (300 mg/9 mg). Therefore, the presence of AP135 in the Applicant's approved product SUSTOL does not qualify the levels of this excipient in HTX-011.

The Applicant conducted nonclinical studies with APF18A as part of the development of SUSTOL, including repeat-dose toxicity studies, a genetic toxicity assessment (bacterial forward mutation, mouse lymphoma, and forward mutation assays) and reproductive toxicity studies (fertility and early embryonic development in rat, embryo-fetal development in rat and rabbit, and pre- and post-natal development in rat, see table below). The genetic toxicity studies were negative for mutation or clastogenicity. The only treatment-related effects observed in the reproductive studies were local tissue effects at the injection site. Local effects were characterized in more detail in the repeat-dose studies, including histopathological analysis of the injection sites. The repeat-dose studies also characterized the systemic effects of APF18A and the relevance of the local and systemic findings are discussed below. In addition to nonclinical studies with APF18A, the Applicant also characterized the local and systemic effects of AP135 as part of the HTX-011 vehicle used in multiple studies for the current NDA.

Table 4: Reproductive Toxicology Studies with APF18A

Study #	Type	AP135 (mg/mL)	AP135 (mg/kg)	HED (mg)	Systemic Safety margin¹
27-02	Fertility and early embryonic development (rat)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
25-09	Embryo-fetal development (rat)				
25-10	Embryo-fetal development (rabbit)				
27-03	Pre- and post-natal development				

¹ Calculated as a ratio of the non-clinical human equivalent dose divided by the amount of AP135 at the maximum recommended human dose (b) (4) mg AP135).

Local effects:

The 90-day studies with the APF18A polymer were conducted in rat and dog (25-07 and 25-08). Dosage was by subcutaneous administration (0.25 mL in rats and 1 mL in dogs) every 24 hours across six dosage sites (16 injections total per site). Local injection site reactions observed consistently in both studies included dermal findings of

erythema and edema, macroscopic observations of thickening of the skin, and multiple microscopic findings (chronic inflammation of multiple regions, necrosis/degeneration, fibrosis, and edema). Other findings seen primarily in one species included erosion, ulceration, hyperplasia, and hemorrhage. In general, findings were mostly no longer present following a 6-week recovery period, except for edema and erythema in rats, which was still present, but the severity and incidence was greatly reduced.

Similar injection site findings were seen with the HTX-011 vehicle (HTX-024-002) in 28-day repeat-dose rat and dog studies (33-85 and 33-86) by subcutaneous injection (0.13-0.19 mL in rats and 5.2-8.9 mL in dogs) every 48 hours across six dosage sites (2-3 injections total per site). In rats, scabbing was seen at the injection sites and microscopic observations included chronic and granulomatous inflammation. In dogs, edema, swelling, and scabs were observed at the injection site and microscopic observations included chronic inflammation, granulomatous inflammation, fibrosis, and hemorrhage. Of note, in both studies chronic inflammation was still present at the end of the study, suggesting that it persists for up to 26-28 days.

When interpreting results from these studies, it must be kept in mind that the studies were conducted under exaggerated conditions; the 90-day study had a total of 16 injections per injection site and the 28-day studies had a total of 2-3 injections per injection site. This exceeds the single dose of HTX-011 that would be administered clinically. However, similar local site reactions were seen following a single subcutaneous dose of the HTX-011 vehicle in rats (33-48 and 33-55) suggesting that local effects of the polymer seen in repeat-dose studies are likely relevant for the clinical dosing scheme.

The results from both studies together suggest that AP135 contributes to a local tissue reaction. This is not unexpected as the response appears to be a typical inflammatory response to a foreign material. However, some of the findings suggest that the effects may be more adverse than a typical inflammatory reaction, such as the observations of necrosis, erosion, and ulceration. These effects were seen in the APF18A studies, which were of a longer duration and used a higher concentration of AP135 as compared to repeat-dose studies with the HTX-011 vehicle ((b) (4)). It is unlikely that the longer duration plays a role since necrosis was also observed in single-dose rat studies using the HTX-011 vehicle (33-48 and 33-55). Therefore, it is more likely that the higher dosage of AP135 contributed to the increased severity of findings. In addition to a higher concentration of AP135, it appears increasing the injection volume may also play a role. The single-dose rat studies (33-48 and 33-55) where potentially adverse findings were observed used increased injection volumes (0.5 mL and 2 mL) compared to the rat 28-day repeat-dose study with the HTX-011 vehicle (33-85, 0.13-0.19 mL) where only inflammation was observed microscopically. A similar trend of dose volume and severity/incidence of local reactions was seen in the 28-day dog study with HTX-011 (33-86). Dogs treated with the HTX-011 vehicle (5.2-8.9 mL) had increased incidence and severity of injection site findings compared to animals receiving the HTX-011 drug product (2.3-3.9 mL), despite the two formulations having similar levels of AP135. While increased dosage volume appears to result in a more

severe local reaction, it is difficult to meaningfully translate the dose volumes to the clinical dose.

The majority of the findings observed in studies with both APF18A and the HTX-011 vehicle either completely recovered, or severity and incidence was greatly reduced by the end of the recovery periods. Based on the 28-day studies with the HTX-011 vehicle, chronic inflammation was the longest lasting affect at the injection site, which was still present at some extent around 28 days later. Chronic inflammation was not observed in recovery animals in the APF18A studies, likely due to the extended recovery period in these studies (6 weeks).

In summary, the local toxicity of AP135 was adequately characterized. Treatment with APF18A and the HTX-011 vehicle, resulted in multiple injections site findings. The injection site findings were for the most part consistent with a typical inflammatory reaction to injected material. However, in some cases more severe findings were observed, likely related to the higher concentration of AP135 in APF18A or the use of a large injection volume. In general, the findings recovered with time; the most persistent finding was chronic inflammation, which lasted about 28 days following treatment with the HTX-011 vehicle. It is difficult to put the local findings into context since both adverse (necrosis) and likely tolerable (chronic inflammation) findings were seen with clinically relevant concentrations of AP135. In addition, it is unclear what the clinical significance of chronic inflammation would be as it appears to be a normal, though robust, reaction to foreign material. In that regard, only a minor effect on wound healing was observed in a GLP mini-pig study (33-54). Therefore, we acknowledge that there is clinical experience with HTX-011 from the Phase 2 and 3 studies and defer to the clinical review team in regard to if the local findings in non-clinical studies are acceptable from a risk-benefit perspective considering the clinical data. However, it appears that more severe findings were seen when either the concentration of AP135 exceeded the clinical formulation or an increased volume of injection was used in the animal studies. Therefore, we would recommend caution regarding increased dosing in humans.

Table 5: Summary of Local Effects Following AP135 Treatment

<i>Study</i>	<i>AP135 (W/W)</i>	<i>Injection site</i>	<i>Injection volume</i>
Rat, 90-day, APF18A, 25-07	(b) (4)	Dermal observations: erythema and edema Macroscopic changes: abrasion and thickening Microscopic observations: hyperplasia, chronic inflammation, necrosis, edema, erosion, ulceration, fibrosis	0.25 mL
Dog, 90-day, APF18A, 25-08		Dermal observations: edema Macroscopic changes: thickening Microscopic observations: chronic inflammation, granulomatous inflammation,	1 mL

	(b) (4)	necrosis, edema, hemorrhage, fibrosis	
Rat, 28-day, HTX-011 vehicle, 33-85		Dermal observations: scab Macroscopic changes: scab Microscopic observations: chronic inflammation, granulomatous inflammation	0.13-0.19 mL
Dog, 28-day, HTX-011 vehicle, 33-86		Dermal observations: edema, scab Macroscopic changes: thickening Microscopic observations: chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage	5.2-8.9 mL
Rat, single, HTX-011 vehicle, 33-48		Dermal observations: edema Macroscopic changes: scab, dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, crust	2 mL
Rat, single, HTX-011 vehicle, 33-55		Dermal observations: not conducted Macroscopic changes: dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, fibrosis	0.5 mL

Systemic effects:

The 90-day studies with the APF18A polymer were conducted in rat and dog (25-07 and 25-08). Dosage was by subcutaneous administration (0.25 mL in rats and 1 mL in dogs) every 24 hours across six injection sites (16 injections total per site). Based on body weights at the beginning of the study, rats were exposed to 531-1008.6 mg/kg of AP135 ((b) (4)) and dogs were exposed to 93.6-170.2 mg/kg ((b) (4)). While these studies address the direct exposure to the excipient, a few things must be kept in mind. The first is that the studies were multiple-dose studies, which is exaggerated compared to single-dose exposure from the clinical product. The second is that the route of administration was subcutaneous injection as opposed to direct instillation into the surgical area clinically. It is unclear how the different route of administration would affect systemic exposure to the excipient.

In rats, systemic effects observed following APF18A treatment included increases in hematology parameters (platelets, white blood cells, neutrophils, monocytes, reticulocytes (f), and lymphocytes (f)), decreased clinical chemistry parameters (total protein, albumin, albumin/globulin ratio, and calcium (f)), increased organ weights (spleen, adrenal (f), and ovary (f)), and increased incidence of microscopic findings (spleen hematopoiesis, lung alveolus histiocytes, kidney nephropathy (m), kidney corticomedullary junction mineralization (f), myeloid hyperplasia of the sternum and femur marrow, and lymphoid hyperplasia in multiple lymph nodes). A majority of these findings are consistent with a nonspecific immune system response to a foreign material, likely the result of breakdown products of the polymer entering systemic circulation. Findings in dogs were limited to changes in clinical chemistry parameters

(decreased albumin (m), increased globulin (m), and decreased albumin/globulin ratio). The more limited number of systemic findings in dogs compared to rats is likely related to the dog receiving a much lower mg/kg dose of APF18A.

Similar systemic findings were seen with the HTX-011 vehicle in 28-day repeat-dose rat and dog studies (33-85 and 33-86) by subcutaneous injection (0.13-0.19 mL in rats and 5.2-8.9 mL in dogs) every 48 hours across six injection sites (2-3 injections total per site). This resulted in rats being exposed to 646.4 mg/kg of AP135 (b) (4) and dogs being exposed to 760.52 mg/kg (b) (4). When interpreting the results of these studies it is important to keep in mind that the HTX-011 vehicle contained multiple excipients beyond just the AP135 polymer. In addition, the study was a repeat-dose study by a slightly different route of exposure as was discussed for the APF18A studies.

Findings in the rat study were limited to altered hematology parameters in male rats (increased platelets, neutrophils, eosinophils, and reticulocytes). In dogs, multiple systemic effects were observed including: hypoactivity, decreased body weight (f), altered hematology parameters (increased white blood cells, platelets, leukocytes, eosinophils, neutrophils, monocytes, and reticulocytes; decreased red blood cells, HGB, HCT, and lymphocytes), altered clinical chemistry parameters (increased globulin, decreased albumin (f), decreased albumin/globulin ratio, increased LDH, and decreased creatinine clearance), and increased organ weights (liver, thymus (m), adrenal (f), and kidney (f)). The more robust systemic reaction to the HTX-011 vehicle in dogs is likely related to the large dose of vehicle in this study. This is supported by the observation of very limited systemic findings with the HTX-011 drug product in the same study (limited to increased reticulocytes in males and increased kidney weight in females) which delivered an AP135 dose of 334.62 mg/kg (b) (4).

Taken together, the potential systemic toxicity of AP135 was adequately characterized across multiple studies. Potentially adverse effects (histopathological findings and/or decreased body weight) were seen in the rat study with APF18A at approximately 0.67-1.3x the MRHD (300 mg/9 mg) and in the dog study with the HTX-011 vehicle at a dose of 3.3x the MRHD (300 mg/9 mg). The adverse effects observed in these studies may not be clinically relevant since they are likely the result of repeat dosing and the drug product is indicated for a single treatment. In that regard, these adverse effects were not observed in single-dose studies with the HTX-011 vehicle by subcutaneous injection at approximately 1.2-9.4x the MRHD (300 mg/9 mg). The most consistent systemic effect was an increase in hematology parameters that were seen in dogs with APF18A at approximately 0.41-0.74x, in rats with the HTX-011 vehicle at approximately 0.82x, and in dogs with HTX-011 at approximately 1.4x the MRHD (300 mg/9 mg). Altered hematology parameters were also seen in one of the two single dose rat studies with the HXT-011 vehicle at approximately 1.2-2.0x the MRHD, suggesting they may be clinically relevant even after a single exposure. Changes in hematology parameters were measured in the clinical studies and we defer to the clinical team in terms of

interpreting if the observed effects in non-clinical studies are acceptable from a risk-benefit perspective considering the clinical data.

Table 6: Summary of Systemic Effects Following AP135 Treatment

Study	Dose of AP135 (mg/kg)	Effects	Safety Margin³
Rat, 90-day, APF18A, 25-07	531-1008.6	↑ hematology parameters, altered clinical chemistry, ↑ organ weights, microscopic findings	0.67-1.3x ¹
Dog, 90-day, APF18A, 25-08	93.6-170.2	altered clinical chemistry	0.41-0.74x ¹
Rat, 28-day, HTX-011 vehicle, 33-85	646.4	↑ hematology parameters	0.82x ²
Dog, 28-day, HTX-011 vehicle, 33-86	760.5	↓ activity, ↓ BW, altered hematology parameters, altered clinical chemistry, ↑ organ weights	3.3x ²
Dog, 28-day, HTX-011, 33-86	334.6	↑ reticulocytes	1.4x ²
Rat, single dose, HTX-011 vehicle, 33-55	957.8-1584.4	↑ hematology parameters	1.2-2.0x ¹
Rat, single dose, HTX-011 vehicle, 33-48	3755-7455	none	4.7-9.4x ¹

¹ Safety margins calculated based on initial body weights. The mg/kg dose would decrease over the course of the study as animals weight increased.

² These studies used a constant volume/kg throughout the study.

³ Safety margins are compared to the MRHD of 300 mg/9 mg of bupivacaine/meloxicam.

Overall Conclusion for AP135:

The local and systemic toxicity of AP135 was adequately characterized across multiple animal studies using different formulations. Locally, the primary finding at clinically relevant concentrations was a local inflammatory reaction at the injection site which could be considered a typical response to foreign material. Chronic inflammation persisted for around 28 days. In addition, increased amounts of the excipient (either due to an increased concentration of AP135 or increased dosage volumes) appeared to cause severe local reactions, although it is difficult to translate the increased volumes in a meaningful way to compare to the clinical dose. The only systemic finding after a single exposure was changes in hematology parameters at 1.2-2.0x the level of AP135 at the MRHD (300 mg/9 mg), although no systemic effects were seen in a second single-dose study at 4.7-9.0x the level of AP135 at the MRHD (300 mg/9 mg). In repeat-dose studies, hematology parameters were affected at 0.41-1.5x (dog) and 0.82x (rats) the level of AP135 at the MRHD (300 mg/9 mg). Adverse systemic effects were observed following repeat exposure at 0.67-1.3x the level of AP135 at the MRHD (300 mg/9 mg) and in the dog study with the HTX-011 vehicle at 3.3x the MRHD (300 mg/9 mg); however, these effects are likely the result of exaggerated exposure conditions and

not entirely clinically relevant. Taken together, the data suggest the potential for both local and systemic effects at clinically relevant doses and therefore AP135 is not completely qualified for safety. However, we acknowledge that there is clinical experience from the Phase 2 and 3 studies with the formulation and defer to the clinical review team in terms of interpreting if the observed effects in non-clinical studies are acceptable from a risk-benefit perspective considering the clinical data.

Dimethyl Sulfoxide (DMSO)

Dimethyl sulfoxide (DMSO) was included in the drug product as a (b) (4). The amount of DMSO in the drug product is (b) (4) % w/w or (b) (4) mg/g. This results in a total of (b) (4) mg of DMSO administered at the MRHD ((b) (4) DMSO has been used in multiple approved products based on the CDER Inactive Ingredients Database (IID), primarily dosed by the topical route. Based on the levels of DMSO in this drug product, the use of DMSO in this drug product is novel based on total dose via the SC route of administration. The IID provides the following information on FDA approved drug products that contain DMSO.

Table 7: DMSO FDA-approved products as per IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency Per Unit Dose	Comment on Adequacy
Dimethyl Sulfoxide	IV (Infusion)	Powder, For Injection Suspension, Lyophilized	NA	Inadequate information and route
Dimethyl Sulfoxide	Subcutaneous	Implant	104 mg	Inadequate dose
Dimethyl Sulfoxide	Topical	Dressing	NA	Inadequate information and route
Dimethyl Sulfoxide	Topical	Lotion	45.5% W/W	Inadequate information on exposure via this route
Dimethyl Sulfoxide	Topical	Solution	45.5% W/W	Inadequate information on exposure via this route

Based on the information provided above, the proposed dose of DMSO via the SC route of injection clearly exceeds that of any other FDA approved drug product. In the absence of adequate exposure information, the systemic coverage via the topical drug products is not clear. As such, adequate safety justification is required. The Applicant noted that DMSO is present in the IID as noted in the table above. To support the safety, they reference three publications, summarized below:

Table 8: DMSO Literature Cited by Applicant

Reference	Applicant Conclusion	Doses Tested	Comment on Adequacy
Noel 1975	Based on studies in the dog, pig, rabbit, and rat, the lenticular nucleus of	Various doses in multiple studies summarized.	Inadequate but supportive. Review of studies with a focus on

	the eye is a target organ of toxicity.		lenticular changes. Lacks adequate detail for independent substantive review.
Vogin 1970	Oral NOEL in an 18-month rhesus monkey study was 3 mg/kg/day	HED = 58 mg/60 kg person.	Inadequate dose to inform risk assessment
De la Torre 1981	No significant or long-lasting effects noted following IV administration of 3 g/kg DMSO in a 40% solution for 9 days.	HED = 58064 mg/60 kg person (47 times the MRHD of DMSO).	Inadequate. Supportive at best given study limitations. The study only tested 6 male animals. Battery of tissues examined via histopathology not fully described. May be limited to muscle, lymph nodes, salivary gland and peripheral nerve.

The above published studies were reviewed to determine if these data could be adequate to address the general toxicity of DMSO. Collectively, the data are supportive at best but suggest that DMSO has been tested in numerous species, rodent and non-rodent to inform the potential target organs of toxicity. However, the general toxicity of DMSO was characterized in part via the pivotal toxicology studies testing the drug product formulation.

DMSO was included in HTX-011 and the HTX-011 vehicle that were tested in the 28-day repeat dose rat and dog studies (33-85 and 33-86) by subcutaneous injection and the rat single dose studies (33-55 and 33-48) by subcutaneous injection. These studies can be used to help characterize the presence of DMSO in the drug product from a local and general toxicology perspective. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section.

Nonetheless, the Applicant did not provide any justification or discussion to inform the impact of the proposed dose on reproductive and developmental toxicity endpoints.

Triacetin

(b) (4) The amount of triacetin in the drug product is (b) (4) mg/g. This results in a total of (b) (4) mg of triacetin administered at the MRHD for HTX-011 (b) (4).

Triacetin has been used in multiple approved products, primarily dosed by the oral route, as identified by the IID. The information from the IID is reproduced in the table below:

Table 9: Triacetin in FDA-approved products as per CDER IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency Per Unit Dose
Triacetin	Endocervical	Gel	NA
Triacetin	Oral	Capsule	6.42 mg
Triacetin	Oral	Capsule, Coated Pellets	1.21 mg
Triacetin	Oral	Capsule, Enteric Coated Pellets	5.1 mg
Triacetin	Oral	Capsule, Extended Release	46.7 mg
Triacetin	Oral	Capsule, Sustained Action	2.76 mg
Triacetin	Oral	Concentrate	50 mg/1 mL
Triacetin	Oral	Powder, For Oral Suspension	1.02 mg/5 mL
Triacetin	Oral	Suspension	0.32 mg/1 mL
Triacetin	Oral	Suspension, Extended Release	1.18 mg/5 mL
Triacetin	Oral	Tablet	6 mg
Triacetin	Oral	Tablet (Immed./Comp. Release), Film Coated	0.72 mg
Triacetin	Oral	Tablet, Chewable	1.36 mg
Triacetin	Oral	Tablet, Coated	1 mg
Triacetin	Oral	Tablet, Delayed Action, Enteric Coated	6 mg
Triacetin	Oral	Tablet, Delayed Release	1.82 mg
Triacetin	Oral	Tablet, Extended Release	8 mg
Triacetin	Oral	Tablet, Film Coated	15.12 mg
Triacetin	Oral	Tablet, Sustained Action	1.96 mg
Triacetin	Transdermal	Patch	22.1 mg
Triacetin	Transdermal	Patch, Controlled Release	22.1 mg

Based on the MRHD and route of administration, none of the previously approved drug products provide adequate coverage for the proposed dose of triacetin. The Applicant justifies the safety by stating that triacetin is considered generally recognized as safe (GRAS). However, GRAS determinations for foods are in the context of how the material is used in food and oral toxicity data. No data regarding food consumption or metabolism was provided. The Applicant cited a summary of toxicity findings but provided no references other than an LD₅₀ study in rats and mice. From a general toxicology perspective, the NDA lacks reference to adequate published data to support the systemic safety of the proposed levels of triacetin.

Triacetin was included in HTX-011 and the HTX-011 vehicle that were tested in the 28-day repeat-dose rat and dog studies (33-85 and 33-86) by subcutaneous injection and the rat single-dose studies (33-55 and 33-48) by subcutaneous injection. These studies can be used to help characterize the presence of triacetin in the drug product. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section.

In terms of reproductive and developmental toxicology endpoints, the Applicant provided the following justification in their toxicology written summary: "Triacetin is not mutagenic or genotoxic and has no reproductive or developmental toxicity (Fiume

2003). We reviewed the referenced citation to determine what reproductive and developmental data exists to inform the safety of the proposed drug product. The citation provided actually stated the following: "Published data on the reproductive and developmental toxicity of Triacetin were not found." As such, the Applicant did not adequately address these endpoints for a novel excipient. This is considered a deficiency.

Maleic Acid

(b) (4)

The amount of maleic acid in the drug product is 0.05% w/w or 0.5 mg/g. This results in a total of 6.1 mg of maleic acid administered at the MRHD for HTX-011 (10.5 mL * 0.585 mg/mL). Maleic acid has been used in multiple approved drug products by different routes according to the IID, including intramuscular, intravenous, and oral, as noted in the table below.

Table 10: Maleic Acid FDA-approved products as per CDER IID

Inactive Ingredient	Route	Dosage Form	Maximum Potency per unit dose
MALEIC ACID	INTRAMUSCULAR	INJECTION	0.01%W/V
MALEIC ACID	INTRAMUSCULAR	POWDER, FOR INJECTION SOLUTION	NA
MALEIC ACID	INTRAVENOUS	INJECTION	0.01%
MALEIC ACID	IV(INFUSION)	INJECTABLE	0.01%
MALEIC ACID	IV(INFUSION)	INJECTION	ADJ PH
MALEIC ACID	ORAL	CAPSULE	2MG
MALEIC ACID	ORAL	SYRUP	1.73MG/5ML
MALEIC ACID	ORAL	TABLET	4MG

ADJPH = adjust PH as necessary

Of the products listed above, the oral syrup product can be dosed up to 12 teaspoons per day (~59 mL), which would correlate with a maximum daily dose (MDD) of 20.41 mg maleic acid. This exceeds the amount of maleic acid that a patient would be exposed to at the MRHD (6.1 mg). However, the oral route is not ideal for justifying the clinical route of exposure due to potential differences in absorption and metabolism and therefore the approved products do not qualify this excipient for general systemic exposure.

Approved products identified in the IID had a lower concentration of maleic acid than HTX-011 (0.1 mg/mL vs. 0.585 mg/mL in HTX-011). Thus, maleic acid in HTX-011 cannot be qualified for local safety based on the approved products.

Maleic acid was included in HTX-011, but not the HTX-011 vehicle, that was tested in the 28-day repeat-dose rat and dog studies (33-85 and 33-86) by subcutaneous

injection. The studies using HTX-011 can be used to help characterize the presence of maleic acid in the drug product. The ability of the nonclinical studies to characterize the excipients is discussed at the end of this section.

The Applicant cited a publicly available EPA action memorandum to address the systemic safety of the proposed dose of maleic acid (EPA 2006). The EPA reference cited studies with maleic anhydride but that the results could be applied to maleic acid due to rapid hydrolysis of maleic anhydride to maleic acid. The EPA reference contained results of a multi-generation reproduction study and a teratogenic study in rats. The study reports were not provided by the Applicant. According to the action memorandum, in a multigenerational study in rats, maleic anhydride was administered by oral gavage at 0, 20, 55, or 150 mg/kg/day. Renal lesions were noted at all doses. A LOAEL for the study was described as 20 mg/kg/day. The EPA memo states that there were no effects noted on fertility at doses up to 55 mg/kg/day. Further, no adverse effects on litter size and pup survival were noted at doses up to 150 mg/kg/day. In a separate dedicated embryo-fetal development study, the EPA memo noted that there were no external abnormalities, soft tissue abnormalities or skeletal abnormalities when female rats were treated with 0, 30, 90, or 140 mg/kg/day maleic anhydride from Gestation Day 6 to 15. The memo reported that the maternal NOAEL was the high dose of 140 mg/kg.

