

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

210136Orig1s000

NON-CLINICAL REVIEW(S)



FDA Center for Drug Evaluation and Research
Division of Pharmacology/Toxicology for Neuroscience
10903 New Hampshire Avenue, Silver Spring, MD 20993

PHARMACOLOGY TOXICOLOGY NDA REVIEW

NDA number: 210136
Drug Product: BRIXADI (buprenorphine) extended-release injection for subcutaneous use
Applicant: Braeburn Inc.
450 Plymouth Road
Suite 400
Plymouth Meeting, PA 19462
Submission Type: Commercial
Supporting Document: 129
Submission Date/Receipt: June 15, 2021
Reviewer name: Grace S. Lee, PhD, DABT
Supervisor: Jay H. Chang, PhD
Clinical Review Division: Division of Anesthesiology, Addiction Medicine, and Pain Medicine
Clinical Division Director: Rigoberto Roca, MD
Project Manager: Rita K. Joshi, PharmD
Review completion date: November 19, 2021

The Applicant, Braeburn Inc., submitted a 505(b)(2) NDA in support of their depot buprenorphine drug product BRIXADI, referred to as CAM2038 during development, for the treatment of opioid use disorder. The NDA relies upon an FDA-previous finding of safety for Subutex (buprenorphine sublingual) and literature, and Applicant-conducted studies. There are two drug product formulations in this NDA, a once weekly [BRIXADI (weekly)] and a once monthly [BRIXADI (monthly)] formulations. These formulations primarily differ (b) (4)

At the time of original NDA submission on July 19, 2017, the drug product contained two new excipients, namely glycerol dioleate (GDO) and N-methylpyrrolidone (NMP). Both formulations contain GDO, whereas only the once monthly injection formulation contains NMP. NMP was considered to be used in a new manner via this product (NMP was previously used in an FDA-approved product for acute dental use and a product indicated for the treatment of advanced cancer). The NDA was not approved during the first review cycle and the complete response letter dated January 19, 2018 listed numerous deficiencies, including several regarding the safety qualification of the new excipients.

The NDA was resubmitted on June 26, 2018, and the safety of the CAM2038 formulations were also reviewed during the second review cycle. A tentative

approval was granted on December 21, 2018. In the Tentative Approval letter, four nonclinical studies were recommended as post-marketing requirements (PMRs) as follows.

1. Evaluate elemental impurity levels in at least three batches of drug product on stability at 12 and 24 months or provide adequate extraction data to characterize the elemental impurities that could be leached from the container closure system using suitable solvents (e.g., nitric acid for elementals from glass).
2. Conduct a study to confirm, using validated methods, the identity of the unspecified (b) (4) the unidentified compound with relative retention time (RRT) of (b) (4) minutes, the unknown compound containing (b) (4) with RRT of (b) (4) min, and the unknown compound with (b) (4) (b) (4) with RRT of (b) (4) min that were detected in your leachable studies above the safety concern threshold of 5 mcg/day and the levels of the identified leachables (b) (4). Evaluate at least three batches of your to-be-marketed drug product at multiple timepoints over the course of your stability studies to identify trends in leachable levels over time. Base the final safety assessment on the maximum predicted levels of leachables identified in individual batches to determine the safe level of exposure via the label-specified route of administration. Do not combine samples from different batches. Once chemical identification is confirmed for the unknown compounds, provide a toxicological risk assessment for each of these compounds and any other compounds detected at ≥ 5 mcg/day.
3. Conduct a fertility and early embryonic development study in female rats testing NMP administered via the subcutaneous route.
4. Conduct a pre- and post-natal development study in rats testing NMP administered via the subcutaneous route.

In addition, the Division requested to add the statement regarding the pre- and postnatal effects of NMP in the Section 8.1 Pregnancy of the labeling as follows.

“Decreased pup survival was noted following oral treatment of female rats prior to and during gestation with NMP, an excipient in BRIXADI (monthly) at doses 242 times the MDD of NMP delivered by BRIXADI (monthly) on an AUC basis. A No Adverse Effect Level (NOAEL) was not demonstrated.”

In the second re-submission (SD 113 dated June 1, 2020), the Applicant requested Final Approval of NDA 210136 and submitted two of the nonclinical post-marketing requirement studies, which include a fertility and early embryonic

development (FEED) study in female rats testing subcutaneous doses of NMP and a pre- and post-natal development (PPND) study in rats testing subcutaneous doses of NMP. Additionally, the Applicant submitted a nose-only inhalation toxicokinetic study of NMP in pregnant rats to determine the systemic exposure of NMP in the published embryo-fetal toxicity studies in rats administered inhaled doses of NMP. With these new data provided in the third submission, the Reviewer concluded that the overall reproductive and developmental toxicity assessment of NMP has been adequately evaluated for use of NMP as an excipient in BRIXADI (monthly) at the human maximum daily dose (MDD) and suggest significant safety margins compared to the published literature. Accordingly, it was concluded that the previously recommended statement (b) (4) may be removed in the drug label of BRIXADI. There were no other nonclinical recommendations on the drug labeling.

However, this NDA was not approved during the third review cycle due to deficiencies identified during the recent inspection for this application of the manufacturing and testing facility and the warehouse and testing facility (see Complete Response [CR] letter dated December 1, 2020). In this CR letter, the Division included the proposed revisions of the drug labeling, and in the revised labeling, the statement (b) (4) has been removed.

In the current NDA amendment (SD 129 dated June 15, 2021), the Applicant submitted a complete response to the deficiencies outlined in the CR letter dated December 1, 2020. In the Applicant's draft labeling included in this third re-submission, the statement (b) (4) is removed to reflect the Division's recommended labeling.

In addition, the Applicant is asking for Agency's feedback on the PMR for elemental impurities based on questions included in the current CR submission, and their rationale is as follows.

“In review of available extraction data on the container closure system, an extractable study was performed (b) (4) (Ref. S52 REP S279_01). Using ICP/MS, elemental impurities were quantified using three solvents, (b) (4). The CAM2038 drug product is comprised of buprenorphine, polar and non-polar lipids (b) (4). Therefore, extractions with (b) (4) are considered to appropriately cover the drug product in terms of lipophilicity. The study also quantified elements in accordance with ICH Q3D and USP <232> for parenteral products (b) (4).

(b) (4) referenced above. There were no reportable elements found in the (b) (4) extracts. For the (b) (4) extracts, the LOQ was lower than (b) (4) mcg/L for all elements (b) (4). The data indicate that all elements were (b) (4). For the (b) (4) extracts, the LOQ was higher (b) (4) but all elements were (b) (4).

The Applicant asked whether the Agency agrees that based on the data provided in S52 REP S279_01, combined with the results of testing both Q1W and Q4W drug products after 6 months of storage at 40°C/75% RH [refer to 3.2.P.5.5 Characterization of Impurities (Q1W) and 3.2.P.5.5 Characterization of Impurities (Q4W)] and the Elemental Impurities Risk Assessment that the level of elemental impurities observed do not require any further control and these data satisfy the Agency's request for additional data to fulfill the PMR.

The extraction study [S52 REP S279_0] mentioned above was previously reviewed by the CMC team, and the CMC team concluded that the conditions used in this extraction study (IPA at room temperature up to 14 days) was not considered adequate [see Drug Product Review dated December 6, 2018 by Dr. Valerie Amspacher and Nonclinical PharmTox Review dated December 7, 2018 by Drs. Bolan, Bond, and D'Agostino]. Apparently, the Applicant has not submitted new data and accordingly, the Agency does not agree with the Applicant's conclusion that the level of elemental impurities observed do not require any further control and these data satisfy the Agency's request for additional data to fulfill the PMR. Thus, the Applicant still needs to fulfill the PMR regarding the elemental impurities and our response to the Applicant's question regarding this PMR will be conveyed to the Applicant.

The current submission also states that Braeburn has completed the risk assessment of (b) (4) impurities for both weekly and monthly drug products in accordance with FDA Guidance for Industry (b) (4) (b) (4) and concluded that there is no risk associated with possible presence of (b) (4) its family of impurities. Braeburn's risk assessment report is available upon request, but at this moment, the CMC team has determined not to request further information regarding the control of (b) (4) impurities [Email conversation with Dr. Valerie Amspacher on September 17, 2021].

Overall, there are no nonclinical issues with this current submission, but the Applicant still needs to provide the information on elemental impurity levels in at least three batches of drug product on stability at 12 and 24 months (or adequate extraction data to characterize the elemental impurities) and identification and evaluation of the unspecified (b) (4). These data may be submitted as post-marketing requirements as noted above. As the NDA will not be approved during this review cycle, formal PMRs will not be issued at this time. Nevertheless, we

will convey the following comment to the Applicant to address their question regarding their elemental impurity evaluation.

Comment to the Applicant:

Regarding your question about the adequacy of your elemental impurity evaluation, we do not agree you have provided adequate information to evaluate impurity levels in your drug product. As communicated to you in a recent information request, we do not agree that (b) (4) (b) (4) used in report Ref. S52 REP S279_01 are the appropriate solvents for elemental impurities extraction. We refer you to USP <233> for possible concentrated acids to use as extraction solvents. The recommendation to evaluate elemental impurities, as noted in the tentative approval letter dated 12/21/2018, may be submitted as a post-marketing requirement.

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

/s/

GRACE S LEE
11/19/2021 04:23:36 PM

JAY H CHANG
11/19/2021 04:35:12 PM

**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 210136
Supporting document/s:	113
Applicant's letter date:	June 1, 2020
CDER stamp date:	June 1, 2020
Product:	BRIXADI (weekly and monthly)
Indication:	Opioid use disorder
Applicant:	Braeburn Inc.
Clinical Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine
Reviewer:	Grace S. Lee, PhD, DABT
Supervisor/Team Leader:	R. Daniel Mellon, PhD/ Jay H. Chang, PhD
Clinical Division Director:	Rigoberto Roca, MD
Project Manager:	Matthew W. Sullivan, MS, RAC

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 210136 are owned by Braeburn Inc. or are data for which Braeburn Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 210136 that Braeburn Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 210136.

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1 Executive Summary

1.1 Introduction

The Applicant Braeburn Inc. submitted a 505(b)(2) NDA in support of their depot buprenorphine drug product BRIXADI, referred to during development as CAM2038, for the treatment of opioid use disorder. The NDA relies upon an FDA-previous finding of safety for Subutex (buprenorphine sublingual) and literature. There are two drug product formulations in this NDA, a once weekly [BRIXADI (weekly)] and a once monthly [BRIXADI (monthly)] formulation. These formulations primarily differ (b) (4)

(b) (4) At the time of original NDA submission on July 19, 2017, the drug product contained two new excipients, namely glycerol dioleate (GDO) and N-methylpyrrolidone (NMP). Both formulations contain GDO, whereas only the once monthly injection formulation contains NMP. NMP was considered to be used in a new manner via this product (NMP was previously used in an FDA-approved product for acute dental use and a product indicated for the treatment of advanced cancer). The NDA was not approved during the first review cycle and the complete response letter dated January 19, 2018 listed numerous deficiencies, including several regarding the safety qualification of the new excipients.

The NDA was resubmitted on June 26, 2018, and the safety of the CAM2038 formulations were also reviewed during the second review cycle. A tentative approval was granted on December 21, 2018. In the Tentative Approval letter, four nonclinical studies were required as postmarketing requirements as follows.

1. Evaluate elemental impurity levels in at least three batches of drug product on stability at 12 and 24 months or provide adequate extraction data to characterize the elemental impurities that could be leached from the container closure system using suitable solvents (e.g., nitric acid for elementals from glass).
2. Conduct a study to confirm, using validated methods, the identity of the unspecified (b) (4) the unidentified compound with relative retention time (RRT) of (b) (4) minutes, the unknown compound containing (b) (4) with RRT of (b) (4) min, and the unknown compound with (b) (4) with RRT of (b) (4) min that were detected in your leachable studies above the safety concern threshold of 5 mcg/day and the levels of the identified leachables (b) (4)

Evaluate at least three batches of your to-be-marketed drug product at multiple timepoints over the course of your stability studies to identify trends in leachable levels over time. Base the final safety assessment on the maximum predicted levels of leachables identified in individual batches to determine the safe level of exposure via the label-specified route of administration. Do not combine samples from different batches. Once chemical identification is confirmed for the unknown compounds, provide a

toxicological risk assessment for each of these compounds and any other compounds detected at ≥ 5 mcg/day.

3. Conduct a fertility and early embryonic development study in female rats testing NMP administered via the subcutaneous route.
4. Conduct a pre- and post-natal development study in rats testing NMP administered via the subcutaneous route.

In the current NDA amendment (SD 113 dated June 1, 2020), the Applicant submitted a request for Final Approval of NDA 210136, and submitted two of the nonclinical postmarketing requirement studies, which include a fertility and early embryonic development (FEED) study in female rats testing subcutaneous doses of NMP and a pre- and post-natal development (PPND) study in rats testing subcutaneous doses of NMP. In addition, the Applicant submitted a nose-only inhalation toxicokinetic study of NMP in pregnant rats to determine the systemic exposure of NMP in the published embryo-fetal toxicity studies in rats administered inhaled doses of NMP. These three animal studies are evaluated in this review.

1.2 Brief Discussion of Nonclinical Findings

In the GLP rat FEED toxicity study conducted by the Applicant, female Sprague Dawley rats were administered NMP via once daily subcutaneous (SC) injection at doses of 0 (vehicle), 50, 100, or 250 mg/kg/day. Females were administered once daily for 14 days prior to cohabitation and continued through Gestation Day (GD) 7, and laparohysterectomies were performed on GD 15. There were no NMP-related effects on female reproductive parameters and embryonic developmental parameters in this study. The no observed adverse-effect level (NOAEL) for maternal toxicity, female fertility, and early embryonic developmental was the high-dose of 250 mg/kg/day, corresponding to the AUC value of 2495 mcg•h/mL in female rat (377 times the human maximum daily dose [MDD] of NMP delivered via BRIXADI (monthly) on an AUC basis).

In the GLP pre- and postnatal developmental (PPND) toxicity study by the Applicant, time-mated Sprague Dawley rats (F_0 ; 25/group) were administered NMP via once daily SC injection at doses of 0 (vehicle), 50, 100, or 250 mg/kg/day from GD 6 through Lactation Day 21. There was a decrease in body weight gain in F_0 females at 250 mg/kg between GD 6 and 20 (-17% decrease, relative to the control group). The F_1 females at 250 mg/kg exhibited delayed vaginal patency (PND 35.2 with the mean body weight of 130 g), compared to the control group (PND 31.4 with the mean body weight of 114.3 g). These high mean values in the 250 mg/kg group (day and body weight of acquisition) were due to six females with delayed vaginal patency with relatively high body weight. However, these females and all other females in the 250 mg/kg group exhibited normal estrous cycle lengths and mating performance and thus, the finding of delayed vaginal patency in the 250 mg/kg group was not considered by this reviewer to be adverse. There were no other NMP-related effects on pre- and postnatal development and reproductive performance in F_1 animals. There were no NMP-related

effects on viability of F₂ fetuses in any dose groups, as examined on GD 15. The NOAEL for maternal toxicity of F₀ animals was the mid-dose of 100 mg/kg/day, corresponding to the AUC value of 709 mcg•h/mL (107 times the human MDD of NMP delivered by BRIXADI (monthly) on an AUC basis) because of the decreased body weight gain at 250 mg/kg during the gestation period. The NOAEL for pre- and postnatal development of F₁ animals was the high-dose of 250 mg/kg/day, corresponding to an AUC value of 2495 mcg•h/mL which is 377 times the human MDD of NMP delivered by BRIXADI (monthly) on an AUC basis. Because of the delayed vaginal patency at 250 mg/kg/day, the NOEL for pre- and postnatal development was the mid-dose of 100 mg/kg/day.

In the rat inhalation TK study, pregnant Sprague Dawley rats were administered NMP at target doses of 51, 89, or 165 ppm via nose-only inhalation for 6 hours daily during GDs 6–10. The AUC_(0-t) values of NMP were 99.7, 301, and 4510 mcg•h/mL for the target dose level of 51, 89, and 165 ppm, respectively. The AUC increase was 3-fold from 51 ppm to 89 ppm (1.7-fold increase in dose level) and 15-fold from 89 ppm to 165 ppm (1.9-fold increase in dose level), indicating that the increase in exposure to NMP via the route of inhalation is not linear. These data confirm sufficiently large exposure margins for potential embryo-fetal development reported in the literature following administration of NMP via the inhalation route.

The submitted PPND study in rats following the SC injection of NMP determined the NOAEL and provided a sufficient safety margin for the developmental toxicity of NMP. Similarly, the submitted FEED study in female rats also identified the NOAEL and provided a sufficient safety margin for female fertility and early embryonic developmental toxicity of NMP. Overall, the reproductive and developmental toxicity assessment of NMP has been adequately evaluated for use of NMP as an excipient in BRIXADI (monthly) at the human MDD and suggest significant safety margins compared to the published literature. Thus, the previously recommended statement (b) (4) may be removed in the drug label of BRIXADI. There were no other nonclinical recommendations on the drug labeling.

1.3 Recommendations

1.3.1 Approvability

During the previous review cycle, we recommended that the NDA may be approved with postmarketing requirements to conduct studies to evaluate the effects of NMP on fertility and early embryonic development and pre- and post-natal reproductive development. These studies were submitted with this request for final approval and the data demonstrated that there were no adverse NMP-related effects on female reproductive parameters, embryonic developmental parameters, and pre- and postnatal development parameters at up to 377 times the human exposure. Therefore, from a nonclinical perspective, the NDA may be approved without any additional postmarketing requirements to conduct these studies.

1.3.2 Additional Nonclinical Recommendations

None

2 Drug Information

2.1 Drug

CAS Registry Number: 52485-79-7

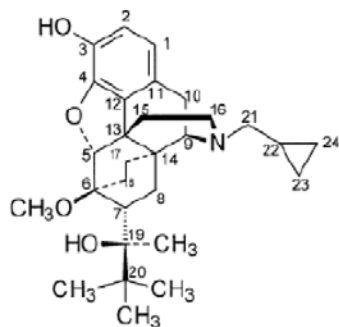
Generic Name: Buprenorphine base

Code Name: N/A

Chemical Name: (2S)-2-[-(-)-(5R,6R,7R,14S)-9 α -cyclopropylmethyl-4,5-epoxy-6,14-ethano-3-hydroxy-6-methoxymorphinan-7-yl]-3,3-dimethylbutan-2-ol

Molecular Formula/Molecular Weight: C₂₉H₄₁NO₄

Structure:



Pharmacologic Class: Partial opioid agonist (FDA Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs, and DMFs

IND/NDA/MF	Drug/Compound	Sponsor/Applicant	Division	Status
NDA 20732	Subutex	Indivior	DAAP	Approved 10/8/2002
IND 114082	CAM2038	Braeburn	DAAP	Active

2.3 Drug Formulation

BRIXADI is available as two different formulations, one for weekly [BRIXADI (weekly) or CAM2038 q1w] and one for monthly [BRIXADI (monthly) or CAM2038 q4w], extended-release lipid-based subcutaneous depots containing 50 and 356 mg/mL buprenorphine base, respectively. The proposed strengths of the single-use prefilled syringes for

BRIXADI (weekly) are 8 mg, 16 mg, 24 mg, and 32 mg and the strengths for BRIXADI (monthly) are 64 mg, 96 mg, and 128 mg. (b) (4)

The injection depot platform contains soybean phosphatidylcholine (SPC), glycerol dioleate (GDO), and a (b) (4) and is referred to by the Applicant as “Fluid Crystal” technology. The two formulations (b) (4) with the weekly product containing ethanol and the monthly formulation containing N-methyl-2-pyrrolidone (NMP). According to the Applicant, “once injected, CAM2038 spontaneously transforms from a low-viscous solution to a highly viscous crystalline gel that encapsulates the BUP and releases it at a steady rate as the depot degrades.” The quantitative composition of each strength for the weekly and monthly BRIXADI formulations is outlined in Tables 1 and 2, respectively.

Table 1 Composition of BRIXADI (weekly) [CAM2038 q1w Drug Product]

Component	Standard	Function	Composition (mg) per dose			
			8 mg/ 0.16 mL	16 mg/ 0.32 mL	24 mg/ 0.48 mL	32 mg/ 0.64 mL
Buprenorphine Base (BUP)	Ph. Eur.	Active ingredient	8	16	24	32
Ethanol Anhydrous (Dehydrated alcohol) (EtOH)	USP	(b) (4)	(b) (4)			
Soybean Phosphatidylcholine (SPC)	In-house	(b) (4)	(b) (4)			
Glycerol Dioleate (GDO)	In-house	(b) (4)	(b) (4)			
Total weight (mg) for all components per dose	Not Applicable	Not Applicable	(b) (4)			

Table 2 Composition of BRIXADI (monthly) [CAM2038 q4w Drug Product]

Component	Standard	Function	Composition (mg) per dose			(b) (4)
			64 mg/ 0.18 mL	96 mg/ 0.27 mL	128 mg/ 0.36 mL	
Buprenorphine Base (BUP)	Ph. Eur.	Active ingredient	64	96	128	
N-Methyl-2-Pyrrolidone (NMP)	USP					(b) (4)
Soybean Phosphatidylcholine (SPC)	In-house					
Glycerol Dioleate (GDO)	In-house					
Total weight (mg) for all components per dose	Not Applicable	Not Applicable				(b) (4)

2.4 Comments on Novel Excipients

The BRIXADI formulations contain two “novel” excipients as follows: glycerol dioleate (GDO) in both weekly and monthly formulations and N-methyl-2-pyrrolidone (NMP) in the monthly formulation only. Glycerol dioleate has not been previously used in an FDA-approved drug product. The use of NMP in this drug product formulation is technically novel by the SC route of administration. NMP was used as an excipient in two FDA-approved drug products, one of which is approved for the periodontal route for an acute indication and the other for the subcutaneous route for the palliative treatment of prostate cancer. The novelty of NMP for a chronic non-oncology indication was discussed with the Applicant over the course of development and the Agency noted that the Applicant would have to address the safety for this indication. The Applicant’s safety assessment of these two excipients has been submitted in the original and second NDA submissions as well as the recent NDA amendment (SD 113) currently under review. For the detailed information, see the Nonclinical Pharmacology Toxicology Reviews dated December 22, 2017 and December 7, 2018 and also this review below.

2.5 Comments on Impurities/Degradants of Concern

See Nonclinical Pharmacology Toxicology Reviews dated December 22, 2017 and December 7, 2018.

2.6 Proposed Clinical Population and Dosing Regimen

BRIXADI is indicated for the treatment of moderate to severe opioid use disorder in patients who have initiated treatment with a single dose of a transmucosal buprenorphine product or who are already being treated with buprenorphine.

The depot formulation should ensure medication adherence, ultimately improving treatment effectiveness.

2.7 Regulatory Background

- NDA 210136 was originally submitted July 9, 2017 and priority review was granted.
- A complete response action was taken on January 19, 2018 listing numerous deficiencies.
- A Type A post action meeting was held with the Applicant on May 2, 2018.
- The NDA was resubmitted on June 26, 2018, and a tentative approval was granted on December 21, 2018.

3 Studies Submitted

3.1 Studies Reviewed

Study title: A Nose-Only Inhalation Toxicokinetic Study of N-methyl-2-pyrrolidone (NMP) in Female Sprague Dawley Rats [Testing Facility Study No 00371513; Braeburn Study No PK-18-624]

Study title: A Subcutaneous Injection Study of the Effects of N-methyl-2-pyrrolidone (NMP) on Fertility and Early Embryonic Development to Implantation in Female Rats [Testing Facility Study No 00371510; Braeburn Study No TO-18-620]

Study title: A Subcutaneous Injection Study of the Effects of N-methyl-2-pyrrolidone (NMP) on Pre- and Postnatal Development, Including Maternal Function in Rats [Testing Facility Study No 00371511; Braeburn Study No TO-18-619]

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

NDA 210136 Primary Pharmacology Toxicology Review dated December 22, 2017
NDA 210136 Primary Pharmacology Toxicology Review dated December 7, 2018

4 Pharmacology

4.1 Primary Pharmacology

No primary pharmacology studies submitted.

4.2 Secondary Pharmacology

No secondary pharmacology studies were submitted.

4.3 Safety Pharmacology

No safety pharmacology studies were submitted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No pharmacokinetics/ADME studies were submitted.

5.2 Toxicokinetics

A toxicokinetics study for the excipient NMP was submitted under SD 113 and is reviewed herein.

Study title: A Nose-Only Inhalation Toxicokinetic Study of N-methyl-2-pyrrolidone (NMP) in Female Sprague Dawley Rats

Study no.:	PK-18-624 [Testing Facility Study No 00371513]
Study report location:	SD 113; 6/1/2020; EDR 4.2.2.7; \\CDSESUB1\evsprod\nda210136\01113\m4\42-stud-rep\422-pk\4227-other-pk-stud\pk-18-624\pk-18-624.pdf
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	June 1, 2018
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	N-methyl-2-pyrrolidone (NMP), Batch 0002171585, and 99.9% purity

The objective of this study is to provide NMP exposure information for published embryo-fetal development (EFD) studies conducted by the inhalation route of administration. Although whole-body inhalation was used in the EFD studies, the nose-only exposure regimen was selected in this TK study to allow for sampling of blood during exposure for bioanalysis of NMP.

Dose levels for the female rats were selected based on previously reported inhalation studies conducted at target exposure concentrations of up to 165 ppm. To bridge the toxicokinetic exposure data to doses in published inhalation developmental toxicity

studies, the target exposure concentrations for this study were selected to be 51, 89, and 165 ppm.

Method: Pregnant rats (8/group) were dosed via nose-only inhalation for 6 hours daily during Gestation Days (GD) 6–10 [Study Days 0–4]. The evaluated endpoints in this study consisted of clinical signs, body weights, body weight gains, toxicokinetic (TK) parameters, and pregnancy status.

Blood for TK analysis was collected via a jugular vein for the timepoints as shown in Table 3. Blood samples were analyzed by the LC-MS/MS method, and the lower limit of quantitation (LLOQ) for NMP was 1.0 mcg/mL. A typical inhalation study includes characterization of aerosol/particle of test article generated from solution or dry materials, and particle size such as the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) is determined. However, this study did not characterize test article vapor generated and used for the study. Because this TK study was conducted to obtain the systemic exposure to inhaled NMP to bridge the inhalation doses used in the EFD studies and not to characterize the respiratory effects of inhaled NMP, it is acceptable not to determine particle size of the test article vapor in this study.

Table 3 TK blood collection time points

No. of Females	Sample Collection Time Points (Time Post-initiation of Exposure) on Gestation Day 10							
	1 hr	2 hr	4 hr	6 hr	8 hr	12 hr	16 hr	24 hr
4/group	X	-	X	-	X	-	X	-
4/group	-	X	-	X	-	X	-	X

X = Samples collected for up to 4 females/group; - = sample not collected.

Taken directly from the study report, p 12

Result: All females survived to the scheduled sacrifice and were gravid. There were no remarkable clinical signs, except for a single occurrence of yellow material on the ventral trunk in one female in the 165 ppm group. All females in the 51, 89, and 165 ppm groups gained body weight between GD 6–10.

Toxicokinetic data of NMP are shown in Table 4. Systemic exposure, expressed by $AUC_{(0-t)}$ and C_{max} , increased as dose increased in a greater than dose-proportional manner. The $AUC_{(0-t)}$ values in pregnant Sprague Dawley rats were 99.7, 301, and 4510 mcg•h/mL at 51, 89, and 165 ppm, respectively on GD 10. A 3-fold increase in target exposure concentration from 51 to 165 ppm, resulted in $AUC_{(0-t)}$ and C_{max} exposure that was greater than dose proportional by 45- and 38-fold, respectively. Specifically, a 1.75-fold increase in target exposure concentration from 51 to 89 ppm resulted in a 3.0- and 2.6-fold increase in $AUC_{(0-t)}$ and C_{max} exposure, respectively, whereas a 3.24-fold increase in target dose level from 89 to 165 ppm resulted in a 15.0- and 14.5-fold increase in $AUC_{(0-t)}$ and C_{max} exposure, respectively.

Table 4 Toxicokinetic data of NMP in plasma from pregnant rats following nose-only inhalation of NMP at 51, 89, or 165 ppm

Group	Target Exposure Concentration (ppm)	Actual Exposure Concentration (ppm)	AUC(0-t) ($\mu\text{g}\cdot\text{h}/\text{mL}$)	AUC(0-t)/D	C _{max} ($\mu\text{g}/\text{mL}$)	C _{max} /D	T _{max} (h)	T _{1/2} (h)
1	51	49	99.7	2.03	14.8	0.303	6	NC
2	89	88	301	3.42	38.5	0.437	4	1.91
3	165	175	4510	25.7	557	3.18	8	NC

Units for AUC(0-t)/D and C_{max}/D, where D = Actual Exposure Concentration (ppm), are ($\mu\text{g}\cdot\text{h}/\text{mL}$)/(ppm) and ($\mu\text{g}/\text{mL}$)/(ppm), respectively.

NC = Not calculable, due to limited data in the terminal elimination phase.

Taken directly from the study report, p 303

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicity studies were submitted.

6.2 Repeat-Dose Toxicity

No repeat-dose toxicity studies were submitted.

7 Genetic Toxicology

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

No Ames studies were submitted.

7.2 *In Vitro* Assays in Mammalian Cells

No *in vitro* genotoxicity studies in mammalian cells were submitted.

7.3 *In Vivo* Clastogenicity Assay in Rodent

No *in vivo* genotoxicity studies were submitted.

7.4 Other Genetic Toxicity Studies

No other genotoxicity studies were submitted.

8 Carcinogenicity

No carcinogenicity studies were submitted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

A fertility and early embryonic developmental (FEED) toxicity study for the excipient NMP was submitted under SDN 113 and is reviewed herein.

Study title: A Subcutaneous Injection Study of the Effects of N-methyl-2-pyrrolidone (NMP) on Fertility and Early Embryonic Development to Implantation in Female Rats

Study no.:	TO-18-620 [Testing Facility Study No 00371510]
Study report location:	SD 113; 6/1/2020; EDR 4.2.3.5.1; \\CDSESUB1\evsprod\nda210136\0113\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42351-fert-embryo-dev\to-18-620\to-18-620.pdf
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	May 2, 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	N-methyl-2-pyrrolidone (NMP), Batch 0002171585, and 99.9% purity

Key Study Findings

- In the fertility and early embryonic developmental (FEED) toxicity study, female Sprague Dawley rats were administered NMP at subcutaneous doses of 0 (vehicle), 50 (LD), 100 (MD), or 250 (HD) mg/kg/day. Females were administered once daily for 14 days prior to cohabitation and continued through Gestation Day 7 and laparohysterectomies were performed on GD 15.
- Increased incidences of erythema, edema, hemorrhages, and scabbing in the injection sites were observed in the test article-dosed groups, but these findings were not dose-dependent and higher incidences of these findings were noted in the LD group.
- There were no NMP-related effects on female reproductive parameters and embryonic developmental parameters in this study.
- The NOAEL for maternal toxicity, female fertility, and early embryonic development is the high-dose of 250 mg/kg/day.

Methods

Doses: 0 (control), 50 (LD), 100 (MD), and 250 (HD) mg/kg/day; see Table 5

Frequency of dosing: Once daily for 14 days prior to cohabitation and continued through Gestation Day (GD) 7

Dose volume: 5 mL/kg

Route of administration: Subcutaneous (SC) injection

Formulation/Vehicle: Deionized water

Species/Strain: Crl:CD(SD) rats

Number/Sex/Group: 25 /female/group

Satellite groups: None

Study design: The day of confirmed mating was designated Gestation Day 0 (GD 0).

Deviation from study protocol: There were no deviations that negatively impacted the integrity of the study data.

Table 5 Experimental design of the FEED study

Group Number	Treatment	Dosage Level (mg/kg/day) ^a	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
					Male ^b	Female
1	Vehicle	0	0	5	25	25
2	N-methyl-2-pyrrolidone	50	10	5	25	25
3	N-methyl-2-pyrrolidone	100	20	5	25	25
4	N-methyl-2-pyrrolidone	250	50	5	25	25

^a No correction factor was used.

^b Males were not administered the test or vehicle control article and were used for breeding purposes only.

Taken directly from the study report, p 14

Observations and Results**Mortality**

Viability was checked twice daily.

All animals survived to the scheduled necropsy.

Clinical Signs

A detailed clinical observation was performed once daily throughout the study. During the dosing period, these observations were performed twice (prior to dosing and approximately 1 h postdose).

There were no adverse test article-related clinical signs, but there were slightly increased incidences in scabbing in the dorsal trunk in all test article-dosed groups and dried red material around nose in the MD and HD groups.

Injection Site Scoring

The detailed clinical observations recorded daily included an examination of the injection sites for erythema, swelling, and other dermal findings beginning on Study Day 9.