No reproductive or developmental effects were seen at human equivalent doses of 532 mg (reproductive) and 1354.8 mg (teratogenic), which is 87x and 220x times the levels a patient would be exposed to at the MRHD, respectively. Normally, the Agency does not accept summary information to support the safety novel excipient use. However, because the EPA is part of the U.S. Federal Government, we will accept these summary data based on their conclusions.

The Applicant did not submit any discussion of embryo-fetal development in the rabbit. The Applicant should address this aspect of standard new excipient qualification data.

Characterization of the excipients by nonclinical data

As noted above, DMSO, triacetin, and maleic acid in the drug product were not characterized for either local or systemic safety based on use in other approved products. However, the Applicant did test these excipients as part of the drug product and vehicle in the nonclinical studies. An important distinction is that the HTX-011 vehicle did not contain maleic acid, but the drug product itself did.

In terms of local toxicity, the HTX-011 vehicle (containing DMSO and triacetin) resulted in an inflammatory reaction that is typical to a reaction to foreign material. This was characterized as chronic inflammation that lasted for up to 28 days. A similar reaction was seen with the HTX-011 drug product (containing DMSO, triacetin, and maleic acid). Based on studies with APF18A, the inflammatory reaction is very likely driven by the presence of AP135, although a contribution of the other excipients cannot be completely

ruled out. More severe findings, such as necrosis, were seen in the single-dose rat studies but this is possibly due to the increased injection volume used.

In the worst-case scenario, the excipients could have contributed to the observed local inflammatory reaction. It is unclear what the clinical significance of chronic inflammation would be as it appears to be a normal reaction to a foreign material. In that regard, there did not appear to be a significant effect on wound healing in a GLP mini-pig study (33-54). We also acknowledge that there is clinical experience with the excipients from the Phase 2 and 3 studies and defer to the clinical review team regarding if the local findings in non-clinical studies would be of a safety concern. If the clinical team finds the chronic inflammation as tolerable, then the excipients are characterized for local safety at a margin of 1x, as the concentrations of DMSO, triacetin, and maleic acid in the nonclinical studies are the same as those used in the clinical formulation.

In terms of systemic effects, the primary findings with the HTX-011 vehicle (containing DMSO and triacetin) were changes in hematology parameters with similar effects observed with HTX-011 (containing DMSO, triacetin, and maleic acid). Again, it is likely that these changes are the result of AP135 as similar findings were seen with APF18A, although a contribution of the other excipients cannot be completely ruled out. More severe effects (decreased body weights and decreased activity) were seen in the repeat-dose dog study with the HTX-011 vehicle (33-86) but these were not seen in rat studies or when HTX-011 was given at a lower dose. In the worst-case scenario, the excipients contributed to the hematology findings, which could be considered an adverse effect. We acknowledge that there is clinical experience with the excipients from the Phase 2 and 3 studies, which included some monitoring for hematology changes, and defer to the clinical review team regarding if the changes in non-clinical studies would be of a safety concern. If the clinical team finds the changes in hematology parameters tolerable, then the excipients are characterized for general systemic safety at a margin of up to 1.4x (see table below).

While the above studies help characterize the general systemic effects of the excipients, no studies were conducted with any of the individual excipients, HTX-011, or the HTX-011 vehicle to address the potential reproductive toxicology of DMSO, maleic acid, and triacetin. The Applicant did provide studies to address reproductive toxicology for AP135 as described above. The Applicant also cited literature references to help address the reproductive toxicity of triacetin (Fiume 2003) and maleic acid (EPA 2006). These data were discussed above for both excipients. The Applicant did not address the lack of a rabbit embryo-fetal development study for maleic acid. Therefore, based on the lack of a reproductive data, DMSO, triacetin and maleic acid are not fully characterized. These needs to be addressed by the Applicant.

Table 11: Summary of Local Effects Potentially Associated with Excipients

<i>Study</i>	<i>Excipients</i>	<i>Injection site</i>	<i>Injection volume</i>
Rat, 28-day, HTX-011 vehicle, 33-85	DMSO: 10% Triacetin: 25%	Dermal observations: scab Macroscopic changes: scab	0.13-0.19 mL

		Microscopic observations: chronic inflammation, granulomatous inflammation	
Dog, 28-day, HTX-011 vehicle, 33-86	DMSO: 10% Triacetin: 25%	Dermal observations: edema, scab Macroscopic changes: thickening Microscopic observations: chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage	5.2-8.9 mL
Rat, single, HTX-011 vehicle, 33-48	DMSO: 10% Triacetin: 25%	Dermal observations: edema Macroscopic changes: scab, dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, crust	2 mL
Rat, single, HTX-011 vehicle, 33-55	DMSO: 10% Triacetin: 25%	Dermal observations: not conducted Macroscopic changes: dark focus Microscopic observations: inflammation, necrosis, edema, hemorrhage, fibrosis	0.5 mL

Table 12: Summary of Systemic Effects Potentially Associated with Excipients

<i>Study</i>	<i>Excipients (mg/kg)</i>	<i>Effects</i>	<i>Safety Margin³</i>
Rat, 28-day, HTX-011 vehicle, 33-85	DMSO: 99.5 Triacetin: 248.6	increased hematology parameters	0.78x ²
Dog, 28-day, HTX-011 vehicle, 33-86	DMSO: 117 Triacetin: 292.5	decreased activity, decreased BW, altered hematology parameters, altered clinical chemistry, increased organ weights	3.2x ²
Dog, 28-day, HTX-011, 33-86	DMSO: 51.5 Triacetin: 128.7 Maleic acid: 0.26	Increased reticulocytes	1.4x ²
Rat, single dose, HTX-011 vehicle, 33-55	DMSO: 147-244 Triacetin: 368-609	Increased hematology parameters	1.2-1.9x ¹
Rat, single dose, HTX-011 vehicle, 33-48	DMSO: 578-1147 Triacetin: 1444-2868	none	4.6-9.0x ¹

¹ Safety margins calculated based on initial body weights. The mg/kg dose would decrease over the course of the study as animals weight increased.

² These studies used a constant volume/kg throughout the study.

³ Safety margins are compared to the MRHD of 300 mg/9 mg of bupivacaine/meloxicam.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Specifications

Bupivacaine

The drug substance specifications for bupivacaine base are shown below. At the MRHD of 300 mg bupivacaine, according to ICH Q3A(R2), the appropriate qualification threshold is 0.15% or 1 mg per day intake (whichever is lower). Based on this the Applicant's specifications for (b) (4) and any unspecified impurity are acceptable.

The drug substance contains one residual solvent, (b) (4) is listed in ICH Q3C as a (b) (4) with a concentration limit of (b) (4) ppm so the Applicant's proposed specification of (b) (4) ppm is acceptable.

(b) (4)



Table 13: Bupivacaine Drug Substance Specifications

Attribute		Acceptance Criteria	Test Method Reference	
Appearance		White to off-white crystalline powder or crystals or granules	Visual	
Identification	IR	Complies with reference spectrum	USP <197A>, Ph Eur 2.2.24	
	Chromatographic	Complies with reference	TLC	
Water content (% w/w)		(b) (4)	USP <921>, Ph Eur 2.5.12	
Residue on ignition/sulfated ash (% w/w)			USP <281>, Ph Eur 2.4.14	
Loss on drying (% w/w)			USP <731>, Ph Eur 2.2.32	
Assay (% w/w)			Titration	
Chromatographic purity (% w/w)	(b) (4)		HPLC	
	Any unspecified impurity			
	Total impurities			
(b) (4)			GC	
			GC	
			GC	
Bacterial endotoxins (EU/g)			USP <85>, Ph Eur 2.6.14	
Microbiological limits (CFU/g)	Total aerobic microbial count ^a	(b) (4)	USP <61>, Ph Eur 2.6.12	
	Total combined yeasts/molds count ^a			

Abbreviations: IR, infrared; USP, United States Pharmacopeia; Ph Eur, European Pharmacopoeia; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; GC, gas chromatography; % w/w, percent weight/weight; ppm, parts per million; EU, endotoxin units; CFU, colony forming units; NMT, not more than
^a Per USP <1111> and Ph Eur 5.1.4; 10² is 200 CFU/g.

Table from page 1 of the Applicant's Specification document (3.2.S.4.1)

Meloxicam

The drug substance specifications for meloxicam are shown below. At the MRHD of 9 mg meloxicam, according to ICH Q3A(R2), the appropriate qualification threshold is 0.15% or 1 mg per day intake (whichever is lower). Based on this the Applicant's specifications for all impurities are acceptable. All of the specifications for the residual solvents are within ICH Q3C. ICH Q3C does not specifically delineate (b) (4) but refers to (b) (4) which, as per the guidance, (b) (4)

(b) (4) ICH Q3C notes that the PDE for (b) (4) is (b) (4) ppm concentration. The proposed specification of NMT (b) (4) ppm (b) (4) would result in (b) (4) mcg of (b) (4) at the MRHD of (b) (4) mg meloxicam via this product which is below the amount of (b) (4) present at the PDE of (b) (4) mg (b) (4). The specification is acceptable.

Table 14: Meloxicam Drug Substance Specifications

Attribute		Acceptance Criteria	Test Method Reference
Appearance		Pale yellow powder	Visual
Identification	IR	Complies with reference spectrum	USP <197K>, Ph Eur 2.2.24
	HPLC	The retention time of the major peak of the sample corresponds to that of the reference standard	USP monograph
Loss on drying (% w/w)		NMT 0.5	USP <731>, Ph Eur 2.2.32
Residue on ignition/sulfated ash (% w/w)		NMT 0.1	USP <281>, Ph Eur 2.4.14
Assay, HPLC (% w/w on dried basis)		98.0 – 102.0	USP monograph
Assay, titration (% w/w on dried basis)		99.0 – 101.0	Ph Eur monograph
Related substances (HPLC) (% w/w)	USP related compound A and Ph Eur impurity A ^a	NMT 0.1	HPLC, USP monograph Procedure 1, Ph Eur monograph
	USP related compound B and Ph Eur impurity B ^b	NMT 0.1	
	USP methyl meloxicam and Ph Eur impurity C ^c	NMT 0.05	
	USP ethyl meloxicam and Ph Eur impurity D ^d	NMT 0.05	
	Any unspecified impurity	NMT 0.10	
	Total impurities	NMT 0.3	
Residual solvents (ppm)	(b) (4)	GC	
Bacterial endotoxins (EU/g)	(b) (4)	USP <85>, Ph Eur 2.6.14	

Table from page 1 the Applicant's Specification document (3.2.S.4.1).

Drug Product

The specifications for the drug product are located in the tables below. At the MRHD of 300 mg bupivacaine, according to ICH Q3B(R2), the appropriate qualification threshold is 0.2% or 3 mg per day intake (whichever is lower). Based on this, the Applicant's specifications for any unspecified impurity is acceptable. The specification for (b) (4) of NMT (b) (4) % is higher than ICH Q3B(R2). The Applicant believes this is justified as it is an intermediate metabolite in the metabolism of bupivacaine. No data were submitted to support this statement and therefore this specification is not acceptable.

At the MRHD of 9 mg meloxicam, according to ICH Q3B(R2), the appropriate qualification threshold is (b) (4) mcg per day intake (whichever is lower). Based on this, the Applicant's specifications for all meloxicam-related substances are acceptable.

(b) (4)

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(b) (4)

Table 15: HTX-011 Drug Product Specifications (Part 1)

Attribute	Acceptance Criteria	Method Reference
Appearance (Visual)	(b) (4)	
Dynamic Viscosity at 25°C		
Molecular Mass Distribution (GPC)		
Particulate Matter by Light Obscuration		
Identification of Bupivacaine and Meloxicam by HILIC ^a		
Identification of Bupivacaine and Meloxicam by HPLC ^a		
Bupivacaine Assay (HPLC)		
Bupivacaine Related Substances (Area % by HPLC)		

Table from page 1 of the Applicant's Specification document (3.2.P.5.1)

Table 16: HTX-011 Drug Product Specifications (Part 2)

Attribute	Acceptance Criteria	Method Reference
Bupivacaine Dose Uniformity ^a	(b) (4)	
Meloxicam Assay (HPLC)		
Meloxicam Related Substances (Area % by HPLC)		
Meloxicam Dose Uniformity ^a		
In Vitro Release (see Table 2 for additional Level 2 and Level 3 acceptance criteria)		
(b) (4)		
Container Closure Integrity (Dye Ingress) ^b		
Bacterial Endotoxins		

Table from page 2 of the Applicant's Specification document (3.2.P.5.1)

(b) (4)

(b) (4)

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Container Closure System

HTX-011 is a solution filled into a 10 or 20 mL (b) (4) clear glass vial with a 20 mm opening ((b) (4) The vials are closed with 20 mm (b) (4) (b) (4) coated with (b) (4) and capped with 20 mm (b) (4) aluminum overseas

The preparation and administration components include syringes, a vented vial spike, Luer lock applicators, tip caps, and instructions for use. The sealed glass vial is considered the primary container closure.

Table 18: Primary Container Closure

Description	Manufacturer	DMF	Specification Number
10 mL (b) (4) clear glass vial with a 20 mm opening	(b) (4)	(b) (4)	(b) (4)
20 mL (b) (4) clear glass vial with a 20 mm opening			
20 mm (b) (4) gray (b) (4) stopper with (b) (4)			
20 mm Aluminum overseas with a matte flip-off cap ^a			

Abbreviations: DMF, Drug Master File; NA, not applicable

^a Different flip-off cap colors are used for each of the product configurations (Table 3).

^b Aluminum overseas with matte flip-off cap is not in direct contact with the product.

Table from page 3 of the Applicant's Container Closure System document (3.2.P.7)

Table 19: Preparation and Administration Components

Description	Manufacturer	Manufacturer Part Number	510(k) Clearance Number	Shelf Life	Storage Condition
VVS	(b) (4)				
2 mL (3 mL) ^a Luer lock syringe					
10 mL (12 mL) ^a Luer lock syringe					
Syringe tip cap					
LLA					

Abbreviations: VVS, vented vial spike; LLA, Luer lock applicator; NDA, New Drug Application

^a The first number indicates the nominal volume of the syringe, and the number in parentheses indicates the maximum volume.

^b-Refer to [Section 3.2.P.8.1.6](#).

Table from page 4 of the Applicant's Container Closure System document (3.2.P.7)

Extractables/Leachables Evaluation

To justify the safety of leachables arising from the container closure system or from (b) (4) processes that are in direct contact with the drug product, the Applicant performed extractables and leachables studies, which are described in the following documents:

- Module 3.2.P.2 Container Closure System
- Module 3.2.P.2 Manufacturing Process Development

The Applicant conducted three extractable/leachable studies, and the relevant reports are included in the table below.

38 Page(s) has been Withheld in Full as B5 immediately following this page

Extractable/Leachable summary:

The Applicant conducted multiple extractables studies to identify potential leachables from the (b) (4) and the container closure system. The first two extraction studies identified a small set of potential leachables from the (b) (4). In general, the studies appeared to be well conducted. However, a third study using more sensitive methodology was conducted and identified a larger set of potential leachable compounds. Therefore, from the nonclinical perspective, the third study appears to be the most robust extractable study and the data from this study should have been used to inform the leachable assessment.

It appears that the Applicant did not perform leachable assessment of the extractables from the (b) (4) identified in Extractable Study 3. Instead, they only focused on the extractables that were identified from the (b) (4). While there was some data from previous leachable studies (Leachables Study 1 and 2), they did not look for the full list of potential extractables identified in Extractable Study 3. Therefore, from the nonclinical perspective, Leachable Studies 1 and 2 did not fully address the safety of all compounds that could be in the final drug product. The fact that the Applicant did not look for the full list of potential extractables in multiple lots of the final drug product at multiple times is a major deficiency.

The Applicant did test six samples for leachables (b) (4) identified in the Extractables Study 3. Each sample was from a different lot at a single time point. Of these samples, only three were in the commercial container closure. In addition, an aged bulk drug product not stored in a container closure sample was tested. A total of six leachable compounds were identified in these samples. The level of the leachables were not consistent (up to 10-fold variability) across the samples. Furthermore, the samples were only late time points ((b) (4) months) and no trends with time could be established and no early timepoints were assessed. Early time points are important because in this case the data suggests that the leachables are coming from

the (b) (4) (b) (4)

Taken together, there is no confidence that the values reported by the Applicant represent the worst-case scenario and a toxicological risk assessment on the numbers reported by the Applicant may not represent what is actually in the drug product. The Applicant should reanalyze at least three batches of their to-be-marketed drug product at multiple time points over the course of stability in order to identify trends in leachables over time.

Since there is no confidence that the quantitative values of the leachables in the drug product reported by the Applicant are the highest possible values, the risk assessment provided by the Applicant was not reviewed in detail. However, the surrogate approach for (b) (4) was evaluated. Based on the data provided by the Applicant, the use of the (b) (4) as a surrogate for the (b) (4) is not fully supported. The approach could be acceptable, however, the Applicant must provide additional support for the assumption, such as further literature references or QSAR data.

2.6 Proposed Clinical Population and Dosing Regimen

HTX-011 is indicated for application into the surgical site to reduce postoperative pain for 72 hours (b) (4). The Applicant proposed a limitation of use since safety and efficacy have not been established in highly vascular surgeries. Beyond this limitation of use, the Applicant is proposing a broad indication. However, based on internal discussions and the data available, the Division believes that only the following surgeries are supported: bunionectomy and herniorrhaphy. See the clinical review for additional information.

The Applicant proposed a maximum dose of 400 mg/12 mg (14 mL). However, based on internal discussions and the data available, it appears that the Division would only be willing to approve up to 300 mg/9 mg dosing (10.5 mL). Please see clinical review for more information.

2.7 Regulatory Background

HTX-011 was originally developed under IND 125927, which was opened on May 7th, 2015. NDA 211988 was submitted on September 21st, 2018 and the NDA was fileable from the nonclinical perspective with no comments for the 74-day letter.

3 Studies Submitted

The Applicant submitted multiple nonclinical studies to support HTX-011 and are summarized in the tables below.

Table 61: Pharmacology Studies Submitted

Study Number	Type of Study	Test System	Method of Administration	Testing Facility
<i>Primary Pharmacodynamics</i>				
33-12	POP ^a	Domestic Piglet	Local Administration into the incision site	(b) (4)
33-14	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-13	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-24	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-61	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-68	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-71	POP ^a	Domestic Piglet	Local Administration into the incision site ^b	
33-87	Incision Model	Domestic Piglet	Local Administration into the incision site	
33-23	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-25	POP ^a	Domestic Piglet	Local Administration into the incision site	
33-29	POP ^a	Domestic Piglet	Local Administration into the incision site	
<i>Secondary Pharmacodynamics</i>				
NA				
<i>Safety Pharmacology</i>				
33-62	In vitro	CHO	In vitro	(b) (4)
<i>Pharmacodynamic Drug Interactions</i>				
NA				

Abbreviation: POP, postoperative pain.

^a Incisional POP employing Von Frey methods for assessment of analgesia.

^b Eloxiject™ (meloxicam) 5 mg/mL Solution for Injection.

Table from Page 2 of the Applicants Pharmacology Tabulated Summary (2.6.3)

Table 62: Pharmacokinetics Studies Submitted

Overview			Test Article: HTX-011-56	
Type of Study	Species	Method of Administration	Testing Facility	Heron Study No.
Absorption				
	Rat	Subcutaneous	(b) (4)	33-79
	Dog	Subcutaneous		33-36
Excretion				
	Minipig sow and neonate piglet	Subcutaneous		33-77
Pharmacokinetic Drug Interactions				
	Minipig	Subcutaneous		33-78
Other				
	Dog	Subcutaneous		33-47
	Dog	Subcutaneous		33-69

Table from Page 2 of the Applicants Pharmacokinetics Tabulated Summary (2.6.5)

Table 63: Pharmacokinetic Studies Submitted (Continued)

			Test Article: APF18A (TEG-POE Polymer)
Distribution			
	Rat	Subcutaneous	(b) (4) 24-21 ^a
Excretion			
	Rat	Subcutaneous	24-21 ^a

Abbreviations: No., number.

^a Study 24-21 of the vehicle control APF18A was performed as part of the NDA for SUSTOL[®] (aranesetron) extended-release injection (NDA 022445).

(b) (4) The vehicle control data from the SUSTOL NDA were submitted to the HTX-011-56 IND (IND 125927).

Sources: Module 4, Section 4.2.2.2, Study Report 33-79, Study Report 33-36; Module 4, Section 4.2.2.5, Study Report 33-77; Module 4, Section 4.2.2.6, Study Report 33-78; Module 4, Section 4.2.2.7, Study Report 33-47, Study Report 33-69, Study Report 24-21.

Table from Page 3 of the Applicants Pharmacokinetics Tabulated Summary (2.6.5)

Table 64: Toxicology Studies Submitted-Part 1

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Single Dose Toxicity							
Single Dose Toxicity (HTX-011-56, HTX-002-14)	Rat (SD)	IP	Single Dose	BPV: 0, 15 mg/kg (M) MLX: 0, 0.5 mg/kg (M)	GLP	(b) (4)	33-37
Single Dose (HTX-002)	Rat (SD)	SC	Single Dose	BPV: 0, 7, 42, 83 mg/kg (M); 0, 11, 63, 126 mg/kg (F) MLX: 0 mg/kg (M/F)	GLP	(b) (4)	33-48
Extended Single Dose (HTX-009-005)	Rat (SD)	SC	Single Dose	BPV: 0 mg/kg (M/F) MLX: 0, 0.2, 0.6, 1.2 mg/kg (M); 0, 0.3, 0.8, 1.6 mg/kg (F)	GLP	(b) (4)	33-55
Repeated Dose Toxicity							
28-Day Toxicity Study (HTX-011-56)	Rat (SD)	SC	28 Days (every 48 hours)	BPV: 0, 0, 12.5, 25 mg/kg MLX: 0, 0, 0.38, 0.75 mg/kg	GLP	(b) (4)	33-85
28-Day Toxicity Study (HTX-011-56)	Dog (Beagle)	SC	28 Days (every 48 hours)	BPV: 0, 0, 10, 13 mg/kg MLX: 0, 0, 0.3, 0.39 mg/kg	GLP	(b) (4)	33-86
Genotoxicity							
Ames Assay (HTX-011-15)	S. typhimurium and E. coli strains	In vitro	Incubated for 48 – 72 hours	Up to 5,000 µg/plate	GLP	(b) (4)	33-11

Table from Page 4 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 65: Toxicology Studies Submitted-Part 2

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Local Tolerance							
Bone Healing Model (HTX-011-19)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 0, 17.80, 44.45, 88.98 mg/kg (M) 0, 0, 21.21, 53.04, 106 mg/kg (F) MLX: 0.0, 0.55, 1.37, 2.74 mg/kg (M) 0, 0, 0.65, 1.63, 3.26 mg/kg (F)	Non-GLP	(b) (4)	33-15
Bone Healing Model (HTX-11-56, HTX-002-14)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 50 mg/kg (F) MLX: 0, 1.5 mg/kg (F)	Non-GLP		33-39
Bone Healing Model (HTX-011-56)	Rat (SD)	Instillation in the incision near the bone defect	Single Dose	BPV: 0, 14.6 mg/kg (F) MLX: 0, 0.44 mg/kg (F)	GLP		33-83
Hernia Repair Model (HTX-11-56, HTX-002-14)	Rabbit (NZW)	Test article was administered onto the mesh prior to closure of the SC tissues and skin at the incision site	Single Dose	BPV: 0, 10 (M); 0, 9 mg/kg (F) MLX: 0, 0.3 (M); 0, 0.3 mg/kg (F)	Non-GLP		33-38
Hernia Repair Model (HTX-011-56)	Rabbit (NZW)	Test article was administered onto the mesh prior to closure of the SC tissues and skin at the incision site	Single Dose	1.0 mL/site BPV: 29.25 mg/mL MLX: 0.88 mg/mL	GLP		33-80

Table from Page 5 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 66: Toxicology Studies Submitted-Part 3

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Wound Healing Model (HTX-11-56, HTX-002-14)	Minipig (Gottingen)	Dermal	Single Dose	BPV: 0, 2.3, 4.6 mg/kg (M); 0, 2.3, 4.5 mg/kg (F) MLX: 0, 0.1 mg/kg (M); 0, 0.1 mg/kg (F)	GLP	(b) (4)	33-41
Wound Healing Model (HTX-002-13), HTX-009-05)	Minipig (Gottingen)	Dermal	Single Dose	BPV: 0, 4.6 mg/kg (M/F) MLX: 0, 0.1 mg/kg (M/F)	GLP		33-54
Brachial Plexus Nerve Block Model (HTX-024-02, HTX-002-14, HTX-011-56)	Dog (Beagle)	Intra-plexus	Single Dose	BPV: 0, 1.4, 8.3 (M); 0, 1.8, 10.5 (F) MLX: 0, 0.2 (M); 0, 0.3 (F)	GLP		33-42
Perineural Toxicity Study (HTX-011-56)	Dog (Beagle)	Perineural injection to femoral nerve	Single Dose	BPV: 0, 2, 7, 14.1 (M) MLX: 0, 0.2, 0.4 (M)	GLP		33-63
Other Toxicity Studies Conducted with APF18A Supporting the TEG-POE Polymer							
Repeat Dose Toxicity (APF18A)	Rat/Crl:CD [®] (SD)	SC	Up to 93 days	APF18A: 0, 295 mg/dose	Yes		25-07
Repeat Dose Toxicity (APF18A)	Dog/Beagle	SC	Up to 93 days	APF18A: 0, 1180 mg/dose	Yes		25-08

Table from Page 6 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 67: Toxicology Studies Submitted-Part 4

Test Article: HTX-011-56 or Supporting Formulations							
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number
Genotoxicity (APF18A)	S. typhimurium strains TA98, TA100, TA1535 and TA1537 E. coli strain WP2uvrA	In vitro	52±4 hour incubation	APF18A: ± S9: 0 (DMSO), 100, 333, 1000, 3330 and 5000 µg APF18A/plate	Yes	(b) (4)	24-17
Genotoxicity (APF18A)	Mouse lymphoma L5178Y cells	In vitro	Initial assay: 4 hour incubation Confirmatory assay: 24-hour treatment period	APF18A: ±S9: 0 (DMSO), 78.5, 157, 313, 625, 1250, 2500, 4000 and 5000 µg APF18A/mL	Yes		24-19
Genotoxicity (APF18A)	Rat/Crl:CD (SD)BR	SC	Single dose Animals sacrificed 24 and 48 hours post dose	APF18A: 0 (saline), 750, 1500 and 3000 mg/kg	Yes		24-16
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Males: beginning 28 days before cohabitation through cohabitation Females: beginning 15 days before cohabitation till Gestation Day 7	APF18A: 0 (0.9% NaCl), 0.142 and 0.295 g APF18A/day	Yes		27-02
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Gestation Days 7-17 (0, 0.14 and 0.295 g/day) Gestation Days 7, 10, 13 and 16 (0.295 g/day)	APF18A: 0 (0.9% NaCl), 0.14 and 0.295 g APF18A/day and 0.295 g APF18A/every 72 hours)	Yes		25-09

Table from Page 7 of the Applicants Toxicology Tabulated Summary (2.6.7)

Table 68: Toxicology Studies Submitted-Part 5

Test Article: HTX-011-56 or Supporting Formulations								
Type of Study (Relevant Dose Formulation)	Species and Strain	Method of Administration	Duration of Dosing	Doses ^a	GLP Compliance	Testing Facility	Study Number	
Reproductive and Developmental Toxicity (APF18A)	Rabbit/New Zealand white	SC	Gestation Days 7-19 (0.59 and 1.18 g/day) Gestation Days 7, 10, 13, 16 and 19 (1.18 g/day)	APF18A: 0 (0.9% NaCl), 0.59 and 1.18 g APF18A/day and 1.18 g APF18A/every 72 hours)	Yes	(b) (4)	25-10	
Reproductive and Developmental Toxicity (APF18A)	Rat/Crl:CD (SD)	SC	Gestation Day 7 to Lactation Day 20	APF18A: 0 (0.9% NaCl), 0.142 and 0.295 g APF18A/day	Yes		27-03	
(Q)SAR Assessment for HTX-011-56 Drug Product Impurities								
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No		33-98	
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No	33-99		
Other (Qualification of Drug Product Impurities)	NA	NA	NA	NA	No	34-10		

Abbreviations: BPV, bupivacaine; DMSO, dimethyl sulfoxide; F, female; GLP, Good Laboratory Practice; IP, intraperitoneal; M, male; MLX, meloxicam; NA, not applicable; NZW, New Zealand White; (Q)SAR, Quantitative Structure Activity Relationship; SC, subcutaneous; SD, Sprague Dawley; TEG-POE, tri(ethylene glycol)-based poly(orthoester) polymer.

^a Unless otherwise specified. For Repeated Dose Toxicity, the highest No Observed Adverse Effect Level (NOAEL) is underlined. Doses listed specify the approximate mg/kg doses of the active pharmaceutical ingredients (APIs), bupivacaine and meloxicam. Studies utilized a variety of vehicles for HTX-011.

Table from Page 8 of the Applicants Toxicology Tabulated Summary (2.6.7)

3.1 Studies Reviewed

The Applicant conducted multiple nonclinical studies, many with previous formulations of the drug product. Study Numbers 33-85 and 33-86 were formally reviewed as these were the pivotal studies for justifying the safety of the clinical HTX-011 formulation, including the levels of bupivacaine and excipients. Special toxicity studies for hernia repair (33-38), bone healing (33-39), and wound healing (33-54) were also reviewed as these studies supported the use of HTX-011 in the various surgeries used clinically. In addition, Study Numbers 33-48, 33-55, 24-17, 24-19, 24-16, 27-02, 25-09, 25-07, 25-08, 25-10, and 25-03 were reviewed to justify the excipients, especially the AP135 polymer. While a formal review of these studies is not included below, summaries of the results can be found in comments in the excipients (Section 2.4) section of the review above.

3.2 Studies Not Reviewed

The remaining studies in the Applicant's tables above were not reviewed.

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

HTX-011 contains bupivacaine, an amide-type local anesthetic, and meloxicam, an NSAID. (b) (4)



(b) (4)



4.2 Secondary Pharmacology

Secondary pharmacology studies with HTX-011 were not conducted.

4.3 Safety Pharmacology

The only safety pharmacology study conducted by the Applicant was an in vitro study on cardiovascular targets, including the hERG channel. Meloxicam did not cause a meaningful inhibition of any cardiovascular targets. A 20.5% inhibition of the hERG channel was detected at 30 mcM meloxicam.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Exposure to bupivacaine and meloxicam was assessed following a single subcutaneous administration of HTX-011 in rats and dogs (33-79 and 33-36) and the pharmacokinetic

parameters from those studies are included below. In rats, bupivacaine was observed in plasma for 72 to 144 hours with the low dose (25 mg/kg) and for 144 to 168 hours with the high dose (50 mg/kg). In dogs, bupivacaine was observed in the plasma for 96 to 120 hours.

Excretion was measured in minipig sows following a single subcutaneous dose of HTX-011 (33-77). Concentrations of bupivacaine, meloxicam, and the water-soluble end products of AP135 (b) (4) were measured in milk, plasma, and urine. The same compounds were measured in the plasma and urine of untreated neonatal piglets. Bupivacaine and meloxicam were found in the plasma, milk, and urine of the sows, while only bupivacaine was found in the urine and plasma of piglets. The water-soluble end products were found in the milk, plasma, and urine of sows, while only (b) (4) was present in piglet plasma. All four products were found in pooled urine from piglets.

The potential for drug interactions between bupivacaine, meloxicam, and lidocaine were assessed in minipigs (33-78). There was no meaningful impact on the pharmacokinetic profiles of any of the compounds when co-administered.

Table 69: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Rats

Dose of HTX-011-56 (mg/kg)	Analyte	Sex	N	t _{1/2} (h)	T _{max} (h)	C _{max} (ng/mL)	AUC _{inf} (h·ng/mL)
<i>Low dose</i>							
25	Bupivacaine	M	9	20.8	6.00	276	7,260
		F	9	13.6	0.50	321	6,240
0.75	Meloxicam	M	9	26.3	24.00	1,860	175,000
		F	9	NC ^a	72.00	3,770	NC ^a
<i>High dose</i>							
50	Bupivacaine	M	9	18.1	4.00	474	16,400
		F	9	16.2	1.00	510	15,000
1.5	Meloxicam	M	9	36.5	72.00	3,480	340,000
		F	9	40.5	72.00	7,180	962,000

Abbreviations: AUC_{inf}, area under the concentration-time curve from time 0 to infinity; C_{max}, maximum observed concentration; F, female; M, male; NC, not calculated; PK, pharmacokinetic; SC, subcutaneous; t_{1/2}, apparent terminal half-life; T_{max}, time of maximum observed concentration.

^a Not calculated due to insufficient number of samples.

Source: Module 4, Section 4.2.2.2, Study Report 33-79, Appendix 5, Table 5.

Table from page 23 of the Applicant's Nonclinical Overview (2.4)

Table 70: Pharmacokinetic Parameters for Bupivacaine and Meloxicam in Dogs

Dose of HTX-011-56 (mg/kg)	Analyte	Sex	N	t _{1/2} (h)	T _{max} (h)	C _{max} (ng/mL)	AUC _{last} (h·ng/mL)	AUC _{inf} (h·ng/mL)
6.96	Bupivacaine	M	5	35.6	1.80	321	9,527	10,495
6.66		F	4 ^a	36.5	2.50	228	8,230	9,458
0.209	Meloxicam	M	5	154	36.0	301	28,474	69,649
0.200		F	4 ^a	94.1	48.0	257	23,778	44,894

Abbreviations: AUC_{inf}, area under the concentration-time curve from time 0 to infinity; AUC_{last}, area under the concentration-time curve from time 0 to last quantifiable sampling time point; C_{max}, maximum observed concentration; F, female; M, male; PK, pharmacokinetic; SC, subcutaneous; t_{1/2}, apparent terminal half-life; T_{max}, time of maximum observed concentration.

Note: the dose level in mg/kg for the active ingredients was calculated based on the nominal concentration of bupivacaine or meloxicam including the density of 1.17 g/mL, the dose volume administered, and an average body weight for each sex.

^a One female received only half of the targeted dose. The plasma concentration-time data from this female was excluded from the analysis.

Source: [Module 4, Section 4.2.2.2, Study Report 33-36, Table 1.](#)

Table from page 24 of the Applicant's Nonclinical Overview (2.4)

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose studies with the HTX-011 vehicle were summarized as part of the Comments on Novel Excipients Section (Section 2.4).

6.2 Repeat-Dose Toxicity

Study title: HTX-011: 28-Day Intermittent Subcutaneous Toxicity and Toxicokinetic Study in Sprague-Dawley Rats (with a 2-week Recovery)

Study no.: 33-85
 Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\33-85\33-85.pdf>
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 1/17/2018
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: HTX-011, Lot 026517, 99.9% bupivacaine and 97.3% meloxicam

Key Study Findings

- All animals survived until necropsy.
- No treatment related effects were observed on body weight, food consumption, ophthalmic parameters, clinical chemistry parameters, organ weights, or urinalysis parameters.
- An increased incidence of scabbing was seen at the injection site of some animals receiving HTX-011 or the vehicle at some injection sites. The incidence of scabbing was reduced following recovery.
- In males, increased platelets, neutrophils, eosinophils, and reticulocytes were observed in all treated groups (including the vehicle) compared to saline. Increases in hematology parameters were no longer present following recovery.
- Histopathological findings that appeared to be related to systemic exposure were limited to minimal mononuclear cell infiltrates in the liver of high dose males and minimal accumulation of erythrocytes in the thymus of low and high dose males.
- Injection site histopathological findings included chronic inflammation, hemorrhage, and presence of test material following treatment with the vehicle and HTX-011. Treatment with HTX-011 resulted in an increase in the severity or incidence of some findings. Chronic inflammation was still present in recovery animals, although the overall incidence was reduced in animals that had a longer recovery time.
- Bupivacaine and meloxicam toxicokinetics were measured on Days 1, 5, and 27. For both compounds, AUC increased linearly with dose. AUCs also appeared to increase with time suggesting some accumulation. Bupivacaine T_{max} values were generally short (0.1-1 hours), while meloxicam T_{max} values were longer (0.5-48 hours) but with more variance. C_{max} values for bupivacaine were highly variable and no trends could be deciphered. In contrast, meloxicam C_{max} values followed a linear trend with dose and increased with time.
- Polymer breakdown products were evaluated in urine on Day 20. (b) (4)
[REDACTED]
were all detected at levels above the calibration range.
- In terms of systemic effects, findings were limited to males and included effects on hematology parameters in all treated animals (vehicle and both doses of HTX-011) and potentially associated histopathological findings of infiltration of mononuclear cells into the liver (high dose of HTX-011) and accumulation of erythrocytes in the thymus (low and high dose of HTX-011). Changes in hematology parameters were small and did not follow a dose response. The observed histopathological findings in the liver and thymus were of minimal

severity and had a small incidence (30%). They were not considered toxicologically relevant.

- A local inflammatory reaction was observed following treatment with the vehicle and HTX-011. The effects are consistent with a typical biological response to injection of foreign material. The majority of the effects resolved as indicated by the lack of findings in recovery animals. However, chronic inflammation was still present in recovery animals up to 26 days following the last injection. Effects were at times worse (increased incidence and/or severity) with HTX-011 compared to the vehicle suggesting that bupivacaine or meloxicam may have exacerbated the effects of the vehicle. There was no dose response, except for the presence of test material, which is expected since the dose was controlled by injecting different volumes of the clinical formulation (same concentration of drug substance and excipients). The Applicant claims that the effects are non-adverse and therefore the NOAEL is the highest dose tested. While the effects observed are not unexpected, it is difficult to ascertain the adversity and clinical relevance of the observed persisting chronic inflammation. Therefore, in terms of local effects, this study demonstrates that the clinical formulation causes chronic inflammation up to 26 days that is likely the result of the vehicle.

Methods

Doses:	Saline, vehicle control, 12.5/0.38 mg/kg bupivacaine/meloxicam, and 25/0.75 bupivacaine/meloxicam (see table below)
Frequency of dosing:	Once every 48 hours
Route of administration:	Subcutaneous injection
Dose volume:	0.85 mL/kg (saline, vehicle, and 25/0.75 mg/kg) or 0.43 mL/kg (12.5/0.38 mg/kg)
Formulation/Vehicle:	HTX-011-056 (HTX-011): 25% triacetin, 10% DMSO, 62.375% AP135 polymer, 0.05% maleic acid, 2.5% bupivacaine, and 0.075% meloxicam HTX-024-002 (vehicle): 25% triacetin, 10% DMSO, and 65% AP135 polymer
Species/Strain:	Sprague-Dawley rat
Number/Sex/Group:	10 for the main experiment, 5 for recovery, and 3 (saline and vehicle) or 27 (low and high dose) for toxicokinetics
Age:	8-9 weeks old
Weight:	193-227 g (male) and 148-193 g (female)
Satellite groups:	Toxicokinetic and recovery groups
Unique study design:	None
Deviation from study protocol:	None that impacted study results

Experimental Design

Group ^a	Description	Number of Rats/Sex/Group			Dose Level		Dose Volume (mL/kg)	Dose Concentration ^d	
		Core	Recovery	TK	bupivacaine (mg/kg/day)	meloxicam (mg/kg/day)		bupivacaine (mg/mL)	meloxicam (mg/mL)
1 ^b	Saline Control	10	5	3	0	0	0.85	0	0
2 ^c	Vehicle Control	10	5	3	0	0	0.85	0	0
3	Low Dose	10	5	27	12.5	0.38	0.43	29.25	0.88
4	High Dose	10	5	27	25	0.75	0.85	29.25	0.88

^aAnimals were dosed once every 48 hours ($\pm$ 1 hour) for a total of 14 doses. Animals were dosed on Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27.

^bGroup 1 was administered saline control (sterile saline) only.

^cGroup 2 was administered vehicle control (HTX-024-002) only.

^dConcentrations of bupivacaine and meloxicam were 2.5% and 0.075% w/w (or 25 mg/g and 0.75 mg/g), respectively. When corrected for density (1.17 g/mL) the final concentrations of bupivacaine and meloxicam were 29.25 mg/mL and 0.88 mg/mL, respectively.

Injection Site Rotations

Dose Site	Location	Injection Day	Dose Free Period to Terminal Necropsy
A	Left Cranial Back	1, 13, and 25	4
B	Right Cranial Back	3, 15, and 27	2
C	Left Mid Back	5 and 17	12
D	Right Mid Back	7 and 19	10
E	Left Caudal Back	9 and 21	8
F	Right Caudal Back	11 and 23	6

Observations and Results

Mortality

All animals survived until scheduled necropsies.

Clinical Signs

Dosing sites were scored using a modified Draize scoring system prior to dosing and 2-4 hours post dose. Detailed clinical examinations were performed weekly during the study and cage-side observations were performed at the time of dose site observations.

No evidence of edema or erythema was observed during the study. The most remarkable finding was scabbing at the injection sites. Findings were seen with both the vehicle and HTX-011 in males and females, with an increasing incidence of findings in the low and high dose groups in some cases. Scabs were most prevalent at the injection sites that were injected three times (A and B). However, Site C also had a large amount of scabbing and it was only injected a total of two times. Furthermore, Site A and B both had scabbing occur prior to the third injection and in some cases prior to the second injection. The incidence of scabbing was greatly reduced in recovery animals with only a few sporadic findings.

Table 71: Incidence of clinical observations in males

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Scab, A, small, 1-10 mm	1	2	5	8
Scab, B, small, 1-10 mm	-	6	6	6
Scab, C, small, 1-10 mm	-	-	4	6
Sore, C, small 1-10 mm	-	-	-	3

N = 15

Table 72: Incidence of clinical observations in females

	Female			
	Saline	Vehicle	12.5/0.38	25/0.75
Scab, A, small, 1-10 mm, multiple	-	1	-	3
Scab, A, small, 1-10 mm	-	9	7	9
Scab, B, small, 1-10 mm	-	5	5	10
Scab, B, medium, >10-20 mm	-	1	1	3
Scab, C, small, 1-10 mm	-	1	5	5
Scab, D, small, 1-10 mm, multiple	-	1	4	5
Scab, D, small, 1-10 mm	-	1	5	8

N = 15

Body Weights

Body weights were recorded prior to randomization, prior to treatment, and weekly for the remainder of the study.

There were no differences in body weights between groups.

Feed Consumption

Food consumption was measured in conjunction with body weight.

Rats of all groups consumed a similar amount of food.

Ophthalmoscopy

Indirect funduscopy examinations were performed pretest and on the main experiment animals one week prior to the end of treatment.

There were no treatment-related ophthalmic changes observed.

Hematology

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of hematology and coagulation parameters was evaluated.

Changes in hematology parameters compared to the saline control were limited to male animals and included increased absolute platelets (all treated groups), increased neutrophils as a percentage of red blood cells (high dose), increased absolute neutrophils (all treated groups), increased absolute eosinophils (all treated groups), increased absolute and as a percentage of red blood cells reticulocytes (all treated groups), and increased fibrinogen (all treated groups). Following recovery, all hematology parameters were similar between the saline control and treated groups. Overall, the changes were relatively small and the fact that the changes were seen in all treated groups indicated that the effects are likely secondary to the local inflammatory reaction.

Table 73: Notable changes in hematology parameters in male rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Platelets (x 10 ³ cells/mL)	1170 ± 107	1312 ± 135* (12)	1285 ± 112 (10)	1331 ± 131* (14)
Neutrophils (%)	13.4 ± 4.4	14.0 ± 5.8 (4)	14.2 ± 4.9 (6)	16.5 ± 4.4 (23)
Neutrophils (x 10 ³ cells/mL)	1.11 ± 0.34	1.33 ± 0.56 (20)	1.52 ± 0.60 (37)	1.45 ± 0.47 (31)
Eosinophils (x 10 ³ cells/mL)	0.10 ± 0.02	0.12 ± 0.04 (20)	0.14 ± 0.04* (40)	0.11 ± 0.04 (10)
Reticulocytes (x 10 ⁹ cells/L)	196.5 ± 28.3	228.8 ± 29.0 (16)	223.5 ± 33.5 (14)	257.8 ± 47.0* (31)
Reticulocytes (%)	2.37 ± 0.41	2.72 ± 0.43 (15)	2.81 ± 0.51 (19)	3.18 ± 0.55* (34)
Fibrinogen (mg/dL)	327 ± 31	390 ± 28* (19)	391 ± 31* (20)	394 ± 19* (20)

* Significantly different from saline control, p ≤ 0.05

Values in () represent % change compared to saline.
N = 10

Clinical Chemistry

Blood was collected prior to scheduled necropsy from the abdominal aorta in deeply anesthetized (sodium pentobarbital) and fasted rats. A complete battery of clinical chemistry parameters was evaluated.

A statistically significant decrease in albumin was reported in males receiving the vehicle and high dose of HTX-011. The magnitude of change was small and there was no difference following the recovery period, therefore the change is not likely toxicologically relevant.

A large increase in aspartate aminotransferase (AST) was noted in one male receiving the low dose of HTX-011, which caused the average AST level in this group to be elevated. Since the increase in AST was only seen in one animal across the entire study, this was considered an incidental finding not related to treatment.

Table 74: Notable changes in clinical chemistry parameters in rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Albumin (g/dL)	2.8 ± 0.1	2.6 ± 0.1* (7)	2.7 ± 0.1 (4)	2.6 ± 0.1* (7)
Aspartate aminotransferase (%)	119 ± 20	133 ± 25 (12)	168 ± 110 (41)	130 ± 13 (9)

* Significantly different from saline control, $p \leq 0.05$

Values in () represent % change compared to saline.

N = 10

Urinalysis

Urine was collected from fasted main study and recovery rats the day prior to terminal sacrifice. A complete battery of urinalysis parameters was evaluated.

There were no treatment-related changes in urinalysis parameters.

Gross Pathology

Following sacrifice, all main study and recovery animals received a complete necropsy.

Gross observations were primarily at the injection site and consisted of scabs and accumulations in animals receiving HTX-011 or the vehicle. In males, findings were primarily at Injection Site A and B, which were the injection sites with the least time between the last injection and sacrifice (4 and 2 days, respectively). In females, findings were also seen at Injection Site C, which had the longest time between the last injection and sacrifice (12 days). There was no clear dose-response to the findings,

although the findings were overall more prevalent in animals who received HTX-011 than the vehicle. The lack of a dose response between the two HTX-011 doses is not unexpected since the gross observations represent a local effect and dose was controlled by injecting different volumes of the same concentration of HTX-011. Scabbing and accumulation were not observed in recovery animals.

Table 75: Gross observations in male rats

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Accumulation (A)	-	-	-	1
Accumulation (B)	-	1	3	1
Scab (A)	-	-	2	-
Scab (B)	-	1	2	-

Table 76: Gross observations in female rats

	Female			
	Saline	Vehicle	12.5/0.38	25/0.75
Accumulation (A)	-	-	-	2
Accumulation (B)	-	-	3	2
Accumulation (C)	-	1	2	-
Scab (A)	-	2	-	1
Scab (B)	-	-	1	3
Scab (C)	-	-	3	-

N = 10

Organ Weights

The following tissues were weighed:

Adrenal Glands	Liver	Testes
Brain	Ovaries	Thymus
Epididymides	Pituitary gland	Thyroid
Heart	Prostate gland	Uterus
Kidneys	Spleen	

There were no treatment-related changes in organ weights.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Tissue Collection Table

Adrenals*	Pancreas
Aorta	Pituitary*
Brain* (entire)	Prostate* (with seminal vesicles/ coagulating gland/empty bladder)*
Epididymides*	Salivary glands (mandibular)
Esophagus	Sciatic nerve
Eyes with optic nerve	Skeletal muscle
Femur with marrow	Skin (with mammary gland for females)
Harderian gland	Spinal cord (cervical, thoracic, lumbar)
Heart*	Spleen*
Intestines (gut-associated lymphoid tissue, examined only if present in plane of section)	Sternum (bone with marrow)
Cecum	Stomach
Colon	Testes*
Duodenum	Thymus*
Ileum	Thyroid* (parathyroids, examined if present in plane of section)
Jejunum	Tongue
Rectum	Trachea
Kidneys*	Uterus* (with cervix/empty bladder)
Larynx	Vagina
Liver*	Gross lesions/masses
Lungs with bronchi	Identification (ear tag)
Lymph nodes (mandibular and mesenteric)	Injection sites A, B, C, D, E, F (full thickness at each site; including underlying muscle)
Nasal Cavity	
Ovaries* (with oviducts)	

Histological Findings

Histopathological findings related to systemic exposure were only seen in male animals and included minimal mononuclear cell infiltrates in the liver at the high dose and minimal accumulation of erythrocytes in the thymus at the low and high doses. These effects were not seen in recovery animals. These findings were not considered adverse due to the minimal severity and low incidence.

The remaining histopathological findings were seen at the injection sites with the vehicle and HTX-011. While not consistent, treatment with HTX-011 did result in a greater incidence and or severity of some findings compared to the vehicle control. Observations included chronic inflammation, granulomatous inflammation, hemorrhage, and the presence of test material. Granulomatous inflammation was seen at all injection sites with an average severity between minimal and mild but was no longer

seen in the recovery groups indicating that it resolved by 14 days post treatment. Findings of hemorrhage were more prevalent at the injection sites that were more recently injected although there were some exceptions. Hemorrhage was not seen in the recovery animals suggesting it resolves after 16 days.

Chronic inflammation was described in the report as either "active" or having no additional description. Active chronic inflammation appeared to be most prevalent at Injection Site B, where there was only 2 days following injection before necropsy. In general, the incidence of active chronic inflammation decreased with time after injection, although there were some exceptions. Active chronic inflammation was not observed in recovery animals. In contrast, chronic inflammation was most prevalent at Injection Site C, where there was 12 days following injection before necropsy. Based on the incidence data for both types of chronic inflammation, it appears that it is active following injection and eventually transitions over to chronic inflammation, although it is difficult to determine the precise time course based on the available data. What is clear is that for up to 12 days post injection there is some form of chronic inflammation at the injection site. Chronic inflammation, but not active chronic inflammation, was observed in recovery animals. While still present, the incidence was reduced in the animals which had the longest recovery time (26 days).