Increased incidences of erythema, edema, hemorrhages, and scabbing in the injection sites were observed in the test article-dosed group, but these findings were not dose dependent and higher incidences of these findings were noted in the LD group.

Body Weight

Females were weighed twice weekly during the pre-mating period. Females with evidence of mating were then weighed on GD 0, 3, 7, 10, 13, and 15 during the gestation period, whereas females with no evidence of mating were weighed twice weekly until euthanasia.

There were no test article-related body weight changes in females during the pre-mating period. There was a slight decrease in body weight gain in the MD and HD groups between GD 0 and 7 (10% decrease, relative to the control group), but a slight increase was observed in these group between GD 7 and 15. Because the magnitude of the decrease in body weight gain between GD 0 and 7 was very slight, this decrease was not considered adverse.

Food Consumption

Food consumption was measured weekly (prior to treatment) or twice weekly during the pre-mating period and GD 0, 3, 7, 10, 13, and 15 for females with evidence of mating and twice weekly until euthanasia for females with no evidence of mating.

There were no test article-related effects on food consumption in females.

Toxicokinetics

Toxicokinetic analysis was not performed in the study, but the Applicant previously included a TK study in SD rats that were subcutaneously administered NMP [Study TO-15-540] in the NDA re-submission in 2018. The AUC values from this TK study are presented in the section of Integrated Summary and Safety Evaluation below.

Dosing Analysis

The dosing formulations were prepared approximately weekly. Samples from dosing formulations prepared on May 22, 2018 and June 13, 2018 were analyzed for concentrations.

Results of the concentration analyses showed that the analyzed dosing formulations

ranged 103-107% of the target concentrations. The test article was not detected in the analyzed vehicle formulation for the control group (Group 1).

Estrous Cycles

Vaginal lavages were performed daily, and the slides were evaluated microscopically to determine the stage of the estrous cycle of each female for 14 days prior to test article administration and continuing until evidence of copulation was observed or until termination of the mating period for females with no evidence of mating.

The estrous cycle data are shown in Table 6. There were no test article-related effects on estrous cycles.

Table 6 Summary of estrous cycle data

GROUP:	0 MG/KG/DAY	FEMALES 50 MG/KG/DAY	100 MG/KG/DAY	250 MG/KG/DAY
ESTROUS CYCLE LENGTH (DAYS)				
MEAN	4.1	4.0	4.2	4.1
S.D.	0.44	0.61	0.57	0.56
N	25	25	25	25

None significantly different from control group
ESTROUS CYCLE LENGTH CALCULATED FOR FEMALES HAVING ONE FULL ESTROUS CYCLE

Taken directly from the study report, p 28

Necropsy

All animals were euthanized by carbon dioxide inhalation at the scheduled necropsy, and the gross necropsy was performed on all females.

The Cesarean section was performed on GD 15 for each female with evidence of mating. The females without evidence of mating were euthanized following completion of postmortem examinations of the females with evidence of mating. The organ weights for the brain, pituitary gland, and ovaries were recorded. The uteri of females without macroscopic evidence of implantation were opened and placed in 10% ammonium sulfide solution for detection of early implantation loss.

There were no test article-related macroscopic findings or changes in organ weights in females at necropsy.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Mating, fertility, and copulation/conception indices were determined. For pregnant females, uterus and ovaries were removed, and number of corpora lutea on each ovary, the number and location of all embryos, early resorptions, and the total number of implantation sites were recorded. Viability of the embryos was determined.

Mating, fertility, and copulation/conception indices are presented in Table 7, and embryonic data are presented in Table 8. There were no test article-related effects on

any reproductive and embryonic developmental parameters evaluated in this study. Any differences observed in this study were due to background variations.

Table 7 Summary of female reproductive performance

DOSE GROUP	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
FEMALE MATING INDEX	25/25	100.0	25/25	100.0	25/25	100.0	25/25	100.0
FEMALE FERTILITY INDEX	25/25	100.0	24/25	96.0	24/25	96.0	24/25	96.0
FEMALE CONCEPTION INDEX	25/25	100.0	24/25	96.0	24/25	96.0	24/25	96.0
MEAN PRECOITAL INTERVALS								
DAYS	2.8	NA	2.4	NA	2.6	NA	2.9	NA
S.D.	2.78	NA	1.41	NA	1.36	NA	2.55	NA
N	25	NA	24	NA	25	NA	25	NA
FEMALE MATING INDEX (%) =	NO. OF FEMALES WITH EVIDENCE OF MATING (OR CONFIRMED PREGNANCY)							X 100
	TOTAL NO. OF FEMALES USED FOR MATING							
FEMALE FERTILITY INDEX (%) =	NO. OF FEMALES WITH CONFIRMED PREGNANCY							X 100
	TOTAL NO. OF FEMALES USED FOR MATING							
FEMALE CONCEPTION INDEX (%) =	NO. OF FEMALES WITH CONFIRMED PREGNANCY							X 100
	NO. OF FEMALES WITH EVIDENCE OF MATING (OR CONFIRMED PREGNANCY)							

1- 0 MG/KG/DAY 2- 50 MG/KG/DAY 3- 100 MG/KG/DAY 4- 250 MG/KG/DAY
 PRECOITAL INTERVALS NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING DUNNETT'S TEST
 MATING, FERTILITY AND COPULATION/CONCEPTION INDICES NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING CHI-SQUARE TEST
 NA = NOT APPLICABLE

Taken directly from the study report, p 40

Table 8 Summary of embryonic data at scheduled necropsy (% per litter)

Parameter	0 (control)	50 mg/kg/day (LD)	100 mg/kg/day (MD)	250 mg/kg/day (HD)
No of litters	25	23	24	24
Mean litter no. of corpora lutea	16.5	15.9	16.3	16.4
Mean litter no. of implantation site	15.8	15.0	15.0	15.4
Total resorption per litter (%)	2.5	5.7	5.8	7.1
Live fetuses per litter (%)	97.5	94.3	94.2	92.9
Mean preimplantation loss per litter (%)	4.1	4.8	8.2	5.6
Mean postimplantation loss per litter (%)	2.5	5.7	5.8	7.1

Taken directly from the study report, p 45

9.2 Embryonic Fetal Development

No embryonic fetal developmental (EFD) toxicity study was submitted in the SD 113 NDA amendment.

9.3 Prenatal and Postnatal Development

A pre- and postnatal developmental (PPND) toxicity study for the excipient NMP was submitted under SD 113 and is reviewed herein.

Study title: A Subcutaneous Injection Study of the Effects of N-methyl-2-pyrrolidone (NMP) on Pre- and Postnatal Development, Including Maternal Function in Rats

Study no.: TO-18-619 [Testing Facility Study No 00371511]

Study report location: SD 113; 6/1/2020; EDR 4.2.3.5.3; \\CDSESUB1\evsprod\nda210136\0113\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42353-pre-postnatal-dev\to-18-619\to-18-619.pdf (b) (4)

Conducting laboratory and location: [REDACTED]

Date of study initiation: May 16, 2018

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: N-methyl-2-pyrrolidone (NMP), Batch 0002171585; 99.9% purity

Key Study Findings

- In the pre- and postnatal developmental (PPND) toxicity study, time-mated Sprague Dawley rats (F₀) were administered NMP via once daily subcutaneous injection at doses of 0 (vehicle), 50 (LD), 100 (MD), or 250 (HD) mg/kg/day from Gestation Day [GD] 6 through Lactation Day (LacD) 21 (day of confirmed mating = GD 0).
- The incidence and severity of the injection site findings, including erythema, edema, and scabbing, were slightly increased in the test article-dosed groups, compared to the control group.
- There were decreased body weight gain in F₀ females in the HD group between GD 6 and 20 (-17% decrease, relative to the control group).
- The F₁ females in the HD group exhibited delayed vaginal patency (PND 35.2 with the mean body weight of 130 g), compared to the control group (PND 31.4 with the mean body weight of 114.3 g). These high mean values in the HD group (day and body weight of acquisition) were due to six females with delayed vaginal patency with relatively high body weight. However, these females and all other females in the HD group exhibited the normal estrous cycle lengths and mating performances and thus, the finding of the delayed vaginal patency in the HD group was considered test article-related, but not adverse. There were no other test article-related effects on pre- and postnatal development and reproductive performance in F₁ animals.

- There were no test article-related effects on viability of F₂ fetuses in any dose groups, as examined on GD 15.
- The NOAEL for maternal toxicity of F₀ animals was the mid-dose of 100 mg/kg/day because of the decreased body weight gain at 250 mg/kg during the gestation period. The NOAEL for pre- and postnatal development of F₁ animals was the high-dose of 250 mg/kg/day. The NOEL for pre- and postnatal development was the mid-dose of 100 mg/kg/day because of the delayed vaginal patency at 250 mg/kg/day.

Methods

Doses:	0 (control), 50 (LD), 100 (MD), and 250 (HD) mg/kg/day; see Table 9
Frequency of dosing:	Once daily from GD 6 through Lactation Day (LacD) 20
Route of administration:	Subcutaneous (SC) injection; the injection site was rotated between 4 distinct sites in the scapular/lumbar regions between doses.
Formulation/Vehicle:	Deionized water
Species/Strain:	CrI:CD(SD) rats
Number/Sex/Group:	25 mated females/group
Satellite groups:	None
Study design:	The day of mating evidence was designated Gestation Day (GD) 0. The day parturition was initiated was designated Lactation Day (LacD) 0 (Postnatal Day [PND] 0 for pups).
Deviation from study protocol:	There were no deviations that affected the integrity of the study or impacted the study design.

Table 9 Experimental design of the PPND rat study

Group Number	Treatment	Dosage Level ^a (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	Number of F ₀ Females
1	Vehicle	0	0	5	25
2	NMP	50	10	5	25
3	NMP	100	20	5	25
4	NMP	250	50	5	25

^a No correction factor was used.

Taken directly from the study report, p 20

Observations and Results

F₀ Dams**Survival:**

Viability was checked twice daily, once in the morning and once in the afternoon.

There was no test article-related mortality.

Clinical signs:

The animals were removed from the cage, and a detailed clinical observation was performed once daily throughout the study. During the dosing period, the clinical observations were performed prior to dosing and 1-2 h postdose. The detailed clinical observations recorded daily included an examination of the injection sites for erythema, swelling, and other dermal findings beginning on GD 6.

There were no adverse test article-related clinical signs. The incidence and severity of the injection site findings, including erythema, edema, and scabbing, were slightly increased in the test article-dosed groups, compared to the control group.

Dilated pupil was observed in one female in the MD group and two females in the HD with one or two incidences. Because of the low incidences, the finding is not considered test article-related.

Body weight:

Body weights were measured on GDs 0, 6, 9, 12, 15, 18, and 20, and LacDs 1, 4, 7, 10, 14, 17, and 21.

There were decreased body weight gain in the HD group between GD 6 and 20 (17% decrease, relative to the control group). The body weight gain between GDs 6 and 20 was 116 g, 109 g, 108 g, and 96 g in the control, LD, MD, and HD groups, respectively. However, during the lactation period, there was a slight increase in mean body weight gain in the HD group so that mean body weight on LacD 21 in the HD group was comparable to the control group.

Food consumption:

Food consumption was quantitatively measured on GDs 6, 9, 12, 15, 18, and 20 and on LacDs 1, 4, 7, 10, 14, 17, and 21.

There was a slight decrease in mean food consumption in the MD and HD group between GD 9 and 18 (1 or 2 g/animal/day less than in the control group).

Necropsy observation:

A complete necropsy examination was conducted for all F₀ females, and tissues with gross lesions along with corresponding sections from the animals from the control group were collected. All surviving F₀ females were euthanized by carbon dioxide inhalation following weaning or euthanasia of the F₁ pups on LacD 21, and the numbers of former implantation sites were recorded for females that delivered. The number of unaccounted-for sites was calculated for each female by subtracting the number of pups born from the number of former implantation sites observed. Females that failed to deliver were euthanized on Postmating Day 25, and uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss.

There were no test article-related macroscopic findings. Slightly increased incidences of dark red areas in apparently injection sites were observed in the test article-dosed groups, but the magnitude was slight.

Reproductive assessment:

During the period of expected parturition, females were observed twice daily for initiation and completion of parturition and for dystocia or other difficulties. All females were allowed to deliver naturally. Beginning on the day parturition was initiated, the numbers of stillborn and live pups were recorded. Individual gestation length was calculated using the date delivery was first observed. The dam and litter remained together until weaning on PND 21.

Female 5285 in the 50 mg/kg/day group had a total litter loss and was subsequently euthanized on LacD 2.

Reproductive data of F₀ females and pup survival rate during the pre-weaning period are shown in Table 10. There were no test article-related effects on the mean gestation lengths, number of F₁ pups born, live litter size, percentage of males per litter at birth, and postnatal survival.

Table 10 Reproductive assessment of F₀ females from the PPND rat study

Parameter	0 (control)	50 mg/kg/day (LD)	100 mg/kg/day (MD)	250 mg/kg/day (HD)
No. of pregnant rats	25	25	25	25
No. of live litters delivered	25	24	25	25
Mean gestation period (days)	21.7	21.6	21.7	21.8
Litter mean no. of implantation sites	12.2	12.6	12.4	12.6
Litter mean no. of total pups born	11.9	11.9	11.8	11.4

Parameter	0 (control)	50 mg/kg/day (LD)	100 mg/kg/day (MD)	250 mg/kg/day (HD)
Litter mean no. of live pups on PND 0	11.9	11.8	11.8	11.4
Sex at birth (%males per litter)	48.4	50.4	54.4	48.5
PND1 mean body weight (g) - males	7.6	7.6	7.7	7.4 (-2.6% ^a)
PND1 mean body weight (g) - females	7.3	7.3	7.3	7.1 (-2.7% ^a)
Postnatal survival on PND 0 (% per litter)	98.7	98.8	99.6	99.2
Postnatal survival on PND 0 to PND 1 (% per litter)	99.7	96.3	99.7	98.0
Postnatal survival on PND 1 to PND 4 (pre-selection; % per litter)	100.0	96.0	99.3	98.9
Postnatal survival on PND 4 (post-selection) to PND 21 (% per litter)	100.0	100.0	100.0	100.0

^a Difference from the control group

Toxicokinetics: Toxicokinetic (TK) analysis was not performed in the study, but the Applicant previously included a TK study in SD rats that were subcutaneously administered NMP [Study TO-15-540] in the NDA re-submission in 2018. The AUC values from this TK study are presented in the section of Integrated Summary and Safety Evaluation below.

**Dosing
Formulation
Analysis:**

Duplicate sets of samples (1.0 mL) for each sampling time point were collected, and samples from the dose formulations prepared on May 18, 2018, June 6, 2018, and June 24, 2018 were analyzed for concentration.

Results of the concentration analyses showed that the concentrations of the prepared dosing formulations ranged from 100% to 105% of the target concentrations. The test article was not detected in the analyzed vehicle formulation for the control group (Group 1).

F₁ Generation**Survival:**

Litters were observed for general health/mortality and moribundity twice daily, once in the morning and once in the afternoon. A daily record of litter size was maintained. On PND 4, litters were culled to keep eight offspring (4 males and 4 females in each litter if possible). Offspring were weaned on PND 21.

For the F₁ generation, 1 male and 1 female/litter were randomly selected prior to weaning for evaluation of attainment of developmental landmarks, neurobehavioral evaluations, and breeding.

There was no test article-related effect on the survival rate of F₁ offspring.

Clinical signs:

The animals were removed from the cage, and a detailed clinical observation was performed on PND 1, 4, 7, 10, 14, 17, and 21 and then weekly following weaning on PND 21.

There were no test article-related clinical signs.

Body weight:

Pups were weighed individually on PND 1, 4, 7, 10, 14, 17, and 21. Following weaning, F₁ animals were weighed individually weekly. Once evidence of mating was observed, female body weights were recorded on GDs 0, 6, 9, 12, and 15.

There were no test article-related effects on mean body weight or body weight gain throughout the study.

The mean pup body weight on PND 1 was slightly lower in the HD group (-2.7%, relative to the control group); however, the decrease was very slight and was not considered test article related.

The mean body weight gain in the HD females was slightly lower after the weaning period (8% decrease relative to the control group between PND 21 and 84), but slightly higher during the gestation period (10% increase relative to the control group between GDs 0 and 15). Because there was no change in males and females in the test article groups during the pre-weaning period in the test article-exposed groups and in males during the post-weaning period, and thus, the slight decrease in the HD female group during the post-weaning period was considered not test article related.

Food

consumption: Not evaluated

Neurological assessment:

- Post-weaning neurobehavioral tests were performed as follows:
- (1) Auditory startle response on PND 60: For each trial, auditory startle responses were recorded during the first 100 ms following the onset of the startle stimulus. Each test session consisted of 50 trials, with 8 sec between each trial. Startle response data were analyzed in 5 blocks of 10 trials each. Startle response measurements obtained were PEAK (peak response amplitude) and Tpeak (latency to PEAK).
 - (2) Motor activity on PND 61: The animals were tested using a series of infrared photobeams to quantify each animal's motor activity. Data were collected in 5 min epochs over a 60-min period, with reporting in 10-min subintervals for total motor activity (defined as a combination of fine motor skills; i.e., grooming; interruption of 1 photobeam) and ambulatory motor activity (e.g., interruption of 2 or more consecutive photobeams).
 - (3) Learning and memory beginning on PND 62: Swimming ability and learning and memory were assessed using a water-filled 8-unit T-maze. Learning and memory testing regimen was presented below. For the learning and memory phases, the time required to traverse the maze and the numbers of entries into an error zone for all trials were recorded. The time to escape (all phases) and the number of errors (Phases 2 and 3) were evaluated.

Learning and memory testing regimen

Testing Phase	Testing Day	Test(s) Performed
Phase 1	Day 1	4 trials to escape from a straight channel
Phase 2	Days 2 and 3	2 trials/day in path A
	Days 4, 5, and 6	2 trials/day in path B (reverse of path A)
Phase 3	Day 7	2 trials in path A

There were no test article-related effects on any neurobehavioral assessments evaluated in the study.

Reproduction: For sexual maturation assessment, each male pup was observed for balano-preputial separation beginning on PND 35. The age and body weight at which balano-preputial separation was first observed were recorded for each pup. Similarly, each female pup was observed for vaginal perforation beginning on PND 25. The age and body weight at which vaginal patency was first observed were recorded for each pup.

Vaginal lavages were performed daily for 14 days prior to the initiation of the mating period and continuing until evidence of mating was observed or until the end of the mating period. The slides were

microscopically examined to determine the stage of the estrous cycle. The average cycle length was calculated for complete estrous cycles.

The F₁ animals were paired on a 1:1 basis within each group, avoiding sibling mating. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. Vaginal lavages were performed daily during the mating period until evidence of mating was observed. If evidence of mating was not apparent after 7 days, the female was placed with another male that had successfully mated for an additional 7 days. If evidence of mating was not apparent after 14 days of cohabitation, the animals were separated without further opportunity for mating.

The females in the HD group exhibited delayed vaginal patency (PND 35.2 with the mean body weight of 130 g), compared to the control group (PND 31.4 with the mean body weight of 114.3 g; Table 11). These high mean values in the HD group (day and body weight of acquisition) were due to six females with delayed vaginal patency with relatively high body weight as shown in Table 13. However, these females exhibited normal estrous cycle lengths and mating performance, except for one female with a high pre-implantation loss.

Vaginal patency is an indicator of pubertal onset, which is positively correlated with body weight, and substantial changes in growth can alter the day of acquisition. Aside from effects on growth, estrogenic and anti-estrogenic compounds can dramatically influence the day on which vaginal opening is first observed (Beck et al., 2012). For instance, testosterone has been demonstrated to significantly delay vaginal patency and increase body weights at vaginal patency and also cause marked effects on estrous cycles and other reproductive parameters (Kim et al., 2002). In contrast to the effects of testosterone on female reproductive parameters, NMP only resulted in delayed vaginal patency at the HD, without affecting any other reproductive parameters. Thus, delayed vaginal patency observed at the NMP HD is considered test article related, but not adverse.

There were no other test article-related effects on sexual maturation or mating parameters examined in the study (see Tables 12 and 13).

Table 11 Mean data of vaginal patency and estrous cycle lengths from the PPND study and the conducting facility's historical control data (HCD)

Parameter	0 (control)	50 mg/kg/day (LD)	100 mg/kg/day (MD)	250 mg/kg/day (HD)	HCD Mean (min/max)
N	25	24	25	25	
Vaginal Patency (PND)	31.4	32.5	32.6	35.2**	32.7 ^a (31.1/34.4)
Body weight at acquisition (g)	114.3	118.7	120.4	130.5**	114.3 ^a (99.3/131.9)
Estrous cycle lengths (days)	4.1	4.2	4.3 ^b	4.1	4.3 ^c (3.7/ 5.0)

** = Significantly different from the control group at 0.01

^a Data from 56 studies with 110 control groups

^b The mean estrous cycle length was obtained from 24 females in the MD group.

^c Data from 55 studies with 57 control groups

Table 12 Individual data of F₁ females in the HD group (250 mg/kg/day) with delayed vaginal patency

Animal No	5241-09	5267-07	5271-09	5269-10	5286-11	5299-10
Vaginal Patency (PND)	165.0	154.8	171.8	161.4	144.9	194
Body weight at acquisition (g)	39	38	46	37	37	51
Mean length of estrous cycle (days)	4.0	4.0	4.0	4.0	4.0	5.0
No of corpora lutea	16.0	15.0	16.0	20.0	16.0	16.0
No of implantation site	16.0	14.0	16.0	19.0	14.0	6.0
% of total resorption	0	14.3	6.3	10.5	0	0
% of live embryo	100	85.7	93.8	89.5	100	100
% of pre- implantation loss	0	6.7	0	5.0	12.5	62.5
% of post- implantation loss	0	14.3	6.3	10.5	0	0

Table 13 Reproductive assessment of F₁ animals from the PPND study

Parameter	0 (control)	50 mg/kg/day (LD)	100 mg/kg/day (MD)	250 mg/kg/day (HD)
Male mating index (%) ^a	92.0 (23/25)	95.8 (23/24)	95.8 (23/24)	100 (24/24)
Female mating index (%) ^a	100 (25/25)	100 (24/24)	100 (25/25)	100 (25/25)

Male fertility index (%) ^b	84.0 (21/25)	91.7 (22/24)	95.8 (23/24)	95.8 (23/24)
Female fertility index (%) ^c	92.0 (23/25)	95.8 (23/24)	100 (25/25)	96.0 (24/25)
Mean litter no. of corpora lutea	16.8	17.3	16.8	16.3
Mean litter no. of implantation site	14.7	15.8	15.5	14.1
Total resorption per litter (%)	3.6	3.8	5.1	4.0
Live fetuses per litter (%)	96.4	96.2	94.9	96.0
Mean preimplantation loss per litter (%)	13.4	7.6	7.3	12.5
Mean postimplantation loss per litter (%)	3.6	3.8	5.1	4.0

^a Mating index (%) = (No. of males (females) with evidence of mating (or confirmed pregnancy)/No. of males (females) used for mating) x 100

^b Male fertility index (%) = (No. of males siring a litter/Total no. of males used for mating) x 100

^c Female fertility index (%) = (No. of females with confirmed pregnancy/Total no. of females used for mating) x 100

Other: A necropsy was conducted for animals that died on study. Intact offspring that were found dead during PND 0-4 were necropsied using a fresh dissection technique, which included examination of the heart and major vessels. Unselected offspring on PND 4 were euthanized by intraperitoneal injection of sodium pentobarbital and discarded. A gross necropsy was performed on any pup found dead after PND 4.

On PND 21, each non-selected F₁ pup was euthanized by carbon dioxide inhalation and was subject to a complete necropsy examination. Gross lesions were collected and preserved in 10% neutral buffered formalin. Females with no evidence of mating were euthanized on post-cohabitation Day 7.

All surviving females were euthanized by carbon dioxide inhalation on GD 15 and the males were euthanized thereafter. All animals were subject to a complete necropsy examination, and uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss. Tissues with gross lesions along with the corresponding tissues from the control group were collected.

There were no test article-related macroscopic findings.

For the F₂ generation, only viability of fetuses was evaluated on GD 15 as shown in Table 13, and there was no test article-related effect on F₂ fetal viability.

11 Integrated Summary and Safety Evaluation

BRIXADI, referred to during development as CAM2038, was developed for the treatment of opioid use disorder by Braeburn Inc. A 505(b)(2) NDA for BRIXADI was originally submitted on July 9, 2017, but the NDA was not approved due to numerous deficiencies. Subsequently, the NDA was resubmitted on June 26, 2018, and after the review of the resubmission, there still remained several outstanding nonclinical issues, including the lack of an adequate female fertility study for NMP, pre- and post-natal development (PPND) study for NMP, an adequately rigorous elemental impurity assessment for the container closures system, and several outstanding issues with the extractable leachable studies, including unidentified leachables from the container closure system. Note that the Applicant had originally submitted only published and unpublished studies with NMP in lieu of conducting new studies to address the reproductive and developmental toxicology requirements for this program. The published studies employed oral or inhalation routes but not the clinical route of administration and demonstrated that NMP can have adverse effects on male and female fertility and fetal development. During the second review cycle, oral PK data were provided in the rat that suggested that the adverse effects in males occurred at doses greater than 108 times the level of NMP at the maximum recommended human dose (MRHD) of BRIXADI based on AUC comparison. While this adequately addressed the male fertility assessment, definitive studies to address female fertility and PPND endpoints were still considered warranted. However, given the reasonably high exposure margins based on the data available at that time, and the risk:benefit of this drug product, the outstanding issues were allowed to be completed as postmarketing requirements. A tentative approval was granted for BRIXADI on December 21, 2018 with these nonclinical studies to be addressed as postmarketing requirements.

In the current NDA amendment (SD 113 dated June 1, 2020), the Applicant submitted a request for Final Approval of NDA 210136. With this amendment, the Applicant submitted two nonclinical postmarketing requirement studies, which include the fertility and early embryonic development study in female rats and a pre- and post-natal development (PPND) study in rats testing subcutaneous doses of NMP. In these studies, TK analyses were not included, but the Applicant previously included a TK study in SD rats that were subcutaneously administered NMP [Study TO-15-540] in the NDA resubmission in 2018. The AUC data from this TK study are presented in Table 14.

Table 14 Mean AUC values of NMP from Study TO-15-540

Group	Dose (mg/kg)	Gender	AUC _{0-t} (hr*ug/ml)	SE_AUC _{0-t} (hr*ug/ml)	AUC _{inf} (hr*ug/ml)	AUC _{inf} /D (hr*kg*ug/ml/mg)
1	100	Male	431	49	433	4.33
		Female	709	124	710	7.10
		Both	570	76	571	5.71
2	250	Male	1468	66	1468	5.87
		Female	2495	276	2496	9.99
		Both	1984	230	1986	7.94
3	500	Male	4002	258	4004	8.01
		Female	5674	572	5676	11.35
		Both	4838	403	4840	9.68

n=5/sex for each study Group. Blood sampling for toxicokinetics was performed using a spares sampling scheme with n=3/sex/time point for each Group.

In addition, the Applicant submitted a nose-only inhalation toxicokinetic (TK) study of NMP in pregnant rats to determine the systemic exposure of NMP in the published embryo-fetal toxicity studies in rats administered inhaled doses of NMP in the current NDA amendment. These three submitted animal studies were evaluated in this review, and the overall reproductive and developmental toxicity data that are supported for use of NMP in the BRIXADI (monthly) formulation are summarized herein. For the purposes of safety margin comparison, the AUC value of 6.61 mcg•h/mL, which was estimated human exposure to NMP at the maximum recommended human dose (MRHD) of 128 mg BRIXADI (monthly) by the Applicant, is used in this review. The new information submitted under SD 113 is described in **bold font**.

Male Fertility:

The male fertility assessment of NMP was reviewed during the first review cycle, and in the second cycle, the Applicant completed an oral rat PK bridging study to estimate systemic exposures via the oral route [Study PK-18-611]. In the published study by Sitarek & Stetkiewicz, 2008, male rats were orally administered NMP at doses of 100, 300, or 1000 mg/kg/day for 10 weeks prior to mating, and males were mated with untreated females then necropsied and epididymis and testis were fixed for histopathological evaluation. The individual stages of spermatogenesis were assessed. Viability and development of offspring were observed up to 28 days. The doses of 100 and 300 mg/kg did not significantly alter fertility or spermatogenesis. However, significantly lower viability of the offspring in the first four days of life was seen at 300 mg/kg and above. Infertility and extensive damage to the seminiferous epithelium and the seminal tubule of the testis were observed at 1000 mg/kg (1573 times the human maximum daily dose (MDD) of NMP delivered by BRIXADI (monthly) at the MRHD on an AUC basis). The NOEL for male fertility is 300 mg/kg (464 times the human MDD) although the NOEL for paternal effects of prenatal development of the progeny is 100 mg/kg which is 108 times the human MDD. This study appears to contain all the primary endpoints of a male fertility study. No additional male fertility data were deemed necessary after the second cycle review.

Female Fertility:

With this amendment, the Applicant submitted a newly conducted GLP rat fertility and early embryonic development (FEED) toxicity study [Study TO-18-620] in which female Sprague Dawley rats (25/sex/group) were administered NMP at subcutaneous doses of 0 (vehicle), 50, 100, or 250 mg/kg/day. Females were administered once daily for 14 days prior to cohabitation and continued through GD 7, and laparohysterectomies were performed on GD 15. There were no NMP-related effects on female reproductive parameters and embryonic developmental parameters in this study. The NOAEL for maternal toxicity, female fertility, and early embryonic development was the high-dose of 250 mg/kg/day, corresponding to an AUC value of 2495 mcg•h/mL in female rat (377 times the human MDD of NMP delivered by BRIXADI (monthly) on an AUC basis).

Published female fertility studies were submitted in previous review cycles. The Solomon et al. 1995 study (Solomon, Burgess, Kennedy, & Staples, 1995) evaluated female fertility as part of their two-generation study in rat. Females were exposed to whole-body inhalation of 10, 51, or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg/day) for 6 h/day, 7 day/week, from age 34 days to weaning with interruption between GD 20 to Day 4 post-partum (134 days total). Treated males were mated with treated females. Female mating and fertility indices were assessed, and both were based only on reproductive performance (i.e., number of rats copulating or bearing litters). The uterus was weighed, and reproductive organs were examined for gross lesions. Live and dead fetuses, resorptions, corpora lutea, and fetal weights were evaluated. The female fertility parameters that were evaluated appeared to be adequate in this study and no NMP-related effects were observed. The NOEL for female fertility is the high-dose of 116 ppm, and based on the PBPK modeling, the exposure of 116 ppm would correlate with an AUC value of 642.2 mcg•h/mL, which would be 97 times the human MDD of NMP via BRIXADI (monthly) on an AUC comparison.

In the Sitarek et al., paper (Sitarek, Stetkiewicz, & Wasowicz, 2012), treated female rats were treated with oral NMP 150, 450, or 1000 mg/kg/day for 5 days/week for 2 weeks before mating, during mating, and through gestation and lactation. The fertility index was significantly lower at 450 and 1000 mg/kg. No standard early embryonic endpoints were examined (only fertility index = percentage of pregnant females in mating female evidence of early resorptions indicating intrauterine mortality of the embryos). The NOEL for fertility in this study is 150 mg/kg. The authors note the 150 mg/kg dose “slightly toxic” (body weight changes) to the dams. It should be noted that although there was no effect on female fertility parameters at 150 mg/kg, pup survival was decreased at this dose. Both the index of viability (% of pups born alive that survived to 4 days) and index of lactation (% of pups alive at 4 days that survived to 21 days) were significantly lower than control for all treated groups. The NOEL for female fertility is 150 mg/kg, which is 242 times the AUC based on the PK bridging data. No NOAEL for pup effects can be reported. Given the limited endpoints, this study is technically not an adequate full assessment of early embryonic development.

Note that the dosing periods of the published papers describing above were much longer than the standard FEED study like the one that was submitted under SD 113. Thus, the exposure margin determined by the newly submitted FEED study should be more appropriate for female fertility and early embryonic developmental risk assessment of NMP. Given the large safety margin established by the GLP female fertility study at 377 times the human exposure, no labeling language is warranted.

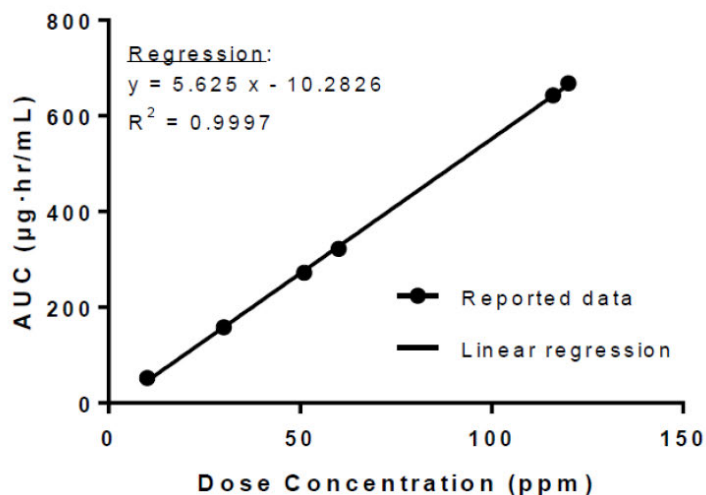
Embryofetal Development (EFD) in Rat:

The Applicant provided published studies with the original NDA submission to address the embryofetal developmental toxicity of NMP. The published studies in rat employed the dermal, oral, and inhalation routes of administration and demonstrated that NMP causes embryo- and fetotoxicity as well as malformations. NMP also causes maternal toxicity. In the second cycle, the Applicant completed an oral rat TK study to estimate exposures via the oral route [Study PK-18-611] and attempted to estimate inhalation exposure using a PBPK model. With this resubmission (SD 113), the Applicant submitted a bridging rat inhalation TK study [Study PK-18-624] to assess systemic exposure levels at the doses tested in the published inhalation EFD study which could be used to derive exposure margins for the adverse findings observed in the animal studies relative to the levels of NMP in the maximum recommended human dose (MRHD) of BRIXADI. The newly conducted bridging inhalation TK study is described below and then the published studies are summarized with updated margins based on AUC comparison.

In the newly submitted bridging inhalation TK study [Study PK-18-624], pregnant Sprague Dawley rats (8/group) were administered NMP at the target doses of 51, 89, or 165 ppm via nose-only inhalation for 6 hours daily during GDs 6–10. These doses were tested by Hass et al (1995); however, the inhalation rat studies of NMP by Hass et al. used whole-body exposure. Whole-body inhalation would have other routes of exposure such as oral (fur licking) and dermal routes, and thus overall systemic exposure to test article tends to be higher for the whole-body inhalation, compared to the nose-only inhalation (Wong, 2007). Because nose-only inhalation was used for the bridging TK study, the systemic exposure obtained from this bridging study was likely underestimated for the systemic exposure that would be obtained from the whole-body inhalation studies at the same dose level if the exposure condition such as particle sizes, drug concentrations, and drug formulations was comparable [email communication with Dr. Pei Luqi from FDA]. Thus, the Reviewer concludes that it is acceptable to use the TK data from the bridging TK study for the exposure margin calculations of NMP. The $AUC_{(0-t)}$ values of NMP were 99.7, 301, and 4510 mcg•h/mL for the 51, 89, and 165 ppm dose levels, respectively. The AUC increase was 3-fold from 51 ppm to 89 ppm (1.7-fold increase in dose level) and 15-fold from 89 ppm to 165 ppm (1.9-fold increase in dose level), indicating that the increase in exposure to NMP via the route of inhalation is not linear. Notably, during the second review cycle, the Applicant provided estimated exposure values of NMP in rats following nose-only inhalation using a physiologically-based pharmacokinetic (PBPK)

model, and these data showed a linear increase in AUC (see the below figure), which was not observed in the actual TK data provided in SD 113.

Relationship between NMP AUC and Dose Based on a Physiologically-Based Pharmacokinetic Model after Inhalation Dosing



Based on the rat bridging inhalation TK data and available human PK data, we can now estimate that the preimplantation losses, delayed ossification and reduced fetal body weights reported by Hass et al. following treatment of pregnant rats during gestation with NMP via inhalation at 165 ppm (**corresponding AUC value of 4510 mcg·h/mL**) **occurred at approximately 682** times the human MDD of NMP delivered by BRIXADI (monthly) on an AUC basis. Similar findings of reduced ossification and decreased fetal body weights reported in a published report by Exxon Biomedical Sciences (1992) at a 125 mg/kg oral dose, or 159 times the MDD of NMP based on AUC comparison. In addition, fetal malformations and resorptions were observed following oral administration of 250 mg/kg (388 times the human MDD) in a published study by Saillenfait et al. (2002). These individual studies are briefly summarized in the following paragraphs.

Hass et al. treated rats 0 or 165 ppm NMP via whole body inhalation on GD 4-20 (Hass et al., 1995) for 6 hours per day. Increased pre-implantation loss, delayed ossification of the skeleton and decreased fetal body weight was observed in the absence of maternal toxicity. No NOAEL can be established in this study. Previously, using the PBPK model, the exposure to 165 ppm would correlate with an AUC of 918 mcg·h/mL (139 times the human MDD based on AUC). **However, according to the TK data from the bridging inhalation TK study, the target inhaled dose of 165 ppm yielded an AUC value of 4510 mcg·h/mL, which is 682 times the human MDD of NMP delivered by BRIXADI (monthly) on an AUC basis.**

Exxon Biomedical Sciences (1992) conducted an embryofetal development study in which pregnant female rats were administered NMP via oral gavage at doses of 40,125,

or 400 mg/kg/day from GD 6 to 15. There was no clear evidence of maternal toxicity at any dose although the 400 mg/kg group did have reduced uterine weights which indirectly impacted overall body weight gain in that group. Fetal body weights were reduced in high-dose animals by approximately 10-11% compared to controls. Mid-dose fetal body weights were reduced by 3%. There was also an increase in the number of “stunted fetuses” in the 400 mg/kg group compared to controls and reduced ossification in the hindlimb proximal phalanges in the 400 mg/kg group as well. The NOAEL for maternal and fetal effects was reported to be the mid-dose of 125 mg/kg (the AUC value of 1050 mcg•h/mL; 159 times the human MDD based on AUC). A conservative evaluation given the slight decrease in fetal body weights in the mid-dose group would declare the low-dose of 40 mg/kg the NOAEL (the estimated AUC value of 380 mcg•h/mL; 57 times the human MDD based on AUC).

Saillenfait et al. orally administered 125, 250, 500, or 750 mg/kg/day NMP to pregnant rats from GD 6-20 (Saillenfait et al., 2002). Maternal toxicity (decreased body weight gain and food consumption) was evident at 500 and 750 mg/kg. NMP increased resorptions at these same doses. Fetal malformations (imperforate anus, absence of tail, anasarca, malformations of the great vessels and cervical arches) were noted at 500 and 750 mg/kg as well and one fetus in the 250 mg/kg group showed similar evidence of teratogenicity. Decreased fetal weights were evident at 250 mg/kg and above. The maternal NOAEL was 250 mg/kg (388 times based on an AUC basis); however, the developmental NOAEL was 125 mg/kg (159 times the human MDD based on an AUC basis).

Although the cited studies were technically not GLP, collectively they provide replicative data to suggest that the adverse effects of NMP occur at very high exposures compared to the levels that appear to occur with the BRIXADI (monthly) drug product. The nonclinical team has concluded that no further rat EFD studies are necessary given these data.

Embryofetal Development in Rabbit:

The Applicant submitted a 1991 study report by the International Research and Development Corporation of a rabbit study which tested 0, 55, 175, and 540 mg/kg/day oral NMP daily from GD 6-18 with the original NDA submission. The study was conducted in accordance with GLPs and appeared to evaluate the appropriate embryofetal development endpoints and the line listings of the data were provided. In the second cycle submission, the Applicant submitted an oral PK study in pregnant rabbits [Study PK-18-610]. The AUC values in pregnant rabbits were 124, 775, and 3860 mcg•h/mL at 55, 175, and 540 mg/kg oral doses, respectively. Maternal toxicity (statistically significant decreases in body weights, reduced food consumption) was observed at 175 and 540 mg/kg. Postimplantation loss, increased incidence of cardiovascular malformations and skull malformations were observed at 540 mg/kg (584 times the human MDD of NMP via BRIXADI ONCE MONTHLY based on an AUC comparison). The NOEL for developmental toxicity in this study is 175 mg/kg, which is 117 times the human MDD of NMP via BRIXADI (monthly) based on an AUC comparison. Maternal toxicity was present at this dose. No new EFD studies were

submitted with this amendment as the published information previously submitted were considered adequate.

Pre- and Postnatal Development in Rat:

With this amendment, the Applicant submitted a newly conducted GLP pre- and postnatal developmental (PPND) toxicity study [Study TO-18-619] in which time-mated Sprague Dawley rats (F₀; 25/group) were administered NMP via once daily SC injection at doses of 0 (vehicle), 50, 100, or 250 mg/kg/day from GD 6 through Lactation Day (LacD) 21. There were decreased body weight gain in F₀ females at 250 mg/kg between GD 6 and 20 (17% decrease, relative to the control group). The F₁ females at 250 mg/kg exhibited delayed vaginal patency (Postnatal Day [PND] 35.2 with the mean body weight of 130 g), compared to the control group (PND 31.4 with the mean body weight of 114.3 g). These high mean values in the 250 mg/kg group (day and body weight of acquisition) were due to six females with delayed vaginal patency with relatively high body weights. However, these females and all other females in the 250 mg/kg group exhibited normal estrous cycle lengths and mating performances and thus, the finding of the delayed vagina patency in the 250 mg/kg group was not considered adverse. There were no other NMP-related effects on pre- and postnatal development and reproductive performance in F₁ animals. There were no NMP-related effects on viability of F₂ fetuses in any dose groups, as examined on GD 15. The NOAEL for maternal toxicity of F₀ animals was the mid-dose of 100 mg/kg/day, corresponding to the AUC value of 709 mcg•h/mL (107 times the human MDD of NMP delivered by BRIXADI (monthly) on an AUC basis) because of the decreased body weight gain at 250 mg/kg during the gestation period. The NOAEL for pre- and postnatal development of F₁ animals was the high-dose of 250 mg/kg/day, corresponding to an AUC value of 2495 mcg•h/mL which is 377 times the human MDD of NMP delivered by BRIXADI (monthly) on an AUC basis. Because of the delayed vaginal patency at 250 mg/kg/day, the NOEL for pre- and postnatal development was the mid-dose of 100 mg/kg/day.

The Hass et al. study treated pregnant rats via whole body inhalation to 0 or 150 ppm NMP for 6 h/day on GD 7-20 (Hass, Lund, & Elsner, 1994). The authors state that this dose level was chosen so as not to cause maternal toxicity or decrease viability of the offspring. Although the study did not include exposures during lactation, the offspring were allowed to be born and were evaluated via functional assessments, similar to a PPND study. Exposed offspring showed decreases in body weight and had delayed physical development. Performance in tasks including the reversal procedure (retesting with a new platform location) in the Morris water maze and operant delayed spatial alteration (Skinner boxes) was impaired in exposed offspring. No differences between NMP exposed animals and control animals were seen in motor function (rotarod), activity level (open field), and learning tasks of low complexity. No NOAEL could be established for PPND parameters. Based on the PBPK modeling, the exposure to 150 ppm for 6 h/day is estimated to result in an AUC of 833 mcg•h/mL which is 126 times the human MDD of NMP via BRIXADI (monthly). **However, in the inhalation TK bridging study in pregnant rats, the AUC_(0-t) value of NMP at 165 ppm was 4510**

mcg•h/mL, which provides 682 times the human MDD. The NMP dose of 150 ppm via whole-body inhalation would likely to yield the AUC value close to 4510 mcg•h/mL rather than the estimated AUC value of 833 mcg•h/mL from the PBPK modeling.

Sitarek et al. treated female rats with oral NMP 150, 450, or 1000 mg/kg/day for 5 days/week 2 weeks before mating, during mating, and through gestation and lactation (Sitarek et al., 2012). The authors considered the 150 mg/kg dose “slightly toxic” (body weight changes) to the dams. The incidence of stillbirths was increased at 1000 mg/kg. At 150 mg/kg there was no effect on fertility, but pup survival was decreased. As noted above, both the index of viability (% of pups born alive that survived to 4 days) and index of lactation (% of pups alive at 4 days that survived to 21 days) were significantly lower than control for all groups. In terms of typical PPND endpoints the offspring were only evaluated for general appearance, litter weight, mean pup weight, and mortality from birth to PND 21. As such, there are limited PPND endpoints in this report. The low-dose of 150 mg/kg was 242 times the AUC based on the TK bridging data. However, no NOAEL for these PPND endpoints was noted in this study.

Note that all three studies that evaluated pre- and post-natal developmental toxicity of NMP summarized here have different dosing durations. In the Applicant’s conducted PPND study, pregnant rats were subcutaneously administered from GD 6 to LacD 21, and the NOAEL for pre- and postnatal development was 250 mg/kg (377 times the human MDD). In the Hass et al study, pregnant rats were administered NMP via whole body inhalation to 0 or 150 ppm from GD 7 to GD 20, and no NOAEL could be established for PPND parameters at 150 ppm (likely slightly lower than 682 times the human MDD). In the Sitarek et al study, female rats were orally administered NMP at 150, 450, or 1000 mg/kg for 5 days/week 2 weeks before mating, during mating, and through gestation and lactation, and no NOAEL could be determined for PPND parameters. Even at the low dose of 150 mg/kg, the exposure margin only provided 242 times the human MDD on an AUC comparison, which is lower than the exposure margin at the NOAEL in the subcutaneous PPND study (377 times the human MDD), but note that F₀ females in the Sitarek study were dosed considerably longer than F₀ females in the PPND study.

When the exposures are compared between the subcutaneous PPND study and the oral EFD rat studies in the literature, the NOAEL of 250 mg/kg in the subcutaneous PPND study yielded the AUC value of 2495 mcg•h/mL, and this was higher than the AUC levels that caused fetal toxicity in the Exxon study. In this Exxon study, where the pregnant rats were orally dosed NMP from GD 6 to 15, there were an increase in the number of “stunted fetuses” and decreased fetal body weights (~10-11% compared to controls) at 450 mg/kg and decreased fetal body weight (3%) at 125 mg/kg (the AUC value of 1050 mcg•h/mL; 159 times the human MDD based on AUC). A conservative evaluation given the slight decrease in fetal body weights in the MD group declared the LD of 40 mg/kg the NOAEL (the estimated AUC value of 380 mcg•h/mL; 57 times the human MDD based on AUC). In the Saillenfait et al. study in which pregnant rats were orally administered from GD 6-20, NMP caused increased resorptions, decreased fetal

weight, and fetal malformations (imperforate anus, absence of tail, anasarca, malformations of the great vessels and cervical arches) at 500 and 750 mg/kg. At 250 mg/kg (the AUC value of 2570 mcg•h/mL; 389 times the human MDD based on AUC), one fetus also showed similar malformations and decreased fetal weights (~10%) were also observed. The NOAEL for embryo-fetal development was 125 mg/kg (159 times the human MDD based on an AUC basis). Collectively, the NMP dose of 250 mg/kg (AUC value of ~ 2500 mcg•h/mL) started to manifest test article-related effects that could be defined LOAEL even though very slight decrease in fetal body weight was noted at the oral dose of 125 mg/kg. At higher doses, NMP causes developmental toxicity, including malformation and decreases in survival and offspring body weights, delayed physical development, and impaired learning and memory.

During the previous review cycles, we recommended that the following statement regarding NMP be included in labeling under the 8.1 Pregnancy section based on the information available at that time as follows:

“Decreased pup survival was noted following oral treatment of female rats prior to and during gestation with NMP, an excipient in BRIXADI (monthly) at doses 242 times the MDD of NMP delivered by BRIXADI (monthly) on an AUC basis. A No Adverse Effect Level (NOAEL) was not demonstrated.”

The above statement describes the findings from the Sitarek et al. (2012) study, and in this study, female rats were orally administered NMP for 5 days/week 2 weeks before mating, during mating, and through gestation and lactation. As such, the dosing duration of this study essentially encompassed all dosing periods that three typical segmented studies covered. The segmented developmental toxicity studies of NMP tended to manifest toxicity at higher dose levels because the dosing duration was shorter than the Sitarek et al. study, and some of these segmented studies identified the NOAELs. **Specifically, the submitted PPND study in rats following the SC injection of NMP determined the NOAEL and provided a sufficient safety margin for the developmental toxicity of NMP. Similarly, the submitted FEED study in female rats following the SC injection of NMP identified the NOAEL and provided a sufficient safety margin for female fertility and early embryonic developmental toxicity. In the previous submission, the EFD studies of NMP in rats and rabbits also identified the NOAEL. Overall, the reproductive and developmental toxicity assessment of NMP has been adequately evaluated for use of NMP as an excipient in BRIXADI (monthly) at the human MDD.** (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 REVIEW AND EVALUATION

Application number: 210136

Supporting document/s: SDN 1, 2, 9, 10, 29, 79, and 81

Applicant's letter date: 5/8/2017, 7/19/2017, 8/23/2017, 8/24/2017,
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CDER stamp date: 5/8/2017, 7/19/2017, 8/23/2017, 8/24/2017,
10/13/2017, 5/23/2018, and 6/5/2018

Product: CAM2038 (q1w and q4w)

Indication: Opioid use disorder

Applicant: Braeburn Pharmaceuticals, Inc.

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

Reviewer: Elizabeth Bolan, PhD, Gary Bond, PhD, and
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Disclaimer

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1 Executive Summary

1.1 Introduction

Braeburn Pharmaceuticals submitted a 505(b)(2) NDA in support of their depot buprenorphine drug product BRIXADI, referred to during development as CAM2038, for the treatment of opioid use disorder. The NDA relies upon an FDA-previous finding of safety for Subutex (buprenorphine sublingual) and literature. There are actually two drug product formulations in this NDA, a once weekly (q1w) and a once monthly (q4w) formulation. The formulations (CAM2038 q1w and CAM2038 q4w) primarily differ (b) (4). During development, the Division noted that as per any depot formulation development program, the fate of the injected material should be adequately characterized and any novel excipients should be adequately justified for safety as per the FDA guidance document on new excipients. At the time of original submission, the drug product contained two new excipients. First, the once monthly injection contains N-methyl-pyrrolidone (NMP) which during development was considered to be used in a new manner via this product (NMP was previously used in an FDA-approved product for acute dental use and a product indicated for the treatment of advanced cancer). Both formulations contain glycerol dioleate (GDO), a diglyceride, which has also not previously been used in an FDA-approved drug product. Of note, the Agency recently approved a chronic depot injection product for a non-oncology indication that contains NMP at levels approximately (b) (4) times higher than this drug product with post-marketing requirements and labeling for potential adverse effects due to the NMP in that formulation. This review focuses on the safety of the CAM2038 formulations, and the two novel excipients (which are reviewed in Appendix 1 and 2).

1.2 Brief Discussion of Nonclinical Findings

There are no safety concerns with the drug substance or drug product specifications.

The components of the container closure system that contact the drug product primarily include a (b) (4) glass syringe with a (b) (4) rubber plunger stopper. Importantly, the drug products include (b) (4)

In the first cycle, some extractables and leachables data were submitted to characterize and justify the safety of potential leachables arising from the to-be-marketed container closure system and from manufacturing components that directly contact the drug product. However, working closely with the CMC review team, we concluded that the extraction studies with the container closure system were not performed appropriately to give us confidence that the potential leachables profile of the container closure system was fully characterized. In the second cycle, the Applicant submitted new extractable and leachable data to address the deficiencies identified in the first cycle. Overall, the studies were more robust than the previous submissions and took into account our

recommendations from the first cycle review. The extractable study was used to inform the leachables study and the safety of the majority of the leachables identified was adequately addressed. However, the extractable leachable testing was still considered deficient in three major regards: 1) the Applicant did not conduct new extractable/leachable testing for inorganic compounds; instead they are relying on their previous analysis which we identified as inadequate (incorrect solvent for glass) and 2) there were at least four unidentified compounds above the safety concern threshold of 5 mcg but adequate justification for the efforts made to identify their structures and their safety were not provided, and 3) we cannot rely on the reported levels of two identified compounds detected above 5 mcg/day because validated methods were not employed. While these deficiencies need to be addressed by the Applicant, they can be addressed as a post marketing requirement because: 1) the majority of the identified leachables were adequately qualified, 2) the levels of the unidentified compounds in the formulations were predicted to be low ($\leq$ (b) (4) mcg/dose), 3) the product is not dosed daily (either once a week or once a month), 4) the product has the potential to help address a significant public health crisis, and 5) while inorganic extractable/leachable data was not ideal, data from the 6-month stability time point are available with the drug products indicating that the levels of inorganic compounds were within acceptable levels in the drug product and (b) (4) are not expected to significantly increase over time.

In terms of general toxicity, the Applicant is relying upon the Agency previous finding of safety of Subutex to address general toxicity of buprenorphine. As noted in the clinical pharmacology review, technically, the 128 mg (b) (4) (b) (4) proposed buprenorphine drug product q4w result in systemic exposures to buprenorphine that exceed the referenced drug product Subutex. In general, this would require general toxicity studies in two species (b) (4)

The 9-month dog study with the clinical formulation discussed below does provide general toxicity data for buprenorphine in the nonrodent species. The Applicant did not provide a chronic rat toxicology study for buprenorphine. Although the 128 mg q4w dose also exceeds the exposure from 24 mg dose of Subutex, the Applicant cited the fact that the referenced drug product Subutex contains long-term carcinogenicity data in mice and rats. The Division has concluded that there are adequate clinical data on buprenorphine such that general toxicity in two species are not necessary to support this proposed dose.

As with any depot formulation, the Applicant was required to address the fate of the injected materials. The Applicant evaluated the effects of q1w and q4w following SC injection in the dog model and q1w in the minipig model. In addition, local effects were also recorded in the 9-month dog study discussed below. The results indicate that following injection of either formulation primarily swelling at the injection site and tenderness was observed with little evidence of erythema or hemorrhage. Swelling lasted around 2-3 weeks in most cases and was resolving by around 30 days; however, longer duration reactions were also noted in some animals. Microscopically, the injection sites were characterized by mononuclear cell infiltration, cystic spaces at times

surrounded by inflammatory cells, fibrosis, hemorrhage and eosinophilic material. Collectively, the data suggest that the depot can last up to 30 days or longer and local tissue reactions are consistent with a foreign body reaction and the evidence that the body is attempting to clear the material from the injection site.

The Applicant conducted a 9-month subcutaneous repeat-dose toxicology study in the dog model testing both the q1w and the q4w formulations injected over rotating sites which provides adequate local and systemic safety justification for the proposed drug product in this model. From a local tissue perspective, CAM2038 q1w and q4w product local injection site produced swelling and thickening of the skin with both drug products and drug product vehicles. The swelling was the result of both the remaining depot material as well as the expected local tissue response to the depot (inflammatory cell infiltrates). Microscopically, the local injection site demonstrated minimal fibrosis and mononuclear cell infiltrates surrounding cystic spaces (likely residual injection depot matrix material) and evidence of chronic inflammation. In addition, there were changes in the axillary (draining) lymph nodes that included small cystic spaces (likely lipid) with "little to no" associated tissue response. The chronic tissue response at older injection sites consisted of cavitation of the tissue with mononuclear and multinuclear giant cells. Collectively, these changes are consistent with a foreign body reaction to a depot injection.

Of note, over the course of development, the Applicant did not evaluate the impact of repeated injections for the q4w formulation into the same site. Rather, they established a dosing rotation scheme to prevent the material from being injected into the same site prior to adequate local tissue assessments. The proposed labeling, however, states that there is no rotation schedule necessary for the q4w product as follows:

SELECTING AN INJECTION SITE

The areas for subcutaneous injection are highlighted in Figure 5. BRIXADI should not be administered to the same site of injection for at least 8 weeks for (b) (4) No injection site rotation is required for (b) (4)

BRIXADI should be injected slowly, into the subcutaneous tissue (b) (4)

The proposed injection site rotation recommendations for q4w and q1w as stated in the proposed label are supported by local safety data provided by the 9-month SC toxicity study in dogs, which was submitted with the NDA. This study established adequate local safety margins for the proposed clinical dosing regimen with q1w, which was administered repeatedly to the same local site every 8 weeks at least 5 times, and with q4w, which was administered repeatedly to the same site every month for 3 months.

To address the remaining nonclinical requirements for the new excipient GDO, the Applicant submitted PK studies in the rat that demonstrated that SC injection of GDO resulted in no detectable levels of GDO in the plasma over background levels. They

also submitted a chronic toxicology study in the rat testing GDO and leveraged the findings in the 9-month dog study with CAM2038 q4w to address chronic toxicity. The results suggest expected local tissue reactions and evidence of systemic inflammation, also not unexpected for a lipid. Adequate NOAELs were established to support safety. The Applicant conducted the full genetic toxicology battery of studies with GDO which indicated no risk for genotoxicity. The Applicant submitted a rabbit embryo-fetal development study (second cycle) and a rat pre- and post-natal development study with GDO (first cycle) which demonstrated adequate safety margins. To address the remaining standard reproductive and developmental toxicology study requirements, they submitted published literature testing diacyl glycerol (DAG) oil containing significant levels of GDO to address fertility and early embryonic development and embryofetal development studies in the rat and conducted a pre- and post-natal study on GDO. Adequate NOAELs were established to support safety. The Applicant also cited published carcinogenicity studies with DAG oil to address the requirement for carcinogenicity of GDO. These data suggest that the risk of carcinogenicity of DAG oil is no different than the risk of triglycerides. Nonetheless, as it was not clear how much GDO was present in the DAG oil tested in these articles, and the Applicant did not provide an adequate scientific bridge (justification) to definitively leverage those data in the first cycle submission. As discussed in the post-action meeting, the Agency agreed that if the company can provide data to document that the DAG oil studies contained adequate levels of GDO in the material tested and provided adequate coverage for the safety of the GDO administered, these data would be adequate to support the new excipient. In the second cycle, the Applicant submitted a literature report that determined the amount of DAG and oleic acid residues in the DAG oil (b) (4) (b) (4). Although definitive analytical data to determine the GDO content of the DAG oil was not provided, the Applicant leveraged the published analytical data and provided a risk assessment based on the probability of the DAGs in the DAG oil to contain two oleic acid residues. The basis of this assessment and the calculations to estimate the GDO content were reviewed and considered adequate. The studies for DAG oil also suggested significant safety margins for the predicted levels of GDO. As such, the collective data to support the safety of the novel excipient GDO has been provided and deemed adequate.

In addition to the 9-month dog study that contained NMP in the formulation tested, the Applicant also submitted a literature review to support the safety of NMP in this drug product formulation. Based on PK studies in humans following administration of CAM2038 q4w, the NMP in this formulation appears to be cleared from plasma within 24 hours of drug product injection. NMP has been tested in several published subchronic toxicology studies using the oral or inhalation route of administration and although some adverse effects (mostly hepatocellular hypertrophy) were reported in these studies, NOAELs that established reasonable safety margins were demonstrated for this product suggesting limited concern. NMP was deemed overall negative in terms of genotoxic potential. Published carcinogenicity studies with NMP do suggest the potential for NMP-induced liver tumors in mice which may not be relevant to human risk assessment; however, a mode of action assessment was not provided in the first cycle submission to permit dismissal of the findings in the study as not relevant to human risk

assessment. As such, the Division required a mode of action assessment to clarify human relevance. This mode of action assessment has been reviewed during this second cycle and although the data suggest limited concern in human, the assessment lacks key data points to be conclusive. As such, these findings should be included in labeling, unless clearly demonstrated to be irrelevant to human risk via an adequate mode of action assessment. Given the exposure margins for this product, further studies will not be required.

The Applicant submitted published and unpublished reproductive and developmental studies with NMP in lieu of new studies. The published studies employed the oral (gavage/dietary) or inhalation routes and did not test the clinical route of administration. These studies indicate that NMP can have adverse effects on male and female fertility and fetal development. Adverse effects on male reproductive tissues were noted following oral administration of NMP at doses greater than 26 times the human MDD based on BSA with a NOAEL of 8.6x the MDD based on BSA. In an unaudited draft report testing dietary NMP in both males and female rats suggest a conservative NOAEL of approximately 4.3x for male and female fertility effects based on body surface area. In other published studies with fertility endpoints testing NMP via the oral and inhalation routes, there was no impact on fertility indices at doses in the rat that were 5.9 times and 38.7 times the MDD of NMP based on BSA; however, all standard endpoints were not included in these studies. Given the lack of clarity on the impact of NMP on fertility, the lack of studies via the clinical route, and the potential for adverse effects, definitive studies via the SC route were considered warranted at the end of the first review cycle. In the second cycle submission, oral pharmacokinetic data were provided in the rat which suggest adverse effects in males occurred only at doses that are greater than 108 times the human maximum daily dose of NMP based on AUC. Despite the new PK data, there is still a lack of studies that include all standard female and early embryonic development endpoints. Given the exposure margins estimated to date, a definitive fertility and early embryonic development study should still be completed as a post-marketing requirement if the CAM2038 q4w product can be approved this cycle.

The published and unpublished embryofetal development data (EFD) for NMP submitted by the Applicant and identified by the Agency suggest the potential for adverse effects of NMP on embryofetal development in the rat that includes preimplantation losses, delayed ossification, and decreased fetal weight at doses 8.4 times the maximum daily dose (MDD) via this product based on body surface area. At higher doses, increased resorptions and fetal malformations were reported at 21.5x and fetal lethality and malformations have been reported at 64.6x the MDD based on body surface area. An audited draft report of an embryofetal development study in rabbits was also submitted in the first cycle. This report suggested greater safety margins in this species; however, the study was not a final report and it is not clear how the oral route exposures compare to SC exposures via this product. Collectively, although there are several publications in the literature on NMP, the Applicant did not provide an adequate scientific bridge to put these findings into perspective, and the unpublished draft report in the rabbit was not acceptable to qualify a new excipient. The final report

was submitted in the second cycle and was deemed acceptable. In addition, the Applicant submitted new rat and rabbit oral pharmacokinetic data to bridge to the referenced published oral reproductive and developmental toxicology studies. These data suggest significantly larger exposures margins than predicted by the body surface area comparisons. The Applicant did not conduct nonclinical pharmacokinetic bridging data to support their reliance upon published inhalation toxicity data. Rather, they cite a published physiologically based pharmacokinetic (PBPK) modeling paper that predicts exposures in the pivotal reproductive and developmental toxicology studies based on the findings noted and other key aspects of their PBPK model. Although not definitive, the PBPK modeling supports the conclusions from the oral toxicology data suggesting relatively large safety margins for embryo-fetal effects compared to the maximum NMP exposure via the drug product. Collectively, these studies and bridging PK data are deemed adequate to address the embryo-fetal development study requirements for NMP for this drug product. No further EFD studies for NMP are necessary.

Finally, several published reports on the impact of NMP on pre- and postnatal development (PPND) were reviewed in support of the original application. The data suggested that NMP can result in decreased pup survival at doses 12.9 times the MDD and developmental delays at 7.6 times the MDD based on body surface area. A NOAEL was not determined in this study. The oral PK data submitted in the second cycle support, however, suggest adverse effects occurred at 242 times the MDD of NMP based on AUC. Given the lack of a NOAEL, a definitive study should still be completed. However, given the data to date and inclusion of the findings in labeling, should this NDA be approved this cycle, a definitive PPND study for the SC route could be completed as a post-marketing requirement.

There are limited data to inform what could occur should someone attempt to misuse the drug product intravenously. In a non-GLP rat intravenous toxicity study in 3 males, administration of the drug to the tail vein resulted in occlusion of the vessel to the base of the tail with no clear evidence of distribution to other tissues. Lungs, however, could not be evaluated as the animals were sacrificed via CO₂ which results in lung pathology itself. The data suggest that if the drug product were to be administered intravenously, it will either gel rapidly and potentially block the injected vessel as it apparently did in the rat tail vein, or if the injected vein is larger and the product does not gel quickly, it could likely result in a lung emboli or eventually be lodged in other small capillaries. The clinical significance of any blockade of blood vessels would depend upon the redundancy of vascular perfusion and degree of blockade, but it could result in tissue hypoxia and necrosis with an adverse impact on tissue functionality. Given the existing evidence of prolonged tissue residency, the vascular blockade of any tissue would be expected to take considerable time to clear.

1.3 Recommendations

1.3.1 Approvability

There are several outstanding issues remaining including the lack of an adequate female fertility study and pre- and post-natal development study for NMP, an adequately

rigorous elemental impurity assessment for the container closures system, and several outstanding issues with the extractable leachable studies, including unidentified leachables from the container closure system. Therefore, from a nonclinical pharmacology toxicology perspective, the NDA may only be approved if the risk:benefit of this drug product is deemed appropriate. If approved, the above outstanding issues should be addressed via post-marketing requirements.