There did not appear to be a clear difference between the low and high dose of HTX-011 in terms of incidence or severity for histopathological findings, except for the presence of test material. This is not unexpected since the histopathological observations represent a local effect and the dose was controlled by injecting a different volume of the same concentration of HTX-011. However, the presence of test material would be expected to be related to the volume injected as a higher volume would result in a larger absolute amount of test material.

Table 77: Systemic histopathological observations

	Male			
	Saline	Vehicle	12.5/0.38	25/0.75
Liver-mononuclear cell infiltrate	1 (1)	1 (1)	1 (1)	3 (1)
Thymus-accumulation of erythrocyte	0	0 (1)	2 (1)	3 (1)

N = 10

Table 78: Chronic Inflammation

Inj Site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	2 (2)	1 (1)	2 (1.5)	2 (1)	1 (1)	2 (1)	1 (1)	3 (1.3)
A [4]	2 (1)	1 (2)	3 (2)	4 (1.3)	1 (1)	4 (1)	6 (1.7)	6 (1.5)
F [6]	0	2 (1)	2 (2)	6 (2)	0	4 (1)	4 (1)	2 (2.5)
E [8]	0	4 (1.5)	4 (2)	2 (2.5)	1 (1)	4 (1.3)	4 (1.5)	2 (2.5)
D [10]	0	2 (1.5)	6 (2)	4 (1.5)	0	4 (1.8)	6 (1.7)	4 (2.3)
C [12]	0	8 (1.1)	7 (1.7)	7 (1.4)	0	4 (1)	4 (1.3)	9 (1.4)

N = 10

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 79: Chronic Inflammation, active

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	3 (1)	7 (1.7)	8 (2.1)	8 (2)	1 (1)	5 (1.6)	9 (1.7)	7 (1.9)
A [4]	0	5 (2.2)	5 (2.4)	3 (1.7)	0	3 (2)	2 (1.5)	4 (1.5)
F [6]	0	3 (2.7)	5 (2.6)	3 (2.3)	0	0	1 (3)	3 (3)
E [8]	0	2 (1.5)	4 (2.5)	6 (2)	0	2 (1.5)	1 (1)	5 (2.2)
D [10]	0	2 (2)	1 (2)	5 (2.4)	0	0	3 (2)	2 (1)
C [12]	0	0	1 (3)	2 (1.5)	0	1 (2)	0	1 (1)

N = 10

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 80: Granulomatous inflammation

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	2 (1)	1 (2)	0	0	0	0	1 (1)
A [4]	0	3 (1)	2 (1.5)	1 (1)	0	2 (1)	0	1 (1)
F [6]	0	2 (1.5)	2 (1.5)	2 (1)	0	2 (1)	1 (1)	3 (1.7)
E [8]	1 (2)	1 (1)	4 (1)	5 (1)	0	1 (1)	1 (1)	5 (1.4)
D [10]	0	3 (1)	2 (1.5)	4 (1)	0	1 (1)	2 (1)	3 (1.7)
C [12]	0	0	4 (1)	4 (1)	0	2 (1.5)	0	3 (1)

N = 10

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 81: Hemorrhage

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	1 (2)	8 (2.9)	6 (1.8)	0	2 (1)	3 (2)	4 (1.8)
A [4]	0	2 (1.5)	2 (3)	2 (1)	0	2 (1.5)	5 (1.2)	2 (1)
F [6]	0	1 (4)	1 (2)	5 (1.8)	0	0	1 (3)	1 (2)
E [8]	1 (2)	0	2 (2)	4 (1)	0	0	0	2 (1)
D [10]	0	1 (3)	1 (1)	1 (1)	0	1 (1)	1 (1)	1 (2)
C [12]	1 (1)	1 (3)	0	2 (3)	0	1 (2)	0	0

N = 10

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 82: Presence of Test Material

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [2]	0	0	4 (2.8)	5 (3)	0	2 (1)	3 (1.7)	3 (1.3)
A [4]	0	1 (1)	1 (3)	3 (2.7)	0	2 (2)	2 (1.5)	1 (1)
F [6]	0	1 (1)	0	2 (2.5)	0	0	0	3 (3)
E [8]	0	0	2 (2)	3 (2.7)	0	0	1 (1)	2 (3.5)
D [10]	0	0	0	3 (2)	0	0	2 (1)	0
C [12]	0	0	0	1 (3)	0	1 (2)	0	0

N = 10

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 83: Chronic inflammation, recovery animals

Inj site	Male				Female			
	Saline	Vehicle	12.5/0.38	25/0.75	Saline	Vehicle	12.5/0.38	25/0.75
B [16]	0	1 (1)	3 (1.3)	2 (1.5)	0	1 (1)	1 (1)	2 (1.5)
A [18]	0	0	2 (1)	5 (1.4)	0	2 (1)	2 (2)	0
F [20]	0	0	0	0	0	1 (1)	1 (1)	1 (1)
E [22]	0	4 (1)	4 (1.5)	4 (1.3)	0	3 (1.3)	0	3 (1.7)
D [24]	0	2 (1.5)	2 (1)	1 (1)	0	2 (1)	3 (1)	1 (1)
C [26]	0	2 (2)	1 (1)	1 (2)	0	2 (1)	1 (1)	0

N = 5

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Toxicokinetics

Toxicokinetic animals (3 rats/sex/timepoint) were bled via the sublingual vein on Days 1, 5, and 27. Urine was collected from vehicle and high-dose toxicokinetic animals in Week 3 for analysis of polymer breakdown products.

Bupivacaine

Bupivacaine AUC followed a nearly linear dose response on Day 1 and Day 5, but not on Day 27. It appeared that there was some accumulation of bupivacaine over time as AUC increased with time in males, but not females, with both doses. By Day 27, the AUC was 1.8-2.3 times higher in males compared to AUC on Day 1. In addition, there were still small, but detectable, amounts of bupivacaine in plasma at 48 hours after dosing in both males and females. Bupivacaine appeared to release quickly with T_{max} s ranging from 0.5 – 1 hour. Bupivacaine C_{max} values were highly variable and it is difficult to interpret any patterns. In general, C_{max} , T_{max} , and AUC were comparable between males and females.

Table 84: Bupivacaine toxicokinetic parameters

Dose (mg/kg/dose)	Day	Sex		C_{max} (ng/mL)	t_{max} (h)	AUC _{0-48h} (ng•h/mL)	R ^a	$C_{max}/Dose$	AUC _{0-48h}/Dose}
12.5	1	Male	Mean	348	0.5	2570	NA	27.8	206
			SE	235		278			
		Female	Mean	303	0.5	3220	NA	24.3	258
			SE	45.2		420			
	5	Male	Mean	1250	0.5	3640	1.42	100	291
			SE	852		498			
		Female	Mean	326	1	3000	0.930	26.1	240
			SE	94.4		302			
	27	Male	Mean	470	0.5	5860	2.28	37.6	469
			SE	149		938			
		Female	Mean	487	1	4650	1.44	38.9	372
			SE	188		605			
25	1	Male	Mean	254	6	4580	NA	10.1	183
			SE	86.5		478			
		Female	Mean	718	0.5	6600	NA	28.7	264
			SE	184		574			
	5	Male	Mean	328	0.5	6360	1.39	13.1	254
			SE	104		487			
		Female	Mean	558	1	7290	1.10	22.3	292
			SE	80.1		1250			
	27	Male	Mean	948	1	8120	1.77	37.9	325
			SE	367		753			
		Female	Mean	566	1	6490	0.980	22.6	260
			SE	211		465			

NA: Not Applicable; SE: standard error (C_{max} and AUC_{0-48h} only)

$C_{max}/Dose$ units: [(ng/mL)/(mg/kg/dose)]

AUC_{0-48h}/Dose units: [(ng•h/mL)/(mg/kg/dose)]}

^a R (Accumulation Ratio) = Day 5 or Day 27 AUC_{0-48h}/Day 1 AUC_{0-48h}}}

Taken from page 552 of Study report 33-85

Meloxicam

Meloxicam AUC followed a linear dose response on all days for both sexes. Meloxicam AUC increased with time; by Day 27 AUC was 2.7-3.9 times higher than on Day 1.

There were still high levels of meloxicam in plasma 48 hours after dosing, which is reflected by the high T_{max} values ranging from 0.5-48 hours. C_{max} values also followed a linear dose response and increased with time. For both C_{max} and AUC, the values were higher in females compared to males. The T_{max} values were highly variable in both sexes but generally seemed comparable.

Table 85: Meloxicam toxicokinetic parameters

Dose (mg/kg/dose)	Day	Sex		C_{max} (ng/mL)	t_{max} (h)	AUC _{0-48h} (ng•h/mL)	R ^a	C_{max} /Dose	AUC _{0-48h} /Dose
0.38	1	Male	Mean	597	48	25600	NA	1570	67400
			SE	75.8		989			
		Female	Mean	1200	48	42300	NA	3170	111000
			SE	119		4540			
	5	Male	Mean	1340	6	50100	1.96	3510	132000
			SE	473		3240			
		Female	Mean	3080	12	112000	2.65	8110	295000
			SE	457		11200			
	27	Male	Mean	1810	12	70200	2.74	4770	185000
			SE	219		4040			
		Female	Mean	4190	12	163000	3.85	11000	429000
			SE	280		22400			
0.75	1	Male	Mean	1280	24	49000	NA	1710	65300
			SE	159		2520			
		Female	Mean	2740	48	79500	NA	3660	106000
			SE	198		8840			
	5	Male	Mean	2770	6	109000	2.22	3700	145000
			SE	318		6370			
		Female	Mean	5200	36	209000	2.63	6930	279000
			SE	1520		28800			
	27	Male	Mean	3680	6	142000	2.90	4900	189000
			SE	89.9		8190			
		Female	Mean	7710	0.5	289000	3.64	10300	385000
			SE	647		39900			

NA: not applicable; SE: standard error (C_{max} and AUC_{0-48h} only)

C_{max} /Dose units: [(ng/mL)/(mg/kg/dose)]

AUC_{0-48h}/Dose units: [(ng•h/mL)/(mg/kg/dose)]

^a R (Accumulation Ratio) = Day 5 or Day 27 AUC_{0-48h}/Day 1 AUC_{0-48h}

Taken from page 556 of Study report 33-85

Polymer breakdown products

Urine collected on Day 20 was analyzed for the presence of breakdown products of the polymer (AP135) using a non-validated method for exploratory purposes. The breakdown products measured were (b) (4)

(b) (4) For all compounds, the levels were over the calibration range and extrapolated values were reported when available. For (b) (4) the values were above the calibration range (b) (4) mcg/mL) but estimated values were not reported. Excluding (b) (4) the most prevalent breakdown product was (b) (4) followed by (b) (4) and then (b) (4) Levels of (b) (4) were similar between males and females while levels of (b) (4) and (b) (4) were higher in males.

Table 86: Levels of polymer breakdown products in the urine

Dose Group	Animal ID	Sex
Group 2 Vehicle Control	507	Male
	508	Male
	509	Male
	510	Female
	511	Female
	512	Female
Group 4 High Dose	567	Male
	568	Male
	569	Male
	594	Female
	595	Female
	596	Female

(b) (4)

^a The assays used to measure the concentrations were not validated, and all samples were above the calibration range. The extrapolated values are shown for informational purposes only, when available.

Taken from page 28 of Study report 33-85

Dosing Solution Analysis

The vehicle was analyzed at the end of the study (March 6, 2018) to compare to the results from the Certificate of Analysis provided by the Applicant dated March 19th, 2018. There were no differences between the CoA and the analysis. Meloxicam and bupivacaine were not detected in the vehicle.

HTX-011 was not analyzed. The Applicant provided characterization of the test substance including stability data. The CoA for Lot 026517 indicated that the test substance had 99.9% bupivacaine and 97.3% meloxicam with a retest date of November 2018 (24 months after manufacture), that is later than the end of Study 33-85.

Study title: HTX-011: 28-day intermittent subcutaneous toxicity and toxicokinetic study in Beagle dogs (with a 2-week recovery)

Study no.: 33-86
Study report location: SDN 1, Module 4
<\\cdsesub1\levsprod\nda211988\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\33-86\33-86.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/22/2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: HTX-011, Lot 026517, 99.9% bupivacaine and 97.3% meloxicam

Key Study Findings

- All animals survived until necropsy.
- No treatment related effects were observed on ophthalmic parameters, electrocardiography parameters, or urinalysis parameters.
- There was an increased incidence of edema at the injection site following vehicle and HTX-011 treatment. Incidence and severity were highest with the vehicle, while both doses of HTX-011 caused similar incidence and severity scores that were on average lower than the vehicle. Edema was not seen in recovery animals.
- Treatment with the vehicle also resulted in hypoactivity, dermal scabs, and swollen sites. These were not observed in recovery animals.
- Decreased body weight was observed following treatment with the vehicle. This did not appear to be toxicologically relevant in males due to lower weight prior to treatment and similar body weight gain to saline control. However, the effect was toxicologically relevant in females where body weight gain was greatly reduced over the course of the study.
- Treatment with the vehicle affected multiple hematology parameters while treatment with HTX-011 only resulted in increased reticulocytes. There was no difference in any hematology parameters in recovery animals.
- Treatment with the vehicle affected multiple clinical chemistry parameters while treatment with HTX-011 only resulted in increased glucose at the interim sacrifice. There was no difference in any clinical chemistry parameters in recovery animals.

- Gross pathologic inspection revealed thickening at the injection site in vehicle treated animals and accumulation in HTX-011 and vehicle treated animals. No treatment related macroscopic findings were present in recovery animals.
- Changes in organ weights were observed in vehicle treated animals compared to saline control. This included increased absolute and relative liver weights, increased absolute and relative thymus weights (males only), increased absolute and relative adrenal weights (females only), and increased absolute and relative kidney weights (females only). Most of these changes were also observed in recovery animals.
- Injection site histopathological findings included chronic inflammation, granulomatous inflammation, fibrosis, hemorrhage, and presence of test material following treatment with the vehicle and HTX-011. In general, findings were more prevalent or more severe in female animals and vehicle treated animals. Multifocal chronic inflammation, multifocal granulomatous inflammation, and multifocal fibrosis were still present in recovery animals at reduced severity and/or incidence up to around 28 days.
- Bupivacaine and meloxicam toxicokinetics were measured on Days 1, 9, and 27. Bupivacaine and meloxicam C_{max} and AUC increased with time and were similar between the two dose groups. T_{max} values for both compounds were highly variable.
- Polymer breakdown products were detected in urine on Day 22. (b) (4)
[REDACTED]
were all detected at levels above the calibration range.
- Effects that could be potentially related to systemic exposure included hypoactivity, decreased body weight, altered hematology parameters, altered clinical chemistry parameters, and increased organ weights. All of these effects were observed following treatment with the vehicle and not saline or HTX-011. The majority of findings were similar in both males and females (except for effects on body weight). No histopathological effects on any organs were observed and most effects (except organ weight changes) were reversible. Overall, the data supports that the excipients in the vehicle cause a systemic response.
- The injection volume used for the vehicle was larger than that for HTX-011, therefore vehicle animals received higher exposure to certain excipients which may have caused the systemic effects. The vehicle was not a perfect match for the drug products as it does not contain maleic acid and contains more AP135 (650 mg/g vs. 623.75 mg/g). Compared to HTX-011, the vehicle resulted in about twice as much exposure to DMSO (117 vs. 51.5 mg/kg), triacetin (292.5 vs. 128.7 mg/kg), and AP135 (760.5 vs. 321.11 mg/kg). Compared to human

exposure at the potentially approvable dose of 300 mg/9 mg, HTX-011 showed limited systemic effects (increased reticulocytes in females) with an exposure margin of ~1.4x for DMSO, triacetin, and AP135, while the vehicle showed systemic effects with an exposure margin of ~3.2 for DMSO, triacetin, and AP135.

- A local inflammatory reaction was observed following treatment with the vehicle and HTX-011. The effects are consistent with a typical biological response to injection of foreign material. The majority of the effects resolved following a recovery period as indicated by the lack of those findings in recovery animals. However, multifocal chronic inflammation, granulomatous inflammation, and fibrosis were still present in recovery animals up to 28 days following the last injection. Local effects were predominantly in females and were worse with vehicle compared to HTX-011, likely due to the increased injection volume used for the vehicle. In addition, there appeared to be more effects with the high dose of HTX-011 compared to the low dose. The increased local findings with the vehicle and HTX-011 high dose is slightly unusual as generally the concentration of the formulation is considered the dose metric for local effects. However, the results in dogs support that the absolute amount of the excipients delivered to the local tissue seems to be the driving force to the local effects.
- The Applicant claims that the effects are non-adverse and therefore the NOAEL is the highest dose tested. While the effects observed are not unexpected, it is difficult to ascertain the adversity and clinical relevance of the observed persisting chronic inflammation, granulomatous inflammation, and fibrosis. Therefore, in terms of local effects, this study demonstrates that the clinical formulation causes a local inflammatory response up to 28 days that is likely the result of the vehicle. The total amount of excipients seems to be a driving force in the local reaction. However, if that is the case, it is difficult to compare the local effects in animals to humans since the dosing in the animal study is different from the clinical dosing regimen and it is unclear how to translate the volume of injection into a HED dose.

Methods

Doses: Saline, vehicle control, 10/0.30 mg/kg bupivacaine/meloxicam, and 13/0.39 bupivacaine/meloxicam

Frequency of dosing: Once every 48 hours

Route of administration: Subcutaneous injection

Dose volume: 0.44 mL/kg (saline and 13/0.39 mg/kg), 1 mL/kg for vehicle (start of study), 0.5 mL/kg for vehicle (after seeing decreased body weight and injection site findings), or 0.34 mL/kg (10/0.30 mg/kg)

Formulation/Vehicle: HTX-011-056 (aka HTX-011): 25% triacetin, 10% DMSO, 62.375% AP135 polymer, 0.05% maleic acid, 2.5% bupivacaine, and 0.075% meloxicam
HTX-024-002 (vehicle): 25% triacetin, 10% DMSO, and 65% AP135 polymer

Species/Strain: Beagle dog

Number/Sex/Group: 4 for the main experiment, and 2 for recovery

Age: 5-6 months old

Weight: 6.4-8.9 kg (male) and 5.2-7.2 kg (female)

Satellite groups: Recovery groups

Unique study design: None

Deviation from study protocol: None that impacted study results

Experimental Design

Group ^a	Description	Bupivacaine Dose Level (mg/kg/day)	Meloxicam Dose Levels (mg/kg/day)	Dose Volume (mL/kg)	Bupivacaine Conc. ^d (mg/mL)	Meloxicam Conc. ^d (mg/mL)	Number of Dogs/Group	
							Core	Recovery
1 ^b	Saline Control	0	0	0.44	0	0	4/sex	2/sex
2 ^c	Vehicle Control	0	0	1/0.5 ^e	0	0	4/sex	2/sex
3	Low Dose	10	0.30	0.34	29.25	0.88	4/sex	2/sex
4	High Dose	13	0.39	0.44	29.25	0.88	4/sex	2/sex

^aAnimals will be dosed once every other day for a total of 14 doses, on Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27.

^bGroup 1 will be administered saline control (sterile saline) only.

^cGroup 2 will be administered vehicle control (HTX-024) only.

^dConcentrations of bupivacaine and meloxicam will be 2.5 and 0.075% w/w (or 25 mg/g and 0.75 mg/g), respectively. When corrected for density (1.17 g/mL) the final concentrations of bupivacaine and meloxicam will be 29.25 mg/mL and 0.88 mg/mL, respectively.

^eDue to decreased body weights and adverse injection site findings, the dose volume for the Vehicle Control group was decreased to 0.5 mL/kg for Days 21, 23, 25, and 27.

Injection Site Rotations

Dose Site	Location	Injection Day	Dose Free Days to Terminal Necropsy
A	Left Cranial Back	1 and 13	16
B	Right Cranial Back	3 and 15	14
C	Left Mid Back	5 and 17	12
D	Right Mid Back	7 and 19	10
E	Left Caudal Back	9, 21, and 25	4
F	Right Caudal Back	11, 23, and 27	2

Observations and Results

Mortality

All animals survived until scheduled necropsies.

Clinical Signs

Dosing sites were scored using a modified Draize scoring system prior to dosing and two hours post dose. Detailed clinical examinations were performed weekly during the study in conjunction with body weight. Cage-side observations were performed at the time of dose site/dermal observations.

The primary finding at the dermal site was increased edema. The largest effect was seen with the vehicle treatment resulting in edema scores of four (severe) at most time points. Treatment with either HTX-011 dose resulted in less frequent and less severe observations of edema than the vehicle, but they were still increased compared to the saline control. Edema scores were highest two days after dosing. There was no dose response to the incidence or severity; however, this is not unexpected since the doses are very similar and similar volumes were injected. At the end of the recovery period, the incidence and severity of edema was greatly reduced and no difference between treatment groups was observed.

The dose volume of the vehicle control was reduced due to the observation of injection site findings. After reducing the volume from 1 mL/kg to 0.5 mL/kg (similar to the high dose group), the edema scores were reduced as well.

Some of the animals receiving the vehicle control exhibited serosanguinous fluid with expulsion from the injection site. The fluid was tested for bacteria and did not contain any. Additional findings only seen in the vehicle control animals were hypoactivity, dermal scabs, and swollen sites.

Table 87: Incidence of edema at the injection site

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
A	0	6 (4)	6 (2)	5 (2)	0	6 (4)	6 (2)	6 (2)
B	0	6 (4)	6 (1)	6 (1)	0	6 (4)	6 (1)	6 (1)
C	0	6 (4)	3 (1)	3 (1)	0	6 (4)	2 (1)	6 (1)
D	0	6 (4)	5 (1)	6 (2)	0	6 (4)	5 (1)	5 (1)
E	0	6 (4)	6 (2)	6 (2)	0	6 (4)	4 (2)	6 (2)
F	0	6 (4)	6 (2)	6 (2)	0	6 (4)	6 (3)	6 (2)

N = 6

Values in () represent average severity: 1 = very slight, 2 = well-defined, 3 = moderate, 4 = severe.

Table 88: Average severity of edema in vehicle control group

Inj site	Day 11 (1 ml/kg) M/F	Day 13 (1 ml/kg) M/F	Day 23 (0.5 ml/kg) M/F	Day 25 (0.5 ml/kg) M/F
E	3/3.8	-	2.8/2.3	-
F	-	3.7/3.7	-	2.7/2.7

Body Weights

Body weights were recorded prior to randomization, prior to treatment, and weekly for the remainder of the study.

Body weights in male vehicle treated animals were less than saline control. However, it appears that this group of animals weighed less than the saline control prior to treatment (Day 0). Furthermore, the group as a whole had a similar level of body weight gain over the first 19 days of treatment (0.28 kg for both the saline and vehicle control group) compared to the saline control group.

Body weight was also decreased in female vehicle treated animals compared to the saline control. Similar to males, it appears that this group of animals weighed less than the saline control prior to treatment (Day 0). In contrast to males, there did appear to be an effect on body weight by the vehicle treatment as the % difference between the two groups increased over the first 19 days of treatment. This can also be seen by comparing body weight gain over the 19 days between the saline control (0.31 kg) and the vehicle control (-0.15 kg).

No differences between the HTX-011 groups and the saline control were observed.

Table 89: Body weights

Day	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
0	7.78	91.9%	98.2%	96.5%	6.32	96.2%	98.6%	101.3%
1	7.69	91.7%	98.5%	97.1%	6.18	96.4%	97.6%	100.8%
7	7.90	89.4%	98.7%	99.0%	6.32	94.3%	102.5%	106.3%
13	7.81	92.2%	102.2%	100.5%	6.50	90.0%	99.2%	103.2%
19	8.06	92.2%	102.7%	100.4%	6.62	89.6%	99.2%	104.8%
25	8.34	91.7%	100.2%	99.4%	6.74	93.6%	100.3%	103.0%
27	8.00	95.0%	103.4%	100.8%	6.67	95.8%	100.9%	105.4%

N = 6

Ophthalmoscopy

Indirect fundoscopic examinations were performed pretest, at the end of the treatment period, and during recovery.

There were no treatment-related ophthalmic changes observed.

Electrocardiography

A six-lead electrocardiogram was performed on each dog prior to treatment, on Day 27, and during the second week of recovery. ECGs were evaluated by a qualified veterinary cardiologist.

There were no treatment-related electrocardiographic effects.

Hematology

Blood was collected from the jugular vein prior to treatment, at about 3 weeks, at end of the treatment period, and the end of recovery. A complete battery of hematology and coagulation parameters was evaluated.