1.3.2 Additional Nonclinical Recommendations

If approved this cycle, the following studies are recommended as post-marketing requirements to support both CAM2038 q1w and q4w:

1. Conduct a study to evaluate elemental impurity levels in at least three batches of drug product on stability at 12 and 24 months or provide adequate extraction data to characterize the elemental impurities that could be leached from the container closure system using suitable solvents (e.g., nitric acid for elementals from glass).
2. Conduct a study to confirm, using validated methods, the identity of the unspecified (b) (4) the unidentified compound with relative retention time (RRT) of (b) (4) minutes, the unknown compound containing (b) (4) with RRT of (b) (4) min, and the unknown compound with (b) (4) with RRT of (b) (4) min that were detected in your leachable studies above the safety concern threshold of 5 mcg/day and the levels of the identified leachables (b) (4). Evaluate at least three batches of your to-be-marketed drug product at multiple timepoints over the course of your stability studies to identify trends in leachable levels over time. Base the final safety assessment on the maximum predicted levels of leachables identified in individual batches to determine the safe level of exposure via the label-specified route of administration. Do not combine samples from different batches. Once chemical identification is confirmed for the unknown compounds, provide a toxicological risk assessment for each of these compounds and any other compounds detected at ≥ 5 mcg/day.

If approved this cycle, to support the CAM2038 q4w drug product, the following additional studies are recommended as post-marketing requirements:

3. Conduct a fertility and early embryonic development study in female rats testing NMP administered via the subcutaneous route.
4. Conduct a pre- and post-natal development study in rats testing NMP administered via the subcutaneous route.

The following comment is intended as an additional nonclinical recommendation regardless of whether the NDA is approved or given a complete response action.

5. Your assessment to establish a mode of action for the NMP-induced tumorigenesis observed in the 18-month study in B6C3F1 mice did not include adequate information to clearly demonstrate that the findings are not human relevant. The information submitted was inadequate for us to conclude that the NMP-induced effects are potentially attributed entirely to a PPAR alpha-mediated mechanism as proposed. However, because a NOEL was established in the mouse study that provided an 8x safety margin, we acknowledge that the risk to humans may not be significant. Therefore, no additional data will be required, but the findings must be included in labeling. If you want to remove the language from labeling, you must submit a revised MOA assessment with additional data to bolster the weight of evidence to argue that the tumorigenesis is driven by a PPAR alpha-mediated pathway. Refer to Klaunig et al., PPAR alpha Agonist-Induced Rodent Tumors: Modes of Action and Human Relevance. Critical Reviews In toxicology 33(6): 655-780. 2003.

1.3.3 Labeling

The labeling recommendations below have not been discussed with the team or the Applicant and may not reflect final labeling recommendations. For the final labeling, the reader is referred to the Action Letter.

<i>Applicant's proposed label language: 2nd cycle</i>	<i>Reviewer's recommended changes</i>	<i>Comments and rationale for recommended changes</i>
8.1 Pregnancy <u>Risk Summary</u> The data on use of buprenorphine, the active ingredient in BRIXADI in pregnancy, are limited; however, these data do not indicate an increased risk of major malformations specifically due to buprenorphine exposure. There are limited data from randomized clinical trials in women maintained on buprenorphine that were not designed appropriately to assess the risk of major malformations [see Data]. (b) (4) Embryo fetal death was observed in rats and rabbits administered buprenorphine during the period of organogenesis at doses (b) (4)	8.1 Pregnancy <u>Risk Summary</u> The data on use of buprenorphine, the active ingredient in BRIXADI, in pregnancy, are limited; however, these data do not indicate an increased risk of major malformations specifically due to buprenorphine exposure. There are limited data from randomized clinical trials in women maintained on buprenorphine that were not designed appropriately to assess the risk of major malformations [see Data]. (b) (4) Embryofetal death was observed in both rats and rabbits administered buprenorphine during the period of organogenesis at doses	The dose for 32 mg CAM2038 q1w and 128 mg CAM2038 q4w were expressed as the mean daily dose of 4.6 mg (32 mg/7 days= 4.6; 128 mg/28 days=4.6). The human AUC data are from Study HS-15-549. At steady state, exposure is highest from 12-36 h post-dose. The Applicant provided mean partial AUC (24-hour interval) at steady state to provide the highest exposure (32 mg q1w= 149 mg*h/mL; 128 mg q4w= 242 ng*h/mL). These values will be used in

(b) (4) Pre- and post-natal development studies in rats demonstrated increased neonatal deaths attributed to reduced milk production at clinical or (b) (4) No teratogenic effects were seen when buprenorphine was administered during organogenesis with a range of doses (b) (4)	approximately 21 times and equal to, respectively, the mean daily dose of 4.6 mg buprenorphine delivered by either 32 mg BRIXADI (weekly) or 128 mg BRIXADI (monthly). Pre- and post-natal development studies in rats demonstrated increased neonatal deaths at doses approximately equal to and above and dystocia at approximately 11 times the mean daily dose of 4.6 mg buprenorphine. No clear teratogenic effects were seen when buprenorphine was administered during organogenesis with a range of doses 4 times and greater than the mean daily dose of 4.6 mg of buprenorphine. However, increases in skeletal abnormalities were noted in rats and rabbits administered buprenorphine daily during organogenesis at doses 2 and 21 times the mean daily dose of 4.6 mg of buprenorphine, respectively. In a few studies, some events such as acephalus and omphalocele were also observed but these findings were not clearly treatment-related [see Data]. Based on animal data, advise pregnant women of the potential risk to a fetus.	the exposure margin calculations that use AUC comparisons.
<i>Animal Data</i> (b) (4)	<i>Animal Data</i> The exposure margins below are based on the mean daily dose of 4.6 mg buprenorphine delivered by 32 mg BRIXADI (weekly) or 128 mg BRIXADI (monthly on body surface area comparisons, unless otherwise noted.	
Buprenorphine was not teratogenic in rats or rabbits (b) (4)	No definitive drug-related teratogenic effects were observed in rats and rabbits at IM doses up to 30 mg/kg/day (approximately 64 times and 127 times, respectively,	Exposure margins were updated and the omitted text from the Subutex label that was omitted by the

(b) (4)	on a mg/m² basis). Maternal toxicity resulting in mortality was noted in these studies in both rats and rabbits. Acephalus was observed in one rabbit fetus from the low-dose group and omphalocele was observed in two rabbit fetuses from the same litter in the mid-dose group; no findings were observed in fetuses from the high-dose group. Maternal toxicity was seen in the high-dose group but not at the lower doses where the findings were observed. Following oral administration of buprenorphine to rats, dose-related post-implantation losses, evidenced by increases in the numbers of early resorptions with consequent reductions in the numbers of fetuses, were observed at doses of 10 mg/kg/day or greater (21 times on a mg/m² basis). In the rabbit, increased post-implantation losses occurred at an oral dose of 40 mg/kg/day (169 times on a mg/m² basis). Following IM administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 mg/kg/day (approximately 64 times and 127 times, respectively, on a mg/m² basis). Buprenorphine was not teratogenic in rats and rabbits after subcutaneous (SC) doses of up to 5 mg/kg/day (9 and 5 times in rat, and 12 and 7 times in rabbit the highest daily exposure from 32 mg BRIXADI [weekly] or 128 mg BRIXADI [monthly], respectively, on an AUC basis), after intramuscular (IM) doses of up to 5 mg/kg/day (11 and 21 times on a mg/m² basis), after IV doses up to 0.8 mg/kg/day (2 and 3 times on a mg/m² basis), or after oral doses up to 160 mg/kg/day in rats (338 times on a mg/m² basis) and 25 mg/kg/day in rabbits (106 times on a mg/m² basis). Significant increases in skeletal abnormalities (e.g., extra thoracic vertebra or thoraco-lumbar ribs) were noted in rats after SC	Applicant was added back in.

	administration of 1 mg/kg/day and up (2.4 or 1.5 times the highest daily exposure from 32 mg BRIXADI [weekly] or 128 mg BRIXADI [monthly], respectively, on an AUC basis), but were not observed at oral doses up to 160 mg/kg/day (338 times on a mg/m² basis).	
(b) (4)	Increases in skeletal abnormalities in rabbits after IM administration of 5 mg/kg/day (21 times on a mg/m² basis) in the absence of maternal toxicity or oral administration of 1 mg/kg/day or greater (4 times on a mg/m² basis) were not statistically significant. In rabbits, buprenorphine produced statistically significant pre-implantation losses at oral doses of 1 mg/kg/day or greater (4 times on a mg/m² basis) and post-implantation losses that were statistically significant at IV doses of 0.2 mg/kg/day or greater (approximately equal on a mg/m² basis). No maternal toxicity was noted at doses causing post-implantation loss in this study.	Exposure margins were updated and the omitted text from the Subutex label that was omitted by the Applicant was added back in.
	Dystocia was noted in pregnant rats treated intramuscularly with buprenorphine from Gestation Day 14 through Lactation Day 21 at 5 mg/kg/day (11 times on a mg/m² basis). Fertility and pre- and post-natal development studies with buprenorphine in rats indicated increases in neonatal mortality after oral doses of 0.8 mg/kg/day and up (2 times on a mg/m² basis), after IM doses of 0.5 mg/kg/day and up (approximately equal on a mg/m² basis), and after SC doses of 0.1 mg/kg/day and up (0.4 or 0.3 times the highest daily exposure from 32 mg BRIXADI [weekly] or 128 mg BRIXADI [monthly], respectively, on an AUC basis). An apparent lack of milk production during these studies likely contributed to the decreased	Exposure margins were updated and the omitted text from the Subutex label that was omitted by the Applicant was added back in.

	pup viability and lactation indices. Delays in the occurrence of righting reflex and startle response were noted in rat pups at an oral dose of 80 mg/kg/day (169 times on a mg/m² basis).	
	Decreased pup survival was noted following oral treatment of female rats prior to and during gestation with NMP, an excipient in BRIXADI (monthly) at doses 242 times the MDD of NMP delivered by BRIXADI (monthly) on an AUC basis. A No Adverse Effect Level (NOAEL) was not demonstrated.	No NOAEL was demonstrated in the study by Sitarek. Given the revised exposures margins, the findings were noted at a very high exposure margin. As such, the utility for labeling is questionable. Nonetheless, until a NOAEL is defined, the findings should be included in labeling and a definitive pre- and post-natal study should be completed as a PMR.
8.3 Females and Males of Reproductive Potential <u>Infertility</u> Chronic use of opioids may cause reduced fertility in females and males of reproductive potential. It is not known whether these effects on fertility are reversible [see <i>Adverse Reactions (6.2), Clinical Pharmacology (12.2), Nonclinical Toxicology (13.1)</i>].	8.3 Females and Males of Reproductive Potential <u>Infertility</u> Chronic use of opioids may cause reduced fertility in females and males of reproductive potential. It is not known whether these effects on fertility are reversible [see <i>Adverse Reactions (6.2), Clinical Pharmacology (12.2), Nonclinical Toxicology (13.1)</i>].	No changes were made to this section.
12.1 Mechanism of Action BRIXADI contains buprenorphine, a partial agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor.	12.1 Mechanism of Action BRIXADI contains buprenorphine, a partial agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor.	No changes were made to this section.
13 NONCLINICAL TOXICOLOGY 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenicity</u>	13 NONCLINICAL TOXICOLOGY 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenicity</u> Carcinogenicity studies of buprenorphine were conducted in Sprague-Dawley rats and CD-1 mice. Buprenorphine was administered in the diet to rats for 27 months at doses of 0.6, 5.5, and 56 mg/kg/day (0.3, 1.4 and 11.3	Exposure margins were updated based on AUC data. Omissions were added back in.

(b) (4)	times the highest daily exposure from 32 mg BRIXADI WEEKLY on an AUC basis and 0.2, 0.9 and 6.9 times the highest daily exposure from 128 mg BRIXADI MONTHLY on an AUC basis). A statistically significant dose-related increase in Leydig cell tumors occurred. In an 86-week study in CD-1 mice, buprenorphine was not carcinogenic at dietary doses up to 100 mg/kg/day (7 and 4 times the highest daily exposure from 32 mg BRIXADI [weekly] or 128 mg BRIXADI [monthly], respectively, on an AUC basis). NMP, an excipient in BRIXADI (monthly) produced an increase in hepatocellular adenomas and carcinomas in male and female mice at 47 and 60 times, respectively, the maximum daily dose (MDD) of NMP via 128 mg BRIXADI (monthly) on a mg/m² basis. The clinical significance of these findings is unclear. No tumors were noted in male or female mice at 7.4 and 9.5 times the MDD on a mg/m² basis. In 2-year inhalation and dietary studies in rats, NMP did not result in evidence of carcinogenicity.	
Mutagenicity Buprenorphine was studied in a series of tests utilizing gene, chromosome, and DNA interactions in both prokaryotic and eukaryotic systems. Results were negative in yeast (<i>S. cerevisiae</i>) for recombinant, gene convertant, or forward mutations; negative in <i>Bacillus subtilis</i> "rec" assay; negative for clastogenicity in CHO cells, Chinese hamster bone marrow and spermatogonia cells, and negative in the mouse lymphoma L5178Y assay. Results were equivocal in the Ames test: negative in studies in two laboratories, but positive for frame shift mutation at a high dose (5 mg/plate) in a third study. Results were positive in the Green-Tweets (<i>E. coli</i>) survival test, positive in a	Mutagenicity Buprenorphine was studied in a series of tests utilizing gene, chromosome, and DNA interactions in both prokaryotic and eukaryotic systems. Results were negative in yeast (<i>S. cerevisiae</i>) for recombinant, gene convertant, or forward mutations; negative in <i>Bacillus subtilis</i> "rec" assay, negative for clastogenicity in CHO cells, bone marrow and spermatogonia cells, and negative in the mouse lymphoma L5178Y assay. Results were equivocal in the Ames test: negative in studies in two laboratories, but positive for frame shift mutation at a high dose (5 mg/plate) in a third study. Results were positive in the Green-Tweets (<i>E. coli</i>) survival test, positive in a DNA synthesis inhibition (DSI) test	Minor edits were made to reflect the most updated version of the referenced label.

DNA synthesis inhibition (DSI) test with testicular tissue from mice, for both in vivo and in vitro incorporation of [³ H] thymidine, and positive in unscheduled DNA synthesis (UDS) test using testicular cells from mice.	with testicular tissue from mice, for both in vivo and in vitro incorporation of [³ H]thymidine, and positive in unscheduled DNA synthesis test using testicular cells from mice.	
<u>Impairment of Fertility</u> Reproduction studies of buprenorphine in rats demonstrated no evidence of impaired fertility at (b) (4)	<u>Impairment of Fertility</u> Reproduction studies of buprenorphine in rats demonstrated no evidence of impaired fertility at daily oral doses up to 80 mg/kg/day (169 times the mean daily dose of 4.6 mg buprenorphine as delivered by 32 mg BRIXADI [weekly] or 128 mg BRIXADI [monthly] on a mg/m ² basis) or up to 5 mg/kg/day IM (11 times the mean daily dose of 4.6 mg buprenorphine as delivered by 32 mg BRIXADI [weekly] or 128 mg BRIXADI [monthly] on a mg/m ² basis) or 5 mg/kg/day SC (16 or 10 times the highest daily exposure from 32 mg BRIXADI [weekly] or 128 mg BRIXADI [monthly], respectively, on an AUC basis).	Exposure margins were updated.

The following table summarizes the exposure margins calculated based on a comparison of AUC levels achieved in animal PK studies compared to the partial AUC values reported from Clinical Study HS-15-549. The values provided by the Applicant to address Deficiency 8 of the action letter were reanalyzed by Dr. Suresh Narahariseti from OCP. Dr. Narahariseti concurred with the assessment of the data provided by the Applicant. Refer to Dr. Narahariseti's review dated 11/27/18 for details. The mean $\pm$ SD of buprenorphine AUC_{SS,24h} for 32 mg q1w is 149 $\pm$ 56 ng•h/mL and for 128 mg q4w is 242 $\pm$ 113 ng•h/mL. These AUC values are at steady state and represent the highest exposures over a given 24 h which is the 0-24 h period 12-36 h post-dose. The AUC_{0-24h} values for the nonclinical studies and the exposure margins are presented in the table below. Reviews of the nonclinical studies are in the PK section of this review.

Table 1. Exposure Margins Based on AUC for Reproductive and Developmental Toxicology and Carcinogenicity Studies

Species and Route of Administration	Study Number	Study Day	Dose of Bup (mg/kg)	Animal AUC _{0-24h} (ng•h/mL)	Animal/Human Exposure Margins based on AUC	
					32 mg q1w ^a	128 mg q4w ^b
Rat (gravid), Subcutaneous	2335-003	12	0.1	66	0.4	0.3
			1	351	2.4	1.5
			5	1290	8.7	5.3
Rat (males and	2335-003	14	5	2330	15.6	9.6

nongravid females), Subcutaneous						
Rabbit (gravid), Subcutaneous	2335-004	12	5	1750	11.7	7.2
Rat, Dietary	2335-001	28	0.6	42	0.3	0.2
			5.5	208	1.4	0.9
			56	1680	11.3	6.9
Mouse, Dietary	2335-002	28	100	1050	7.0	4.3

a. Human $AUC_{ss,24h}$ for 32 mg q1w= 149 ng•h/mL from Study HS-15-549

b. Human $AUC_{ss,24h}$ for 128 mg q4w= 242 ng•h/mL from Study HS-15-549

Table 2. Labeling Exposure Margins Based on BSA

Species	Route of Administration	Animal dose, mg/kg	Animal BSA, mg/m ²	Current Label: Safety Margin (BSA) ^a
Rat	IM	0.5	6	1
	IV	0.8	4.8	2
	IM	5	30	11
	PO	10	60	21
	IM	30	180	64
	PO	80	480	169
	PO	160	960	338
Rabbit	IV	0.2	2.4	0.9
	IV	0.8	9.6	3
	PO	1	12	4
	IM	5	60	21
	PO	25	300	106
	IM	30	360	127
	IM	40	480	169

a. The human dose of 4.6 mg/day buprenorphine (2.84 mg/m²) for either the weekly or monthly product was used for the calculation of the exposure margins.

2 Drug Information

2.1 Drug

CAS Registry Number: 52485-79-7

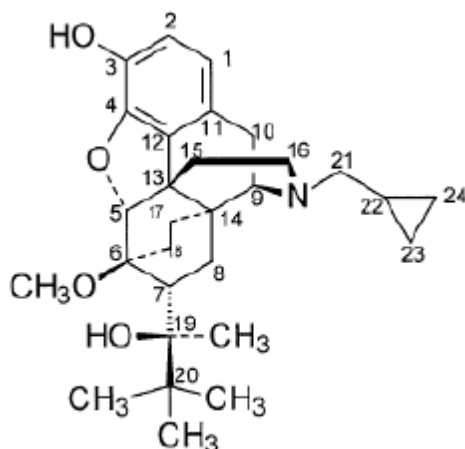
Generic Name: Buprenorphine base

Code Name: N/A

Chemical Name: (2S)-2-[-(-)-(5R,6R,7R,14S)-9 α -cyclopropylmethyl-4,5-epoxy-6,14-ethano-3-hydroxy-6-methoxymorphinan-7-yl]-3,3-dimethylbutan-2-ol

Molecular Formula/Molecular Weight: C₂₉H₄₁NO₄

Structure:



Pharmacologic Class: Partial opioid agonist (FDA Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

<i>NDA</i>	<i>Name</i>	<i>Division</i>	<i>Strength (route)</i>	<i>Marketing Status</i>	<i>Approval Date</i>	<i>Indication</i>	<i>Applicant</i>
20732	Subutex	DAAAP	SL	Approved	10/8/2002	Opioid Use Disorder	Indivior

<i>IND</i>	<i>Drug</i>	<i>Status</i>	<i>Division</i>	<i>Indication</i>	<i>Stamp Date</i>	<i>Sponsor</i>
114082	CAM2038	Active	DAAAP	Opioid dependence	5/9/2015	Braeburn

<i>DMF</i>	<i>Subject</i>	<i>Holder</i>	<i>Status</i>	<i>Reviewer's Comments</i>
		(b) (4)	Active since 4/13/2010	Referenced in multiple FDA-approved drug products
			Active since 3/25/1992	Not previously reviewed formally Not referenced via other FDA-approved applications
			Active since 4/24/2007	
			Active since 10/4/2013	
			Active since 12/14/2015	Referenced by multiple FDA-

(b) (4)		approved drug products
	Active since 8/9/20047	Referenced by other FDA-approved products
	Active since 1/30/2015	Not formally reviewed to date.

2.3 Drug Formulation

The CAM2038 q1w and CAM2038 q4w formulations are a once-weekly and once-monthly, respectively, extended-release lipid-based subcutaneous depots containing 50 mg/mL buprenorphine base. The proposed strengths of the single-use prefilled syringes for the q1w product are 8 mg, 16 mg, 24 mg, and 32 mg and the strengths for the q4w product are 64 mg, 96 mg and 128 mg (b) (4)

The injection depot platform contains soybean phosphatidylcholine (SPC), glycerol dioleate (GDO) and a (b) (4) and is referred to by the Applicant as “FluidCrystal” technology. The two formulations (b) (4) with the q1w product containing ethanol and the q4w formulation containing N-methyl-2-pyrrolidone (NMP). According to the Applicant, “once injected, CAM2038 spontaneously transforms from a low-viscous solution to a highly viscous crystalline gel that encapsulates the BUP and releases it at a steady rate as the depot degrades.” The quantitative composition of each strength for the two products are outlined in the tables below.

Table 3. Composition of CAM2038 q1w Drug Product

Component	Standard	Function	Composition (mg) per dose			
			8 mg/ 0.16 mL	16 mg/ 0.32 mL	24 mg/ 0.48 mL	32 mg/ 0.64 mL
Buprenorphine Base (BUP)	Ph. Eur.	Active ingredient	8	16	24	32
Ethanol Anhydrous (Dehydrated alcohol) (EtOH)	USP	(b) (4)	(b) (4)			
Soybean Phosphatidylcholine (SPC)	In-house	(b) (4)	(b) (4)			
Glycerol Dioleate (GDO)	In-house	(b) (4)	(b) (4)			
Total weight (mg) for all components per dose	Not Applicable	Not Applicable	(b) (4)			

Table 4. Composition of CAM2038 q4w Drug Product

Component	Standard	Function	Composition (mg) per dose		
			64 mg/ 0.18 mL	96 mg/ 0.27 mL	128 mg/ 0.36 mL
Buprenorphine Base (BUP)	Ph. Eur.	Active ingredient	64	96	128
N-Methyl-2-Pyrrolidone (NMP)	USP	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Soybean Phosphatidylcholine (SPC)	In-house	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Glycerol Dioleate (GDO)	In-house	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Total weight (mg) for all components per dose	Not Applicable	Not Applicable	(b) (4)	(b) (4)	(b) (4)

2.4 Comments on Novel Excipients

There were two “novel” excipients in the drug product formulations covered by this NDA at the time of the original submission.

N-methyl-2-pyrrolidone (NMP) - Only the CAM2038 q4w contains NMP (b) (4). As discussed during development, the use of NMP in this drug product formulation is technically novel by the SC route of administration. According to the Inactive Ingredients Database (IID, December 12, 2017), NMP was used as an excipient in two FDA-approved drug products, one of which is approved for the periodontal route for an acute indication and the other for the subcutaneous route for the palliative treatment of prostate cancer.

The novelty of NMP for a chronic non-oncology indication was discussed with the Applicant over the course of development and the Agency noted that the Applicant would have to address the safety for this indication. Literature could be used to support the standard requirements for a novel excipient; however, the adequacy of the literature could only be determined during the NDA review. At the preNDA meeting in 2017, the Division noted that there was technically no chronic rodent toxicology study with NMP; however, we acknowledged that the carcinogenicity studies with NMP may be adequate to address this requirement for any novel chronic excipient.

Since the original submission, NMP was also listed in the IID as being present in a subcutaneously administered product at levels higher than that of the current formulation.

Refer to Appendix 1 in Section 12 for more information on NMP and to Section 1.3 Recommendation for proposed regulatory activity regarding NMP and this NDA submission.

Glycerol dioleate (GDO) - Glycerol dioleate (GDO) is included in both the CAM2038 q1w and q4w formulations. The highest proposed level is (b) (4) mg/32 mg dose via the weekly dosed product. The proposed concentration is (b) (4) mg/mL or (b) (4) % w/v. GDO is not listed in the FDA IID and is therefore considered a novel excipient that must be qualified for safety per the guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*. Diglycerides, like GDO, are endogenously formed via the hydrolysis of triglycerides. Diglycerides are metabolized by lipases to the free fatty acids and glycerol. The fatty acids are further metabolized via beta-oxidation or utilized as substrates for cellular metabolism.

Toxicological safety data was submitted by the Applicant for GDO (see Appendix 2). The Applicant also references published studies with diacyl glycerol oils (DAG) as a surrogate molecule for GDO. According to the Applicant, DAG oils are largely GDO but also contain linoleic acids, and other fatty acids attached to glycerol. In the second cycle, the Applicant provided adequate bridging data to support their conclusion that data with DAG oil provided adequate support for the safety of GDO. Collectively, the data support the use of this novel excipient.

Refer to Appendix 2 in Section 12 for more information on GDO and to Section 1.3 Recommendation for proposed regulatory activity regarding GDO and this NDA submission.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Specifications

The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substance for a maximum daily dose (MDD) of ≤ 2 g/day is 0.15% or 1 mg/day intake, whichever is lower. The Applicant has set the specifications for impurities in the buprenorphine drug substance obtained from (b) (4) (DMF (b) (4)) at NMT (b) (4) % and no qualification will be necessary. It is noted that several additional impurity specifications are listed in (b) (4) DMF (b) (4). The CMC team will determine whether additional drug substance specifications are necessary for buprenorphine in this NDA.

Table 5. Drug Substance Impurities for Buprenorphine in CAM2038 q1w and CAM2038 q4w

<i>Impurity</i>	<i>Proposed Specification</i>	<i>ICH Qualification Thresholds or Limits</i>	<i>Comments</i>
(b) (4)	$\leq$ (b) (4) %	0.15% or 1 mg/day	Acceptable

Drug Product Specifications

For the CAM2038 q1w product (highest strength 32 mg BUP; 32 mg/7 days = 4.6 mg/day) and the CAM2038 q4w product (highest strength 128 mg BUP; 128 mg/28 days = 4.6 mg/day) the qualification threshold according to the ICH Q3B(R2) guidelines for impurities/degradants in the drug product for an MDD between <10 mg of BUP administered per day is 1% or 50 mcg TDI, whichever is lower. In the first cycle, the

Applicant listed (b) (4) at a specification (b) (4) % (b) (4). In the second cycle, the Applicant identified an additional impurity (b) (4) and set a specification (b) (4) %. Both specifications meet ICH Q3B(R2) and are considered acceptable.

The Applicant provided data to show that (b) (4) is detected in human plasma with a total exposure corresponding to 22-26% of the total AUC_{inf} following SC injection of both the CAM2038 q1h and q4h products (Study HS-13-487). Additionally, the Applicant provided a computational toxicology analysis for the drug product impurity, (b) (4) (Study TO-18-007). The impurity is predicted to be negative for mutagenic potential using both rule-based and statistical-based platforms. The review of the computational toxicology analyses can be found below in Special Toxicology Studies.

Table 6. Drug Product Degradants for CAM2038 q1w

Degradant	Proposed Specification	ICH Qualification Thresholds or Limits	Comments
(b) (4)	NMT (b) (4) %	1% or 50 mcg	Acceptable
(b) (4)	NMT %	1% or 50 mcg	Acceptable

Table 7. Drug Product Degradants for CAM2038 q4w

Degradant	Proposed Specification	ICH Qualification Thresholds or Limits	Comments
(b) (4)	NMT (b) (4) %	1% or 50 mcg	Acceptable
(b) (4)	NMT %	1% or 50 mcg	Acceptable

(b) (4)

Elemental Impurities

The Applicant analyzed the CAM2038 q1w and q4w drug products for elemental impurities as per ICH Q3D. CMC has concurred with the analysis submitted by the Applicant. No elemental impurities exceed the permitted daily exposures for a parenteral drug product, therefore no specifications are required to be set. Tables detailing the elements analyzed in the CAM2038 q1w and CAM2038 q4w products are below. Refer to the review by the chemist Dr. Valerie Amspacher for details.

Table 8. Permitted Daily Exposure and Actual Elemental Impurities in the CAM2038 q1w Drug Product, Batch 23505.04: 6-month Storage at 40°C/75% RH

Element	Permitted daily exposure		Found amount
	(µg/day)	(ppm, injection volume (b) (4) mL)	(ppm)

(b) (4)

(b) (4)

Table 9. Permitted Daily Exposure and Actual Elemental Impurities in CAM2038 q4w Drug Product Batch 23518.03 (Accelerated Conditions)

Element	Permitted daily exposure		Found amount
	(µg/day)	(ppm, injection volume ^{(b) (4)} mL)	(ppm)



Of note, the data on elemental impurities is only at a single time point, 6 months, and not the proposed end of shelf-life. This is reasonable for the drug product formulation as elemental impurities should not increase with time. However, it does not adequately address the potential for increases in elemental impurities that are derived from the container closure system as leachables.

Container Closure System

The CAM2038 q1w and CAM2038 q4w drug products are presented as pre-filled syringes (PFS). ^{(b) (4)} syringes and ^{(b) (4)} ^{(b) (4)} plunger stoppers are the primary container closure. The drug product-filled syringe is inserted in a safety device assembly for post injection needle stick prevention. The individual syringe system components and the safety device assembly are shown in the figure below.



CAM2038 q1w and CAM2038 q4w drug products are packaged into a (b) (4) (b) (4) 1 mL (b) (4) glass syringe sealed with a sterile (b) (4) rubber plunger stopper. The individual components selected for, and in direct contact with, the drug product are detailed in the table below.

Component	Description	Specification	Manufacturer
(b) (4)	Syringe, staked-in needle, 1 mL long with 23G ½" 3B TW, with RNS inserted (b) (4) Grey		
Syringe	1 mL long Glass (b) (4)		
Needle	Staked 23G thin wall (TW) ½" Stainless steel		
Needle shield	Rigid needle shield (RNS) Rubber formulation (b) (4)		
Plunger stopper	(b) (4) elastomer		

The labeled (drug product filled) syringe is inserted into the safety device assembly. The assembly consists of the safety device body and finger flange. The individual components selected for the combination product are detailed in the table below.

Component	Description	Specification	Manufacturer
CAM2038 q1w or CAM2038 q4w	Drug Product -Filled Syringe	(b) (4)	
Safety Device (Body)	(b) (4)		
Finger Flange			

The product kit includes the product, pre-printed carton with (b) (4) tray, plunger rod, Medication Guide, Prescribing Information (PI), Instructions for Use (IFU) and (b) (4). All the components are packaged as a single "Combination Product Kit". An example of a CAM2038 product kit is illustrated in the figure below.



CAM2038 q1w and CAM2038 q4w drug product filled syringes (b) (4)

Extractables/Leachables Evaluation

To justify the safety of leachables arising from the container closure systems or from (b) (4) manufacturing processes that are in direct contact with the drug product, the

Applicant performed extractables and leachables studies, which are described in the following documents and reports:

- Module 3.2.P.2.4 Container Closure System (CAM2038 q1w and CAM2038 q4w)
- A Discussion of the Extractables Data for CAM2038 Drug Products' Packaging Components ((b) (4) -17-010 and (b) (4) -17-010 Amendment No. 1)
- Screening and Profiling Drug Product Leachables Over Shelf Life ((b) (4) -18-012)
- Toxicological Patient Safety Risk Assessment Life ((b) (4) -18-014)

Extractables Testing on Container Closure System

Title: A Discussion of the Extractables Data for CAM2038 Drug Products' Packaging Components

Study Number: (b) (4) -17-010 and (b) (4) S-17-010 Amendment No. 1

Conducting Lab: (b) (4)

Report (b) (4) -17-010 and (b) (4) -17-010 Amendment No. 1 is a summary report of all the extractable testing conducted by the Applicant. The report is prepared by (b) (4) (b) (4) and references the following extractable studies conducted by (b) (4)

- Extractables study: Determination of the extractable chemical compounds present in and on (b) (4) rigid needle shields. Extraction conditions: (b) (4)
- Extractables study: Determination of the extractable chemical compounds present in and on (b) (4) staked needle glass syringes. Extraction conditions: (b) (4)
- Extractables study: Determination of the extractable chemical compounds present in and on (b) (4) rubber stoppers. Extraction conditions: (b) (4)
- Extractables study: Determination of the extractable chemical compounds present in and on (b) (4) staked needle glass syringes. Extraction conditions: (b) (4)

- Extractables study: Determination of the extractable chemical compounds present in and on (b) (4) rigid needle shields. Extraction conditions: (b) (4)
- Extractables study: Determination of the extractable chemical compounds present in and on (b) (4) rubber stoppers. Extraction conditions: (b) (4)
- Extractables study: Determination of the extractable chemical compounds present in and on (b) (4) rigid needle shields. Extraction conditions: (b) (4)
- Extractables study: Determination of the extractable chemical compounds present in and on (b) (4) staked needle glass syringes. Extraction conditions: (b) (4)

Components tested:

The syringe barrel (Lot 30688-2), needle shield (Lot 30688-2), and plunger stopper (Lot 30157-3) from the Applicant's table below were tested, while the needle was not.

Table 10. Components of the Pre-Filled Syringe

Component	Description	Material	Manufacturer
Syringe Barrel	1 mL long	Glass (b) (4)	(b) (4)
Needle	Staked 23G thin wall (TW) ½"	Stainless steel	
Needle shield	Rigid needle shield (RNS)	Rubber formulation (b) (4)	
Plunger stopper	1 mL long	(b) (4) elastomer, (b) (4) Gray	

Taken from page 5 of (b) (4)-17-010 Amendment No. 1

Solvents and Extraction Conditions:

Summary:

Overall the extractable testing appeared adequately designed to inform the leachable testing. Adequate SCTs were established and a number of compounds were identified above the SCTs. The Applicant's assessment of the leachable study results has been considered adequate by the CMC review team. Although the Applicant only targeted two compounds, they provided adequate information to the CMC review team to support their conclusion that their screening of the leachable study materials would detect any unspecified compound above 5 mcg/day. The CMC review team concurred with this Applicant. However, there were a few deficiencies identified in the Applicant's extractables testing:

- The Applicant's extraction study only addressed the potential for organic compounds to be extracted. No analysis for inorganic compounds, such as trace elements or metals, was performed or addressed in this report.

- No information regarding the structure of the unidentified compound and what efforts to identify the compound were provided.

In regards to the analysis of elements and metals, the Applicant has indicated that they have conducted adequate analysis for metals and elements based on testing in extractable report CAM2038 (Buprenorphine FluidCrystal® Injection depot): Extractables Study of (b) (4) Prefilled Syringe (Report S.52.REP.S279_01) and that since no such leachables were detected above the Limit of Quantitation ((b) (4) mcg/syringe) further leachable testing of elements and metals is not required. However, the conditions used in this extraction study (b) (4) (b) (4) was not considered adequate by the CMC review team as was communicated to the Applicant as part of the previous complete response. Taken together, the Applicant has not adequately addressed the potential for metals or elements to be extracted from the drug product container closure system.

Regarding the structure of the unknown, the only information provided was a mass spectrum in the final reports. Based on the mass spectrum, it is likely that the unknown compound is a (b) (4) structure. However, the Applicant did not provide sufficient information describing how they tried to identify the compound and why they were unable. The unknown compound was not seen in subsequent leachable testing (see below for leachable results). If the methods for detection of this compound are deemed adequate to ensure that not more than 5 mcg/day of the compound would be in the material, further efforts to identify the structure of this compound are not necessary.

Leachables Testing on Container Closure System

Title: A Discussion of the Leachables Data for CAM2038 Drug Products and Packaging System (pre-filled syringe)

Study Number: (b) (4) -18-012

Conducting Lab: (b) (4)

Report (b) (4) -18-012 is a summary report of all the leachable testing conducted by the Applicant. The report is prepared by (b) (4) and references the following leachable studies conducted by (b) (4)

- Method validation of a GC/MS-method to be used in a leachables study on pre-filled syringes sealed with rubber plungers in contact with the pharmaceutical matrix "CAM2038 Q1W" (b) (4)
- Method validation of a GC/MS-method to be used in a leachables study on pre-filled syringes sealed with rubber plungers in contact with the pharmaceutical matrix "CAM2038 Q4W" (b) (4)
- Leachables study: Determination of the leachable amount of chemical compounds present in CAM2038 Q1W (non-aqueous drug product) after contact

with prefilled glass syringes sealed with (b) (4) rubber stoppers and (b) (4) needle shields (b) (4)

- Leachables study: Determination of the leachable amount of chemical compounds present in CAM2038 Q4W (non-aqueous drug product) after contact with prefilled glass syringes sealed with (b) (4) rubber stoppers and (b) (4) needle shields (b) (4)
- Leachables study: Determination of the leachable amount of chemical compounds present in CAM2038 Q1W (non-aqueous drug product) after contact with prefilled glass syringes sealed with (b) (4) rubber stoppers and (b) (4) needle shields (combined report; (b) (4))
- Leachables study: Determination of the leachable amount of chemical compounds present in CAM2038 Q4W (non-aqueous drug product) after contact with prefilled glass syringes sealed with (b) (4) rubber stoppers and (b) (4) needle shields (combined report (b) (4))

Overview

Analytical methods were developed to quantitate the levels (b) (4) (b) (4) in both formulations (targeted method). Analytical methods were also developed to screen both formulations for unspecified leachables at levels equal to or exceeding the analytical evaluation threshold (AET) (screening method). In the initial testing, q1w and q4w drug products (b) (4) were tested using both the targeted and screening methods for leachables. A second set of testing was conducted with q1w and q4w drug products (b) (4) using the targeted and screening methods.

Drug products tested in the study:

The drug products used in the study are shown in the two Applicant's tables below. The first table describes the drug products used in the initial testing using pre-filled syringes of q1w and q4w that were retained from stability studies (b) (4) (b) (4). Lot and batch number information were not provided for these drug products.

The second and third tables below show the q1w and q4w drug products used for the migration studies. (b) (4)

(b) (4) This is in contrast to the recommendation made by the Division to evaluate at least three batches and based the risk assessment on the maximum level detected at any given timepoint in these three batches. The method employed by the Applicant would result in a lower estimate of the peak leachable level and is not considered acceptable. However, given the potential impact of this product on a significant current public health crisis and the PMR to appropriately test and

validate the assessment of the leachables that is recommended by the review team, the approach will not be deemed an approval deficiency for this application if the risk:benefit is deemed appropriate.

Table 20. Test Articles for End of Shelf Life Leachable Testing

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Taken from page 10 of (b) (4)-18-012

Table 21. Test Articles for Targeted Leachable Migration Study

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Taken from page 13 of (b) (4)-18-012

Table 22. Test Articles for Screening Leachable Migration Study



(b) (4)

Taken from page 14 of (b) (4)-18-012

Analytical methods:

The targeted method was a gas chromatography (GC)/mass spectrometry (MS) (single ion monitoring) method developed and optimized to quantitate (b) (4). (b) (4) The screening method consisted of three analytical methods to screen for leachables at or above the AET, consisting of GC/MS with headspace sampling for VOCs, direct injection GC/MS for SVOCs, and Ultra Performance Liquid Chromatography/MS for NVOCs. The analytical methods that were part of the screening method were optimized based on a group of surrogate extractables. These surrogate extractables were spiked into the drug products to assess the method's ability to recover them (see table below). Since the methods were not designed for any specific leachables, screening is accomplished by comparing the response from the stability samples to responses from blanks. Any unique or increased responses in the stability samples are then considered potential leachables. See CMC review for the determination of the adequacy of this method to detect any compound above 5 mcg/day in the drug product.

Table 23. Surrogate Compounds and Their Recovery

MST Target Compound	CAS #	Most Appropriate Analytical Method	Results					
			CAM2038 Q1W Product			CAM2038 Q4W Product		
			Spiked (µg/mL)	Measured (µg/mL)	% Recovery	Spiked (µg/mL)	Measured (µg/mL)	% Recovery
(b) (4)								

Taken from page 9 of (b) (4)-18-012

Analytical Evaluation Threshold (AET):



(b) (4)

(b) (4)

Summary:

The Applicant conducted leachable testing based on their extractable studies. Overall, many of the compounds identified in the extractables studies were not seen in the leachable testing (b) (4) all of which were targeted in some way (see summary table below). Validated methods were employed to measure two targeted leachables only. However, as per the CMC review team, the description of the leachable screening methods that they employed should have been able to detect any of the extractables at levels above the AET.

Overall, a relatively small number of leachables were detected above the AET in the leachables studies. For some leachables, the mcg/day exposure from the drug product was below the general toxicity SCT of 5 mcg/day despite exceeding the AET. This was the case because a worst-case AET based on the highest volume formulation was applied to all samples. For other leachables, adequate justification was provided based on a risk assessment and calculation of a PDE. All of the leachables with identified structures were justified for safety. However, as per the CMC review team, because the screening method used to evaluate the product over the course of stability was not validated for anything other than the two compounds noted above, the values provided by the Applicant are only estimates and cannot be considered definitive until the assays are validated.

In addition, there were four compounds whose structures were not completely identified and adequate justification for what efforts were made to identify the compounds, why they could not be identified, or justification for their safety were not provided. This included two compounds which the Applicant did not even indicate were above the AET in the summary report. The Applicant should provide detailed information regarding the efforts made to identify these compounds and a robust justification for the compounds safety since they exceed the SCT. This is considered a deficiency. Given the potential benefit of the drug product to help address a significant public health crisis and the existing estimated safety justifications, confirmation of identity and validation for all leachables could be completed as a post-marketing requirement if the risk:benefit is deemed acceptable.

Table 40. Summary of Extractables and Leachables Above 5 mcg/day (Values = mcg/day)

Compound	Extractable	Leachable q1w (targeted)	Leachable q4w (targeted)	Leachable q1w (screening)	Leachable q4w (screening)
(b) (4)					

(b) (4)



2.6 Proposed Clinical Population and Dosing Regimen

CAM2038 q1w and CAM2038 q4w are indicated for (b) (4) treatment of opioid use disorder in adults and should be administered once-weekly and once-monthly, respectively. The depot formulation should ensure medication adherence, ultimately improving treatment effectiveness.

2.7 Regulatory Background

Pre-IND meeting were held for IND 114082 on April 12, 2012 and March 31, 2015. The original IND was submitted April 9, 2015. This IND was given fast-track designation on July 9, 2015. A Pre-NDA meeting was held on March 16, 2017. NDA 210136 was submitted July 9, 2017 and priority review was granted.

A complete response action was taken on January 19, 2018 listing numerous deficiencies, including the following pharmacology toxicology deficiencies.

6. You have not provided adequate extractables studies to fully characterize the potential leachables profile of the proposed container closure system. Specifically, the studies submitted with the NDA did not employ sufficiently exhaustive condition (b) (4)

(b) (4)

We acknowledge that your TTCs were based on concepts outlined in ICH M7, and this guidance notes that the acceptable daily intake for genotoxic impurities may be based on total number of dosing days over a lifetime. However, your product is intended to provide continuous exposure to the API for the duration of dosing. Due to the way in which the drug depot is intended to form in subcutaneous tissue after drug administration, leachables arising from the container closure system are likely to be incorporated into the depot (b) (4)

Consequently, patients may be exposed to leachables over the same duration the API is released; at least 7 days and 28 days for the weekly and monthly formulations, respectively. Moreover, treatment for opioid abuse disorder may exceed 10 years. Therefore, your AETs must be able to detect any compound that could be exposed at 1.5 mcg/day or greater. We acknowledge that you have initiated new extractables studies to address these concerns. However, these new data were not provided in time for review for this action.

Information needed to resolve deficiency

Submit final study reports for extraction studies testing (b) (4) (b) (4) employing rigorous extraction techniques in accordance with *USP <1663>: Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems* and ensure that your analytical methods are capable of detecting any compound over 1.5 mcg/day. Use these data to inform which compounds are to be monitored in the leachables studies.

7. You have not provided an adequate leachables evaluation to justify the safety of the proposed container closure system. Specifically, your leachables evaluation did not 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 as advised at the March 16, 2017, Pre-NDA meeting, and in accordance with best practices per *USP <1664>: Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems*.

Information needed to resolve deficiency

Conduct a new leachables study that evaluates at least three batches of the to-be-marketed weekly and monthly drug products and include assessments at multiple timepoints over the course of stability studies (beginning, middle, and end of proposed shelf-life) in order to identify trends in leachable levels over time. In the materials tested, include any secondary container closure systems, if present, and subject these materials to the same sterilization methods, as appropriate. Use the results of the extraction studies 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 upstream manufacturing processes that suggest the potential for additional leachable compounds in the final drug product formulation. For all drug products, establish the AET to be able to detect any compound that could be present at 1.5 mcg/day or greater. If you cannot meet these thresholds, safety evaluations should be based on the limits of quantitation (LOQ). 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. Provide a toxicological risk assessment that qualifies any leachable detected at or greater than 1.5 mcg/day from a genotoxicity perspective and any leachable detected at or greater than 5 mcg/day from a general toxicity perspective. Include copies of all referenced studies upon which a safety assessment is based. We acknowledge that you initiated new leachables studies during this review cycle; however, these new data were not provided in time for review for this action. Submit final study reports for leachables studies that take into consideration the comments outlined above with your resubmission.

8. You have not provided adequate pharmacokinetic data to appropriately calculate exposure margins for buprenorphine in your labeling. Although you have provided AUC_{0-24h} data in animals, you have only provided human AUC_{ss} data. The exposure margins based on AUC in the labeling are to be based on the worst-case exposures in humans over the treatment period at steady state.

Information needed to resolve deficiency

Provide mean partial AUC (24-hour interval) data in humans at steady state that represents the highest exposures over any given 24-hour period to inform labeling.

9. You have not provided adequate justification that the cited published data on the reproductive and developmental effects of NMP adequately address all standard toxicological endpoints for reproductive and developmental toxicity studies. Further, you have not provided adequate data to bridge the published and unpublished studies which employed routes other than the proposed clinical route. Although the data suggest some degree of safety following the different routes of administration, lack of adequate toxicokinetic data and inclusion of all standard endpoints in these studies preclude definitive conclusions regarding the potential risk of NMP via your drug product on these endpoints.

Information needed to resolve deficiency

Conduct definitive reproductive and developmental studies with NMP administered via the SC route. Alternatively, provide adequate PK bridging studies to the existing studies and clearly delineate how the referenced studies address all standard endpoints for fertility and early embryonic development, embryofetal development in two species, and pre- and postnatal development. Draft unpublished study reports are not acceptable as they may not reflect the final presentation of the data. Should you elect to conduct bridging studies, your resubmission must also include a discussion regarding the impact of the safety margins for reported effects of NMP given the known risks to these endpoints from buprenorphine.

10. You have not provided an adequate scientific bridge to the referenced published studies testing diacylglycerol oil (DAG) as a surrogate chemical solution for glycerol dioleate (GDO) for the effects of this novel excipient on rat fertility and early embryonic development, rat embryofetal development, rodent general toxicity, or rat and mouse carcinogenicity.

Information needed to resolve deficiency

As discussed at the February 24, 2015, End-of-Phase 2 meeting, provide data to support your conclusion that the DAG oil tested in these publications contains GDO at levels which provide an adequate characterization of the effects of GDO on these required studies.

11. You have not provided a rabbit embryo-fetal development study for the new excipient GDO.

Information needed to resolve deficiency

Either conduct an EFD study in the rabbit testing GDO or provide adequate justification why a study is not necessary.

12. You have not provided adequate justification for your conclusion that there is no carcinogenic potential of NMP even though your toxicological risk assessment of NMP noted hepatic tumors in mice.

Information needed to resolve deficiency

Conduct a mode of action assessment for N-methyl-pyrrolidone-induced mouse hepatocellular adenomas and carcinomas to inform the human risk assessment for NMP.

A Type A post action meeting was held with the Applicant on May 2, 2018. The NDA was resubmitted on June 26, 2018.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study Number	Location
Buprenorphine 8-Week Subcutaneous Toxicity Study in Beagle Dogs (CAM2038 q1w and q4w)	TK-12-448	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tk-12-448\tk-12-448.pdf
A 4-Weeks Pharmacokinetic Study in Minipigs (not GLP)	PK-08-313	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4222-absorp\pk-08-313\pk-08-313-fr.pdf
Intramuscular Local Tolerability of CAM2038 Drug Products in Dogs	TO-15-537	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4231-single-dose-tox\to-15-537\to-15-537.pdf
Non-GLP Evaluation of the Effects of Buprenorphine, Norbuprenorphine, and Naltrexone on Cloned HERG Channels Expressed in Human Embryonic Kidney (HEK293) Cells	TO-17-589	\\cdsesub1\evsprod\nda210136\0002\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\to-17-589\to-17-589.pdf
Effects of Naltrexone HCl and Norbuprenorphine HCl on Cloned hNAV1.5 Sodium Peak and hNAV1.5 Sodium Late Current, hCAV1.2 Calcium, and hKvLQT1/hminK Potassium Channels Expressed in Mammalian Cells	TO-17-594	\\cdsesub1\evsprod\nda210136\0002\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\to-17-594\to-17-594.pdf
9-Month Subcutaneous Toxicity	TO-15-531	\\cdsesub1\evsprod\nda210136\0001\m4\42-

Study of CAM2038 Drug Products in Dogs		stud-rep\423-tox\4232-repeat-dose-tox\to-15-531\to-15-531-2335012.pdf
Intravenous Administration of CAM2038 Q1W in rats (non-GLP)	P-17-598	IND 114082
A 12 to 14-Day Subcutaneous Pharmacokinetic Study of Buprenorphine in Gravid and Non-Gravid Sprague-Dawley Rats	2335-003	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\2335-003\2335-003.pdf
A 12-Day Subcutaneous Pharmacokinetic Study of Buprenorphine in Gravid New Zealand White Rabbits	2335-004	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\2335-004\2335-004.pdf
A 28 Day Dietary Pharmacokinetic Study of Buprenorphine in Sprague-Dawley Rats	2335-001	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud\2335-001\2335-001.pdf
A 28-Day Dietary Pharmacokinetic Study of Buprenorphine in CD1 Mice	2335-002	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud\2335-002\2335-002.pdf
A 26 Week Toxicity Study of Glycerol dioleate and FluidCrystal® Vehicle by Subcutaneous Injection in Rats	TO-10-405	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-10-405\to-10-405.pdf
Glycerol Dioleate: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow: 0 h and 24 h Subcutaneous Injection Dosing and 48 h Sampling	TO-12-446	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-12-446\to-12-446.pdf
(b) (4) (Glycerol dioleate, $\frac{w}{4}$ %) Ames Test	TO-04-141	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-04-141\to-04-141.pdf
(b) (4) (Glycerol dioleate, $\frac{w}{4}$ %) Ames Test	TO-04-146	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-04-146\to-04-146.pdf
(b) (4) (Glycerol dioleate, $\frac{w}{4}$ %) In Vitro Mammalian Cell Gene Mutation Test Performed with Mouse Lymphoma L5178Y Cells	TO-04-142	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-04-142\to-04-142.pdf
An Embryofetal Development Study of Glycerol Dioleate (GDO) by Subcutaneous Injection in Rabbits	TO-18-607	\cdsesub1\evsprod\nda210136\0079\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\to-18-607\to-18-607.pdf
Glycerol Dioleate (GDO): A Subcutaneous Pre- and Post-Natal Reproductive and Developmental Toxicity Study in Female Sprague-Dawley Rats	TO-16-569	\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-16-569\to-16-569.pdf
Glycerol Dioleate Formulation Review	TO-18-625	\cdsesub1\evsprod\nda210136\0079\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-18-625\to-18-625.pdf

A single dose study in rat evaluating circulating plasma glycerol dioleate levels following s.c. administration of FluidCrystal® Injection depot placebo formulation or glycerol dioleate	PK-15-539	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4227-other-pk-stud\pk-15-539\pk-15-539-fr2.pdf
Exploratory evaluation of NMP (N-methyl-2-pyrrolidinone) levels in plasma from CAM2038 q4w treated healthy volunteers from Study HS-13-487	PK-15-551	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4227-other-pk-stud\pk-15-551\pk-15-551-fr.pdf
NMP (N-methyl-2-pyrrolidinone) Toxicokinetic Study in Rats	TO-15-540	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4227-other-pk-stud\to-15-540\to-15-540-77246-fr.pdf
NMP (N-methyl-2-pyrrolidinone) Toxicokinetic Study in Rabbits	TO-15-541	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4227-other-pk-stud\to-15-541\to-15-541-77247-fr.pdf
An Oral (Gavage) Toxicokinetic Study of N-methyl-2-pyrrolidinone (NMP) in New Zealand White Rabbits	PK-18-610	\\cdsesub1\evsprod\nda210136\0079\m4\42-stud-rep\422-pk\4227-other-pk-stud\pk-18-610\pk-18-610.pdf
An Oral (Gavage) Toxicokinetic Study of N-methyl-2-pyrrolidinone (NMP) in Sprague-Dawley Rats	PK-18-611	\\cdsesub1\evsprod\nda210136\0079\m4\42-stud-rep\422-pk\4227-other-pk-stud\pk-18-611\pk-18-611.pdf
Projection of NMP Exposures in Rats after Inhalation of Various Doses	PK-18-623 (b) (4)	\\cdsesub1\evsprod\nda210136\0079\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\pk-18-623\pk-18-623.pdf
Multi-Generation Rat Reproductive Study with N-Methylpyrrolidinone	Exxon (1991) Project Number 236535 Draft Report	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\exxon-1991.pdf
Developmental Toxicity Study in Rat with N-Methylpyrrolidinone	Exxon (1992) Project Number 236534 Draft Report	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\exxon-1992.pdf
Report on Testing of N-Methylpyrrolidinone for Possible Mouse Teratogenicity	BASF (1970a) Study XIX-422	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\basf-1970a.pdf
NMP (N-methyl-2-pyrrolidinone): Developmental Toxicity Study in New Zealand White Rabbits	IRDC (1991)	\\cdsesub1\evsprod\nda210136\0079\m4\43-lit-ref\irdc-1991.pdf
Oral, Dermal, and Inhalation Pharmacokinetics and Disposition of [2- ¹⁴ C] NMP in the Rat	Dupont (1995b)	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\dupont-1995b.pdf
Report on Testing of Pyrrolidinone for any Teratogenic Effect in the Mouse and Report on the Testing of Pyrrolidinone for any Teratogenic Effect in the Rat after Oral Administration	BASF (1970b) Study XIX-421	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\basf-1970b.pdf
Mutagenicity Test on N-Methylpyrrolidinone in the	Hazelton Laboratories	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\hazelton-1988a.pdf

CHO/HGPRT Forward Mutation Assay	(1988a)	
Mutagenicity Test on N-Methylpyrrolidone in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay	Hazelton Laboratories (1988b)	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\hazelton-1988b.pdf
Evaluation of Three Compounds for their Mutagenic Potential	(b) (4)	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\ (b) (4) .pdf
Toxicological Review and Risk Analysis of N-Methyl-2-Pyrrolidone	TO-16-570	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-16-570\to-16-570.pdf
Toxicological Review and Risk Analysis of Glycerol Dioleate	TO-16-581	\\cdsesub1\evsprod\nda210136\0002\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-16-581\to-16-581.pdf
Computational Mutagenicity Analysis of CAM2038 Impurity	(b) (4) -18-007	\\cdsesub1\evsprod\nda210136\0079\m4\42-stud-rep\423-tox\4237-other-tox-stud\42376-imp (b) (4) 18-007 (b) (4) 18-007.pdf

In addition, the Applicant submitted an extensive literature search to justify the safety of NMP and GDO. These studies are reviewed and included in the literature cited sections of Appendix 1 and 2.

3.2 Studies Not Reviewed (Formally)

The studies listed in the table below were not formally reviewed because there were longer term studies that served as the pivotal supporting data.

Study Title	Study Number	Location
7 Month Repeat Dose Toxicity Study including Toxicokinetics and Local Tolerance of Long-acting Release FluidCrystal® Injection Depot Product of Leuprolide in Beagle Dogs	TO-13-490	\\cdsesub1\evsprod\nda210136\0002\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-13-490\to-13-490-526468-fr.pdf
Buprenorphine A Toxicology and Toxicokinetics Study on Single and Repeat Dosing in Dogs	TO-08-323	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\to-08-323\to-08-323-68434-fr.pdf
CAM2038: 18 Week Subcutaneous Administration Repeat Toxicity Study including Toxicokinetics and Local Tolerance of Longacting Release FluidCrystal® Injection Depot Products of Buprenorphine in Beagle Dogs	TO-13-489	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\to-13-489\to-13-489-8293608-fr.pdf
(b) (4) (Glycerol dioleate, (b) (4) %). Acute Single Dose Study in Rats	TO-05-153	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-05-153\to-05-153.pdf
(b) (4) (Glycerol dioleate, (b) (4) %). A Repeat Dose Subcutaneous Study in Rats	TO-05-156	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-05-156\to-05-156.pdf

(b) (4) (glycerol dioleate, $\frac{w}{(4)}\%$). A 28 Days Toxicity Study in Rats	TO-05-177	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-05-177\to-05-177.pdf
(b) (4) (Glycerol dioleate, $\frac{w}{(4)}\%$). A Single Dose Study in Rats with Recovery	TO-0-189	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-05-189\to-05-189.pdf
4050-K-Placebo Toxicity and Local tolerance of FluidCrystal® vehicle Injection depot formulation – Evaluation of vehicle treated dogs from an 8-Week Subcutaneous Toxicity Study in Dogs with an 8-Week Recovery Period	TO-10-395	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-10-395\to-10-395.pdf

3.3 Previous Reviews Referenced

No other reviews are referenced.

4 Pharmacology

4.1 Primary Pharmacology

No pharmacology studies were conducted for the CAM2038 products. Buprenorphine is a synthetic opioid agonist that is 10-20 times more potent than morphine with a very long duration of action. It acts as a partial mu opioid receptor agonist and a kappa opioid receptor antagonist. The referenced product labeling includes the following information:

12.1 Mechanism of Action

SUBUTEX sublingual tablet contains buprenorphine, a partial agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor.

4.2 Secondary Pharmacology

The secondary pharmacologic effects of a mu opioid agonist such as BUP include dysphoria, euphoria, and sedation. These as well as primary pharmacodynamic effects are summarized in the referenced product labeling under 12.2 as follows:

12.2 Pharmacodynamics

Subjective Effects

Comparisons of buprenorphine to full opioid agonists such as methadone and hydromorphone suggest that sublingual buprenorphine produces typical opioid agonist effects which are limited by a ceiling effect. Opioid agonist ceiling-effects were also observed in a double-blind, parallel group, dose-ranging comparison of single doses of buprenorphine sublingual solution (1, 2, 4, 8, 16, or 32 mg), placebo and a full agonist control at various doses. The treatments were given in ascending dose order at intervals of at least one week to 16 opioid-experienced subjects

who were not physically dependent. Both active drugs produced typical opioid agonist effects. For all measures for which the drugs produced an effect, buprenorphine produced a dose-related response. However, in each case, there was a dose that produced no further effect. In contrast, the highest dose of the full agonist control always produced the greatest effects. Agonist objective rating scores remained elevated for the higher doses of buprenorphine (8-32 mg) longer than for the lower doses and did not return to baseline until 48 hours after drug administration. The onset of effects appeared more rapidly with buprenorphine than with the full agonist control, with most doses nearing peak effect after 100 minutes for buprenorphine compared to 150 minutes for the full agonist control.

Physiologic Effects

Buprenorphine in IV (2, 4, 8, 12 and 16 mg) and sublingual (12 mg) doses has been administered to opioid-experienced subjects who were not physically dependent to examine cardiovascular, respiratory and subjective effects at doses comparable to those used for treatment of opioid dependence. Compared to placebo, there were no statistically significant differences among any of the treatment conditions for blood pressure, heart rate, respiratory rate, O₂ saturation, or skin temperature across time. Systolic BP was higher in the 8 mg group than placebo (3-hour AUC values). Minimum and maximum effects were similar across all treatments. Subjects remained responsive to low voice and responded to computer prompts. Some subjects showed irritability, but no other changes were observed. The respiratory effects of sublingual buprenorphine were compared with the effects of methadone in a double-blind, parallel group, dose ranging comparison of single doses of buprenorphine sublingual solution (1, 2, 4, 8, 16, or 32 mg) and oral methadone (15, 30, 45, or 60 mg) in non-dependent, opioid experienced volunteers. In this study, hypoventilation not requiring medical intervention was reported more frequently after buprenorphine doses of 4 mg and higher than after methadone. Both drugs decreased O₂ saturation to the same degree. Androgen Deficiency Chronic use of opioids may influence the hypothalamic-pituitary-gonadal axis, leading to androgen deficiency that may manifest as low libido, impotence, erectile dysfunction, amenorrhea, or infertility. The causal role of opioids in the clinical syndrome of hypogonadism is unknown because the various medical, physical, lifestyle, and psychological stressors that may influence gonadal hormone levels have not been adequately controlled for in studies conducted to date.

4.3 Safety Pharmacology

The effects of BUP on critical physiological systems are well-known and extensive clinical experience exists with BUP. Two new safety pharmacology studies were completed by the Applicant in response to questions from the Agency about the

potential impact of buprenorphine on the QT prolongation. In addition, several cardiovascular parameters were built into the toxicology studies, which are also summarized below. These data are consistent with the known effects of buprenorphine and suggest minimal risk for an impact on the receptors examined as the responses were in the micromolar range. The reader is referred to the review by the QT IRT team for specific details regarding the potential impact of buprenorphine on the QT interval.

The summary of these studies, as provided by the Applicant, is reproduced below:

Table 2.6.3.4-1 Safety Pharmacology							
Test Article: CAM2038 q1w and CAM2038 q4w							
Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses	Gender/ No. per Group	Noteworthy Findings	GLP Compliance	Report No.
I _{Kr} potassium ion channel hERG	Stably expressed HEK293 cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 1, 3, 10, 30 μM NBPN: 3, 10, 30, 100 μM NTX: 3, 10, 30, 100 μM Cisapride (positive control) 0.1 μM	3-4 cells per dose	BPN IC ₅₀ 11 μM; IC ₂₀ 2.5 μM NBPN IC ₅₀ 70 μM; IC ₂₀ 11 μM NTX IC ₅₀ 76 μM; IC ₂₀ 11 μM Cisapride ~82% inhibition (laboratory mean historical control value is 79.89%)	No	TO-17-589
Peak hNav1.5 sodium ion channel	Stably expressed HEK293 cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 3, 10, 30, 100 μM NBPN: 1, 3, 10, 30, 100 μM NTX: 3, 10, 30, 100 μM Lidocaine (positive control) 2 mM	3 cells per dose	BPN IC ₅₀ 13.8 μM; IC ₂₀ 4.9 μM NBPN IC ₅₀ 7.9 μM; IC ₂₀ 2.5 μM NTX IC ₅₀ >100 μM; IC ₂₀ <100 μM Lidocaine 91.6% inhibition	No	TO-17-594
Late hNav1.5 sodium ion channel	Stably expressed HEK293 cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 1, 3, 10, 30 μM NBPN: 1, 3, 10, 30 μM NTX: 3, 100 μM Ranolazine (positive control) 100 μM	3 or 5 cells per dose	BPN IC ₅₀ 7.3 μM; IC ₂₀ 2.1 μM NBPN IC ₅₀ 8.7 μM; IC ₂₀ 1.8 μM NTX IC ₅₀ >100 μM; IC ₂₀ <100 μM Ranolazine 74% inhibition	No	TO-17-594
hCav1.2 (L-type) calcium ion channel	Stably expressed CHO cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 1, 3, 10, 30 μM NBPN: 1, 3, 10, 30 μM NTX: 30, 100 μM Nifedipine (positive control) 0.1 μM	3 cells per dose	BPN IC ₅₀ 8.6 μM; IC ₂₀ 2.6 μM NBPN IC ₅₀ 29.7 μM; IC ₂₀ 10.5 μM NTX IC ₅₀ >100 μM; IC ₂₀ <100 μM Nifedipine 94.7% inhibition	No	TO-17-594

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses	Gender/ No. per Group	Noteworthy Findings	GLP Compliance	Report No.
hKvLQT1/hmin K (I _{Ks}) potassium ion channel	Stably expressed HEK293 cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 3, 10, 30, 100 μM NBPN: 3, 10, 30, 100 μM NTX: 30, 100 μM Chromanol (positive control) 30 μM	3-5 cells per dose	BPN IC ₅₀ 34.8 μM; IC ₂₀ 5.9 μM NBPN IC ₅₀ 29.7 μM; IC ₂₀ 10.5 μM NTX IC ₅₀ >100 μM; IC ₂₀ >100 μM Chromanol 68.8% inhibition	No	TO-17-594
Cardiovascular system	Dog/Beagle	SC	Saline (weekly dosing) CAM2038 q1w – 32 mg, CAM2038 q1w Placebo CAM2038 q4w – 128, 160, 192 mg, CAM2038 q4w Placebo	MF/4	Repeat dose toxicity for 9 months (once-weekly CAM2038 q1w, once-monthly CAM2038 q4w); ECG was measured after the first (Day 1) and last dose. Day 1 post-dose interval consisting of a mild slowing of the heart rate and moderate lengthening of the QT and QTc intervals for both drug products compared to the respective placebo and saline control. Changes not observed at termination of the study.	Yes	TO-15-531
Cardiovascular system	Dog/Beagle	SC	CAM2038 q1w 32 mg (0.64 mL), FC Placebo 0.36 mL CAM2038 q4w 64, 128 mg (0.18, 0.36 mL)	MF/3	Repeated administration of CAM2038 q4w at 64 or 128 mg monthly, or weekly injections of CAM2038 q1w at 32 mg had no effect on ECGs at Days 71, 92 or 120 in males and females compared to the vehicle-treated group	Yes	TO-13-489

BPN – buprenorphine; CHO – Chinese hamster ovary; HEK293 – human embryonic kidney; NBPN – norbuprenorphine; NTX- naltrexone; SC – subcutaneous

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The Applicant conducted PK assessments following single dosing and repeat dosing in rats, dogs, and minipigs testing various formulations of the drug product. These data are not reviewed formally.