A number of hematology parameters were affected by treatment with the vehicle in both males and females. Consistently effected parameters were increased white blood cells, decreased red blood cells, decreased hemoglobin, decreased hematocrit, increased platelets, increased absolute and relative neutrophils, decreased relative lymphocytes, decreased relative basophils, increased absolute monocytes, increased absolute leukocytes, increased red blood cell distribution width, increased absolute eosinophils, and increased absolute and relative reticulocytes. All parameters were similar to the saline control after a two-week recovery period.

In contrast, there were very few effects on hematology with the drug product. In males an absolute and relative increase in reticulocytes were seen at the interim blood collection with both doses but values were similar at the terminal blood collection. In females, absolute and relative reticulocytes were increased at both the interim and terminal blood collections. All parameters were similar to the saline control after a two-week recovery period.

Table 90: Hematology parameters in male dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
WBC (x10 ³ cells/mcL)	11.05	433.9%*	107.9%	111.9%
	7.99	301.1%*	110.4%	115.5%
RBC (x10 ³ cells/mcL)	6.71	75.3%*	100.4%	99.3%
	6.24	78.5%*	101.0%	96.6%
HGB (g/dL)	15.2	73.7%*	98.0%	95.4%
	13.9	79.1%*	100.7%	94.2%
HCT (%)	46.2	76.6%*	98.7%	95.7%
	43.2	80.0%*	99.1%	93.5%
RDW (%)	14.3	103.5%	104.9%	105.6%
	14.0	111.4%*	104.3%	105.0%
PLAT (x10 ³ cells/mcL)	321	144.2%	101.9%	114.6%
	269	208.9%*	105.9%	110.0%
NEUT (%)	56.5	151.2%*	111.7%	110.3%
	54.9	135.2%*	107.5%	104.6%
LYMP (%)	34.5	20.6%*	84.6%	87.5%
	34.5	47.8%*	93.6%	93.0%
EOS (%)	1.4	28.6%*	114.3%	114.3%
	2.1	104.8%	95.2%	133.3%
MONO (%)	6.7	97.0%	80.6%	79.1%
	7.4	86.5%	77.0%	93.2%
LUC (%)	0.5	100.0%	80.0%	80.0%
	0.7	85.7%	85.7%	85.7%
Retic (%)	0.71	129.6%	163.4%	171.8%
	0.78	239.7%*	101.3%	125.6%
NEUT (x10 ³ cells/mcL)	6.25	657.3%*	120.6%	125.8%
	4.47	410.5%*	116.8%	119.9%
LYMPH (x10 ³ cells/mcL)	3.80	85.8%	91.1%	95.0%
	2.69	133.1%*	105.6%	107.4%
EOS (x10 ³ cells/mcL)	0.15	113.3%	120.0%	126.7%
	0.16	281.3%*	112.5%	168.8%
MONO (x10 ³ cells/mcL)	0.76	415.8%*	84.2%	85.5%
	0.58	256.9%*	86.2%	108.6%
LUC x10 ³ cells/mcL	0.05	460.0%	100.0%	80.0%
	0.05	280.0%*	100.0%	100.0%
Retic (x10 ⁹ cells/L)	47.4	96.6%	165.0%	171.5%*
	48.0	188.1%*	104.6%	123.1%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Table 91: Hematology parameters in female dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
WBC (x10 ³ cells/mcL)	12.82	256.9%*	83.5%	83.5%
	9.47	276.2%*	107.5%	92.8%
RBC (x10 ³ cells/mcL)	7.04	78.6%*	99.3%	98.7%
	6.65	84.1%*	99.7%	98.8%
HGB (g/dL)	15.7	75.8%*	94.9%	100.0%
	14.9	81.9%*	95.3%	98.7%
HCT (%)	48.5	77.5%*	94.8%	98.8%
	45.7	83.6%*	95.6%	99.3%
RDW (%)	14.7	103.4%	104.1%	101.4%
	14.2	109.2%*	107.7%*	102.8%
PLAT (x10 ³ cells/mcL)	392	114.3%	91.8%	91.8%
	333	148.0%*	109.9%	103.0%
NEUT (%)	55.9	143.1%*	106.1%	107.5%
	51.5	146.0%*	121.7%*	112.0%
LYMP (%)	36.1	33.5%*	91.1%	89.2%
	39.5	38.2%*	74.9%*	87.3%
EOS (%)	1.4	71.4%	135.7%	157.1%
	2.2	104.5%	83.4%	127.3%
MONO (%)	5.8	110.3%	87.9%	82.8%
	5.8	117.2%	86.2%	72.4%
LUC (%)	0.5	100.0%	80.0%	80.0%
	0.5	80.0%	80.0%	80.0%
Retic (%)	0.96	94.8%	124.0%	157.3%*
	0.70	198.6%	171.4%	162.9%
NEUT (x10 ³ cells/mcL)	7.24	369.8%*	87.8%	89.1%
	4.92	404.1%*	134.4%	103.9%
LYMPH (x10 ³ cells/mcL)	4.56	78.9%*	77.2%*	75.0%*
	3.71	103.0%	75.2%*	81.1%
EOS (x10 ³ cells/mcL)	0.17	152.9%	117.6%	141.2%
	0.21	261.9%*	85.7%	109.5%
MONO (x10 ³ cells/mcL)	0.73	287.7%*	75.3%	69.9%
	0.54	325.9%*	100.0%	68.5%
LUC x10 ³ cells/mcL	0.06	233.3%*	66.7%	66.7%
	0.05	200.0%*	80.0%	60.0%
Retic (x10 ⁹ cells/L)	67.5	75.6%	123.6%	155.3%*
	46.5	164.7%	173.3%	163.9%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Clinical Chemistry

Blood was collected from the jugular vein prior to treatment, at about 3 weeks, at end of the treatment period, and at the end of recovery. A complete battery of hematology and coagulation parameters was evaluated.

Treatment with the vehicle caused multiple changes in clinical chemistry parameters in male and female animals. Consistent changes in both sexes at both blood collection time points were decreased albumin, decreased ALT, decreased creatinine, decreased glucose, increased LDH, increased globulin, and decreased A/G ratio. Other less

consistent changes included decreased total bilirubin (males at both time points; females at interim only), decreased calcium (males at interim only; females at both time points), increased cholesterol (both sexes at interim only), decreased blood urea nitrogen (males at interim only; females at both time points), decreased potassium (both sexes at interim only), increased CPK (males at terminal only), increased triglycerides (males at terminal only), decreased chloride (males at terminal only), and decreased GGT (females at terminal only). All parameters were similar to the saline control animals following a 2-week recovery period.

The only change observed in animals treated with the drug product was an increase in glucose. This change was seen in both sexes and only at the interim time point and similar to saline treated animals after the 2-week recovery period.

Table 92: Clinical chemistry parameters in male dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Alb (g/dL)	2.9	72.4%	96.6%	96.6%
	2.8	82.1%	100.0%	96.4%
Alt (U/L)	29	34.5%	93.1%	93.1%
	27	48.1%	111.1%	103.7%
Crea (mg/dL)	0.83	43.4%	90.4%	101.2%
	0.59	54.2%	91.5%	98.3%
Glu (mg/dL)	80	55.0%	120.0%	120.0%
	88	71.6%	102.3%	98.9%
LDH (U/L)	72	370.8%	90.3%	102.8%
	112	313.4%	100.9%	127.7%
GLB (g/dL)	2.5	128.0%	96.0%	92.0%
	2.5	124.0%	92.0%	92.0%
A/G	1.2	58.3%	100.0%	100.0%
	1.2	66.7%	100.0%	100.0%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Table 93: Clinical chemistry parameters in female dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Alb (g/dL)	3.1	74.2%	93.5%	96.8%
	3.0	80.0%	96.7%	96.7%
Alt (U/L)	25	48.0%	112.0%	112.0%
	24	54.2%	112.5%	104.2%
Crea (mg/dL)	0.87	51.7%	80.5%	90.8%
	0.59	54.2%	89.8%	84.7%
Glu (mg/dL)	84	79.8%	111.9%	119.0%
	92	80.4%	97.8%	106.5%
LDH (U/L)	78	174.4%	85.9%	91.0%
	100	194.0%	131.0%	93.0%
GLB (g/dL)	2.4	137.5%	95.8%	91.7%
	2.3	130.4%	100.0%	95.7%
A/G	1.3	53.8%	100.0%	107.7%
	1.3	61.5%	100.0%	107.7%

For each row, the top value is for interim sacrifice and the bottom value is for terminal sacrifice
N = 6

Urinalysis

Urine was collected directly from the bladder of animals at scheduled necropsy. A complete battery of urinalysis parameters was evaluated.

There were no treatment-related changes in urinalysis parameters.

Gross Pathology

Following sacrifice, all main study and recovery animals received a complete necropsy.

Macroscopic observations were limited to the injection sites. Accumulations were observed in animals treated with the vehicle and HTX-011. The incidence of accumulations appeared to be higher in HTX-011 dosed animals but a clear dose response was not apparent. Thickening of the injection site was primarily observed in vehicle treated animals with the highest incidences occurring at Injection Sites E and F, the injection sites treated closest to the end of the study. No macroscopic findings were present following the 2-week recovery period.

Table 94: Gross observations in male dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Accumulation (A)	-	1	-	2
Accumulation (B)	-	-	-	1
Accumulation (D)	-	2	-	1
Accumulation (E)	-	1	2	2
Accumulation (F)	-	1	1	3
Thickened (E)	-	3	1	1
Thickened (F)	-	3	-	-

N = 4

Table 95: Gross observations in female dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Accumulation (A)	-	1	-	2
Accumulation (B)	-	1	-	1
Accumulation (D)	-	2	-	-
Accumulation (E)	-	1	3	3
Accumulation (F)	-	-	1	2
Thickened (E)	-	3	1	-
Thickened (F)	-	4	2	2

N = 4

Organ Weights

The following tissues were weighed:

Adrenal Glands	Liver	Testes
Brain	Ovaries	Thymus
Epididymides	Pituitary gland	Thyroid
Heart	Prostate gland	Uterus
Kidneys	Spleen	

Changes in organ weights were primarily seen in the vehicle control group. In males, absolute and relative liver weights were increased. Following recovery, absolute liver weights were still increased but relative liver weights were similar to the saline control. Absolute and relative thymus weights were increased at terminal sacrifice and following recovery.

In females, altered organ weights were observed in the adrenal, liver, and kidney. Absolute and relative adrenal weights were increased but only absolute adrenal weight

was increased following recovery. In the main study animals, only relative liver weight was increased but following recovery an increase in absolute liver weight was also observed. Increased absolute and relative kidney weights were observed in both main study and recovery animals.

Table 96: Organ weights in male dogs

	Male			
	Saline	Vehicle	10/0.30	13/0.39
Liver	225	278 (23%)	246 (9%)	237 (5%)
	2.94	3.88 (32%)*	3.29 (11%)	3.12 (9%)
Thymus	8.7	10.28 (18%)	8.94 (3%)	9.41 (8%)
	0.116	0.143 (23%)	0.118 (2%)	0.125 (8%)
Liver- recovery	204	254 (24%)	228 (12%)	242 (19%)
	2.88	3.00 (4%)	2.91 (1%)	3.08 (7%)
Thymus- recovery	9.63	14.60 (52%)	11.64 (21%)	6.88 (-29%)
	0.131	0.173 (32%)	0.151 (15%)	0.089 (-32%)

For each row, the top value is for absolute weight and the bottom value is for organ weight relative to body weight

N = 4 for main study and N = 2 for recovery

Table 97: Organ weights for female dogs

	Female			
	Saline	Vehicle	10/0.30	13/0.39
Adrenal	1.12	1.48 (32%)*	1.02 (-9%)	1.16 (4%)
	0.017	0.026 (52%)*	0.016 (-6%)	0.018 (6%)
Liver	190	203 (7%)	184 (-3%)	207 (9%)
	2.95	3.54 (20%)*	2.92 (-1%)	3.24 (10%)
Kidney	31.8	37.9 (19%)	33.7 (6%)	38.6 (21%)
	0.494	0.660 (34%)*	0.533 (8%)	0.605 (22%)
Adrenal- recovery	1.08	1.24 (15%)	0.98 (-9%)	0.95 (-12%)
	0.017	0.018 (6%)	0.015 (-12%)	0.013 (-24%)
Liver-recovery	180	224 (24%)	190 (6%)	194 (8%)
	2.85	3.17 (11%)	2.88 (1%)	2.67 (-6%)
Kidney-recovery	28.8	46.0 (60%)	34.9 (21%)	36.2 (26%)
	0.454	0.651 (43%)	0.528 (16%)	0.495 (9%)

For each row, the top value is for absolute weight and the bottom value is for organ weight relative to body weight

N = 4 for main study and N = 2 for recovery

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Tissue Collection Table

Adrenals*	Pancreas
Aorta	Pituitary*
Brain* (entire)	Prostate*
Epididymides*	Salivary glands (mandibular and parotid)
Esophagus	Sciatic nerve
Eyes with optic nerve	Skeletal muscle (biceps femoris)
Femur with marrow	Skin (with mammary gland for females)
Gallbladder	Spinal cord (cervical, thoracic, lumbar)
Harderian gland	Spleen*
Heart*	Sternum (bone with marrow)
Intestines	Stomach
Cecum	Testes*
Colon	Thymus*
Duodenum	Thyroid* (parathyroids if present in the section)
Ileum (with Peyer's patches)	Tongue
Jejunum	Trachea
Rectum	Urinary bladder
Kidneys*	Uterus* (with cervix)
Larynx	Vagina
Liver*	Gross lesions
Lungs with bronchi	Identification (ear tattoo)
Lymph nodes (mandibular and mesenteric)	Injection sites A, B, C, D, E, and F (full thickness at each site, including underlying muscle)
Ovaries* (with oviducts)	

A number of histopathological findings were observed at the injection sites with treatment of either the vehicle or HTX-011 as compared to saline control where few or no findings were observed. These included chronic inflammation, granulomatous inflammation, fibrosis, presence of test material, and hemorrhage. Recovery animals were included who were treated for the same amount of time as the main study animals except they were sacrificed 14 days after treatment was stopped. It is important to note that the decreased number of animals used for recovery (two versus four) made it difficult to compare incidence back to the main experiment animals since a single finding represented 50% of the animals studied.

Chronic inflammation was described as focal, multifocal, or active/multifocal with multifocal and multifocal/active being the more prevalent types of chronic inflammation observed. For active multifocal chronic inflammation, the highest incidences and most severe findings were seen at Injection Sites E and F, the injection sites treated closest to the end of the study, with both the vehicle and HTX-011. Overall, the highest incidences and average severity was observed with treatment of the vehicle and overall incidence for all treatments was higher in females. The overall severity and incidence were lower at injection sites that had a longer time between treatment and necropsy for all treatments. Active chronic multifocal inflammation was not observed at a significant level in recovery animals.

In contrast, multifocal chronic inflammation incidence and severity seemed to be higher at injection sites where there was a longer time between the last injection and necropsy with both the vehicle and HTX-011. As with active chronic multifocal inflammation a higher incidence of findings were observed in females but in this case it appeared that the greatest incidence and severity was seen with the high dose of HTX-011. Chronic multifocal inflammation was also observed in recovery animals. Again, findings were primarily in females and with HTX-011 (both doses). The incidence and severity seemed to decrease at injection sites that had longer time since last injection such that findings were not really seen in either sex around 28-30 days.

Taken together, it appears that active chronic multifocal inflammation is seen early following treatment, which transitions to a chronic multifocal inflammation. Based on the female data, where findings were more prevalent, this occurs around 12 days following injection and the chronic active multifocal inflammation lasts until 28 days following injection.

Granulomatous inflammation was primarily seen in female animals treated with the vehicle (all injection sites) and the high dose of HTX-011 (Injection Sites A, B, E, and F). It was also observed in males receiving the high dose at Sites A, B, C, and D. Overall, the average severity was minimal to mild. It was also observed in recovery females at all injection sites except A, although the overall severity was decreased. Taken together, the data suggests that the incidence and severity of granulomatous inflammation peaks around Day 16 and decreases with time since injection, completely resolving by Day 30.

Fibrosis was described as either focal or multifocal with multifocal being the more prevalent form. Multifocal fibrosis was observed in both sexes with both the vehicle and HTX-011. The overall severity of fibrosis was high at multiple injection sites with average severity values of moderate to marked. Fibrosis was observed at all injection sites in main study animals, suggesting it lasts at least 16 days following injection. Findings of fibrosis were still observed in some recovery animals. Based on the observed injection sites, fibrosis with an average severity of mild to marked was observed in female animals up to 26 days after the last injection. No observations of fibrosis were seen at Injection Sites A or B indicating that fibrosis was completely resolved 28-30 days post injection.

Hemorrhage was observed in both males and females receiving the vehicle and HTX-011. In both sexes, findings were more prevalent with the vehicle and the high dose. The majority of the findings were seen at Injection Sites E and F suggesting that hemorrhage resolved by Day 10-12. Hemorrhage was not observed at a significant level in recovery animals.

The presence of test material at the injection site was seen in some animals and defined as either focal or multifocal, with multifocal being more prevalent. In males, the presence of multifocal test material was seen with an incidence of 50% or more with the vehicle at Injection Site F, and the high dose at Injection Site F and D. In females, the presence of multifocal test material was seen with an incidence of 50% or more with the vehicle at Injection Site E and F, the low dose at Injection Site E, and the high dose at Injection Site E and F. This suggests that the test material was only present at the injection site for up to 10 days. However, test material was seen in individual animals with severity scores ranging from minimal to marked up to 16 days after injection. The test material was not present in recovery animals.

In summary, treatment with the vehicle lead to an increase in incidence/severity of chronic active inflammation, granulomatous inflammation, hemorrhage, and fibrosis compared to the saline control. These findings were primarily in female animals and were mostly not present in recover animals, with some exceptions. In some cases, findings with the vehicle were worse than with HTX-011 (active chronic inflammation, and fibrosis). This is interesting since the vehicle had a similar concentration of excipients as HTX-011 (the vehicle did not contain maleic acid and has slightly less AP135). The major difference was a much larger injection volume of the vehicle at the beginning of the study (1 vs. 0.44 mL/kg). Thus, despite being local effects, the increased absolute amount of the excipients could have played a role in these findings.

Treatment with HTX-011 lead to an increase in incidence/severity of chronic active inflammation, granulomatous inflammation, hemorrhage, and fibrosis compared to the saline control. In general, females were more affected than males but it is unclear why there would be a sex difference for these local effects. For many of the observed findings there was a slight increase in the severity and incidence with the high dose of HTX-011 compared to the low dose of HTX-011. It is unclear why this would be the case since the dose was controlled by injecting different volumes of the same concentration solution. It appears that the local effects are somewhat related to the absolute amount of the solution present which is consistent with the increased findings with the vehicle which had a much larger injection volume. Taken together, it appears that the majority of the findings are likely related to the presence of one or more excipients in the drug product.

Table 98: Percentage of main study animals with chronic multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	25 (1)	0	0	25 (2)	25 (1)	0	25 (1)	25 (1)
E (4)	0	0	0	75 (1.3)	25 (1)	0	50 (1)	50 (1)
D (10)	0	25 (1)	25 (1)	25 (1)	0	25 (1)	0	50 (1)
C (12)	0	25 (1)	25 (1)	25 (2)	25 (1)	50 (1)	50 (1)	75 (1.3)
B (14)	0	0	50 (1.5)	0	0	0	25 (1)	75 (1.7)
A (16)	0	0	75 (1)	0	0	50 (1.5)	25 (1)	100 (2)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 99: Percentage of recovery animals with chronic multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	0	50 (1)	50 (2)	0	50 (1)	50 (2)	50 (1)
E (18)	0	50 (1)	0	50 (1)	0	0	50 (2)	50 (2)
D (24)	0	50 (1)	0	0	0	0	50 (1)	50 (2)
C (26)	0	0	0	50 (1)	0	0	50 (1)	50 (1)
B (28)	0	0	0	0	0	0	50 (1)	0
A (30)	0	0	50 (1)	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 100: Percentage of main study animals with active chronic multifocal Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	75 (3.7)	75 (1.3)	50 (2)	25 (1)	75 (3.3)	25 (3)	75 (3.3)
E (4)	0	75 (1.7)	25 (1)	25 (2)	0	100 (3.8)	50 (2)	50 (2.7)
D (10)	0	25 (2)	0	50 (2.5)	0	50 (2.5)	50 (2)	0
C (12)	0	0	50 (2)	25 (1)	0	25 (3)	0	25 (1)
B (14)	0	0	0	50 (1.5)	0	75 (2.3)	25 (1)	0
A (16)	0	0	0	50 (1.5)	0	75 (2.3)	25 (1)	0

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 101: Percentage of main study animals with multifocal granulomatous Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	0	25 (1)	0	0	50 (1)	0	50 (1.5)
E (4)	0	0	0	0	0	50 (1)	25 (1)	50 (1.5)
D (10)	0	0	0	25 (1)	0	75 (1.7)	25 (1)	0
C (12)	0	0	50 (1)	50 (1)	0	50 (1.5)	0	0
B (14)	0	0	0	25 (1)	0	75 (2)	25 (1)	75 (1.3)
A (16)	0	0	0	25 (2)	0	50 (1)	0	75 (1.7)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 =marked.

Values in [] represent number of days since last injection

Table 102: Percentage of recovery animals with multifocal granulomatous Inflammation

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	0	0	0	0	100 (1.5)	50 (1)	0
E (18)	0	0	0	0	0	0	50 (1)	0
D (24)	0	0	0	0	0	50 (1)	50 (1)	0
C (26)	0	0	0	0	0	50 (1)	50 (1)	50 (1)
B (28)	0	0	0	0	0	0	50 (1)	50 (1)
A (30)	0	0	0	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 =marked.

Values in [] represent number of days since last injection

Table 103: Percentage of main study animals with multifocal fibrosis

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	100 (3.8)	0	0	0	75 (4.3)	50 (3.5)	25 (4)
E (4)	0	100 (4.5)	25 (1)	0	0	100 (3)	25 (2)	75 (2.7)
D (10)	0	100 (1.8)	0	75 (1.3)	0	75 (2.7)	25 (2)	25 (1)
C (12)	0	50 (3)	50 (1.5)	50 (2)	0	75 (1.7)	50 (1.5)	50 (1.5)
B (14)	0	25 (3)	25 (2)	0	0	50 (3.5)	0	75 (2.7)
A (16)	0	0	25 (2)	25 (2)	0	75 (2.3)	0	25 (3)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 =marked.

Values in [] represent number of days since last injection

Table 104: Percentage of recovery animals with multifocal fibrosis

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (16)	0	50 (1)	0	0	0	0	50 (1)	0
E (18)	0	0	0	0	0	50 (4)	0	50 (2)
D (24)	0	50 (1)	0	0	0	100 (2.5)	0	0
C (26)	0	0	0	0	0	0	0	50 (2)
B (28)	0	0	0	0	0	0	0	0
A (30)	0	0	0	0	0	0	0	0

N = 2

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 105: Percentage of main study animals with multifocal hemorrhage

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	25 (3)	0	50 (3)	0	75 (1.7)	50 (1.5)	75 (2)
E (4)	0	75 (1.7)	0	0	0	50 (2.5)	0	50 (1.5)
D (10)	0	25 (1)	0	50 (1.5)	0	0	0	0
C (12)	0	0	25 (1)	25 (1)	0	0	0	25 (1)
B (14)	0	0	0	25 (2)	0	0	0	0
A (16)	0	0	0	0	0	0	0	0

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Table 106: Percentage of main study animals with multifocal presence of test material

Inj site	Male				Female			
	Saline	Vehicle	10/0.30	13/0.39	Saline	Vehicle	10/0.30	13/0.39
F (2)	0	50 (3.5)	25 (2)	75 (4.3)	0	50 (4)	25 (3)	75 (4)
E (4)	0	0	25 (4)	25 (4)	0	50 (4)	50 (3)	50 (3.5)
D (10)	0	25 (2)	0	75 (1.7)	0	25 (4)	25 (1)	0
C (12)	0	0	25 (2)	0	0	0	0	25 (4)
B (14)	0	0	0	0	0	0	0	0
A (16)	0	0	25 (2)	0	0	0	0	25 (4)

N = 4

Values in () represent average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Values in [] represent number of days since last injection

Toxicokinetics

All animals were bled via the jugular vein on Days 1, 9, and 27. Urine was collected from vehicle and high dose animals in week 3 for analysis of polymer breakdown products.

Bupivacaine

Bupivacaine C_{max} increased with time; a large increase was seen between Day 1 and Day 9 and a smaller increase between Day 9 and Day 27. C_{max} values were similar between the low and high dose groups, which isn't surprising considering how closely spaced the doses were. There was a good deal of variability to the T_{max} with values ranging from 1-8 hours across the study. There did not appear to be any time or dose trend to the T_{max} values. Over the course of the experiment, females generally had higher T_{max} values. The AUC values increased with time; again a larger increase was seen between Day 1 and Day 9 compared to Day 9 and Day 27. AUC values were generally comparable between the low and high dose groups in females. In males, AUC in the high dose group tended to be higher (27-61%) and was generally in line with the increased amount of bupivacaine in the high dose (30%).