No new nonclinical studies examining the distribution, protein binding, metabolism, excretion, or drug interactions were conducted or required.

5.2 Toxicokinetics

See results under general toxicology.

In addition, the Applicant conducted two studies to obtain exposure data in pregnant rats and rabbits to attempt to bridge the referenced Subutex reproductive and developmental data in the literature to a prolonged depot injection. The results of these studies are summarized in Section 9 of this review.

The Applicant also conducted two 28-day repeat-dose PK studies in mice and rats to bridge to the referenced carcinogenicity studies. These data are summarized in Section 8 of this review.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: A 4-weeks Pharmacokinetic Study in Minipigs

Study Number: PK-08-313

Study Date: August 12, 2008

Regulatory Status: No GLP statement, presumed nonGLP and not audited.

Test Article: CAM2038 q1w

Methods: One male and one female minipig was injected subcutaneously with 20 mg (0.5 mL) dose of CAM2038 q1w on Day 1. At separate sites q1w vehicle was injected on Day 1, 15, and 22. Animals were euthanized on Day 29 and local tissue injection sites were evaluated macroscopically and microscopically. Blood samples were obtained for PK data.

Injection sites were evaluated for hemorrhage, erythema, swelling and tenderness and scores as per the following scale:

		<u>Score</u>
Not present	-	0
Minimal	-	1
Slight	-	2
Moderate	-	3
Marked	-	4

Microscopic evaluations graded the response on a 5-point system as follows:

Grade 1	-	Minimal/Very few/Very small
Grade 2	-	Slight/Few/Small
Grade 3	-	Moderate/Moderate number/Moderate size
Grade 4	-	Marked/Many/Large
Grade 5	-	Massive/Extensive number/Extensive size
Present	-	Finding present/Severity not scored

Results: There were no clinical signs reported.

At the injection site, swelling was noted with both drug formulation and vehicle. No clear evidence of hemorrhage or erythema was reported. In the Male at the q1w injection site, swelling was scored as slight on Days 1-5, moderate from Days 6 to 16, slight on Days 17-22, and minimal out through Day 30. Similar findings were reported at the vehicle injections sites but they appeared to reach minimal grading earlier (generally by Day 15).

Microscopically, cystic spaces, presumably drug matrix depot, was noted as well as subcutaneous fibrosis and chronic granulomatous inflammation. Findings were more severe on Day 1 than Day 15. Day 29 CAM2038 q1w local findings were similar to Day 22 vehicle findings suggesting that the buprenorphine was contributing to the prolonged local effects.

TK data are summarized below:

Table 2.6.6.2-1: Mean Toxicokinetic Parameters for Buprenorphine in Minipigs Treated with a Single Dose of a CAM2038 q1w Formulation

Dose (mg) (Dose volume)	T _{max} (days) Mean	C _{max} (ng/mL) Mean	AUC _{inf} (ng*d/mL) Mean	t _{1/2} (days) Mean
20 (0.5 mL)	1.0	100	490	2.7

From Study PK-08-313. In the study report, AUC, T_{max} and T_{1/2} are expressed in hours but the values were converted to days by dividing by 24. The CAM2038 q1w formulation was 2038BUP-I (see Table 2.6.1.1-3).

Study title: Buprenorphine 8-Week Subcutaneous Toxicity Study in Beagle Dogs

Study Number: TK-12-488

Study Date: April 25, 2014

Regulatory Status: GLP

Test Article: 2038BUP-BJ = CAM2038 q4w and 21038BUP-G = CAM2038 q1w

Test item	Batch No	BUP free base	SPC	GDO	EtOH	NMP
2038BUP-BJ	B2038BUP(0)-1209-95			(b) (4)	-	30.00
2038BUP-G	B2038BUP(0)-1209-94				10.00	-

BUP = Buprenorphine, SPC = Soy phosphatidylcholine, GDO = Glycerol dioleate, EtOH = Ethanol, and NMP= N-methyl-2-pyrrolidone

Methods: Male and Female beagle dogs were treated subcutaneously with a single dose of CAM2038 q4w or four weekly doses of CAM2038 q1w (Days 1, 8, 15, and 22) at four different injection sites as per the following groupings:

Group	Test item	Dose BUP base* (mg)	Concentration BUP base (mg/mL)	Dose volume test item (mL)	Animal Nos	
					Male	Female
1	2038BUP-BJ	1 x 60	368	1 x 0.16	1-3	4-6
2	2038BUP-G	4 x 15	50	4 x 0.30	7-9	10-12
3	2038BUP-G	4 x 30	50	4 x 0.60	13**-15	16-18

*Material as supplied

**Animal No 13 was only dosed on Day 1.

Animals were euthanized and necropsies on Day 57 (35 Days after the last injection).

Clinical signs were monitored. Local injection site reactions (hemorrhage, erythema, swelling, and tenderness) were scored as per the following:

		<u>Score</u>
Not present	-	0
Minimal	-	1
Slight	-	2
Moderate	-	3
Marked	-	4

Body weight, food consumption, clinical chemistry, hematology, urinalysis, organ weights, macroscopic and microscopic findings and TK were evaluated as per standard procedures. Microscopic evaluations were graded as follows:

Histological alterations were graded on a 5-grade system:

- Grade 1 - Minimal/Very few/Very small
- Grade 2 - Slight/Few/Small
- Grade 3 - Moderate/Moderate number/Moderate size
- Grade 4 - Marked/Many/Large
- Grade 5 - Massive/Extensive number/Extensive size
- Present - Finding present/Severity not scored

Results: Clinical signs consistent with an opioid were observed after injection (e.g., reduced activity, salivation, redness of eyes) which lasted several days in most cases. Other clinical signs included GI effects (soft feces, no feces, vomiting) over the first week.

Hemorrhage was not observed in any group. Erythema was only observed only in a few animals treated with q1w. Tenderness was observed in q4w animals which lasted 2-7

days. In q1w animals, tenderness and swelling was observed in all injection sites and scored minimal to slight. Duration of effects were highly variable with some lasting around 20-35 days.

The size of the depot in q4w animals was generally between 10 and 15 mm in diameter, started to gradually reduce in size after 2-3 weeks and lasted approximately 31 and 37 days (although one was still noted at Day 57). For q1w treated animals, the size of the reaction site was between 15 and 20 mm in diameter, started to reduce in size after about 3 weeks and the last reactions were noted on Days 42-43 but some animals continued to display evidence of the local depot out to Day 57.

At necropsy, subcutaneous nodules were noted at most injection sites in the q1w groups. No macroscopic changes were noted in q4w sites at Day 57.

Microscopically, evidence of mononuclear cell infiltrates, granulomatous inflammation including giant cells and yellow material, cystic spaces, fibrosis, hemorrhage, and eosinophilic materials were noted in the q4w injection sites as per the following table, reproduced from the study report.

Microscopic findings in the subcutaneous injection sites (Group 1)

Dose group	Group 1 2038 BUP-BJ 60 mg BUP Base/0.16 mL	
Injection site (pooled examination of two slides)	No 1	
Days from injection to necropsy	56	
Sex	M	F
Number of animals examined	3	3
Infiltration of mononuclear cells, s.c.		
Total	1	0
Grade 1	1	-
Inflammation, s.c., granulomatous, incl. giant cells & yellow pigment		
Total	2	3
Grade 2	2	3
Cystic spaces, s.c.		
Total	2	3
Grade 1	1	-
Grade 2	1	3
Fibrosis s.c.		
Total	2	3
Grade 2	2	3
Haemorrhage, focal, s.c.		
Total	1	0
Grade 1	1	-
Eosinophilic material, focal, s.c.		
Total	2	3
Grade 1	2	3

Similar microscopic changes were also noted in the q1w treated animals, as per the table below. As expected, the severity of the findings was most notable at Site 4, which had the least amount of recovery time.

Table 7 Microscopic findings in the subcutaneous injection sites (Group 2)

Dose group	Group 2, 2038 BUP-G, 50 mg BUP Base, 0.3 mL/injection							
	Site No 1		Site No 2		Site No 3		Site No 4	
Injection Site (pooled examination of two slides)								
Days from injection to necropsy	56		49		42		35	
Sex	M	F	M	F	M	F	M	F
Number of animals examined	3	3	3	3	3	3	3	3
Infiltration of mononuclear cells, s.c.								
Total	0	0	0	0	0	0	1*	0
Grade 1	-	-	-	-	-	-	1	-
Inflammation, s.c., pyogranulomatous, occasionally incl. necrosis & yellow pigment								
Total	3	3	3	3	3	3	2	3
Grade 1	3	2	-	-	1	-	-	-
Grade 2	-	1	1	2	2	2	-	1
Grade 3	-	-	2	1	-	1	2	2
Cystic spaces, s.c.								
Total	0	2	2	3	2	3	2	3
Grade 1	-	1	-	-	1	-	-	-
Grade 2	-	1	1	2	1	2	2	1
Grade 3	-	-	1	1	-	1	-	2
Fibrosis s.c.								
Total	3	3	3	3	3	3	2	3
Grade 1	2	1	-	-	-	-	-	-
Grade 2	1	2	2	2	3	3	-	2
Grade 3	-	-	1	1	-	-	1	1
Grade 4	-	-	-	-	-	-	1	-
Haemorrhage, focal, s.c.								
Total	0	1	1	0	2	0	1	0
Grade 1	-	1	1	-	2	-	1	-
Basophilic material, focal, s.c.								
Total	0	0	0	1	0	1	0	1
Grade 1	-	-	-	1	-	-	-	-
Grade 2	-	-	-	-	-	1	-	1

*Considered to represent a mis-sampling of the injection site, unsuccessful attempts to locate the centre of the lesion were made

Study title: Intramuscular Local Tolerability of CAM2038 Drug Products in Dogs.

Study no.: TO-15-537
Study report location: EDR 4.2.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: June 3, 2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CAM2038 q1w – Lot 23506.002A, 50 mg buprenorphine/0.64 mL syringe, 101.6%
CAM2038 q4w – Lot 14PII0324-070, 356 mg buprenorphine in 0.54 mL, 99%
CAM2038 q1w placebo – Lot 23513.001A, buprenorphine free
CAM2038 q4w placebo – Batch B2038IAS-1504-119, buprenorphine free

Key Study Findings

- The objective of this study was to evaluate local injection site tolerability following single dose of intramuscular (IM) of CAM2038 q1w or CAM2038 q4w and their respective placebos to Beagle dogs, a potential inadvertent route of dosing.
- Placebo and drug were administered to the same dog.
- For Group 1, 2/sex were sacrificed on days 2 and 29 after dosing (q1w and q1w placebo). For Group 2, 2/sex/group sacrificed on days 2 and 113 after dosing (q4w and q4w placebo).
- There were no definitive buprenorphine-related effects on the local site.
- All observations were related to the vehicle and/or procedure, and were similar for both vehicles on study. In general, injection site microscopic observations were present in both groups and in both the test article and the placebo injection sites, and included one or more of the following: mild cavitation, minimal to moderate myofiber degeneration/necrosis, and minimal to mild mixed leukocyte infiltration, which occasionally extended to mild subacute/chronic inflammation. Cavitation was characterized by variable large clear spaces between myofibers, which were considered to represent test article and placebo depots. In males, there was a higher incidence and severity of myofiber degeneration in sites receiving the test article (Injection Site 2) when compared to placebo (Injection Site 1). However, the injection

site for both placebo and test article-treated females had the same finding and as such, this observation was considered to be likely procedural or vehicle related.

- In conclusion, administration of a single intramuscular dose of CAM2038 q1w at 32mg (0.64 mL) and CAM2038 q4w at 192 mg (0.54 mL) produced expected opioid-like effects on clinical observations, body weight, and food consumption. There were no grossly observed abnormal injection site reactions. No other grossly observed abnormal tissues were observed. Microscopic evaluation was performed on injection sites only. Microscopically, the incidence and/or severity of injection site observations on Day 2 (cavitation, myofiber degeneration/necrosis, and/or mixed leukocyte infiltration) were generally similar between Groups and between placebo and test article injection sites (Injection Sites 1 and 2, respectively).
- Compared with Day 2, microscopic changes were decreased on Day 29 (Group 1) and absent on Day 113 (Group 2), indicating progressing or full recovery over a period of approximately 1 month for q1w and 4 months for q4w.

Methods

Doses:

Group Number	Treatment Identity	Dose Level (mg/dose)	Dose concentration (mg/mL)	Dose volume (mL/dose)	Number of Animals	
					Male	Female
1	CAM2038 q1w	32	50	0.64	4 ^a	4 ^a
	Placebo for CAM2038 q1w	NA	NA	0.64		
2	CAM2038 q4w	192	355.6	0.54	4 ^b	4 ^b
	Placebo for CAM2038 q4w	NA	NA	0.54		

^aTwo animals/sex were euthanized on Day 2 (at least 24 hours postdose). Two animals/sex were maintained for a 4-week (28-day) recovery period.
^bTwo animals/sex were euthanized on Day 2 (at least 24 hours postdose). Two animals/sex were maintained for a 16-week (112-day) recovery period.
 NA – Not Applicable

The dose levels were selected by the Sponsor, or in consultation with the Sponsor, on the basis of available data from previous studies.

Frequency of dosing: Single dose
 Route of administration: Intramuscular
 Dose volume: See table
 Formulation/Vehicle: CAM2038 1w placebo and CAM2038 q4w placebo
 Species/Strain: Beagle dog
 Number/Sex/Group: 4
 Age: 7-8 months
 Weight: 10.90 to 11.70 kg (males) and 9.05 to 10.35 kg

(females)
Satellite groups: Group 1 - 2/sex sacrificed on Days 2 and 29 after dosing
Group 2 – 2/sex/group sacrificed on Days 2 and 113 after dosing
Unique study design: No
Deviation from study protocol: Nothing remarkable

Observations and Results

Mortality

All animals were observed for mortality twice daily.

There were no test article-related deaths. All animals survived to the scheduled necropsies.

Clinical Signs

All animals were observed for morbidity and injury twice daily. A detailed clinical examination of each animal was performed on the day of receipt or transfer, 6 hours post-dose on Day 1, and weekly during the recovery period, at the same time of day ± 90 minutes as the Day 1 observation.

There were no test article-related macroscopic incidences of local tolerability effects on the Day 2, 29, or 113 intervals. Because each animal served as its own control with test article administration occurring in the muscle tissue of the right hind limb and the corresponding placebo administered into the tissue of the left hind limb, distinctions between the placebo and the test articles can be made only with respect to local tissue effects. Although the role of the placebo on systemic effects cannot be identified, clinical observations were generally consistent with the known opioid-like effects of the active component (buprenorphine) and consisted of decreased activity, ataxia, hunched posture, lateral recumbency (one Group 2 female, Animal 106, on Day 1) salivation, and inappetence. The clinical observations tended to be more pronounced for the CAM2038 q4w 192 mg group (Group 2) than for the CAM2038 q1w 32 mg group (Group 1). Except for two Group 2 females noted as thin, potential test article-related clinical observations resolved within approximately a week of dosing.

Dosing and Injection Site Observations

The placebos and test articles were administered once on Day 1 during the study via slow bolus intramuscular injection into the muscle tissue of the hindlimbs.

The placebo was administered into the left hindlimb of each animal (designated as Injection Site 1) and the corresponding test article was administered into the right hindlimb of each animal (designated as Injection Site 2). The injection sites of each animal were evaluated for gross signs of irritation and any other signs of local or systemic effect once pre-dose to 46 minutes post-dose. Additional evaluations were conducted once at 48 and 72 hours post-dose and once weekly thereafter for recovery animals.

No injection site abnormalities were observed. On Day 2, one Group 2 female had a small nodule at Injection Site 2 (CAM2038 q4w), which was likely due to deposition of the gel matrix containing the test article.

Body Weights

Body weights for all animals were measured and recorded on the day of receipt or transfer, prior to the measured-value randomization (Day -3) of animals assigned to study on June 30, 2015, on Day -1, and weekly during the study. The body weights recorded prior to Day -1 are not reported but are maintained in the study file.

Consistent with known opioid-like side effects, administration of CAM2038 q1w 32 mg and CAM2038 q4w 192 mg were associated with body weight loss in the first weeks of the study. Compared with pretest body weights, animals given 32 mg CAM2038 q1w lost up to -14.1% of their body weight 7 to 21 days after dosing. Similarly, animals given CAM2038 q4w 192 mg lost up to -13.7% of their body weight 14 to 35 days post dose. Following the indicated intervals, all animals gained weight. By the end of the recovery period (Days 28 and 112 for Groups 1 and 2, respectively), animals given 32 mg CAM2038 q1w or CAM2038 q4w 192 mg were within 8.8 and 3.5% of pretest body weights, respectively.

Food and Water Consumption

All animals were observed for the availability of food and water twice daily. Food consumption was measured and recorded daily during the study and reported weekly during the recovery period.

Consistent with body weight decreases, food consumption was generally decreased for 1 to 3 weeks following dosing with CAM2038 q1w 32 mg, and 3 to 4 weeks following dosing with CAM2038 q4w 192 mg. Mean Week 1 food consumption was reduced -64.4% to -86.6% compared with food consumption during the week prior to recovery necropsy (Weeks 4 and 16 for Groups 1 and 2, respectively).

Ophthalmoscopy - none

ECG - none**Hematology, Clinical Chemistry, and Urinalysis - none****Gross Pathology**

Postmortem study evaluations were performed on all surviving animals euthanized at the scheduled terminal necropsy (Day 2) and recovery necropsies (Days 29 and 113).

There were mild to moderate focus/foci (red/brown) within the lung on Day 2, and this correlated to foreign material and/or subacute/chronic inflammation microscopically. There were moderate focus/foci (red) in the rectum that correlated to mild hemorrhage microscopically. The role of the test article in these observations could not be delineated because all animals on study received the test article, and systemic toxicity was not a goal of the study.

Organ Weights - none**Histopathology**

Adequate Battery – Yes for local effects.

The following list constitutes the full complement of organs and tissues that were collected, preserved, and examined. Only gross lesions and injection sites were microscopically examined. However, as there is a repeat-dose tox study, this study is only being used to address local effects.

- Adrenal (2) - Aorta - Bone with marrow [femur] - Bone with marrow [sternum] - Bone marrow smear [2 collected]^a - Brain - Epididymis (2) - Eye including optic nerve (2) - Gallbladder - GALT [Gut Associated Lymphoid Tissue] - Gastrointestinal tract: - esophagus - stomach [cardia, fundus, and pylorus] - duodenum - jejunum - ileum - cecum - colon - rectum - Gonads: - ovary (2) with oviduct (2) - testis (2) - Gross lesions[#] - Heart - Injection site [1 and 2][#] - Joint, tibiofemoral	- Kidney (2) - Larynx - Liver [3 sections collected] - Lung with bronchi [collected whole] - Lymph nodes: mandibular [2 collected] and mesenteric - Mammary gland - Pancreas - Pituitary - Prostate - Salivary gland, mandibular [2 collected] - Salivary gland, parotid [2 collected] - Salivary gland, sublingual [2 collected] - Sciatic nerve - Skeletal muscle, biceps femoris - Skin - Spinal cord [cervical, thoracic, and lumbar] - Spleen - Thymus - Thyroid/parathyroid (2) - Tongue - Trachea - Ureter (2) - Urinary bladder - Uterus [both horns]/Cervix - Vagina

^aBone marrow smears were collected at the scheduled necropsies and held.

(2) Paired organ

[#]Microscopically examined

Peer Review – No.

Histological Findings

Day 2 (see table)

There were no definitive buprenorphine-related effects on the local site. All observations were considered to be related to the vehicle and/or procedure, and were similar for both vehicles on study. In general, injection site microscopic observations were present in both groups and in both the test article and the placebo injection sites, and included one or more of the following: mild cavitation, minimal to moderate myofiber degeneration/necrosis, and minimal to mild mixed leukocyte infiltration, which occasionally extended to mild subacute/chronic inflammation. Cavitation was characterized by variable large clear spaces between myofibers, which were considered to represent test article and placebo depots. In males, there was a higher incidence and severity of myofiber degeneration in sites receiving the test article (Injection Site 2) when compared to placebo (Injection Site 1). However, the injection site for both placebo and test article-treated females had the same finding and as such, this observation was considered to be likely procedural or vehicle related.

Injection Site Microscopic Observations – Day 2 Terminal					
Sex		Male		Female	
Dose Group		Group 1	Group 2	Group 1	Group 2
Number Examined		2	2	2	2
Injection site 1 (placebo)					
cavitation					
	-mild	0	0	2	2
degeneration/necrosis, myofiber		0	1	2	2
	-minimal	0	1	2	0
	-mild	0	0	0	2
infiltration, mixed leukocyte		0	1	1	1
	-minimal	0	1	1	0
	-mild	0	0	0	1
inflammation, subacute/chronic					
	-mild	0	0	0	1
Injection site 2 (test article)					
cavitation					
	-mild	2	2	2	2
degeneration/necrosis, myofiber		2	2	2	2
	-minimal	1	0	2	1
	-mild	1	1	0	1
	-moderate	0	1	0	0
infiltration, mixed leukocyte		2	2	1	1
	-minimal	1	0	1	0
	-mild	1	2	0	1
inflammation, subacute/chronic					
	-mild	0	0	0	1

The incidence and/or severity of procedure/vehicle-related findings were generally comparable in Injection Sites 1 (placebo) and 2 (test article) when male and female microscopic data are considered for both test articles.

Day 29 (see table)

There were no Buprenorphine-related effects on the local site. Vehicle-related findings were comparable in Injection Sites 1 (placebo) and 2 (CAM2038 q1w) between males and females and included minimal to mild cavitation, mild to moderate granulomatous inflammation, and minimal to mild pigment. Granulomatous inflammation included scattered macrophages with large cytoplasm (epithelioid) and occasional multinucleated giant cells which indicate a response to exogenous material. Overall, the granulomatous inflammation on Day 29 was interpreted as an attempt to clear the injected material described on Day 2.

Injection Site Microscopic Observations – Day 29 Recovery		
Group	1	1
Sex	Male	Female
Number Examined	2	2
Injection site 1 (placebo)		
cavitation		
-mild	1	2
inflammation, granulomatous	2	2
-mild	1	1
-moderate	1	1
pigment	2	2
-minimal	2	1
-mild	0	1
Injection site 2 (test article)		
cavitation	0	2
-minimal	0	1
-mild	0	1
inflammation, granulomatous		
-mild	2	2
pigment	2	2
-minimal	2	1
-mild	0	1
Group 1 – 32 mg/dose		

Day 113 (only Group 2)

There were no buprenorphine-related effects on the local site. Vehicle-related effects were not observed and were considered fully recovered. The observation of minimal extra medullary hematopoiesis and mild cyst in the pituitary gland of one animal were considered incidental because of the absence in the opposite sex and at other intervals.

Special Evaluation - None other than noted.

Toxicokinetics – none

Dosing Solution Analysis

The Sponsor provided documentation of the strength, purity, composition, stability, and other pertinent information for each batch or lot of the placebos and test articles used on study.

The placebos for CAM2038 q1w and CAM2038 q4w, and test articles, CAM2038 q1w buprenorphine subcutaneous injection (also referred to as CAM2038 q1w) and CAM2038 q4w buprenorphine subcutaneous injection (also referred to as CAM2038 q4w), were administered neat (undiluted), as received from the suppliers.

6.2 Repeat-Dose Toxicity

Study title: 9-Month Subcutaneous Toxicity Study of CAM2038 Drug Product in Dogs

Study no.: TO-15-531 (b) (4) # 2335-012)
 Study report location: EDR 4.2.3.2
 Conducting laboratory and location: (b) (4)
 Date of study initiation: July 29, 2015
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: See table below

Table 41. Summary of Lot and Purity of Drug Products used in 9-month Dog Study

<i>Drug</i>	<i>Lot #</i>	<i>BUP purity</i>
CAM2038 q1w 32 mg	23506.002/23506.002A	101.6 %
CAM2038 q4w 128 mg	23521.001/23521.001A	100.9%
CAM2038 q4w 160 mg	23522.001/23522.001A	100.1%
CAM2038 q4w 192 mg	14PII0325-070	99%

Key Study Findings

Dogs were treated with CAM2038 q1w and q4w for 9 months with the following key study findings:

- The NOAEL in this study is 192 mg, the highest dose tested. The highest dose tested yielded exposure margins in dogs of 16-fold the human systemic exposure of 32 mg CAM2038 q1w based on AUC_{0-168h} comparisons.
- Very slight edema and minimal to moderate swelling/thickening of the skin (with subcutaneous tissue) was observed in all treated groups.
- Microscopic observations of foci of inflammation were observed in all treated groups, independent of BUP level. The localized inflammatory response ranged from acute to chronic inflammation depending on the time between dosing and microscopic evaluation.
- All CAM3028 groups containing BUP showed slowing of heart rate and dose-dependent lengthening of the QT/QTc interval on Day 1 post-dosing but not at the end of the study. This will be addressed clinically because it is a known pharmacologic effect of buprenorphine and is not specific to this formulation.

Methods

Doses: 32, 128, 160, 192 mg BUP
 Frequency of dosing: Saline, CAM2038 q1w vehicle and 32 mg: q1w;
 CAM2038 q4w vehicle, 128 mg, 160 mg and 192 mg: q4w
 Route of administration: SC
 Dose volume: Saline and CAM2038 q1w: 0.64 mL; CAM2038 q4w: 0.58 mL
 Formulation/Vehicle: Saline control, vehicle controls for each presentation outlined in the table below
 Species/Strain: Dog/beagle
 Number/Sex/Group: 4/sex/group
 Age: 12-13 months
 Weight: M: 9-12 lbs; F: 7-10 lbs
 Satellite groups: None
 Unique study design: This study used a saline control and a vehicle control for each presentation (See table below). Injections were rotated across eight sites on back of the dog.
 Deviation from study protocol: None that affected the integrity of the study.

Table 42. Study Design for 9-Month Dog Study

Table A. Study Design							
Group	Test Article				Dose Volume (mL/dose)	Terminal Necropsy	
	Identity	Dose Level (mg/dose)	Dose Frequency	Concentration (mg/mL)		M	F
1	Saline Control	0	Once every week	NA	0.64	4	4
2	CAM2038 q1w Placebo Control	0	Once every week	NA	0.64	4	4
3	CAM2038 q4w Placebo Control	0	Once every 4 weeks	NA	0.58	4	4
4	CAM2038 q1w Drug Product	32	Once every week	50	0.64	4	4
5	CAM2038 q4w Drug Product	128	Once every 4 weeks	355.6	0.36	4	4
6	CAM2038 q4w Drug Product	160	Once every 4 weeks	355.6	0.45	4	4
7	CAM2038 q4w Drug Product	192	Once every 4 weeks	355.6	0.54	4	4

NA: Not applicable
 M – Male
 F – Female

Table 43. Composition of Treatment Groups

	Table B. Composition (mg) per dose					
Identity	CAM2038 q1w Placebo Control ^a	CAM2038 q4w Placebo Control ^a	CAM2038 q1w Drug Product ^b	CAM2038 q4w Drug Product ^c	CAM2038 q4w Drug Product ^c	CAM2038 q4w Drug Product ^c
Dose Group	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Dose Volume	0.64 mL	0.58 mL	0.64 mL	0.36 mL	0.45 mL	0.54 mL
BPN	—	—	32	128	160	192
NMP	(b)(4)					
EtOH						
SPC						
GDO						
<i>BPN = buprenorphine; NMP = N-methyl-2-pyrrolidone; EtOH= ethanol; SPC = soy phosphatidylcholine; GDO = glycerol dioleate</i> ^aThe density of CAM2038 q1w placebo control and CAM2038 q4w placebo control is 0.957 and 0.978 g/mL, respectively. ^bThe density of the CAM2038 q1w drug product is 0.957. ^cThe density of the CAM2038 q4w drug products is 1.051 g/mL						

Dose Site Rotation			
Study Day	Injection Site Used		
	Groups 1, 2, 4	Groups 3, 5, 7	Group 6
1	1	1	1
8	4	-	-
15	5	-	-
22	8	-	-
29	2	4	4
36	3	-	-
43	6	-	-
50	7	-	-
57	1	5	5
64	4	-	-
71	5	-	-
78	8	-	-
85	2	8	8
92	3	-	-
99	6	-	-
106	7	-	-
113	1	2	4
120	4	-	-
127	5	-	-
134	8	-	-
141	2	3 ^a	5
148	3	-	-
155	6	-	-
162	7	-	-
169	1	6	8
176	4	-	-
183	5	-	-
190	8	-	-
197	2	7	4
204	3	-	-
211	6	-	-
218	7	-	-
225	1	7	5
232	4	-	-
239	5	-	-
246	8	-	-
253	2	7	8
260	3	-	-
267	6	-	-

- Dose not administered
^aOn Day 141, one male animal in Group 3 (animal number 224) was administered the placebo into dose site 4, rather than into dose site 3.

Observations and Results

Mortality

All dogs survived until scheduled necropsy.

Clinical Signs

Detailed clinical examinations were conducted weekly during the study and twice on the day of dosing (2 and 6 h post-dose). Injection sites were scored for erythema/eschar and edema using the Draize scale at 4, 24, 48, and 72 h post dose. Reduced incidence of soft feces, decreased appetite and activity and thin appearance were dose-dependently increased in BUP groups. Aggressive behavior and vocalizations were also seen during dosing of BUP groups. These findings are attributed to the known pharmacologic effects of BUP. Increased incidences of injection site scabbing were seen in Group 4 (CAM2038 q1w 32 mg) and Group 7 (CAM2038 q4w 192 mg) but the observations were not dose dependent and were considered secondary to the dosing procedure by the Applicant.

Body Weights

Body weights were measured prior to randomization (Day -6), on Day -1 and weekly, with the exception of Month 2 of the study where they were recorded once (on Day 56). Males in the CAM2038 q4w 192 mg (4.9%) had lower body weight gains at the end of the study as compared to saline and the CAM2038 q4w vehicle (14.8%). The decrease is attributed to the pharmacologic effects of BUP and was not considered adverse. No differences in females in any group were observed.

Food Consumption

Food consumption was measured daily during the study. Overall, no significant effects on food consumption were observed for any group.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted pretest and prior to terminal necropsy. No ophthalmic abnormalities were observed in any group.

ECG

All dogs received ECG examinations pretest, on Day 1 (predose and 4-6 h postdose) and on the last day of dosing (predose and 4-6 h postdose). All CAM3028 groups containing BUP showed slowing of heart rate and dose-dependent lengthening of the QT/QTc interval. The table below shows the magnitude of the changes, which are all greater than 10%. Buprenorphine is a known hERG channel blocker and has effects on QT prolongation in humans (Wedam et al., 2007). The finding is considered treatment-related but is attributed to the known pharmacologic effects of BUP. No ECG abnormalities were detected on the last day of dosing.

Table 44. Summary of QTc Changes on Day 1

Group	CAM2038 Drug Product Dose (mg/dose)	QTc Pretest (sec)	QTc Day 1 Predose (sec)	QTc Day 1 Postdose (sec)	% of Day 1 Predose QTc
1	0 (saline control)	0.228	0.227	0.232	2.20%
2	0 (CAM2038 q1w placebo control)	0.219	0.224	0.232	3.57%
3	0 (CAM2038 q4w placebo control)	0.226	0.223	0.233	4.48%
4	32	0.225	0.220	0.244	10.91%
5	128	0.230	0.227	0.257	13.22%
6	160	0.231	0.225	0.259	15.11%
7	192	0.225	0.225	0.262	16.44%

Hematology

Hematological evaluations were conducted pretest and prior to terminal necropsy. No treatment-related changes in hematological parameters were seen.

Clinical Chemistry

Clinical pathology evaluations were conducted pretest and prior to terminal necropsy. No treatment-related changes in clinical chemistry parameters were observed.

Urinalysis

Urinalysis was conducted pretest and prior to terminal necropsy. No treatment-related changes in urinalysis were seen.

Gross Pathology

Dermal irritation was assessed using the Draize scale. All groups, with the exception of the saline control, showed Grade 1 (“very slight”) edema, which was attributed to the injection depot matrix. No differences were observed between the buprenorphine groups and their corresponding vehicles. Grade 2 (minimal) edema was observed in a few instances but was not dose-related. Grade 3 (moderate) edema was observed in one male dog in the CAM 2038 32 mg group on one day of dosing (Day 8 at a single injection site). The significance of this finding is unclear. No eschar or erythema were observed in any group.

Macroscopic changes were limited to edema, a swelling/thickening of the skin (with subcutaneous tissue) which was observed in all groups with the exception of the saline group. No differences were observed between the buprenorphine groups and their corresponding vehicles. Severity ranged from minimal to moderate and was attributed to the depot matrix and localized inflammatory reaction.

For weekly subcutaneous doses of saline (Group 1), there was no observed increase in edema over the course of dosing. For CAM2038 q1w placebo control or CAM2038 q1w drug product at 32 mg/dose (Groups 2 and 4, respectively) injection site observations consisted primarily of Grade 1 (“very slight”) presence of the injection depot matrix (IDM). Overall, Grade 1 IDM accounted for 41.4% to 100% and 64.1% to 100% of all injection site observations made between any two consecutive injections given at either Site 1 or Site 8 for Groups 2 and 4, respectively. Following the second dose at Sites 1 and 8, there was an increased incidence of Grade 1 IDM ($\geq 73\%$ of all injection site observations) compared with the incidence noted following the first dose at each site. This likely represents the presence of residual IDM from the previous injection. Additionally, there was a small increase in the frequency of Grade 1 IDM observed at Site 8 (distal to the head) compared with Site 1 (proximal to the head), which may reflect differences in the fat content of the subcutaneous space at these two locations.

Consistent with the data from animals dosed once weekly, injection site observations related to administration of CAM2038 q4w placebo control or all dose levels of CAM 2038 q4w drug products consisted primarily of Grade 1 (“very slight”) IDM. Grade 1 IDM accounted for 10.6% to 17.5% of all observations at Site 1 (proximal to the head) and 15.2% to 37.9% of observations at Site 8 (distal to the head). The frequency of Grade 1 IDM had no relationship to dose or the presence of buprenorphine; however, the frequency of Grade 1 IDM was increased at Site 8 compared with Site 1, which may reflect differences in the fat content of the subcutaneous space at these two locations. The frequency of Grade 1 IDM at Site 7, which was dosed three times (Days 197, 225, and 253), ranged from 61.5% to 71.9% (Appendix E, Table E). For CAM2038 q4w drug product at 160 mg/dose (Group 6), the incidence of Grade 1 IDM also increased as dose sites moved from the head to the tail with incidence at Sites 4, 5, and 8 of 29.9%, 37.1%, and 41.1%, respectively, of total injection site observations.

Organ Weights

A statistically significant increase in relative brain to body weight ratio was observed in males in the CAM2038 q1w 32 mg group. No effects were seen on absolute brain weights or body weights or in females of the same dose group. No microscopic correlates were seen in the brain. The finding is not considered to be adverse. No other effects on organ weights were observed.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Minimal fibrosis and minimal to mild mononuclear cell infiltrates were observed in the saline group in response to the injection. In all treated groups, histopathologic changes were observed at the injection sites and in the regional draining lymph node (axillary lymph node). No differences were noted between the CAM2038 q1w and q4w vehicles and the buprenorphine-containing formulations.

Toxicokinetics

Blood samples for TK were collected on Study Days 1, 141 and 225. Toxicokinetic parameters of BUP and nor-BUP are detailed in the tables below. Exposure to BUP and nor-BUP was similar for male and female dogs at all doses. At steady state (Days 141 and 225), BUP and nor BUP had a T_{max} of 8 h for CAM2028 q1w but the T_{max} values for CAM2028 q4w were between 1-2 h. It is not clear why the T_{max} values differ for the two products. The mean C_{max} and AUC_{0-168} values for BUP and nor-BUP were generally dose proportional or slightly greater than dose proportional. Mean AUC_{0-168} values for BUP and nor-BUP were generally similar between CAM2038 q1w 32 mg and CAM2038 q4w 128 mg groups.

The NOAEL in this study is the highest dose tested, 192 mg. The highest dose tested yielded exposure margins in dogs of 16-fold the human systemic exposure of 32 mg CAM2038 q1w based on AUC_{0-168h} comparisons (human data from Study HS-15-549).

The findings observed at necropsy and the clinical observations of edema correlated with microscopic observations of foci of inflammation. The localized inflammatory inflammatory responses ranged from acute to chronic inflammation depending on the time between dosing and microscopic evaluation. Fibrosis occurred with the chronic inflammation. The findings were not dependent on the amount of BUP.

Table 45. Mean BUP and Nor-BUP TK Parameters Following SC Administration of CAM2038 q1w to Dogs (Males and Females Combined)

Group	Day	Analyte	C _{max} (ng/mL)	T _{max} (hr) [day] ^b	C _{trough} (ng/mL)	AUC _{0-168hr} (hr*ng/mL) [day*ng/mL] ^b	AUC _{INF} (hr*ng/mL) [day*ng/mL] ^b	T _{1/2} (hr) [day] ^b	M:P ^a
4	1	buprenorphine	16.4	24 [1]	4.62	1640 [68.33]	1880 [78.33]	76.8 [3.20]	NA
		nor-buprenorphine	0.693	72 [3]	0.146	59.2 [2.47]	35.4 [1.48]	146 [6.08]	0.0406
4	141	buprenorphine	19.7	8 [0.33]	7.08	1850 [77.08]	NA	190 [7.92]	NA
		nor-buprenorphine	0.503	8 [0.33]	0.296	58.1 [2.42]	NA	230 [9.58]	0.0351
4	225	buprenorphine	19.4	8 [0.33]	8.61	2080 [86.67]	NA	138 [5.75]	NA
		nor-buprenorphine	0.600	8 [0.33]	0.283	63.2 [2.63]	NA	77.5 [3.23]	0.0336

NA - Not applicable
Note: AUC_{INF} reported for Day 1 only
a: M:P = (AUC_{0-168hr nor-buprenorphine} x 467.64)/(AUC_{0-168hr buprenorphine} x 413.55)
b: The value in hours was converted to days by dividing by 24.

Table 46. Mean BUP and Nor-BUP TK Parameters Following SC Administration of CAM2038 q4w to Dogs (Males and Females Combined)

Day	Group	Analyte	Dose (mg/dose)	C _{max} (ng/mL)	T _{max} (hr) ^c	C _{trough} (ng/mL)	T _{last} (hr) ^c	AUC _{0-672hr} (hr*ng/mL) ^a	AUC _{0-168hr} (hr*ng/mL) ^a	M:P ^a	AUC _(12h) (hr*ng/mL) ^a	T _{1/2} (hr) ^c
1	5	buprenorphine	128	21.9	24.5 ^b [1.02]	2.97	672 [28.0]	5220 [217.50]	1980 [82.50]	NA	6010 [250.42]	270 [11.25]
		nor-buprenorphine	128	1.06	1 [0.04]	0.0608	552 ^b [23.0]	156 [6.50]	55.7 [2.32]	0.0334	209 [8.71]	133 [5.54]
	6	buprenorphine	160	22.4	72 [3.00]	3.96	672 [28.0]	5920 [246.67]	2160 [90.0]	NA	6990 [291.25]	256 [10.67]
		nor-buprenorphine	160	1.40	25 ^b [1.04]	0.229	672 [28.0]	338 [14.08]	110 [4.58]	0.0599	300 [12.50]	220 [9.17]
	7	buprenorphine	192	23.5	1.5 ^b [0.06]	5.01	672 [28.0]	7050 [293.75]	2360 [98.33]	NA	9490 [395.42]	349 [14.54]
		nor-buprenorphine	192	1.93	1 [0.04]	0.152	672 [28.0]	253 [10.54]	98.9 [4.12]	0.0405	257 [10.71]	145 [6.04]
141	5	buprenorphine	128	38.3	1 [0.04]	6.87	672 [28.0]	6600 [275]	1860 [77.50]	NA	NA	451 [18.79]
		nor-buprenorphine	128	1.41	0.75 ^b [0.03]	0.0628	636 ^b [26.5]	197 [8.21]	59.7 [2.49]	0.0334	NA	174 [7.25]
	6	buprenorphine	160	23.1	2 [0.08]	8.52	672 [28.0]	6780 [282.50]	1750 [72.92]	NA	NA	272 [11.33]
		nor-buprenorphine	160	1.88	2 [0.08]	0.353	672 [28.0]	389 [16.21]	110 [4.58]	0.0605	NA	97.3 [4.05]
	7	buprenorphine	192	60.8	1 [0.04]	10.0	672 [28.0]	10300 [429.17]	3200 [133.33]	NA	NA	324 [13.50]
		nor-buprenorphine	192	3.71	0.75 ^b [0.03]	0.257	672 [28.0]	378 [15.75]	128 [5.33]	0.0420	NA	203 [8.46]
225	5	buprenorphine	128	21.1	1 [0.04]	6.49	672 [28.0]	6780 [282.50]	1620 [67.50]	NA	NA	273 [11.38]
		nor-buprenorphine	128	1.29	1.00 [0.04]	0.0794	672 [28.0]	139 [5.79]	42.0 [1.75]	0.0231	NA	392 [16.33]
	6	buprenorphine	160	24.3	1 [0.04]	14.6	672 [28.0]	8530 [355.42]	2050 [85.42]	NA	NA	459 [19.13]
		nor-buprenorphine	160	2.01	1 [0.04]	0.449	672 [28.0]	345 [14.38]	96.4 [4.02]	0.0464	NA	136 [5.67]
	7	buprenorphine	192	33.0	1 [0.04]	12.3	672 [28.0]	11200 [466.67]	2720 [113.33]	NA	NA	370 [15.42]
		nor-buprenorphine	192	2.57	1 [0.04]	0.269	672 [28.0]	368 [15.33]	90.8 [3.78]	0.0359	NA	151 [6.29]

NA - Not applicable
 Note: AUC_(12h) reported for Day 1 only
 a: M:P = (AUC_{0-672hr} nor-buprenorphine X 467.64)/(AUC_{0-672hr} buprenorphine X 413.55)
 b: Value is not a scheduled collection interval
 c: The value in hours was converted to days by dividing by 24.

Dosing Solution Analysis

All dosing formulation samples had mean concentrations within the acceptance criteria with the exception of two animals in Group 2 (q1w vehicle) that had levels of BUP 1 to 2-fold greater than the LLOQ. No nor-buprenorphine or any other chromatographic peaks were detected in the samples suggesting that the contamination was not in vivo and most likely occurred ex vivo. This is not considered to affect the integrity of the study.

7 Genetic Toxicology

No genetic toxicology studies with BUP were conducted for NDA 210136. The referenced Subutex labeling provides the following information (updated 9/7/2017):

Mutagenicity

Buprenorphine was studied in a series of tests utilizing gene, chromosome, and DNA interactions in both prokaryotic and eukaryotic systems. Results were negative in yeast (*S. cerevisiae*) for recombinant, gene convertant, or forward mutations; negative in *Bacillus subtilis* "rec" assay, negative for clastogenicity in CHO cells, Chinese hamster bone marrow and spermatogonia cells, and negative in the mouse lymphoma L5178Y assay. Results were equivocal in the Ames test: negative in studies in two laboratories, but positive for frame shift mutation at a high dose (5 mg/plate) in a third study. Results were positive in the Green-Tweets (*E. coli*) survival test, positive in a DNA synthesis inhibition (DSI) test with testicular tissue from mice, for both in vivo and in vitro incorporation of [³H]thymidine, and positive in unscheduled DNA synthesis (UDS) test using testicular cells from mice.

The same information should be included in this labeling for this product.

8 Carcinogenicity

No new carcinogenicity studies with BUP were conducted for NDA 210136. The referenced Subutex labeling provides the following information (updated 9/7/2017):

Carcinogenicity

Carcinogenicity studies of buprenorphine were conducted in Sprague-Dawley rats and CD-1 mice. Buprenorphine was administered in the diet to rats at doses of 0.6, 5.5, and 56 mg/kg/day (estimated exposure was approximately 0.4, 3 and 35 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis) for 27 months. As in the buprenorphine/naloxone carcinogenicity study in rat, statistically significant dose-related increases in Leydig cell tumors occurred. In an 86-week study in CD-1 mice, buprenorphine was not carcinogenic at dietary doses up to 100 mg/kg/day (estimated exposure was approximately 30 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis).

The same information should be included in this labeling for this product with exposure margins adjusted accordingly.

To obtain exposure data in mice and rats treated like the above carcinogenicity studies in order to inform labeling, the Applicant conducted 28-day repeat-dose PK studies. The results of these studies are summarized below:

Study Title: A 28 Day Dietary Pharmacokinetic Study of Buprenorphine in Sprague-Dawley Rats

Study Number: 2335-001
Study Completion Date: July 30, 2015

Study Design: Male and female Sprague-Dawley rats were treated via the diet with buprenorphine for 28-days in order to reach steady state.

Group Assignments			
Group Number	Target Dose Level (mg/kg/day)*	Number of Animals	
		Male	Female
1	0.6	9	0
2	5.5	9	0
3	56	9	0
4	0.6	0	9
5	5.5	0	9
6	56	0	9

*Actual dose levels, as determined on Days 1, 14 and 28 from mean food consumption values, are presented in the Pharmacokinetic Analysis Report presented in Appendix H.

Plasma samples were obtained at 1, 2, 4, 12, 13, 14, 16, and 24 hours post dose on Days 1 and 14 and at 1, 2, 4, 12, 13, 16, 24, 40, 56, and 72 hours post dose on Day 28.

PK results are depicted below:

Buprenorphine PK Summary Table								
Day	Target Dose (mg/kg)	Mean Actual Dose (mg/kg)	Mean Actual Dose Ratio	AUC _{0-24hr} (hr*ng/mL)	AUC _{0-24hr} Ratio	C _{max} (ng/mL)	C _{max} Ratio	T _{1/2} (hr)
1	0.6	0.528	1	16.4	1	1.52	1	NA
	5.5	2.75	5.2	90.4	5.5	6.83	4.5	NA
	56	23.6	44.7	700	42.7	56.3	37.0	NA
14	0.6	0.618	1	44.7	1	2.95	1	NA
	5.5	5.47	8.9	306	6.8	16.7	5.7	NA
	56	59.0	95.5	2200	49.2	127	43.1	NA
28	0.6	0.595	1	41.8	1	2.28	1	8.04
	5.5	5.22	8.8	208	5.0	10.4	4.6	10.0
	56	52.8	88.7	1680	40.2	87.3	38.3	17.4
NA – Not Applicable								

Norbuprenorphine TK Summary Table							
Day	Target Dose (mg/kg)	Mean Actual Dose (mg/kg)	AUC _{0-24hr} (hr*ng/mL)	AUC _{0-24h} /Dose (hr*kg*ng/mL/mg)	C _{max} (ng/mL)	C _{max} /Dose (kg*ng/mL/mg)	T _{1/2} (hr)
1	56	23.6	60.9	2.58	4.04	0.171	NA
14	56	59.0	22.3	0.379	1.54	0.0262	NA
28	56	52.8	13.8	0.262	0.893	0.0169	NA
NA – Not Applicable							

Study Title: A 28-Day Dietary Pharmacokinetic Study of Buprenorphine in CD1 Mice

Study Number: 2335-002
 Study Report Date: July 30, 2015

Study Design: Male and female CD1 mice were treated with 100 mg/kg buprenorphine via the diet for a total of 28-days to obtain exposure data in order to better inform the drug product labeling for this depot injection product. This dose was the NOEL for carcinogenicity in the referenced study.

Group Assignments				
Group Number	Target Dose Level (mg/kg/day)*	Number of Animals		
		Male	Female	
1	100	108	0	
2	100	0	108	

** Actual dose levels, as determined on Days 1, 14 and 28 from mean food consumption values for designated TK animals, are presented in the Pharmacokinetic Analysis Report presented in [Appendix H](#).

Plasma samples from 4 animals/group/time point were obtained via cardiac puncture such that data were obtained for 1, 2, 4, 12, 13, 14, 16, and 24 hours post-dosing on Days 1 and 14 and at 1, 2, 4, 12, 13, 14, 16, 24, 40, 56, and 72 hours post dosing on Day 28. The results are depicted below:

Buprenorphine TK Summary Table							
Day	Target Dose (mg/kg)	Mean Actual Dose (mg/kg)	AUC _{0-24hr} (hr*ng/mL)	AUC _{0-24hr} /Dose (hr*kg*ng/mL/mg)	C _{max} (ng/mL)	C _{max} /Dose (kg*ng/mL/mg)	T _{1/2} (hr)
1	100	68.6	817	11.9	47.5	0.693	11.1
14	100	80.1	1040	12.9	56.8	0.709	NA
28	100	84.0	1050	12.4	61.3	0.729	5.90

NA – Not Applicable

9 Reproductive and Developmental Toxicology

No new reproductive and developmental toxicology studies with buprenorphine were conducted for NDA 210136. The referenced Subutex labeling provides the following information (updated 9/7/2017):

Section 13.1

Impairment of Fertility

Reproduction studies of buprenorphine in rats demonstrated no evidence of impaired fertility at daily oral doses up to 80 mg/kg/day (estimated exposure was approximately 50 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis) or up to 5 mg/kg/day IM or SC (estimated exposure was approximately 3 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis).

Section 8 Risk Summary (animal)

Reproductive and developmental studies in rats and rabbits identified adverse events at clinically relevant and higher doses. Embryofetal death was observed in both rats and rabbits administered buprenorphine during the period of organogenesis at doses approximately 6 and 0.3 times, respectively, the human sublingual dose of 16 mg/day of buprenorphine. Pre- and post-natal development studies in rats demonstrated increased neonatal deaths at 0.3 times and above and dystocia at approximately 3 times the human sublingual dose of 16 mg/day of buprenorphine. No clear teratogenic effects were seen when buprenorphine was administered during organogenesis with a range of doses equivalent to or greater than the human sublingual dose of 16 mg/day of buprenorphine. However, increases in skeletal abnormalities were noted in rats and rabbits administered buprenorphine daily during organogenesis at doses approximately 0.6 times and approximately equal to the human sublingual dose of 16 mg/day of buprenorphine, respectively. In a few studies, some events such as acephalus and omphalocele were also observed but these findings were not clearly treatment-related.

Section 8 (Animal Data)

Animal Data

The exposure margins listed below are based on body surface area comparisons (mg/m^2) to the human sublingual dose of 16 mg buprenorphine via SUBUTEX.

No definitive drug-related teratogenic effects were observed in rats and rabbits at IM doses up to 30 mg/kg/day (estimated exposure approximately 20 times and 35 times, respectively, the human sublingual dose of 16 mg). Maternal toxicity resulting in mortality was noted in these studies in both rats and rabbits. Acephalus was observed in one rabbit fetus from the low-dose group and omphalocele was observed in two rabbit fetuses from the same litter in the mid-dose group; no findings were observed in fetuses from the high-dose group. Maternal toxicity was seen in the high-dose group but not at the lower doses where the findings were observed. Following oral administration of buprenorphine to rats, dose-related post-implantation losses, evidenced by increases in the numbers of early resorptions with consequent reductions in the numbers of fetuses, were observed at doses of 10 mg/kg/day or greater (estimated exposure approximately 6 times the human sublingual dose of 16 mg). In the rabbit, increased post-implantation losses occurred at an oral dose of 40 mg/kg/day. Following IM administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 mg/kg/day.

Buprenorphine was not teratogenic in rats or rabbits after IM or subcutaneous (SC) doses up to 5 mg/kg/day (estimated exposure was approximately 3 and 6 times, respectively, the human sublingual dose of 16 mg), after IV doses up to 0.8 mg/kg/day (estimated exposure was approximately 0.5 times and equal to, respectively, the human sublingual dose of 16 mg), or after oral doses up to 160 mg/kg/day in rats (estimated exposure was approximately 95 times the human sublingual dose of 16 mg) and 25 mg/kg/day in rabbits (estimated exposure was approximately 30 times the human sublingual dose of 16 mg). Significant increases in skeletal abnormalities (e.g., extra thoracic vertebra or thoraco-lumbar ribs) were noted in rats after SC administration of 1 mg/kg/day and up (estimated exposure was approximately 0.6 times the human sublingual dose of 16 mg), but were not observed at oral doses up to 160 mg/kg/day. Increases in skeletal abnormalities in rabbits after IM administration of 5 mg/kg/day (estimated exposure was approximately 6 times the human sublingual dose of 16 mg) in the absence of maternal toxicity or oral administration of 1 mg/kg/day or greater (estimated exposure was approximately equal to the human sublingual dose of 16 mg) were not statistically significant. In rabbits, buprenorphine produced statistically significant pre-implantation losses at oral doses of 1 mg/kg/day or greater and post-implantation losses that were statistically significant at IV doses of 0.2 mg/kg/day or greater (estimated exposure approximately 0.3 times the human sublingual dose of 16 mg). No maternal toxicity was noted at doses causing post-implantation loss in this study.

Dystocia was noted in pregnant rats treated intramuscularly with buprenorphine from Gestation Day 14 through Lactation Day 21 at 5 mg/kg/day (approximately 3 times the human sublingual dose of 16 mg). Fertility, and pre- and post-natal development studies with buprenorphine in rats indicated increases in neonatal mortality after oral doses of 0.8 mg/kg/day and up (approximately 0.5 times the human sublingual dose of 16 mg), after IM doses of 0.5 mg/kg/day and up (approximately 0.3 times the human sublingual dose of 16 mg), and after SC doses of 0.1 mg/kg/day and up (approximately 0.06 times the human sublingual dose of 16 mg). An apparent lack of milk production during these studies likely contributed to the decreased pup viability and lactation indices. Delays in the occurrence of righting reflex and startle response were noted in rat pups at an oral dose of 80 mg/kg/day (approximately 50 times the human sublingual dose of 16 mg).

The same information should be included in this labeling for this product with exposure margins adjusted accordingly.

To attempt to bridge the referenced studies and be able to compare these data with the exposures obtained following depot injection of CAM2038 products, the Applicant conducted the following studies. These studies permit some of the above information to

be expressed via exposure margins rather than based on body surface area; however complete PK bridging studies were not completed.

Study Title: A 12 to 14-Day Subcutaneous Pharmacokinetic Study of Buprenorphine in Gravid and Non-Gravid Sprague-Dawley Rats

Report Number: 2335-003
 Study Completion: July 29, 2015
 Study Design: Male and female Sprague-Dawley rats were treated subcutaneously with buprenorphine in 5% dextrose vehicle as follows:

Group Assignments							
Group Number	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Dose Concentration (mg/mL)	Number of Animals			
				Male	Female		
					Non-Gravid	Gravid	
1	0.1	1	0.1	0	0	15	
2	1	1	1.0	0	0	15	
3	5	2	2.5	0	0	15	
4	5	2	2.5	9	9	0	

Plasma samples were collected 0.5, 1, 2, 4, 12, and 24 hours post-dose on Days 1, 8 and 14. The results are depicted below.

Buprenorphine PK Summary Table (Gravid Females)							
Day	Dose (mg/kg)	Dose Ratio	AUC _{0-24hr} (ng*hr/mL)	AUC _{0-24hr} Ratio	C _{max} (ng/mL)	C _{max} Ratio	T _{1/2} (hr)
1	0.1	1	NA	NA	19.8	1	1.47
	1	10	384	1	94.8	4.8	4.93
	5	50	1140	3	229	11.6	9.50
8	0.1	1	77.5	1	24.1	1	2.55
	1	10	367	4.7	70.3	2.9	5.29
	5	50	1420	18.3	197	8.2	11.2
12	0.1	1	66.4	1	24.9	1	2.36
	1	10	351	5.3	63.5	2.6	6.80
	5	50	1290	19.4	171	6.9	30.6
NA – Not Applicable							

As noted in the table above, in gravid females, plasma levels (AUC and C_{max}) were less than dose proportional, and the half-life appeared to increase with increasing during of treatment. Plasma AUC also appeared to be lower in gravid females compared to nongravid male and females combined.

Buprenorphine PK Summary Table (Males and Non-Gravid Females)				
Day	Dose (mg/kg)	AUC _{0-24hr} (ng*hr/mL)	C _{max} (ng/mL)	T _{1/2} (hr)
1	5	1130	245	NA
8	5	1570	241	8.90
14	5	2330	230	39.4
NA – Not Applicable				

Study Title: A 12-Day Subcutaneous Pharmacokinetic Study of Buprenorphine in Gravid New Zealand White Rabbits

Study Number: 2335-004
 Study Completion: July 28, 2015
 Study Design: Pregnant female New Zealand White Rabbits were treated subcutaneously with buprenorphine (5 mg/kg/day) for 12 days. Plasma samples were obtained at 0.5, 1, 2, 4, 12, and 24 hours post injection on Days 1 and 8 and 0.5, 1, 2, 4, 12, 24, 40, 56, and 72 hours post injection on Day 12. Results are summarized below, reproduced from the study report.

Buprenorphine PK Summary Table				
Day	Dose (mg/kg)	AUC _{0-24hr} (ng*hr/mL)	C _{max} (ng/mL)	
1	5	1140	119	
8	5	1400	127	
12	5	1750	145	

10 Special Toxicology Studies

Off-Label Misuse: Intravenous Toxicity

As noted at several opioid drug product advisory committee meetings, there is an increased need to understand the potential adverse effects that could occur if an opioid drug product were to be abused via an unintended route of administration. During development, the Applicant conducted a non-GLP intravenous toxicology study testing one of the two formulations, CAM2038 Q1W. This study is reviewed below:

Study Title: Intravenous Administration of CAM2038 Q1W in Rats

Key Findings: Intravenous injection of CAM2038 Q1W into the tail vein of male Sprague-Dawley rats resulted in partial or complete occlusion of the injected vein with no evidence of blood in the section of the vein even at sacrifice on Day 7. The animals

demonstrated effects consistent with systemic exposure to buprenorphine. No gross observations were made in visceral organs; however, lungs could not be examined due to the method of euthanasia.

Study Number: P-17-598
Regulatory Status: Non-GLP
Test Facility: Camurus AB, Lund Sweden
Study Report Date: October 3, 2017

Methods: GMP manufactured CAM2038 Q1W (entire contents of prefilled syringe = 8 mg CAM2038 in 0.16 mL per rat; ~27 mg/kg buprenorphine) was injected intravenously into the tail vein of 3 male MPF Sprague-Dawley rats obtained from (b) (4) under isoflurane anesthesia. Administration of the test item into the first animal was monitored for an hour to determine if severe or notable findings occurred prior to testing the material via IV administration into the second and third animal. Clinical observations were made over the first hour, hourly for the next six hours, and then at 11-12 hours and 24 hours post-injection. Observations were prolonged to 7 days post dose for Animals 2 and 3 (animals were observed daily during that period). Visual inspection of the injection site was completed during and after the administration. Body weights were recorded prior to dosing and daily thereafter. Animals were euthanized via CO₂ as noted above. At necropsy, injection sites on the tail were examined grossly and macroscopic evaluation of organs was completed in situ (color, shape, size). No microscopic examinations were made.

Results: There were no mortalities or clinical signs. Animals demonstrated expected pharmacological effects of buprenorphine (dazed facial expression, stereotyped repetitive behavior for approximately 11 hours). During injection in one animal, a swelling was observed in the vein at the injection site. This impacted the ability to inject the entire volume in that rat. In another rat, a pale area at the injection site was noted which extended toward the base of the tail during injection.

At necropsy, there were no abnormalities noted in thymus, heart, bladder, liver, kidney, small intestine, colon, or stomach. Lungs could not be evaluated as the animals were sacrificed via CO₂ (expect irregular dark color from method of termination). In all three rats, formulation was found in the tail vein in the form of gel localized in the region of the injection site and extending toward the tail. There was no blood in this region of the tail vein. The test item appears to result in partial or complete occlusion of the vein.

Conclusions and Risk Assessment:

The above data suggest that if the drug product were to be administered intravenously, it will either gel rapidly and potentially block the injected vessel as it apparently did in the rat tail vein, or if the injected vein is larger and the product does not gel quickly, it could result in a lung emboli or lodge in other small capillaries. The clinical significance of any blockade of blood vessels would depend upon the redundancy of vascular perfusion and degree of blockade, but it could result in tissue hypoxia and necrosis with

impact on tissue functionality. Given the existing evidence of prolonged tissue residency, the vascular blockade of any tissue would be expected to take considerable time to clear.

Juvenile Animal Studies and Agreed PSP

The Applicant has requested a waiver of pediatric studies in all age groups citing that the studies would not likely be possible to conduct given the low incidence of patients possible to enroll. See the agreed PSP and clinical review for details. No juvenile animal studies were required given the fact that any pediatric patients who are eligible are already addicted to opioids prior to enrollment in the study.

In Silico Evaluation (b) (4)

Study Title: Computational Mutagenicity Analysis of CAM2038 Impurity

Study Number: (b) (4)-18-007

Key Findings:

The drug product impurity, (b) (4) is predicted to be negative for mutagenic potential using both rule-based and statistical-based platforms in a computational toxicology evaluation.

Summary:

The structure of the drug product impurity, (b) (4) (b) (4) was analyzed for the potential for mutagenicity using two in silico platforms. This impurity does not contain any structural alerts for mutagenicity. The structure is shown in the figure below. The rule-based DEREK Nexus predicted (b) (4) (b) (4) to be negative for mutagenicity (in vitro bacterium) with no misclassified or unclassified features. The statistical-based Leadscope Model Applier predicted (b) (4) (b) (4) to be negative in both the E. Coli-Sal 102 A-T Mut v1 and Salmonella Mut v3 models. The Applicant states that the main features of the structure and several analogs were used to support the Leadscope prediction. The structure of buprenorphine was one of the analogs tested and was predicted to be negative.

Conclusion:

The drug product impurity, (b) (4) (b) (4) is predicted to be negative for mutagenic potential using both rule-based and statistical-based platforms in a computational toxicology evaluation.

Figure 1. Structure (b) (4)



11 Integrated Summary and Safety Evaluation

Braeburn Pharmaceuticals submitted a 505(b)(2) NDA in support of their subcutaneous (SC) depot buprenorphine drug product referred to during development as CAM2038 for the treatment of opioid use disorder. The NDA relies upon an FDA-previous finding of safety for Subutex (NDA 20732 - buprenorphine sublingual) and literature. There are actually two drug product formulations in this NDA, a once weekly (q1w) and a once monthly (q4w) formulation. The formulations (CAM2038 q1w and CAM2038 q4w) primarily differ

(b) (4) During development, the Division noted that as per any depot formulation development program, the fate of the injected material should be adequately characterized and any novel excipients should be adequately justified for safety as per the FDA guidance document on new excipients.

The results of the local tissue reaction assessment suggest that an expected foreign body reaction occurs which prolong localization of the gel matrix which lasts approximately a month or more following SC injection.

The most definitive life span study has been after administration of a single intramuscular (IM) dose of CAM2038 q1w and q4w with observations lasting up to 113 days (4 months). This study was conducted to evaluate effects after inadvertent IM dosing with CAM2038. Microscopically, the incidence and/or severity of injection site local toxicity observations on Day 2 (cavitation, myofiber degeneration/necrosis, and/or mixed leukocyte infiltration) were generally similar between q1w, q4w and their placebo test article injection sites. Compared with Day 2, microscopic changes were decreased on Day 29 (q1w) and absent on Day 113 (q4w), indicating progressing or full recovery over a period of approximately 1 month for q1w and 4 months for q4w. Similar local toxicity reversibility of inflammation was observed after termination of subcutaneous dosing and is expected at an increased rate due to increased vascularity of subcutaneous sites.

The two highest (b) (4) doses of buprenorphine drug product q4w results in exposures to buprenorphine that exceed the referenced drug product Subutex. (b) (4)

The buprenorphine exposure following the 128 mg q4w dose exceeds the exposure at 24 mg via Subutex and therefore toxicology studies in two species were required. The Applicant provided a 9-month dog study only; however, the clinical team has concluded that adequate human experience exists and agreed to waive the 6-month rat study.

The definitive nonclinical study was a SC 9-month study of q1w and q4w in dogs with injections over rotating sites which provided adequate local and systemic safety justification for the proposed drug product. Collectively the local toxicity changes are consistent with a foreign body reaction of inflammatory cell infiltrates to a depot injection. Inflammation was considered reversible after dosing was terminated and was most noticeable in a single-dose intramuscular study in which q1w animals were observed for 29 days after the injection and q4w animals were observed for 113 days after injection. Both local inflammation and depot were either partially (Day 29) or completely (Day 113) in regression/reversed.