Table 107: Bupivacaine PK parameters

Dose (mg/kg/dose)	Day	Sex		C _{max} (ng/mL)	t _{max} (h) ^a	AUC _{0-48h} (ng•h/mL)
10	1	Male	Mean	289	1	7460
			SD	160	1-4	2010
			n	6	6	6
		Female	Mean	315	5	11100
			SD	68.7	1-12	3010
			n	6	6	6
	9	Male	Mean	514	1	13900
			SD	152	1-4	3120
			n	6	6	6
		Female	Mean	567	1	15100
			SD	126	0.5-6	3700
			n	6	6	6
27	Male	Mean	424	2.5	11600	
		SD	152	1-4	4050	
		n	6	6	6	
	Female	Mean	736	1	18400	
		SD	693	0.5-4	13000	
		n	6	6	6	
13	1	Male	Mean	280	4	10500
			SD	80.1	1-24	2910
			n	6	6	6
		Female	Mean	271	8	9890
			SD	53.5	1-48	1760
			n	6	6	6
	9	Male	Mean	581	1	17600
			SD	142	1-1	4330
			n	6	6	6
		Female	Mean	498	4	15600
			SD	106	1-24	2710
			n	6	6	6
27	Male	Mean	639	1	18700	
		SD	183	1-4	6500	
		n	6	6	6	
	Female	Mean	622	2.5	17500	
		SD	312	1-4	8230	
		n	6	6	6	

^a t_{max} is reported as median and minimum-maximum for group summary statistics instead of mean and standard deviation (SD).

Meloxicam

Meloxicam C_{max} increased with time; a large increase was seen between Day 1 and Day 9 and there was essentially no difference between Day 9 and Day 27. C_{max} values were slightly higher with the high dose, which is in line with the small difference between the two dose groups. There was a good deal of variability to the T_{max} with values ranging

from 0.75-48 hours across the study. The majority of the T_{max} values were on the higher end (18-48 hours). There did not appear to be any time, sex, or dose trend to the T_{max} values. The AUC values increased with time; again a larger increase was seen between Day 1 and Day 9 compared to Day 9 and Day 27. AUC values in the high dose group tended to be higher (15-44%) and were generally in line with the increased amount of meloxicam in the high dose (30%).

Table 108: Meloxicam PK parameters

Dose (mg/kg/dose)	Day	Sex		C _{max} (ng/mL)	t _{max} (h) ^a	AUC _{0-48h} (ng•h/mL)
0.30	1	Male	Mean	359	36	13100
			SD	57.0	24-48	2200
			n	6	6	6
		Female	Mean	370	48	12700
			SD	78.1	24-48	2750
			n	6	6	6
	9	Male	Mean	760	18	30900
			SD	146	4-24	6010
			n	6	6	6
		Female	Mean	749	0.75	28700
			SD	212	0.5-24	8100
			n	6	6	6
27	Male	Mean	876	8	35500	
		SD	118	4-24	5480	
		n	6	6	6	
	Female	Mean	728	3.5	30400	
		SD	228	0.5-48	9300	
		n	6	6	6	
0.39	1	Male	Mean	412	36	15200
			SD	33.8	24-48	1630
			n	6	6	6
		Female	Mean	470	48	15500
			SD	95.0	24-48	3480
			n	6	6	6
	9	Male	Mean	1050	24	43100
			SD	342	4-24	15500
			n	6	6	6
		Female	Mean	895	18	33700
			SD	343	0-24	11700
			n	6	6	6
27	Male	Mean	1150	4	45500	
		SD	272	4-24	13000	
		n	6	6	6	
	Female	Mean	1050	36	45400	
		SD	207	0.5-48	8790	
		n	6	6	6	

^a t_{max} is reported as median and minimum-maximum for group summary statistics instead of mean and standard deviation (SD).

Polymer breakdown products

Urine collected on Day 22 was analyzed for the presence of breakdown products of the polymer (AP135) using a non-validated method for exploratory purposes. The breakdown products measured were (b) (4)

(b) (4) For all compounds, the levels were over the calibration range and extrapolated values were reported when available. For (b) (4) the values were above the calibration range (b) (4) mcg/mL) but estimated values were not reported. Excluding (b) (4) the most prevalent breakdown product was (b) (4) followed by (b) (4) and then (b) (4)

Dosing Solution Analysis

The vehicle was analyzed at the end of the study (March 6, 2018) to compare to the results from the Certificate of Analysis provided by the Applicant dated March 19th 2018. There were no differences between the CoA and the analysis. Meloxicam and Bupivacaine were not detected in the vehicle.

HTX-011 was not analyzed. The Applicant provided characterization of the test substance including stability data. The CoA for Lot 026517 indicated that the test substance had 99.9% bupivacaine and 97.3% meloxicam with a retest date of November 2018 (24 months after manufacture) that is later than the end of Study 33-86.

7 Genetic Toxicology

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

Study title:

Study no.: 33-11
 Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\33-11\33-11.pdf>

Conducting laboratory and location:

(b) (4)

Date of study initiation: July 18, 2014
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: HTX-011-15 (10.0% bupivacaine, 1.5% meloxicam, 72.29% APF135, 1.21% maleic acid, (b) (4) Lot 1257021, not applicable since it is a mixture

Key Study Findings

The study was considered invalid due to a very low response from the positive controls that was well below the laboratories historical control values.

Methods

Strains: TA1535, TA1537, TA98, TA100, and WP2 *uvrA*

Concentrations in definitive study: 50, 158, 500, 1581, and 5000 mcg/plate

Basis of concentration selection: Based on maximum recommended dose in ICH S2 (R1)

Negative control: DMSO

Positive control: Sodium azide (TA1535 and TA100, without metabolic activation), 9-aminoacrididine hemihydrate (TA1537 without metabolic activation), 2-nitrofluorene (TA98 without metabolic activation), 4-nitroquinoline N-oxide (WP2 *uvrA* without metabolic activation), 2-aminoanthracene (TA 1535 and WP2 *uvrA*, with metabolic activation), and benzo[a]pyrene (TA1537, TA98, and TA100, with metabolic activation)

Formulation/Vehicle: HTX-011-15 was dissolved in DMSO

Incubation & sampling time: An aliquot of 0.5 mL of S9 mix (+S9) or phosphate buffer 0.1M pH 7.4 (0S9) was combined with 0.1 mL bacterial culture in a sterile container. Appropriate volume, as indicated in the study design, of the test/negative and positive control items was added. Molten top agar (approximately 2 mL) supplemented with 0.05 mM biotin and minimal (0.05 mM) histidine and minimal (0.05 mM) tryptophan was added to the sample. The solution was mixed and overlaid on a minimal glucose plate (1.5% agar, Vogel-Bonner medium E, 2% glucose). After the overlay solidified, the plates were inverted and placed in an incubator set to maintain 37°C for 48 to 72 hours. The sterile containers and the plates were labeled with the ^{(b) (4)} study number and plate identification number.

Study Validity

The following criteria were determined to be valid:

- Selection of bacterial tester strains was adequate based upon Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (ICH-S2(R1); June 2012).
- Historical data were provided.
- Cytotoxicity was properly evaluated.
- The appropriate positive and negative controls were employed according to OECD guideline no. 471
- All strains contain the appropriate genetic markers.
- Dose selection for the plate incorporation were adequate based upon use of the limit dose (i.e., 5000 mcg/plate).
- The S9 concentration was within acceptable limits.

However, the assay was determined to be invalid because the responses from the positive controls were well below the historical averages for the laboratory (see tables below).

Results

Overall there was not a positive response with the HTX-011-15 formulation in the presence and absence of S9. The positive controls did cause a small increase in the number of revertant colonies but in many cases they were barely 2-fold above the vehicle control. Generally a positive response is a doubling or tripling over the negative control. The laboratory included historical control data. The positive controls were far below the historical control values for the laboratory. Therefore, the assay is considered invalid.

Table 109: Plate incorporation in absence of S9

Strains	Dose level (µg/plate of RYR)	Number of revertants			Mean	SD	Background observations			Fold response†
		<i>x1</i>	<i>x2</i>	<i>x3</i>			<i>x1</i>	<i>x2</i>	<i>x3</i>	
TA1535	DMSO	1	1	1	1.0	0.0				
	50	2	1	0	1.0	1.0				1.0
	158	0	2	4	2.0	2.0				2.0 ^b
	500	2	3	1	2.0	1.0				2.0 ^b
	1581	1	2	1	1.3	0.6				1.3
	5000	1	1	1	1.0	0.0				1.0
TA1537	DMSO	12	8	8	9.3	2.3				
	50	10	8	8	8.7	1.2				0.9
	158	7	6	11	8.0	2.7				0.9
	500	9	13	15	12.3	3.1				1.3
	1581	5	5	9	6.3	2.3				0.7
	5000	22	12	12	15.3	5.8				1.6
TA98	DMSO	9	12	6	9.0	3.0				
	50	8	7	10	8.3	1.5				0.9
	158	1	6	7	4.7	3.2				0.5
	500	5	6	10	7.0	2.7				0.8
	1581	7	5	1	4.3	3.1				0.5 ^a
	5000	5	9	6	6.7	2.1				0.7
TA100	DMSO	44	50	32	42.0	9.2				
	50	41	46	66	51.0	13.2				1.2
	158	36	27	54	39.0	13.8				0.9
	500	36	30	41	35.7	5.5				0.9
	1581	20	24	42	28.7	11.7				0.7
	5000	31	21	34	28.7	6.8				0.7
WP2 uvrA	DMSO	4	4	3	3.7	0.6				
	50	3	8	4	5.0	2.7				1.4
	158	5	5	5	5.0	0.0				1.4
	500	4	5	4	4.3	0.6				1.2
	1581	3	6	7	5.3	2.1				1.5
	5000	2	3	2	2.3	0.6				0.6

Comments on plate and/or background lawn (as appropriate) : Normal (no comment), MC: Methylcellulose

†: Fold response in mean revertants compared to concurrent vehicle; SD: Sample standard deviation

^a: Not biologically relevant decrease; ^b: Not biologically relevant positive response

The table above is from page 18 of the study report.

Table 110: Plate incorporation in presence of S9

Strains	Dose level (µg/plate of RYS)	Number of revertants			Mean	SD	Background observations			Fold response†
		<i>x1</i>	<i>x2</i>	<i>x3</i>			<i>x1</i>	<i>x2</i>	<i>x3</i>	
TA1535	DMSO	4	8	2	4.7	3.1				
	50	3	4	3	3.3	0.6				0.7
	158	1	1	3	1.7	1.2				0.4
	500	3	1	7	3.7	3.1				0.8
	1581	3	5	6	4.7	1.5				1.0
	5000	2	3	3	2.7	0.6				0.6
TA1537	DMSO	7	15	13	11.7	4.2				
	50	13	10	9	10.7	2.1				0.9
	158	5	10	10	8.3	2.9				0.7
	500	9	8	7	8.0	1.0				0.7
	1581	13	11	6	10.0	3.6				0.9
	5000	6	5	5	5.3	0.6				0.5T
TA98	DMSO	16	27	23	22.0	5.6				
	50	12	23	16	17.0	5.6				0.8
	158	14	14	18	15.3	2.3				0.7
	500	18	16	18	17.3	1.2				0.8
	1581	12	11	9	10.7	1.5				0.5 ^a
	5000	8	15	13	12.0	3.6				0.6
TA100	DMSO	35	48	51	44.7	8.5				
	50	55	36	54	48.3	10.7				1.1
	158	51	62	43	52.0	9.5				1.2
	500	44	42	66	50.7	13.3				1.1
	1581	39	54	46	46.3	7.5				1.0
	5000	44	38	35	39.0	4.6				0.9
WP2 <i>uvrA</i>	DMSO	10	7	8	8.3	1.5				
	50	6	9	10	8.3	2.1				1.0
	158	5	5	9	6.3	2.3				0.8
	500	6	4	10	6.7	3.1				0.8
	1581	8	6	1	5.0	3.6				0.6
	5000	2	2	4	2.7	1.2				0.3T

Comments on plate and/or background lawn (as appropriate) : Normal (no comment)

†: Fold response in mean revertants compared to concurrent vehicle; SD: Sample standard deviation

T: Toxic; ^a: Non biologically relevant decrease

The table above is from page 19 of the study report

Table 111: Positive control data

Strains	S9	Compound	Dose level (µg/plate)	Number of revertants			Mean	SD	Fold response†
				x1	x2	x3			
TA1535	-	SAZ	0.5	71	78	91	80.0	10.1	80.0
TA1537	-	9AC	20	7	22	26	18.3	10.0	2.0
TA98	-	2NF	1.25	11	29	30	23.3	10.7	2.6
TA100	-	SAZ	0.5	111	117	72	100.0	24.4	2.4
WP2 <i>uvrA</i>	-	NQO	0.5	9	5	11	8.3	3.1	2.3
TA1535	+	2AA	5	35	46	52	44.3	8.6	9.4
TA1537	+	B[a]P	5	34	29	36	33.0	3.6	2.8
TA98	+	B[a]P	2	167	165	174	168.7	4.7	7.7
TA100	+	B[a]P	5	568	586	518	557.3	35.2	12.5
TA100	+	B[a]P	10	497	481	521	499.7	20.1	11.2
WP2 <i>uvrA</i>	+	2AA	15	28	16	27	23.7	6.7	2.9

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

The table above is from page 20 of the study report

Table 112: Historical control data

Vehicle controls, without S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	18.0	11.7	25.0	141.6	26.8
Standard deviation	12.9	3.0	6.4	24.9	7.2
Minimum	6	7	14	105	16
Maximum	64	19	38	185	40
Number of experiments	6	6	6	6	6
Vehicle controls, with S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	15.7	13.5	34.0	141.4	29.2
Standard deviation	5.3	5.7	9.0	25.1	9.2
Minimum	6	4	22	90	14
Maximum	25	28	56	196	52
Number of experiments	6	6	6	6	6
Positive controls, without S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	489.2	327.7	182.6	611.9	140.2
Standard deviation	88.9	166.5	38.5	189.5	51.4
Minimum	400	152	137	400	84
Maximum	626	697	237	875	222
Number of experiments	3	3	3	3	3
Positive controls, with S9Mix					
Strains	TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
Mean revertants per plate	229.3	-	134.0	-	107.9
Standard deviation	98.7	-	28.2	-	28.8
Minimum	91	-	100	-	69
Maximum	347	-	191	-	163
Number of experiments	3	-	3	-	3

Positive controls without S9 Mix

TA1535 SAZ (0.5 µg/plate)
 TA1537 9AC (20 µg/plate)
 TA98 2NF (1.25 µg/plate)
 TA100 SAZ (0.5 µg/plate)
 WP2 *uvrA* NQO (0.5 µg/plate)

Positive controls with S9 Mix

TA1535 2AA (5 µg/plate)
 TA98 B[a]P (2 µg/plate)
 WP2 *uvrA* 2AA (5 µg/plate)

Table above is from page 22 of the study report.

The Applicant is also relying upon the data for the two drug substances as described in the referenced drug product labels.

The referenced Marcaine label (NDA 16964) contains the following information:

The mutagenic potential and the effect on fertility of bupivacaine hydrochloride have not been determined.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

Meloxicam was not mutagenic in an Ames assay, or clastogenic in a chromosome aberration assay with human lymphocytes and an in vivo micronucleus test in mouse bone marrow.

Although the exposure to bupivacaine via this drug product technically exceeds that of the referenced drug product, the increase in exposure is not considered to be of the magnitude that would warrant requiring an Applicant to conduct these studies for an older drug product.

8 Carcinogenicity

Carcinogenicity studies with HTX-011 are not required for an acute-use drug.

The referenced Marcaine label (NDA 16964) contains the following information:

Long-term studies in animals to evaluate the carcinogenic potential of bupivacaine hydrochloride have not been conducted.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

No carcinogenic effect of meloxicam was observed in rats given oral doses up to 0.8 mg/kg/day (approximately 0.4-fold the human dose at 15 mg/day for a 50 kg adult based on body surface area conversion) for 104 weeks or in mice given oral doses up to 8.0 mg/kg/day (approximately 2.2-fold the human dose, as noted above) for 99 weeks.

Exposure margins will be adjusted for this drug product maximum daily dose of meloxicam.

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicity studies were conducted with HTX-011. The Applicant is relying upon the Agency previous finding of safety of bupivacaine and

meloxicam via the 505(b)(2) pathway. The labeling will reflect the existing data in the referenced labels adjusted for dose.

The referenced Marcaine label (NDA 16964) contains the following information:

The mutagenic potential and the effect on fertility of bupivacaine hydrochloride have not been determined.

Pregnancy

Bupivacaine hydrochloride produced developmental toxicity when administered subcutaneously to pregnant rats and rabbits at clinically relevant doses.

Bupivacaine hydrochloride was administered subcutaneously to rats at doses of 4.4, 13.3, & 40 mg/kg and to rabbits at doses of 1.3, 5.8, & 22.2 mg/kg during the period of organogenesis (implantation to closure of the hard palate). The high doses are comparable to the daily maximum recommended human dose (MRHD) of 400 mg/day on a mg/m² body surface area (BSA) basis. No embryo-fetal effects were observed in rats at the high dose which caused increased maternal lethality. An increase in embryo-fetal deaths was observed in rabbits at the high dose in the absence of maternal toxicity with the fetal No Observed Adverse Effect Level representing approximately 1/5th the MRHD on a BSA basis.

In a rat pre- and post-natal development study (dosing from implantation through weaning) conducted at subcutaneous doses of 4.4, 13.3, & 40 mg/kg, decreased pup survival was observed at the high dose. The high dose is comparable to the daily MRHD of 400 mg/day on a BSA basis.

The referenced Bupivacaine label (NDA 18053) and Sensorcaine label (NDA 18304) contain the same information.

The referenced Mobic Label (NDA 20938) contains the following information:

Meloxicam did not impair male and female fertility in rats at oral doses up to 9 and 5 mg/kg/day, respectively (4.9-fold and 2.5-fold the human dose, as noted above). However, an increased incidence of embryoletality at oral doses $\geq$ 1 mg/kg/day (0.5-fold the human dose, as noted above) was observed in rats when dams were given meloxicam 2 weeks prior to mating and during early embryonic development.

Exposure margins will be adjusted for this drug product maximum daily dose of meloxicam.

10 Special Toxicology Studies

Wound and bone healing studies were conducted with HTX-011 and were reviewed during the IND. In summary, there did not appear to be any effect on bone healing or adverse interaction with the Prolene mesh with HTX-011-56. From the nonclinical perspective, no additional testing to assess these parameters are necessary. There was some evidence of a potential effect on wound healing in the rabbit study. However, the minipig study (33-54), which used a higher dose, supported that there was only a

very slight difference in wound healing with HTX-011-56 compared to control treatments. However, it must be kept in mind that when the dose volume was normalized to the size of the incision site (estimated incision sizes for the surgeries proposed in the clinical studies were provided by the clinical team) the minipig study (1 mL/cm) was lower than the proposed clinical surgeries (herniorrhaphy, 3-4 cm, 2.5-3.4 mL/cm; (b) (4)

Study title: HTX-011: A Hernia Repair Model with Mesh in New Zealand White Rabbit

Study no.: 33-38
Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-tol\33-38\33-38.pdf>
Conducting laboratory and location: (b) (4)
Date of study initiation: September 17, 2015
GLP compliance: No
QA statement: No (Study Director Statement only stating the study was conducted with (b) (4) SOPs)
Drug, lot #, and % purity: HTX-011-019, Lot 14072, 97.7% Meloxicam and 101.0% Bupivacaine
HTX-011-49, Lot 15011, 97.9% Meloxicam and 101.5% Bupivacaine
HTX-011-056, Lot 025774, 101.4% Meloxicam and 103.6% Bupivacaine

Key Study Findings

- New Zealand White Rabbits were administered a single application of 25 mg bupivacaine or 25 mg bupivacaine and 0.75 mg meloxicam onto Prolene mesh in a hernia repair model.
- No treatment related mortalities were observed.
- There were no treatment related effects on body weight or food consumption.
- Fecal output was reduced in males receiving the bupivacaine comparator and all three HTX formulations. Fecal output was primarily reduced in females receiving HTX-011-19. This finding was not considered adverse.
- Treatment with the vehicle, bupivacaine comparator, and all three HTX formulations resulted in increased incidence/severity of erythema and edema at the injection site in males and females on Days 3 and 15 although there did not appear to be a consistent pattern across formulation or sex. Overall, there were more findings on the last day of the study in animals treated with the bupivacaine comparator and the HTX formulations than in the saline and vehicle treated groups.
- Treatment with the vehicle, bupivacaine comparator, and all three HTX formulations resulted in increased incidence/severity of wound observations at the injection site in males and females on Days 3 and 15 although there did not appear to be a consistent pattern across formulation or sex. Overall, the most commonly observed effect, hemorrhage, was no longer present in most animals by Day 15 regardless of treatment.
- On Day 3, various effects on hematology parameters were seen following treatment with the various formulations used compared to the saline control, including increased white blood cells, increased neutrophils, decreased monocytes, decreased reticulocytes, and decreased platelets. These changes were less pronounced by Day 15, suggesting recovery.
- Changes in clinical chemistry parameters noted were decreased triglycerides (20-30%) in male animals and decreased phosphorous (13-22%) in female animals. The toxicological significance of both findings is unknown.
- Occasionally, elevations in the number of gross findings at the injection site were increased in some treated groups compared to the saline control.
- On the Day 3 sacrifice, injection site histopathology findings were of similar incidence and severity across the saline control and all treatments.
- On Day 15, there appeared to be some differences compared to the saline control animals in histopathological lesions of the injection site. This included increased incidences/severity of cell debris, cystic change, hemorrhage,

epidermal hyperplasia, dermal granulation, necrosis, epidermal ulcer and subcutaneous/muscular/intermuscular granulation.

- Taken together, the various formulations, including the vehicle control, bupivacaine comparator, and the three HTX formulations, caused increased incidences/severities in dermal, wound site, gross, and histopathological findings throughout the study. The observations at the wound site appeared to be similar to saline control by the end of the study and are likely not adverse. In contrast, the presence of erythema and edema at the end of the study was only seen with the bupivacaine comparator and HTX formulations. Similarly, increased incidence and severity of various histopathological findings were seen at the end of the study with the majority of them occurring with the HTX formulations. This suggests that some shared component of the different formulations may be interfering with wound healing in this study.
- It is unclear if the treatments caused an interaction with the Prolene mesh as no clear evidence of the mesh was seen in any sections, including on Day 3. The Applicant attributed the lack of mesh to loss of the fibers during tissue processing. The similar incidence and severity of histopathological effects on Day 3, especially in cystic change, suggest that the mesh performed similarly in all groups.

Methods

Doses: 25 mg bupivacaine and 0.75 mg meloxicam
 Frequency of dosing: Once prior to closing the subcutaneous tissues and the skin.
 Route of administration: Injection
 Dose volume: 0.5 (HTX-011-19) – 1.0 mL (HTX-024-001, HTX-002-014, HTX-011-49, and HTX-011-56)
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-011-19, HTX-011-49, and HTX-011-56). The vehicle control was HTX-024-001. See table below for further details.
 Species/Strain: Rabbit/New Zealand White, Crl:KBL (NZW) BR
 Number/Sex/Group: 4/sex/group (Main)
 Age: ~4 months
 Weight: 2.5-3.4 kg (m) and 2.6-3.9 kg (f)
 Satellite groups: none
 Unique study design: Hernia repair model using Prolene mesh
 Deviation from study protocol: None that impacted the results of the study.

Table 113: Treatment Groups Hernia Repair Study

Group	Test and Control Items	Active Pharmaceutical Ingredients (APIs)		Excipients				
		Bupivacaine w/w% ¹ (mg/g)	Meloxicam w/w% (mg/g)	Maleic Acid w/w% (mg/g)	^{(b) (4)} w/w% (mg/g)	DMSO w/w% (mg/g)	Triacetin w/w% (mg/g)	API35 w/w% (mg/g)
1	Saline Control	NA	NA	NA	NA	NA	NA	NA
2	HTX-024-001 Vehicle Control	NA	NA	NA	NA	10.00 (100.0)	35.00 (350.0)	55.00 (550.0)
3	HTX-002-014 Comparator	2.50 (25.0)	NA	0.15 (1.5)	NA	8.00 (80.0)	35.00 (350.0)	54.35 (543.5)
4	Test Item HTX-011-19	5.0 (50.0)	0.15 (1.5)	0.60 (6.0)	^{(b) (4)}	NA	NA	79.25 (792.5)
5	Test Item HTX-011-49	2.50 (25.0)	0.075 (0.75)	0.15 (1.5)	NA	8.00 (80.0)	35.00 (350.0)	54.275 (572.75)
6	Test Item HTX-011-56	2.50 (25.0)	0.075 (0.75)	0.05 (0.5)	NA	10.00 (100.0)	25.00 (250.0)	62.375 (623.75)

Data from page 8 of the study report.

Observations and Results

Mortality

One female was found dead during the surgery procedures prior to being dosed and was replaced by a spare animal. Since the animal wasn't dosed this death was not considered treatment related.

All other animals survived until scheduled termination of the study.

Clinical Signs

Cage side observations were conducted once daily throughout the study except on days with detailed clinical observations. Detailed clinical observations were conducted once before treatment, weekly throughout the study period, and before necropsy.

In males, the number of animals with and the total number of occurrences of reduced fecal output were increased slightly in animals who received the bupivacaine comparator, HTX-011-19, HTX-011-49, and HTX-011-56 compared to the vehicle and saline controls. In females, reduced fecal output was seen in all groups except the saline control. The incidence of this finding was higher in the bupivacaine comparator and HTX-011-19 formulation. This finding was not considered adverse due to the sporadic nature, observation in the control animals, and unclear toxicological significance.

All other clinical signs observed were not considered treatment related.

Table 114: Clinical Signs Rabbit Hernia Repair Study

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Reduced fecal output (total # of observations)	3	3	6	7	6	6
Reduced fecal output (# of animals)	3/8	2/8	5/8	4/8	5/8	3/8
Females						
Reduced fecal output (total # of observations)	0	2	3	6	1	1
Reduced fecal output (# of animals)	0/8	1/8	2/8	4/8	1/8	1/8

Dermal Observations

Dermal observations were made daily for the first seven days post-surgery (except Day 1), weekly thereafter, and prior to necropsy. Dermal observations were recorded using a modified Draize scoring system.

In animals sacrificed on Day 3, there were minor increases in the incidence and severity of erythema in males and females compared to the saline control. In males, increased severity was seen with the vehicle, HTX-011-19 and HTX-011-56 formulations while in females increases in severity were seen with the bupivacaine comparator and all three HTX formulations. In animals sacrificed on Day 3, there were also increases in the incidence and severity of edema compared to the saline control in males and females. In males, all treated groups showed an increased incidence compared to saline with the highest incidence and severity in animals who received the vehicle, bupivacaine

comparator, and HTX-011-56. In females, all treated groups showed an increased incidence compared to saline, with the highest severity seen following treatment with the vehicle and HTX-011-56.

In animals sacrificed on Day 15, all groups in males showed an increased severity in erythema compared to the saline treated animals with HTX-011-49 and HTX-011-56 showing the highest increases in severity. In females, an increased incidence of erythema was seen in all treated groups compared to the saline control with the highest severity seen with HTX-011-19. The incidence and severity of edema in males was similar across all treatment groups compared to the saline control, while in females an increase in severity was seen in all groups compared to the saline control. In the saline control groups, findings of erythema (females only) and edema resolved by the middle of the study (Day 6). This was also true for the vehicle treated groups except for one case of edema in a female animal with edema from Day 8-15. For the groups treated with the bupivacaine comparator and the HTX formulations, erythema and edema were more frequently seen on the last day of treatment suggesting a lack of recovery or a prolonged effect.

Overall, the vehicle resulted in more skin observations than the saline control. The bupivacaine comparator displayed a pattern of skin findings very similar to the vehicle control. The three formulations of HTX appeared similar to both the comparator and the vehicle control, although there were some instances where the formulations appeared slightly worse. Taken together, the formulations appeared slightly worse than the saline control, although there did not appear to be a consistent pattern across formulation or sex. In addition, there were more findings at the end of the study with the bupivacaine comparator and the HTX formulations.

Table 115: Dermal Observations of Day 3 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	2 (1)	3 (1)	2 (1)	3 (1.3)	1 (1)	4 (1.5)
Edema	0	3 (1.7)	3 (1.3)	2 (1)	1 (1)	3 (1.7)
Females						
Erythema	2 (1)	1 (1)	3 (1)	2 (1.5)	2 (1.5)	3 (1.3)
Edema	1 (1)	4 (1.5)	3 (1)	3 (1.3)	3 (1.3)	3 (1.7)

N=4

Bold = increased incidence/severity over saline control

Table 116: Dermal Observations of Day 15 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	3 (1.3)	4 (1.3)	4 (1.3)	2 (1.5)	4 (1.8)	3 (2)
Edema	4 (1.8)	4 (2)	4 (2)	3 (2.3)	4 (2)	4 (1.5)
Females						

Erythema	0	3 (1.3)	4 (1.5)	4 (1.8)	4 (1.5)	2 (1.5)
Edema	4 (1.3)	4 (1.8)	4 (2)	3 (1.7)	4 (1.5)	4 (1.5)

N=4

Bold = increased incidence/severity over saline control

Table 117: Dermal Observations on Day 15

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Erythema	0	0	0	0	1	1
Edema	0	0	1	3	3	2
Females						
Erythema	0	0	2	3	2	0
Edema	0	1	2	1	1	1

N=4

Wound Observations

Wound observations were made daily for the first seven days post-treatment, weekly thereafter, and prior to necropsy.

In animals sacrificed on Day 3, there were no clear differences between any treatments in males. In females, a minor increase in severity of hemorrhage was observed in animals receiving the bupivacaine comparator with larger increases in severity in animals receiving the vehicle and HTX-011-56. In addition, females receiving the bupivacaine comparator and HTX-011-49 had increased incidence and severity of exudate.

In animals sacrificed on Day 15, there were increases in the severity of hemorrhage at the wound site in females receiving all treatments compared to the saline control. In males, the severity of hemorrhage was similar across all groups. In females, there was increased severity in exudate in animals treated with the vehicle, HTX-011-19, and HTX-011-49 compared to controls. The severities for the presence of exudate were similar across all groups in males. Males did have an increased incidence of granulation in animals who received the bupivacaine comparator, HTX-011-19 and HTX-011-49.

Since there were several findings in the saline control animals, only the severity of the findings was increased with some treatments and a lack of clear effects across sex and time, it is difficult to make a clear conclusion from the wound observations. The primary finding at the wound site was hemorrhage which was no longer present by Day 15 in most animals, although it was still present in half the female animals who received HTX-011-56. Therefore, while there were slight differences in wound healing during the study, wound healing was on average similar to saline treated animals by the end of the study.

Table 118: Wound Observations of Day 3 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
hemorrhage	4 (2.5)	4 (2.8)	4 (2)	4 (1.3)	3 (1.7)	3 (3)
exudate	1 (1)	1 (1)	0	1 (1)	2 (1)	2 (1)
granulation	0	0	0	0	0	0
Females						
hemorrhage	4 (2)	3 (3)	4 (2.5)	4 (1.3)	3 (2)	4 (2.8)
exudate	1 (1)	1 (1)	2 (2)	2 (1)	3 (1.7)	3 (1)
granulation	0	0	0	0	0	0

N=4

Bold = increased incidence/severity over saline control

Table 119: Wound Observations of Day 15 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
hemorrhage	4 (2.8)	4 (1.8)	4 (3)	3 (3)	4 (3.3)	4 (2.5)
exudate	3 (1.3)	2 (1.5)	4 (1.5)	3 (1)	3 (1.3)	2 (1)
granulation	0	0	1 (2)	2 (2)	1 (3)	0
Females						
hemorrhage	4 (2.3)	4 (3.5)	4 (2.8)	4 (3.3)	4 (3)	4 (2.8)
exudate	3 (1.3)	4 (2)	4 (1)	4 (1.8)	3 (2)	3 (1)
granulation	0	0	1 (1)	1 (1)	0	0

N=4

Bold = increased incidence/severity over saline control

Table 120: Last Day of Wound Hemorrhage

Group	Saline	Vehicle	Bup comparator	HTX-011-19	HTX-011-49	HTX-011-56
Males						
Hemorrhage-last day of finding	8, 6, 6, 7	8, 8, 7, 6	15, 8, 8, 7	6, 5, 6	8, 6, 6, 7	7, 4, 6, 6
Females						
Hemorrhage-last day of finding	6, 6, 8, 8	8, 8, 7, 8	8, 8, 8, 7	8, 8, 8, 7	15, 8, 5, 8	15, 15, 8, 7

Body Weights

Body weights were recorded once prior to group assignment, one week prior to treatment, and on Days -1, 4, 8, and 15.

No treatment-related changes in absolute body weight or body weight gain were

observed.

Food Consumption

Food consumption was measured daily during the last week of the pre-treatment period and throughout the treatment and observation periods.

No treatment-related changes in food consumption were observed.

Hematology

Blood samples were collected from all animals (fasted) on prior to the start of treatment and on necropsy days via the central ear artery. A complete battery of hematology and coagulation parameters was evaluated.

In animals sacrificed on Day 3, there were several effects on hematology parameters with different treatments. In males, treatment with the vehicle lead to decreased platelets, monocytes, and reticulocytes, as well as, increased white blood cells and neutrophils. Similar changes were seen in females, except there was no difference in reticulocyte counts. The bupivacaine comparator caused similar changes as the vehicle control, except that white blood cell counts were only elevated in females. Overall, the three HTX formulations seemed to cause the same effects as the vehicle and the comparator, except that the effects were less consistently observed in both sexes.

Of note, differences of similar magnitude for some parameters were also seen prior to treatment. For example, decreased platelets were seen in males receiving the vehicle, the bupivacaine comparator, HTX-011-19, and HTX-011-56, decreased reticulocytes were seen in males receiving vehicle and the HTX formulations and increased white blood cells were seen in males receiving HTX-011-19. The pretreatment differences could not explain all the changes observed with treatment of the various formulations. Taking the pretreatment changes into consideration, the following effects were still observed:

Vehicle: increased WBC (male/female), increased neutrophils (male/female), decreased monocytes (males)

Bupivacaine comparator: decreased reticulocytes (male), increased neutrophils (male), decreased monocytes (male/female), decreased platelets (female), increased WBC (female)

HTX-011-19: increased neutrophils (male/female), decreased reticulocytes (female), increased WBC (female), decreased monocytes (female)

HTX-011-49: decreased platelets (male), increased neutrophils (male), increased WBC (female)

HTX-011-56: increased WBC (male/female), decreased monocytes (male/female), decreased platelets (female)

Across all formulations and the vehicle, the most consistent findings were increased WBCs, increased neutrophils, and decreased monocytes suggesting these are likely related to the shared excipients.

In animals sacrificed on Day 15, the effects on hematology seen on Day 3 were not as apparent, possibly indicating these effects are recoverable. Still, there were isolated changes in hematological parameters that were consistent with those on Day 3, such as decreased platelets in males receiving HTX-011-56 and females receiving HTX-011-19. Overall, on Day 15, hematology parameters were similar between treated and saline control animals.

Table 121: Hematology Findings on Day 3, Percentage of Saline Control

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
platelets	100	76.7	87.5	90.6	77.5	75.1
Retic (%)	100	76.5	83.8	70.6	75.0	80.9
WBC	100	123.3	102.4	117.6	104.6	112.8
Neutrophil	100	149.9	121.2	127.2	115.4	84.6
Monocytes	100	50.0	61.1	111.1	0	33.3
Females						
platelets	100	89.4	80.0	97.6	99.5	76.9
Retic (%)	100	102.3	88.6	70.5	100.0	127.3
WBC	100	135.9	115.4	132.3	115.1	113.2
Neutrophil	100	150.0	124.3	141.4	101.7	94.9
Monocytes	100	70.0	55.0	65.0	80.0	45.0

Table 122: Hematology Findings on Day 15, Percentage of Saline Control

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						
platelets	100	94.5	105.0	95.0	106.5	88.4
Retic (%)	100	113.3	100.0	100.0	82.2	75.6
WBC	100	92.3	86.6	89.2	105.1	100.8
Neutrophil	100	108.7	104.8	111.9	130.9	130.2
Monocytes	100	58.8	111.8	88.2	123.5	129.4
Females						
platelets	100	90.9	102.8	84.4	106.7	101.2
Retic (%)	100	77.3	122.7	79.5	93.2	90.9
WBC	100	92.5	91.0	88.7	73.3	85.6
Neutrophil	100	115.5	109.2	119.1	75.3	92.4
Monocytes	100	125.0	216.7	158.3	91.7	216.7

Clinical Chemistry

Blood samples were collected from all animals (fasted) on prior to the start of treatment and on necropsy days via the central ear artery. A complete battery of clinical chemistry parameters was evaluated.

The only changes in clinical chemistry parameters noted were decreased triglycerides (20-30%) in male animals treated with the HTX formulations compared to the saline control and decreased phosphorous (13-22%) in females of all groups compared to the saline controls. The toxicological significance of both findings is unknown.

Gross Pathology

A complete necropsy was performed on all animals.

Numerous lesions were observed at the incision site consisting of adhesion, dark area, firm, gelatinous material, mass, soft, pale area, raised area, and thickening. Occasionally, these findings were elevated compared to the saline control in the treated groups as indicated in the tables below.

Table 123: Gross Findings of Day 3 Sacrifice Group

Groups	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f
Number of animals INCISION SITE	Males						Females					
	4	4	4	4	4	4	4	4	4	4	4	4
Adhesion	0	0	0	0	0	1	0	0	0	0	0	0
Dark area	4	3	3	4	3	2	3	4	4	4	4	3
Firm	0	1	3	0	0	0	1	1	0	1	0	0
Gelatinous material	0	1	0	0	0	0	0	0	0	0	0	0
Mass	0	1	0	0	0	0	1	0	0	0	0	0
Pale area	0	1	0	2	1	1	1	1	2	2	1	1
Raised area	1	0	0	0	0	0	0	0	0	0	0	0
Soft	3	0	0	0	0	0	0	2	1	0	0	0
Thickening	3	2	1	3	3	2	2	1	3	2	4	4

^a – Saline Control; ^b – Vehicle Control; ^c – Comparator (HTX-002-014); ^d – HTX-011-19;

^e – HTX-011-49; ^f – HTX-011-56

Table 124: Gross Findings of Day 15 Sacrifice Group

Groups	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f
Number of animals INCISION SITE	Males						Females					
	4	4	4	4	4	4	4	4	4	4	4	4
Adhesion	1	0	0	0	0	0	0	0	0	0	0	0
Dark area	0	0	1	0	2	1	0	1	0	1	0	1
Firm	0	1	1	0	0	1	0	2	2	0	0	1
Gelatinous material	0	0	0	0	1	0	0	0	0	0	0	0
Mass	0	0	1	1	0	0	0	0	0	0	0	0
Pale area	0	1	1	0	1	0	1	0	2	1	1	0
Raised area	1	0	0	0	0	0	0	0	0	0	1	0
Scab	0	0	0	0	0	0	0	0	0	0	1	2
Soft	0	0	0	0	1	0	1	0	1	1	0	0
Thickening	3	4	1	3	3	2	4	4	2	4	1	3

^a – Saline Control; ^b – Vehicle Control; ^c – Comparator (HTX-002-014); ^d – HTX-011-19;

^e – HTX-011-49; ^f – HTX-011-56

Histopathology

Adequate Battery: Yes

Peer Review: No.

Histopathological examinations were only performed on incision sites and gross lesions identified during necropsy.

Histological Findings

On the Day 3 sacrifice, injection site findings consisted of minimal to mild cell debris, minimal to moderate cystic change, minimal to mild epidermal erosion/ulcer, minimal to moderate hemorrhage, minimal epidermal hyperplasia, minimal to moderate subcutaneous acute inflammation, and minimal to mild necrosis. These findings were of similar incidence and severity across the saline control and all treatments. No clear evidence of the mesh material was seen in the sections. However, cystic change was related to the space created by the mesh and since this finding was similar amongst all treatment groups it is unlikely that the mesh performed differently amongst the groups.

On Day 15, similar findings were present at the injection site except both dermal granulation and subcutaneous/muscular/intermuscular granulation were also present. At this time point, there appeared to be some differences compared to the saline control animals. In males, there was an increased incidence of cell debris in animals receiving the HTX formulations, increased incidences of cystic change and hemorrhage in animals of all treatment groups, increased incidence/severity of epidermal hyperplasia with HTX-011-19 and HTX-011-49, and an increased incidence of dermal granulation in animals receiving HTX-011-19. In females, an increased incidence of epidermal ulcer was seen in animals receiving all treatments except the vehicle control, an increased severity of subcutaneous/muscular/intermuscular granulation was observed in animals receiving all treatments except HTX-011-49, an increased incidence of hemorrhage in animals receiving the vehicle, the bupivacaine comparator, and HTX-011-56, and an increased incidence of necrosis in the bupivacaine comparator. Taken together, it appears that there was some impact on wound healing on Day 15 following treatment with the various formulations, primarily in males who received the HTX formulations.

Table 125: Histological Findings of Day 15 Sacrifice Group

Group	Saline	Vehicle	Bup comp	HTX-011-19	HTX-011-49	HTX-011-56
Males						

Cell debris	0	0	0	1 (2)	1 (2)	1 (2)
Cystic change	1 (2)	3 (2)	4 (2)	4 (2)	4 (2)	4 (2)
Epidermal ulcer	0	0	0	2 (1)	0	0
Hemorrhage	2 (1.5)	3 (1.7)	3 (1.7)	3 (1.7)	3 (1.3)	4 (1.5)
Epidermal hyperplasia	1 (1)	2 (1)	2 (1)	2 (1.5)	1 (2)	2 (1)
Dermal granulation	2 (1.5)	4 (1)	3 (1.3)	4 (1.3)	2 (1.5)	3 (1)
Granulation subcutaneous/muscular/intermuscular	4 (2.8)	4 (3)	4 (3)	4 (2.3)	4 (2.8)	4 (2.5)
Necrosis	1 (2)	1 (2)	3 (1.1)	2 (1.5)	2 (1)	2 (1)
Females						
Cell debris	2 (2)	0	1 (1)	2 (1.5)	1 (3)	1 (2)
Cystic change	3 (2.3)	4 (1.8)	4 (2.3)	4 (2)	3 (2)	4 (1.8)
Epidermal ulcer	0	0	1 (1)	3 (1.3)	1 (3)	1 (2)
hemorrhage	0	4 (1.5)	2 (1.5)	1 (1)	1 (1)	2 (1.5)
Epidermal hyperplasia	4 (2.3)	3 (1)	1 (2)	2 (2)	2 (1)	3 (1.7)
Dermal granulation	4 (2)	3 (1.7)	2 (1)	3 (1.3)	3 (2)	4 (2)
Granulation subcutaneous/muscular/intermuscular	4 (1)	4 (1.5)	4 (2)	4 (1.8)	3 (1.3)	4 (1.5)
necrosis	2 (1.5)	3 (1)	4 (1.5)	3 (1.3)	3 (1.7)	3 (1.3)

Bold = increased incidence/severity over saline control

Dosing Solution Analysis

Dosing solution analysis was not conducted by the testing laboratory. Instead, data on the achieved concentrations of the test and control items was provided by the Sponsor in the form of certificates of analysis and stability data to cover the period of dosing.

Study title: Single Subcutaneous Dose Effect of HTX-002-014, HTX-001-49 and HTX-011-56 on Bone Healing in the Cortical Defect Model in Female Sprague Dawley Rats

Study no.: 33-39
Study report location: SDN 1, Module 4
<\\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-to\33-39\33-39.pdf>
Conducting laboratory and location:  (b) (4)
Date of study initiation: September 26, 2015
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: HTX-024-001, Lot 1237047
HTX-002-14, Lot 1273049
HTX-011-49, Lot 15011
HTX-011-056, Lot 025774
Purities were not provided

Key Study Findings

- SD rats were administered a single application of 12.5 mg bupivacaine or 12.5 mg bupivacaine and 0.375 mg meloxicam by subcutaneous injection following surgery to induce cortical defect.
- At least six animals were found to have lameness following surgery. Of these, six animals were sacrificed early due to associated fractures. Since none of these animals were from the control group, the treatment related nature of this finding could not be ruled out.
- Skin lesions (scabs and ulceration) were seen in animals receiving the bupivacaine comparator and HTX formulations.
- There were not treatment related effects on body weight.
- No treatment related effects on bone mineral content, bone mineral density, or the healing stage of the bone were identified throughout the study.
- The study should have been conducted in accordance with Good Laboratory Practices (GLPs). Although this study was not conducted according to GLPs, according to the Applicant the only deviation from GLP was that the study was not quality assured. The results were reviewed, and the study was designed reasonably well. The Division concluded that based on the results obtained, repeating the study in accordance with full GLPs was not likely to impact the clinical assessment of this critical endpoints and would not be required in this unusual circumstance.

Methods

Doses: 12.5 mg bupivacaine and 0.375 mg meloxicam
 Frequency of dosing: Once
 Route of administration: Subcutaneous Injection
 Dose volume: 0.5 mL
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-002-014, HTX-011-49, and HTX-011-56). The vehicle control was HTX-024-001. See table below for further details.
 Species/Strain: Rat/Sprague-Dawley
 Number/Sex/Group: 8/females/group
 Age: 15-16 weeks
 Weight: 293.59 ± 10.93 g
 Satellite groups: none
 Unique study design: Cortical defect surgery and CT scans.
 Deviation from study protocol: Part way through the study, animals began being housed individually to minimize the occurrence of fractures.
 Husbandry checks were only performed once daily instead of twice daily.
 Macroscopic observations were not collected automatically in a way that allowed cross referencing with histological observations.

Group	Test and Control Items	Active Pharmaceutical Ingredients			Excipients		
		Bup	Mel	Maleic Acid	DMSO	Triacetin	AP135
		w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)	w/w% (mg/kg)
1	Saline Control	NA	NA	NA	NA	NA	NA
2	Vehicle Control HTX-024-001	NA	NA	NA	10.00 (100.0)	35.00 (350.0)	55.00 (550.0)
3	Comparator HTX-002-014	2.5 (25.0)	NA	0.15 (1.5)	8.00 (80.0)	35.00 (350.0)	54.35 (543.5)
4	Test Item HTX-011-49	2.5 (25.0)	0.075 (0.75)	0.15 (1.5)	8.00 (80.0)	35.00 (350.0)	54.275 (572.75)
5	Test Item HTX-011-56	2.5 (25.0)	0.075 (0.75)	0.05 (0.5)	10.00 (100.0)	25.00 (250.0)	62.375 (623.75)

Abbreviations: Bup = Bupivacaine; Mel = Meloxicam

Data from page 7 of the study report.

Observations and Results

Mortality

Six animals were identified by CT scan as having fractures. These animals were sacrificed early. All other animals survived to scheduled sacrifice.

Clinical Signs

Animals were monitored daily for lameness, death and morbidity. Animals that showed signs of lameness were x-rayed and/or CT scanned and sacrificed if a fracture was detected.

As described above, at least six animals were found to have lameness and underwent a subsequent CT scan. A total of six animals were identified with a fracture and they consisted of two animals from the vehicle control group, two animals from the bupivacaine comparator group, one animal from the HTX-011-49 group, and one animal from the HTX-011-56 group. Fractures were primarily identified on the right femur (5/6) which is the leg that underwent injection. Since the fractures only occurred in animals receiving one of the formulations and not the saline control, the treatment related nature of this finding could not be ruled out.

Skin lesions at the injection site, consisting of scabs and ulceration, were observed in six animals receiving the bupivacaine comparator, four animals receiving HTX-011-49, and four animals receiving HTX-011-56. These lesions were found during cage side observations on the day of necropsy.

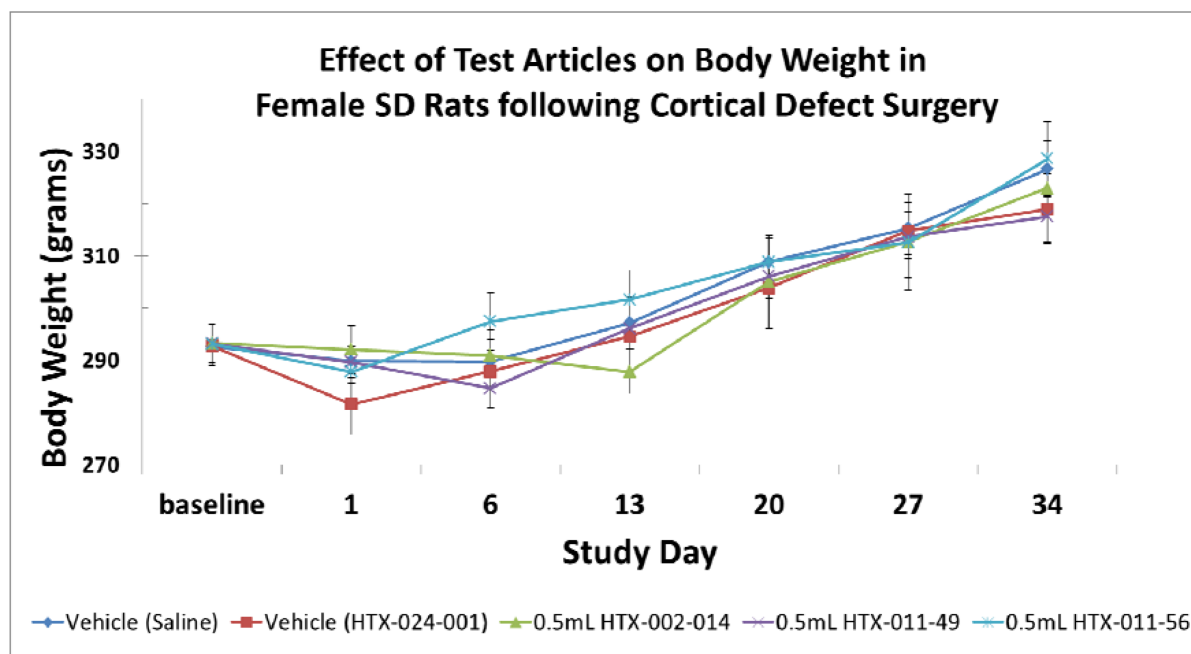
Table 126: Clinical Signs

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Fracture	0/8	2/8 (right, both)	2/8 (right, both)	1/8 (left)	1/8 (right)
Skin lesions	0/8	0/8	6/8	4/8	4/8

Body Weights

Body weights were recorded on Day 5 of the predose phase and on Days 1, 6, 13, 20, and 34 of the dosing phase.

No treatment related differences in absolute body weight were observed.

Figure 1: Body Weight in Rat Bone Defect Study

Data from page 12 of the study report.

CT Imaging

Animals underwent CT imaging on Days 1, 6, 13, 20, and 34 of the dosing phase. Total bone mineral density (BMD) and bone mineral content (BMC) of the callus were measured for both the left and right femur.

No significant differences were noted in total BMD for the left and right femurs and total BMC in the left femurs from Days 1-34. No significant change was observed in total BMC of the right femurs from Days 6 to 34. A significant increase in total BMC was observed in the right femurs of animals receiving HTX-011-56 on Day 1 only. The toxicological significance of this finding is unknown.

Table 127: Bone Mineral Content of Left Femur

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	87.6	84.8	113.8	117.1
Day 6	100	97.7	107.1	95.2	111.7
Day 13	100	112.7	117.8	112.3	147.7
Day 20	100	113.0	123.0	115.6	131.9
Day 34	100	105.6	116.8	108.5	117.5

Table 128: Bone Mineral Content of Right Femur

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	104.5	97.4	116.5	128.8
Day 6	100	104.7	96.6	107.9	119.2
Day 13	100	113.2	83.5	95.3	125.9
Day 20	100	106.1	86.2	91.8	108.9
Day 34	100	99.9	96.8	96.2	103.9

Table 129: Bone Mineral Density of Left Femur

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	89.6	86.4	111.3	110.2
Day 6	100	100.2	108.7	93.0	106.0
Day 13	100	115.6	119.8	110.1	140.5
Day 20	100	116.1	125.7	113.4	125.9
Day 34	100	108.6	119.4	106.5	112.2

Table 130: Bone Mineral Density of Right Femur

Group	Saline	Vehicle	Bup comparator	HTX-011-49	HTX-011-56
Day 1	100	103.7	96.3	109.5	117.4
Day 6	100	103.9	95.7	101.5	109.2
Day 13	100	114.3	82.1	90.8	116.8
Day 20	100	106.3	85.0	86.6	100.6
Day 34	100	100.1	96.0	90.8	96.0

Gross Pathology

A complete necropsy was performed on all animals.

Due to an error, no macroscopic findings were recorded.

Histopathology

Adequate Battery: Not for a full toxicology study, because only left and right femur were analyzed. As this study was intended to characterize the effects on bone, this is acceptable.

Peer Review: No.

Histopathological examinations were only performed on the left and right femur. The stage of the healing process was scored by the pathologist according to a scoring system described by Xu et al., 2011.

Histological Findings

There was no significant difference in the mean healing score for any group compared to the saline control. All animals had a healing score of 4-5, except one animal receiving the vehicle control which had a healing score of 2.

Table 131: Healing Score of Femur

Group	Saline	Vehicle	Bup comp	HTX-011-49	HTX-011-56
Healing score-left	4.00	4.50	4.00	4.00	4.00
Healing score-right	4.13	3.67	4.00	4.14	4.14

Dosing Solution Analysis

Dosing solution analysis was not conducted.

Study title: HTX-002, HTX-009 and HTX-011: A Single Dose 15-Day Wound Healing Dermal Toxicity Study in the Gottingen Minipig

Study no.: 33-54
 Study report location: SDN 1, Module 4
 \\cdsesub1\evsprod\nda211988\0001\m4\42-stud-rep\423-tox\4236-loc-to\33-54\33-54.pdf
 Conducting laboratory and location: (b) (4)
 Date of study initiation: June 20, 2016
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: HTX-002-013, Lot 16007, 2.5% meloxicam, >99.9% purity
 HTX-009-005, Lot 15011, 0.075% meloxicam, purity not reported
 HTX-011-056, Lot 026126, 0.075% meloxicam and 2.5% bupivacaine, purity not reported

Key Study Findings

- HTX-011 caused slightly worse Draize scoring and wound observations compared to saline and sham control.
- Wounds were not completely healed by Day 15 in any group. A slight increase in the severity of histopathological findings was observed at incision sites treated with HTX-011 compared to saline and sham controls.
- Overall the impact on wound healing appeared to be minimal since only small increases in severity were observed by histopathology.
- The doses used in this study are lower than those used in the clinical studies based on normalization to the incision size. The volume per incision size used in this study was 1 mL/cm. Clinical studies are expected to be herniorrhaphy; 2.5-3.4 mL/cm, (b) (4) based on information provided by the clinical team. Because clinical studies were permitted to proceed, the reader is referred to the clinical review for definitive information on the impact of the drug product on soft tissue wound healing in humans.

Methods

Doses: 87.75 mg bupivacaine and 2.63 mg meloxicam
 Frequency of dosing: Once by wound surface application into a surgical incision.
 Route of administration: Wound surface application
 Dose volume: 3 mL
 Formulation/Vehicle: 3 different formulations of the test item were used (HTX-002-013, HTX-009-005, and HTX-011-56). The vehicle control was HTX-024-002. See table below for further details.
 Species/Strain: Gottingen Minipig, *sus scrofa domestica*
 Number/Sex/Group: 3/sex/group (Main)
 Age: 9-10 months
 Weight: 17.8-20.5 kg (m) and 17.9-21.5 kg (f)
 Satellite groups: None
 Unique study design: Wound healing following surgical incision. Each animal received six incisions and received all treatments: saline, HTX-011-056, vehicle, HTX-009-005, HTX-002-013, and sham; one treatment per incision.

Deviation from study protocol: None that impacted the results of the study.

Incision	Test and Control Items	Active Pharmaceutical Ingredients (APIs)		Excipients			
		Bupivacaine w/w% ¹ (mg/g)	Meloxicam w/w% (mg/g)	Maleic Acid w/w% (mg/g)	DMSO w/w% (mg/g)	Triacetin w/w% (mg/g)	API35 w/w% (mg/g)
1	Saline Control	NA	NA	NA	NA	NA	NA
2	Vehicle Control HTX-024-002	NA	NA	NA	10.00 (100.00)	25.00 (250.00)	65.00 (650.00)
3	HTX-002-013	2.50 (25.00)	NA	0.05 (0.50)	10.00 (100.00)	25.00 (250.00)	62.45 (624.5)
4	HTX-011-056	2.50 (25.00)	0.075 (0.75)	0.05 (0.50)	10.00 (100.00)	25.00 (250.00)	62.375 (623.75)
5	HTX-009-005	NA	0.075 (0.75)	NA	10.00 (100.00)	25.00 (250.00)	64.925 (649.25)
6	Sham Control	NA	NA	NA	NA	NA	NA

Data from page 10 of the study report.

Observations and Results

Mortality

No mortalities were observed.

Clinical Signs

Cage side observations were conducted once daily throughout the study except on days with detailed clinical observations. Detailed clinical observations were conducted once before treatment, weekly throughout the study period, and before necropsy.

Bruising, crust, and decreased activity were seen sporadically throughout the study. Since each animal received all treatments, these findings could not be ascribed to any of the test articles.

Dermal Observations

Dermal observations were assessed post-surgery on Days 2-8 and Day 15 for animals being sacrificed on Day 15. Dermal observations were assessed post-surgery on Days 2-4 for animals being sacrificed on Day 4.

In animals sacrificed on Day 4, increased number of days with erythema was observed with treatment of HTX-011 compared to the saline and sham controls in both males and females. Increased number of days with erythema were also observed with treatment of the bupivacaine comparator (male and female) and vehicle (female). The average severity was similar between all groups. For edema, there did not appear to be a major difference in the number of days observed. There was a small increase in the average severity of edema for females at incision sites treated with HTX-011.

In animals sacrificed on Day 15, there did not appear to be a difference in the number of days edema was observed between the different treatments. However, there did appear to be an increase in the incidence of observations on Day 15 compared to the saline and vehicle control with HTX-011.

Taken together, HTX-011 appeared to result in a slightly worse Draize scoring across the study.

Table 132: Dermal Observations in Day 4 Sacrifice Animals, Male

Group	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
Erythema 3 animals	-	1 (1)	3 (1)	3 (1)	2 (1)	5 (1)
Edema 3 animals	8 (1.2)	8 (1)	8 (1.3)	9 (1)	9 (1)	8 (1.2)

Total # of days observed (average severity); n=3 animals

Table 133: Dermal Observations in Day 4 Sacrifice Animals, Female

Group	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
Erythema 3 animals	2 (1)	6 (1)	4 (1)	2 (1)	2 (1)	4 (1)
Edema 3 animals	9 (1.1)	9 (1.3)	9 (1.1)	9 (1.1)	9 (1.2)	9 (1.5)

Total # of days observed (average severity); n=3 animals

Table 134: Dermal Observations in Day 15 Sacrifice Animals, Male

Animal	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
M1	5 (1.2)	6 (1)	7 (1.2)	5 (1.2)	6 (2)	6 (2) Day 15 (2)
M2	6 (1.2) Day 15 (1)	5 (1)	5 (1)	6 (1)	5 (1)	6 (2) Day 15 (1)
M3	6 (2)	1 (2)	1 (1)	5 (1)	5 (1)	6 (1.3) Day 15 (1)

Total # of days observed (average severity); n=3 animals

Table 135: Dermal Observations in Day 15 Sacrifice Animals, Female

Animal	Saline	Vehicle	Bup comparator	Mel Comparator	sham	HTX-011-56
F1	6 (1.8)	6 (1.8)	6 (2)	6 (1.8)	5 (2)	6 (2.2) Day 15 (3)
F2	6 (1.5)	6 (1.8) Day 15 (1)	6 (1.8) Day 15 (1)	6 (1.8)	7 (1.5)	6 (2) Day 15 (1)
F3	6 (2)	6 (2)	6 (1.8)	6 (1.8)	6 (1.8)	6 (2) Day 15 (1)

Total # of days observed (average severity); n=3 animals

Wound Observations

Wound observations were made on Day 4 (all animals), and on Days 8 and 15 for animals in the Day 15 sacrifice group.

On Day 4, there was a slight increase in the appearance of the wound (erythema), condition of the skin surrounding the wound (erythema), and the presence of hemorrhage in males at incision sites treated with HTX-011 compared to saline and sham controls. In females, this was only true for the condition of skin surrounding the wound.

In animals sacrificed on Day 15, increased severity was observed in males on Day 8 for presence of hemorrhage only. No difference in severity for any findings was observed on Day 15.

Table 136: Wound Observations on Day 4

Group	Saline	Vehicle	Bup comp	Meloxicam	Sham	HTX-011-56
Males						
Appearance of Wound and Wound Edge n = 6	6 (1.5)	6 (1.3)	6 (1.8)	6 (1.5)	5 (1.6)	6 (1.8)
Condition of skin surrounding the wound n = 6	5 (1)	5 (1)	5 (1.6)	4 (1)	4 (2)	5 (1.8)
Presence of hemorrhage n = 6	5 (1.8)	5 (1.6)	6 (2.0)	3 (2.3)	4 (1.8)	6 (2.3)
Presence of exudates n = 6	0	1 (2)	1 (3)	0	0	2 (1)
Females						
Appearance of Wound and	6 (2.2)	6 (2.3)	6 (1.7)	6 (2.0)	6 (2.3)	5 (2.2)

Wound Edge n = 6						
Condition of skin surrounding the wound n = 6	6 (1.5)	6 (1.2)	4 (1.5)	4 (1.5)	5 (1.6)	6 (1.8)
Presence of hemorrhage n = 6	6 (2.8)	6 (2.3)	6 (2.7)	6 (2.5)	6 (2.7)	6 (2.5)
Presence of exudate n = 6	4 (2.5)	1 (2)	4 (2.8)	3 (2)	1 (3)	4 (2.3)

of animals with finding (average severity); n = 6/sex

Table 137: Wound Observations on Day 8

Group	Saline	Vehicle	Bup comp	Meloxicam	Sham	HTX-011-56
Males						
Appearance of Wound and Wound Edge (1/3)	3 (1)	3 (1)	3 (1.3)	2 (1.5)	3 (1.3)	2 (1.5)
Condition of skin surrounding the wound (1/3)	3 (1.3)	2 (1)	2 (2.5)	1 (1)	3 (2)	3 (2)
Presence of hemorrhage (3/3)	3 (1.3)	2 (1.5)	3 (2.3)	2 (2)	3 (1)	3 (2.7)

of animals with finding (average severity); n = 6

Gross Pathology

Only the dosing sites were evaluated for gross lesions. There did not appear to be any difference between groups in terms of gross pathological findings at the surgical site.

Table 138: Gross Pathology of the Incision Site, Day 4

Sex	Male						Female					
Incisions *	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Clear fluid	0	1	0	0	1	0	2	2	3	2	0	0
Crepitus	0	0	0	0	0	2	0	0	0	0	0	2
Dark area	2	3	2	3	2	3	3	3	3	3	3	3
Dark material	0	0	0	0	0	0	0	0	1	1	0	0
Gelatinous material	1	1	2	3	3	1	1	0	0	1	0	1
Pale material	0	0	1	1	0	0	0	2	1	0	0	0
Scar	3	2	2	3	3	2	1	1	1	1	1	2
Wound	0	1	1	0	0	1	2	2	2	2	2	1

* Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 29 of the study report

Table 139: Gross Pathology of the Incision Site, Day 15

Sex	Male						Female					
Incisions *	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Crepitus	0	0	0	0	0	0	0	0	0	0	0	1
Dark area	0	0	3	0	0	0	1	2	2	2	1	1
Mass	0	0	0	1	0	0	0	0	0	1	0	0
Pale material	0	0	0	0	0	0	0	0	0	0	1	0
Scar	3	3	3	3	3	3	3	3	3	3	3	3
Thickening	0	0	0	0	0	0	0	0	1	1	0	0

* Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 30 of the study report

Histopathology

Adequate Battery: Yes

Peer Review: No.

Histopathological examinations were only performed on incision sites and gross lesions identified during necropsy.

Histological Findings

Overall, there was a similar incidence of histopathological findings across the different treatments. The exception was that one male on Day 15 had a mild abscess that was not seen with any other treatment group. There were some minor increases in the severity of findings with HTX-011 for a couple of findings, including serro-cellular crust on Day 4, inflammatory cell infiltrates on Day 15, and granulomatous inflammation of the subcutis on Day 15.

Table 140: Summary of Histopathological Findings, Day 4

Sex	Male						Female					
Incisions*	1	2	3	4	5	6	1	2	3	4	5	6
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Cell infiltrate, inflammatory	3	3	3	3	3	3	3	3	3	3	3	3
minimal	0	0	0	0	0	0	2	1	0	1	2	1
mild	3	1	3	2	3	2	1	2	3	2	1	2
moderate	0	2	0	1	0	1	0	0	0	0	0	0
Eosinophilic material	3	3	3	3	3	3	3	3	3	3	3	3
minimal	3	2	2	1	2	2	1	1	0	0	2	1
mild	0	1	1	2	1	1	2	2	3	2	1	1
moderate	0	0	0	0	0	0	0	0	0	1	0	1
Sero-cellular crust	3	3	3	3	2	3	3	3	3	3	3	3
minimal	2	1	2	1	2	1	1	1	1	1	3	2
mild	1	2	0	2	0	2	2	1	1	1	0	1
moderate	0	0	1	0	0	0	0	1	1	1	0	0
Ulcer	3	3	3	2	2	3	3	3	3	3	2	2
minimal	2	2	2	1	2	2	2	3	2	2	2	2
mild	1	1	1	1	0	1	1	0	1	1	0	0

*Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 31 of the study report

Table 141: Summary of Histopathological Findings, Day 15

Sex	Male						Female					
	1	2	3	4	5	6	1	2	3	4	5	6
Incisions*	3	3	3	3	3	3	3	3	3	3	3	3
Number of Animals	3	3	3	3	3	3	3	3	3	3	3	3
Abscess	0	0	0	1	0	0	0	0	0	0	0	0
mild	0	0	0	1	0	0	0	0	0	0	0	0
Cell infiltrate, inflammatory	1	0	0	1	0	0	1	0	0	1	0	0
minimal	1	0	0	0	0	0	1	0	0	0	0	0
mild	0	0	0	1	0	0	0	0	0	1	0	0
Contraction dermal (present)	3	1	1	2	1	3	3	3	3	2	3	2
Inflammation, granulomatous, dermis	3	3	3	3	3	3	3	3	3	3	3	3
minimal	2	2	0	3	2	2	0	1	0	0	0	0
mild	1	1	3	0	1	1	3	0	2	3	2	3
moderate	0	0	0	0	0	0	0	2	1	0	1	0
Inflammation, granulomatous subcutis	3	3	3	3	3	3	3	3	3	3	3	3
minimal	1	2	0	0	1	3	0	0	1	0	0	3
mild	2	1	3	1	2	0	3	2	1	1	3	0
moderate	0	0	0	2	0	0	0	1	1	2	0	0
Sero-cellular crust	3	2	2	3	3	3	3	3	1	1	3	2
minimal	1	1	2	2	2	1	1	3	1	1	3	2
mild	2	1	0	1	1	2	2	0	0	0	0	0

*Incision 1 received Saline; Incision 2 received Vehicle Control (HTX-024-002); Incision 3 received Bupivacaine (HTX-002-013); Incision 4 received Bupivacaine/ Meloxicam mix (HTX-011-056); Incision 5 received Meloxicam (HTX-009-005) and Incision 6 received Sham.

Table above is from page 32 of the study report

11 Integrated Summary and Safety Evaluation

The following integrated summary is in regard to the Applicant's rat bone healing study (33-39), rabbit hernia repair study (33-38), and minipig wound healing study (33-54). Please see the Executive Summary for a discussion of the overall safety evaluation of HTX-011.

The rat bone healing study and the rabbit hernia repair studies were not conducted in accordance to good laboratory practices (GLP). The Applicant claims that the only deviations from GLP were the lack of quality assurance statements and involvement of the Quality Assurance department during study conduct. In addition, the Applicant provided the standard operating procedures of the conducting laboratories, which have been quality assured. Based on the data provided by the Applicant and the study

reports, it is this reviewer's opinion that while the studies were not conducted under ideal GLP conditions, the results of the studies appear to be reliable and repeating the study would be an inappropriate use of animals based on the findings to date. The Applicant was instructed to complete any future studies in accordance with GLPs and was required to include enhanced monitoring in the clinical setting to fully evaluate the potential impact on bone healing.

The main goal of the rat bone healing study was to determine if the clinical formulation would have an adverse impact on bone healing to support the proposed bunionectomy clinical study. In that regard, bone mineral density, bone mineral content, and histopathological evaluation of the bone healing stage were evaluated. No treatment related effects on bone mineral content, bone mineral density, or the healing stage of the bone were identified in this study. Based on the conditions used in this study, there was no difference in bone healing with the clinical formulation compared to the control.

The main goal of the rabbit hernia repair model was to determine the effects of the clinical formulation on Prolene mesh to support its use in the herniorrhaphy clinical study, which also included the use of mesh as part of the surgery. The interaction with the mesh was evaluated by histopathological evaluation. It was difficult to determine how the formulation interacted with the Prolene mesh as no clear evidence of the mesh was seen in any of the sections, including on Day 3, the earliest sacrifice time point. As noted above, the Applicant attributed the lack of evidence of the mesh in the histopathology slides as likely due to loss of the fibers from the mesh during tissue processing. They note that the open spaces in the tissue where the mesh was present were noted, suggesting recent loss. However, the similar incidence of histopathological findings, including in cystic change, on Day 3 suggests that there was no adverse interaction with the mesh.

Across the two studies there was limited evidence of systemic effects following treatment with the HTX-formulations. In the rat bone healing study, no effects related to systemic exposure were observed, although the number of parameters assessed was limited. In the rabbit hernia repair study, the primary systemic effect observed was changes in hematology parameters on Day 3 following treatment with the vehicle, the bupivacaine comparator (HTX-011 without meloxicam), and the different HTX formulations. The most consistent finding across all treatments were increased white blood cells, increased neutrophils, and decreased monocytes, which is consistent with other studies with HTX-011. Additionally, decreased platelets and reticulocytes were seen with some treatments. Most of these changes were not seen on Day 15 suggesting partial to complete recovery. Since effects were seen in the vehicle treated group and across multiple formulations, this suggests that effects on hematology parameters could be related to one or more shared excipients. In this study, the proposed clinical formulation was used at a dose of ~8.3 mg/kg bupivacaine and 0.25 mg/kg meloxicam which is a human equivalent dose of 160.6 mg and 4.8 mg, respectively. Therefore, the effects were observed at a human equivalent dose that is ~2.7 times higher than lowest proposed human dose (60 mg/1.8 mg) and ~2.5 times lower than the highest proposed human dose (400 mg/12 mg).

Dermal and wound site effects were the prominent findings in the two studies. In the rat bone healing study, skin lesions (scab and ulceration) were seen at the injection site on Day 33 in the groups receiving the bupivacaine comparator and both HTX formulations. These were the only local findings reported in this study.

A more detailed analysis of local effects was conducted in the rabbit hernia repair model. In this study, dermal observations, wound observations and histopathological effects were reported. Dermal observations were reported according to the Draize scoring system with the severity and incidence of edema and erythema reported. Increased incidences and or severity of both findings compared to saline control were seen across the vehicle, bupivacaine comparator, and HTX formulations, although there did not appear to be a consistent pattern across formulation or sex.

At the wound site, the most common observation was hemorrhage and was primarily observed in the middle of the study in animals who were sacrificed on Day 15. The severity of hemorrhage was increased in females receiving the vehicle, bupivacaine comparator, and all three HTX formulations. Hemorrhage was not present at the end of the study in most animals, except for 1 female receiving HTX-011-49 and 2 females receiving HTX-011.

Finally, histopathological analysis of the injection sites also revealed increased incidences and severity of findings in treated groups compared to the saline control for animals undergoing necropsy on Day 15. These included the findings of cell debris, cystic change, epidermal ulcer, hemorrhage, epidermal hyperplasia, dermal granulation, and subcutaneous/muscular/intermuscular granulation. There did not appear to be a consistent pattern across formulation or sex making it difficult to interpret if any formulation adversely affected wound healing.

Taken together, it did appear that the vehicle, bupivacaine comparator, and the HTX formulations had an impact at the local injection site compared to the saline control. The effect seemed to be most pronounced at the end of the study (Day 15). Based on the observation of findings with multiple formulations, it suggests that the effects were potentially related to one or more shared excipients. The dose of the proposed clinical formulation used in this study was 25 mg bupivacaine and 0.75 mg meloxicam, which is less than the lowest proposed human dose of 60 mg bupivacaine and 1.8 mg meloxicam. The size of the incisions in the rat and rabbit studies were not reported, so a comparison in terms of volume per cm of incision between the nonclinical and the clinical studies could not be made.

The clinical formulation was also tested for wound healing in minipigs (33-54). In this study, male and female minipigs were given a single 3 mL dose of saline, vehicle, bupivacaine alone, meloxicam alone, and HTX-011. In addition, a sham control was included where an incision was made but no treatment was given. Minipigs were sacrificed on Day 15, the same duration as the rabbit study. There were limited increases in the severity of wound site observations on Day 4 compared to the sham

and vehicle control. Observations included increased redness around the wound site, increased severity of hemorrhage, and increased severity of exudate. These findings were no longer elevated in severity compared to the vehicle or sham control on Day 15. In terms of histopathological findings, there was increased severity of inflammatory cell infiltrate and granulomatous inflammation of the sub cutis on Day 15. Taken together, there was a very slight difference in wound healing in this study. The dose used in this study was 3 mL (75/2.25 mg bupivacaine/meloxicam) with a 3 cm incision or 1 mL/cm. Based on estimated incision sizes provided by the clinical team, for herniorrhaphy (3-4 cm), [REDACTED] (b) (4) the dose used in this study is lower than those proposed in the clinical studies (herniorrhaphy; 2.5-3.4 mL/cm, [REDACTED] (b) (4) [REDACTED] .

In summary, there did not appear to be any effect on bone healing or adverse interaction with the Prolene mesh with HTX-011. From the nonclinical perspective, no additional testing to assess these parameters are necessary. There was some evidence of a potential effect on wound healing in the rabbit study. However, the minipig study, which used a higher dose, supported that there was only a very slight difference in wound healing with HTX-011 compared to control treatments. Even the minipig study did not test local doses as high as those in the Phase 3 studies and therefore the nonclinical data does not fully support these studies. We acknowledge that there is clinical experience with the drug product and defer to the clinical review team in terms if further evaluation of the potential effect on wound healing at clinically relevant doses.

12 Appendix/Attachments

Reference List

(b) (4)



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/s/

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04/17/2019 03:42:17 PM

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04/17/2019 04:21:11 PM
I concur.