In addition to nonclinical test data, the new extractables/leachables studies do not fully characterize the potential leachables from the container closures as noted in Section 2.5 (Container Closure System). Although most of the potential leachables were identified, there were several compounds present at lower levels that were not fully identified and therefore a risk assessment cannot be made definitively. Further, although elemental impurities were measured in the final drug product, they were only measured at 6 months of shelf life and therefore, may not fully reflect the potential contribution of elementals from the container closure over the course of stability. In addition, an adequate risk assessment could not be performed on two identified leachables detected above 5 mcg/day because validated methods were not employed and therefore the levels reported cannot be considered accurate. Given the risk benefit of the drug and specifically in light of the opioid crisis, these may be completed as post-marketing requirements at the discretion of the approving official.

Excipients - The drug product contains two new excipients. First, the once monthly injection q4w contains N-methyl-pyrrolidone (NMP) which during development was considered to be used in a new manner via this product (NMP was previously used in an FDA-approved product for acute dental use and a product indicated for the treatment of advanced cancer). Both formulations contain glycerol dioleate (GDO), a diglyceride, which has also not previously been used in an FDA-approved drug product. Of note, the Agency recently approved a chronic depot injection product for a non-oncology indication that containing NMP at levels approximately (b) (4) times higher than this drug product with post-marketing requirements and labeling for potential adverse effects due to the NMP in that formulation. The review focuses on the safety of the CAM2038 formulations which are reviewed in Sections 4 through 10, and the two novel excipients NMP and GDO (which are reviewed in Appendices 1 and 2, respectively).

12 Appendix/Attachments

Appendix 1: NMP Toxicological Risk Assessment

Review of Toxicity Data for NMP in Braeburn's CAM2038 q4w

CAM2038 q4w is a sterile, non-aqueous solution for subcutaneous injection indicated for the treatment of opioid use disorder. It contains 64 mg, 96 mg, or 128 mg of buprenorphine base (BUP). This delivery system is designed to deliver buprenorphine over 4 weeks after subcutaneous injection. This injection depot platform contains soybean phosphatidylcholine (SPC), glycerol dioleate (GDO) and (b) (4) N-methyl-2-pyrrolidone (NMP) and is referred to by the Applicant as "FluidCrystal" technology. According to the Applicant, "once injected, CAM2038 spontaneously transforms from a low-viscous solution to a highly viscous crystalline gel that encapsulates the BUP and releases it at a steady rate as the depot degrades." In NDA 210136, Braeburn includes two products, CAM2038 q1w for once per week dosing and CAM2038 q4w for once per month dosing. Only the CAM2038 q4w contains NMP (b) (4). The quantitative composition of CAM2038 q4w is outlined in the table below.

Table 47. Composition of CAM2038 q4w Drug Product

Component	Standard	Function	Composition (mg) per dose		
			64 mg/ 0.18 mL	96 mg/ 0.27 mL	128 mg/ 0.36 mL
Buprenorphine Base (BUP)	Ph. Eur.	Active ingredient	64	96	128
N-Methyl-2-Pyrrolidone (NMP)	USP	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Soybean Phosphatidylcholine (SPC)	In-house				
Glycerol Dioleate (GDO)	In-house				
Total weight (mg) for all components per dose	Not Applicable	Not Applicable			

Regulatory History

As discussed during development and at the time of the original NDA submission, the use of NMP in this drug product formulation was technically novel. According to the Inactive Ingredients Database (IID, December 12, 2017), NMP is currently used as an excipient in two FDA-approved drug products, as noted below.

Table 48. FDA Inactive Ingredient Database Listing of N-methyl-2-pyrrolidone

Inactive Ingredient	Route	Dosage Form	Maximum Potency
Methypyrrolidone	Periodontal	Drug Delivery System	NA
Methylpyrrolidone	Subcutaneous	Injection	25.85%

The total daily dose via these drug products is not listed in the IID. According to the Applicant, the SC drug product is approved for an advanced cancer indication. As discussed with the Applicant during development, use in an advanced cancer indication is not adequate justification for the safety of this excipient for a chronic non-oncology indication.

However, since the time of the original submission, the IID has been updated to include the following additional information:

Inactive Ingredient	Route	Dosage Form	Maximum Potency
Methypyrrolidone	Subcutaneous	Injection, suspension	376 mg

Each SC injection of the CAM2038 q4w (b) (4) mg BUP strength drug product contains (b) (4) mg of NMP. CAM2038 q4w is intended to be administered once every 4 weeks with no limitation on the duration of treatment (i.e., chronic/lifetime). Although NMP can be found in previously approved products (one for acute periodontal use only, the second for an advanced cancer indication), the safety has not been characterized for this population (chronic use, not advanced cancer indication). Therefore, the use of NMP in CAM2038 q4w for a chronic non-oncology indication was considered novel at the time of the original NDA submission.

The novelty of NMP for a chronic non-oncology indication was discussed with the Applicant over the course of development and the Agency noted that the Applicant would have to address the safety for this indication. Literature could be used to support the standard requirements for a novel excipient; however, the adequacy of the literature could only be determined during the NDA review. At the preNDA meeting in 2017, the Division noted that there was technically no chronic rodent toxicology study with NMP; however, we acknowledged that the carcinogenicity studies with NMP may be adequate to address this requirement for any novel chronic excipient.

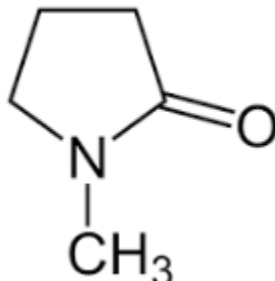
As noted above, recently, the Agency approved a chronic drug product containing NMP for a noncancer indication with postmarketing requirements to provide new data on the reproductive and developmental effects of NMP and a mode of action assessment for mouse liver tumors. As such, although not listed in the IID to date, NMP is technically no longer a new excipient; however, outstanding safety concerns remain to be adequately addressed. These concerns also apply to this drug product.

N-methyl-2-pyrrolidone (NMP)

N-methyl-2-pyrrolidone (CAS No. 872-50-4) is a water-miscible, polar aprotic organic solvent used in the manufacture of paint removers, pesticides, herbicides, fungicides,

pigments, cosmetics and pharmaceuticals. In rats, it has shown to be absorbed rapidly via inhalation, oral and dermal administration (World Health Organization, 2001). The structure is depicted below.

Figure 2. Structure of NMP (C₅H₉NO)



NMP is a commonly used solvent in pharmaceutical manufacturing. In fact, ICH Q3C(R6) specifically discusses NMP as a residual solvent in Part III of the document. The Expert Working Group (EWG) revisited the PDE for NMP in 2000 based on new data (chronic oral carcinogenicity study conducted by the NMP Producers Group (unpublished data) and a reproductive and developmental toxicology study published in 1994 (Hass et al., 1994). As noted in the ICH Q3C document, Hass et al. exposed pregnant Wistar rats to 150 ppm NMP for 6 hours a day from Gestation Day 7-20. The rats were allowed to deliver and offspring were evaluated. There was no evidence of maternal toxicity or physical abnormalities noted. However, there were reduced weight of the offspring, pre-weaning development was impaired, and the pups performed worst at higher cognitive function in solving a difficult task compared to control animals. A NOAEL was not established in this study. The EWG elected to keep NMP in Class 2 residual solvent and set an PDE of not more than (NMT) 5.3 mg/day, as follows:

Figure 3. PDE Calculation for NMP as per ICH Q3C(R6)

$$150 \text{ ppm} = \frac{150 \times 99.13}{24.45} = 608.16 \text{ mg/m}^3 = 0.608 \text{ mg/L}$$

$$\text{For continuous dosing} = \frac{0.608 \times 6}{24} = 0.152 \text{ mg/L}$$

$$\text{Daily dose} = \frac{0.152 \times 290}{0.33} = 133.58 \text{ mg/kg}$$

$$\text{PDE} = \frac{133.58 \times 50}{5 \times 10 \times 1 \times 5 \times 5} = \mathbf{5.3 \text{ mg/day}}$$

$$\text{Limit} = \frac{5.3 \times 1000}{10} = \mathbf{530 \text{ ppm}}$$

This PDE remains in the most current ICH Q3C document (Revision 6, Step 4, dated October 20, 2016).

ADME

NMP is readily absorbed across human skin, the gastrointestinal and respiratory tracts. It is rapidly distributed in the body to most organs including male sex organs. Wells and Digenis reported that after IV administration of 45 mg/kg of radiolabeled NMP to male Sprague Dawley rats, the radiolabel had a plasma half-life of 7-10 hours. Approximately 70% of the label was excreted in urine within 12 hours and 80% by 24 hours. At 6 hours, the highest radioactivity was present in the liver and intestines (Wells and Digenis, 1988).

Sitarek and Kilanowicz also studied the tissue distribution of radiolabeled IP NMP in female and male rats and noted that the highest levels at 4 hours were in the adrenals, kidneys, seminal vesicles, testes, muscles, liver, brain and lungs, as depicted in the tables below (Sitarek and Kilanowicz, 2006). NMP distributes widely to tissues and is cleared fairly rapidly.

Table 1. Radioactivity concentration in different male rat tissues after administration of ¹⁴C-N-methyl-2-pyrrolidone in a single i.p. dose of 250 mg/kg b.w. (350 kBq/male)

Tissue	4 h	8 h	24 h	48 h	72 h
Liver	1.11 ± 0.11 ^a	0.89 ± 0.01	0.05 ± 0.01	0.04 ± 0.001	0.04 ± 0.001
Kidneys	1.40 ± 0.15	1.08 ± 0.02	0.11 ± 0.02	0.04 ± 0.001	0.04 ± 0.001
Spleen	0.69 ± 0.10	0.67 ± 0.10	0.05 ± 0.01	0.03 ± 0.002	0.03 ± 0.0007
Lungs	1.04 ± 0.13	0.86 ± 0.14	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.002
Brain	1.05 ± 0.11	0.91 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.0007
Adrenals	1.42 ± 0.10	1.03 ± 0.10	0.07 ± 0.01	0.06 ± 0.001	0.05 ± 0.0007
Sciatic nerve	0.96 ± 0.04	1.04 ± 0.03	0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.007
Muscles	1.32 ± 0.19	1.44 ± 0.01	0.03 ± 0.001	0.07 ± 0.004	0.02 ± 0.002
Fat	0.83 ± 0.30	0.67 ± 0.10	0.04 ± 0.01	0.06 ± 0.02	0.02 ± 0.001
Epididymis	0.89 ± 0.08	0.82 ± 0.02	0.05 ± 0.02	0.04 ± 0.005	0.03 ± 0.003
Testicles	1.28 ± 0.14	1.08 ± 0.009	0.05 ± 0.02	0.03 ± 0.005	0.03 ± 0.001
Seminal vesicles	1.33 ± 0.14	0.99 ± 0.08	0.09 ± 0.07	0.04 ± 0.003	0.02 ± 0.002
Whole blood	0.87 ± 0.04	0.70 ± 0.05	0.18 ± 0.003	0.17 ± 0.01	0.18 ± 0.01

i.p. – intraperitoneally; b.w. – body weight; a – mean radioactivity (kBq/g tissue) ± SD from 4 male rats at each time.
To assess ¹⁴C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12%, and muscles as 40% of whole b.w. [17].

Table 2. Concentration of radioactivity in different female rat tissues after administration of ¹⁴C-N-methyl-2-pyrrolidone in a single i.p. dose of 250 mg/kg b.w. (350 kBq/female)

Tissue	4 h	8 h	24 h	48 h	72 h
Liver	1.45 ± 0.16 ^a	0.88 ± 0.03	0.06 ± 0.01	0.04 ± 0.005	0.04 ± 0.001
Kidneys	1.70 ± 0.14	1.16 ± 0.22	0.09 ± 0.01	0.05 ± 0.002	0.06 ± 0.007
Spleen	0.87 ± 0.10	0.47 ± 0.05	0.05 ± 0.009	0.04 ± 0.002	0.03 ± 0.004
Lungs	1.70 ± 0.16	1.23 ± 0.06	0.06 ± 0.01	0.05 ± 0.001	0.05 ± 0.002
Brain	1.59 ± 0.19	1.14 ± 0.19	0.02 ± 0.002	0.02 ± 0.002	0.03 ± 0.0007
Adrenals	1.48 ± 0.18	0.98 ± 0.10	0.06 ± 0.005	0.06 ± 0.008	0.05 ± 0.003
Sciatic nerve	1.57 ± 0.16	1.52 ± 0.27	0.05 ± 0.007	0.05 ± 0.003	0.05 ± 0.0007
Muscles	2.02 ± 0.17	1.48 ± 0.12	0.04 ± 0.001	0.07 ± 0.004	0.04 ± 0.007
Fat	0.43 ± 0.08	0.82 ± 0.26	0.06 ± 0.006	0.06 ± 0.004	0.03 ± 0.006
Ovaries	1.66 ± 0.20	0.93 ± 0.16	0.05 ± 0.01	0.05 ± 0.008	0.04 ± 0.005
Uterus	1.19 ± 0.09	0.94 ± 0.03	0.04 ± 0.01	0.04 ± 0.005	0.04 ± 0.007

i.p. – intraperitoneally; b.w. – body weight; a – mean radioactivity (kBq/g tissue) ± SD from 4 female rats at each time.
To assess ¹⁴C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12% and muscles as 40% of whole b.w. [17].

The Applicant conducted their own PK studies for NMP both in humans as well as rats and rabbits, which are reviewed below:

Study Title: Exploratory Evaluation of NMP (N-methyl-2-pyrrolidinone) Levels in Plasma from CAM2038 q4w Treated Healthy Volunteers from Study HS-13-487

Study Number: PK-15-551
Regulatory Status: Non-GLP

Methods: CAM2038 q4w was administered healthy subjects to obtain PK data for buprenorphine. Selected plasma samples were analyzed for NMP concentrations (96 mg q4w and 192 mg of q4w = $\frac{(b)}{(4)}$ mg and $\frac{(b)}{(4)}$ mg NMP, respectively). Samples from Study HS-13-487 analyzed are depicted below:

Treatment group	Treatment	No samples	Time points
D	CAM2038 q4w 96 mg	12/subject	All time points from pre-dose CAM2038 q4w to Day 5 (i.e. the Day 13 sample)
E	CAM2038 q4w 192 mg	12/subject	All time points from pre-dose CAM2038 q4w to Day 5 (i.e. the Day 13 sample)

Plasma samples from 1, 2, 4, 6, 10, 24 hours (Day 9) and 48 hours (Day 10) and also on Days 11, 12, and 13 were examined. The lower limit of quantitation was 30.0 ng/mL.

Results: Individual and mean PK values are presented in the Applicant's figure and table below:

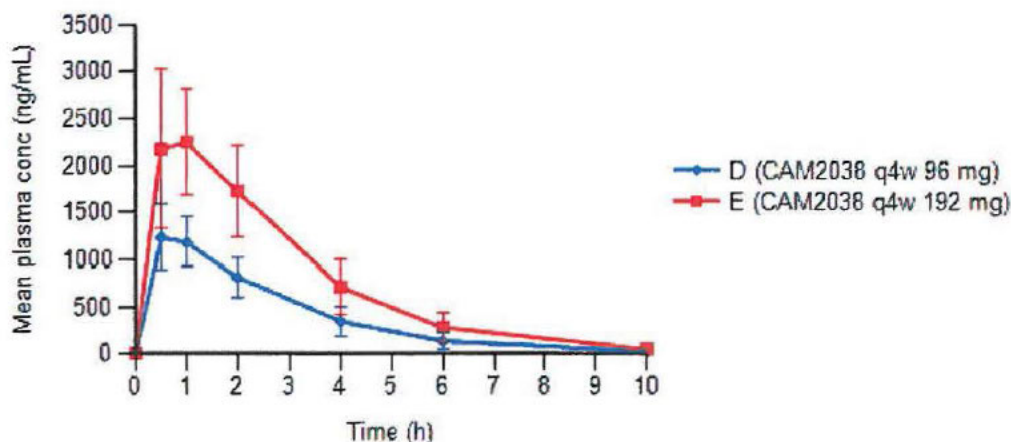


Figure 4. Group mean plasma concentrations of NMP; hours 0-10 (normal scale)

NMP levels were not detected within the LOQ at 24 hours in any individual. The mean AUC values can be used to compare exposures in humans to animals if similar PK data are available.

Table 2. PK parameters determined from NMP concentrations in plasma

Treatment group	Subject	t _{max} (h)	C _{max} (ng/mL)	AUC _{last} (ng/mL*h)	AUC _{inf} (ng/mL*h)	T _{1/2} (h)
D (CAM2038 q4w 96 mg)	258	0.500	1080	4590	4590	1.21
	260	1.03	1300	5310	5310	1.09
	262	0.617	1210	4040	4040	0.932
	264	0.483	962	4800	4800	1.71
	267	0.500	1050	4530	4530	1.77
	269	1.02	1620	8400	8400	1.91
	273	0.517	1970	6920	6920	1.71
	275	0.517	1750	6010	6010	1.67
	277	0.500	1130	3800	3800	1.38
	279	1.00	867	4970	4970	1.78
	281	1.02	1120	5250	5250	1.26
	283	1.03	1360	6180	6180	1.63
	284	0.533	1580	4930	4930	1.22
	287	0.500	1200	4880	4880	1.18
	N	10		10	10	10
Mean	0.698	1300	5330	5330	1.46	
SD	0.251	320	1210	1210	0.311	
Min	0.483	867	3800	3800	0.932	
Median	0.525	1210	4950	4950	1.51	
Max	1.03	1970	8400	8400	1.91	
CV%	36.0	24.6	22.8	22.8	21.3	
E (CAM2038 q4w 192 mg)	259	1.00	1580	6720	6720	1.68
	261	0.433	2150	7730	7730	1.2
	263	1.02	1790	8390	8390	1.72
	265	0.450	4350	14300	14300	2.06
	266	1.00	1660	6910	6910	0.996
	268	0.517	3280	8940	8940	1.42
	270	0.500	1600	5110	5110	0.858
	272	1.00	1420	7700	7700	1.96
	274	0.500	2190	7750	7750	1.23
	276	1.02	2340	9010	9010	1.47
	278	0.983	2980	11400	11400	1.46
	280	0.983	2600	11800	11800	1.4
	282	1.00	3090	11700	11700	1.66
	285	1.00	2680	11500	11500	1.47
	286	0.483	2360	8550	8550	1.51
N	20	20	20	20	20	
Mean	0.792	2400	9170	9170	1.47	
SD	0.264	793	2470	2470	0.324	
Min	0.433	1420	5110	5110	0.858	
Median	0.983	2340	8550	8550	1.47	
Max	1.02	4350	14300	14300	2.06	
CV%	33.4	33.0	26.9	26.9	22.0	

There are no human PK data for NMP exposures at the revised proposed maximum human daily dose of 128 mg CAM2038 q4w. The Applicant interpolated the human NMP AUC_(0-last) exposure between 96 mg CAM2038 q4w (AUC_{last} = 5330 ng•mL/h) and 192 mg CAM2038 q4w (AUC_{last} = 9170 ng•mL/h) to estimate an AUC_{last} at 128 mg CAM2038 q4w of 6610 ng•mL/h). Although not ideal, as the last measurement was taken at 48 hours rather than 24 hours as was done in the animal studies. Given the relatively short half-life of the compound (1.47 h), these data are a reasonable estimate of the exposures that would occur within 24 hours in humans. For the purposes of this review, the estimated human exposure to NMP at the MDD of 128 mg CAM2038 q4w will be 6.61 mcg•h/mL.

Study Title: NMP (N-methyl-pyrrolidone). Toxicokinetic Study in Rats.

Study Number: TO-15-540
 Regulatory Status: GLP, Audited
 Study Report Date: June 17, 2016

Methods: NMP was injected subcutaneously to male and female Sprague-Dawley rats at doses of 100, 250, and 500 mg/kg. Plasma samples were obtained pre-dose, 20 minutes, 1, 2, 4, 8, 12, 24, 48, and 96 hours post dose in cohorts of animals to avoid excessive blood loss.

Results: Mean TK values are depicted below.

Table 4 Mean toxicokinetic parameters of N-methyl-2-pyrrolidone

Group	Dose (mg/kg)	Gender	T _{1/2} (hr)	T _{max} (hr)	C _{max} (ug/ml)	SE_C _{max} (ug/ml)	C _{max} /D (kg*ug/ml/mg)
1	100	Male	1.26	0.3	162	15	1.62
		Female	1.11	0.3	204	21	2.04
		Both	1.25	0.3	183	15	1.83
2	250	Male	1.13	1.0	321	11	1.28
		Female	2.14	1.0	411	8	1.64
		Both	2.28	1.0	366	21	1.46
3	500	Male	1.97	1.0	607	24	1.21
		Female	1.66	1.0	669	16	1.34
		Both	1.74	1.0	638	19	1.28

Group	Dose (mg/kg)	Gender	AUC _{0-t} (hr*ug/ml)	SE_AUC _{0-t} (hr*ug/ml)	AUC _{inf} (hr*ug/ml)	AUC _{inf} /D (hr*kg*ug/ml/mg)
1	100	Male	431	49	433	4.33
		Female	709	124	710	7.10
		Both	570	76	571	5.71
2	250	Male	1468	66	1468	5.87
		Female	2495	276	2496	9.99
		Both	1984	230	1986	7.94
3	500	Male	4002	258	4004	8.01
		Female	5674	572	5676	11.35
		Both	4838	403	4840	9.68

n=5/sex for each study Group. Blood sampling for toxicokinetics was performed using a spares sampling scheme with n=3/sex/time point for each Group.

The Applicant appropriately noted that in this study, female exposures were higher than males, but that for both sexes, NMP could be detected up to 8 to 12 hours after injection pending dose. T_{max} was 20 minutes following a dose of 100 mg/kg but 1 hour for doses of 250 and 500 mg/kg.

Study Title: NMP (N-methyl-2-pyrrolidone). Toxicokinetic Study in Rabbits

Study Number: TO-15-541
 Regulatory Status: GLP, Audited
 Study Date: June 17, 2015

Methods: NMP (50, 150, or 250 mg/kg) was administered via SC injection to White New Zealand Rabbits (five female) and plasma samples were obtained to characterize the PK profile of NMP in this species. Plasma samples were obtained pre-dose, 20 minutes, 1, 2, 4, 8, 12, 24, 48, and 96 hours. For several high dose animals, all time points were not obtained as the animals had to be euthanized early.

Results:

Table 2 Individual toxicokinetic parameters of N-methyl-2-pyrrolidone

Group	Animal	T _{1/2} (hr)	T _{max} (hr)	C _{max} (ug/ml)	C _{max} /D (kg*ug/ml/mg)
1	1	0.91	1.0	62.7	1.25
	2	0.90	1.0	41.9	0.84
	3	0.89	0.3	71.1	1.42
	4	0.87	1.0	66.4	1.33
	5	0.86	1.0	66.2	1.32
	N	5	5	5	5
	Mean	0.89	0.9	61.7	1.2
	SD	0.02	0.3	11.4	0.2
	CV%	2.3	34.6	18.6	18.6
2	6	1.05	1.0	166.0	1.11
	7	1.21	1.0	197.0	1.31
	8	1.21	1.0	87.7	0.58
	9	1.03	1.0	152.0	1.01
	10	1.01	1.0	219.0	1.46
	N	5	5	5	5
	Mean	1.10	1.0	164.3	1.1
	SD	0.10	0.0	50.2	0.3
	CV%	9.0	0.0	30.6	30.6
3	11		2.0	267.0	1.07
	12	2.22	1.0	320.0	1.28
	13		1.0	305.0	1.22
	14		1.0	310.0	1.24
	15	2.20	1.0	348.0	1.39
	N	2	5	5	5
	Mean	2.21	1.2	310.0	1.2
	SD	0.01	0.4	29.2	0.1
	CV%	0.6	37.3	9.4	9.4

Group	Animal	AUC ₀₋₂ (hr*ug/ml)	AUC ₀₋₄ (hr*ug/ml)	AUC _{0-t} (hr*ug/ml)	AUC _{inf} (hr*ug/ml)	AUC _{inf/D} (hr*kg*ug/ml/mg)
1	1	88	141	157	158	3.16
	2	72	134	157	158	3.16
	3	115	177	197	198	3.95
	4	113	183	207	207	4.15
	5	103	177	203	203	4.06
	N	5	5	5	5	5
	Mean	98	163	184	185	3.70
	SD	18	23	25	25	0.49
	CV%	18.5	14.2	13.4	13.4	13.4
2	6	280	523	667	668	4.45
	7	321	630	984	985	6.56
	8	145	284	343	343	2.29
	9	244	492	729	730	4.87
	10	348	647	890	891	5.94
	N	5	5	5	5	5
	Mean	268	515	723	723	4.82
	SD	79	145	247	247	1.65
	CV%	29.5	28.2	34.2	34.1	34.1
3	11	416				
	12	538	1110	1996	1997	7.99
	13	522				
	14	524				
	15	576	1113	1875	1877	7.51
	N	5	2	2	2	2
	Mean	515	1112	1935	1937	7.75
	SD	60	2	85	85	0.34
	CV%	11.6	0.2	4.4	4.4	4.4

Note that animal Nos. 11, 13 and 14 in Group 3 were terminated after 2 hours due to clinical signs.

As noted by the Applicant, NMP was detectable at 4 hours for the 50 mg/kg group and out to 8 and 12 hours for the 150 mg/kg and 250 mg/kg groups, respectively. T_{max} was about 1 hour.

There are limited toxicology data on SC route of exposure. As part of their safety assessment, the Applicant has concluded that the existing data on NMP suggest that comparable exposures occur via any route of administration (IV has slightly higher C_{max}) and therefore, extrapolation across studies testing different routes of administration is appropriate. The data submitted to support that conclusion are summarized in the Applicant's table below:

Table 1: Pharmacokinetic Parameters of Animals Treated with NMP by Oral, IV, SC or Inhalation and Humans Treated SC with CAM2038 q4w (192 mg)

Dose (mg/kg)	C _{max} (µg/mL/mg/kg) ^a	AUC _{0-t} (µg hr/mL/mg/kg) ^a	T _{max} (hr)	T _{1/2} (hr)	Reference
125 (oral) Rat	0.94	5.63	1.00	NR	Canerup et al., 2005
50 (oral) ^b Rat	0.67	3.00	2.25	2.25	DuPont, 1995b
100 (SC) Rat	1.83	5.70	0.30	1.19	CiToxLAB 2016a
50 (SC) Rabbit	1.23	4.18	0.90	0.89	CiToxLAB 2016b
50 (IV) ^b Rat	3.08	9.84	2.85	2.85	DuPont, 1995b
98 (inhalation) ^{b,c} Rat	0.91	5.10	1.55	1.55	DuPont, 1995b
2.8 (SC) Human ^{a,d}	0.86	3.30	0.80	1.47	Camurus, 2016

^aThe C_{max} and AUC values were adjusted based on mg/kg dose.

^bThe mean pharmacokinetic parameters for male and female animals was calculated

^cThe inhalation dose (410 mg/m³) was converted to an oral dose based on Equation 1 cited in the text (see Section 10).

^dThe dose of NMP in the 192 mg CAM2038q4w drug product is [redacted] mg (total dose). This was converted to a mg/kg dose based on a 60 kg individual [redacted] mg/kg).

Additional Information: NR – Not reported; IV - intravenous; SC – subcutaneous

According to the original submission, the PK data were somehow adjusted for dose although the submission did not indicate how this was done. Regardless, review of the above summary table clearly indicates that the AUC in rat following IV administration of 50 mg/kg NMP is 3.3 times higher than the same dose administered via the oral route. C_{max} via IV is 4.6 times higher than oral in the rat. SC would likely be somewhere in between.

The lack of adequate ADME data to bridge the published oral and inhalation reproductive and developmental toxicology data was considered a deficiency in the first cycle. Therefore, in the second cycle, the Applicant conducted two oral pharmacokinetic studies and submitted a published study that reported a PK/PD model used to estimate an acceptable occupational exposure limit for NMP. These are reviewed below and again noted after the relevant toxicology studies.

Study Title: An Oral (Gavage) Toxicokinetic Study of N-methyl-2-pyrrolidone (NMP) in Sprague-Dawley Rats

Study Number: PK-18-611
 Regulatory Status: GLP, Audited
 Study Date: Signed May 7, 2018

In the second cycle, the Applicant completed an oral rat PK study to estimate exposures via the oral route (Study PK-18-611). Male rats were treated with 100, 300, or 1000 mg/kg NMP and pregnant female rats were treated with 125, 250, or 500 mg/kg NMP via oral gavage for 5 consecutive days. Plasma samples were obtained at 0, 1, 2, 4, 8, 12, and 24 hours after dosing. Toxicokinetic data are presented below:

NMP Dose (mg/kg/day):	Males		
	100	300	1000
<u>Parameter (Units)</u>	<u>Day 4</u>		
AUClast (µg·h/mL)	716	3070	10,400
Cmax (µg/mL)	112	364	774
Tmax (h)	1	2	2
	Pregnant Females		
NMP Dose (mg/kg/day):	125	250	500
<u>Parameter (Units)</u>	<u>Gestation Day 10</u>		
AUClast (µg·h/mL)	1050	2570	7010
Cmax (µg/mL)	155	313	612
Tmax (h)	1	2	4

Study Title: An Oral (Gavage) Toxicokinetic Study of N-methyl-2-pyrrolidone (NMP) in New Zealand White Rabbits

Study Number: PK-18-610
 Regulatory Status: GLP, Audited
 Study Date: Signed May 7, 2018

Methods: NMP was administered to pregnant rabbits at doses of 55, 175, and 540 mg/kg/day via oral gavage once daily from GD 7-11. Plasma samples were taken at 0, 0.5, 1, 3, 6, 12, and 24 hours. The PK study results are depicted below, reproduced from the study report:

NMP Dose (mg/kg/day):	55	175	540
<u>Parameter (Units)</u>	<u>Gestation Day 11</u>		
AUClast (µg·h/mL)	124	775	3860
AUClast (µg·h/mL) versus 55 mg/kg/day	NA	6x	31x
Cmax (µg/mL)	48.3	171	584
Tmax (h)	0.875	1.88	3.00
T1/2 (h)	1.12†	7.10†	NC

† = n of 1

Note: *Italicized* value is considered an approximation, due to $R_{sq} < 0.80$.

NC = Not calculable, due to limited data in the terminal elimination phase.

No inhalation PK studies were completed by the Applicant to estimate the exposures that occurred in the published inhalation reproductive and developmental toxicology studies.

Study Title: Using Physiologically Based Pharmacokinetic Modeling and Benchmark Dose Methods to Derive an Occupational Exposure Limit For N-Methylpyrrolidone (Poet et al., 2016).

Poet et al. reported results of a physiologically-based pharmacokinetic (PBPK) model for NMP to estimate short-term and chronic occupational exposure limits. The model employed the published reproductive and developmental toxicity data following oral, inhalation, and dermal routes of exposures. For acute exposures, the model took into consideration skeletal malformations and for repeated exposures the model took into consideration changes in fetal or pup body weights. The tables below, from the publication, summarizes the PK datasets used.

Summary of pharmacokinetic data sets available for NMP and their application to model development.

Species	Data use category for PBPK model	Study	Route	Exposure range	Specific model parameters (if applicable) ^a	
Rat	Calibration	Payan et al. (2002)	iv	0.1–500 mg/kg	Metabolic rate constants ($V_{maxC} = 9 \text{ mg/h/kg} \cdot 0.75$, $K_m = 225 \text{ mg/L}$ for NMP; $V_{maxC2} = 0.09 \text{ mg/h/kg} \cdot 0.75$, $K_{m2} = 4.9 \text{ mg/L}$ for 5HNMP) were optimized to match behavior of NMP in plasma (Fig. A1-1) and first order urinary elimination rates for 5HNMP	
		Midgley et al. (1992)	Oral Gavage	104 mg/kg	Oral absorption terms ($K_{AS} = 1.5 \text{ h}^{-1}$, $K_{AI} = 0.85 \text{ h}^{-1}$, $K_{SI} = 0.006 \text{ h}^{-1}$), first order transfer from stomach to liver, stomach to intestine and intestine to liver) were optimized to match model predictions to NMP plasma data (Fig. A1-2)	
		Payan et al. (2003)	Dermal (liquid)	44–700 μl neat	Dermal absorption ($K_p = 4.3 \times 10^{-3} \text{ cm/h}$) was optimized to match behavior of NMP in plasma (Fig. A1-3)	
	Validation	Wells and Digenis (1988)	iv	45 mg/kg	Metabolic rate constants (V_{maxC} , K_m , V_{maxC2} , K_{m2}) were validated based on predictions of the model to NMP in plasma (Fig. A1-1)	
		Ghantous (1995)	Oral Gavage	54 mg/kg	Oral absorption terms (K_{AS} , K_{AI} , K_{SI}) were validated by comparing model predictions to data for NMP in plasma (Fig. A1-2)	
		Ghantous (1995)	Inhalation, nose only	10, 100 ppm–6 h	Inhalation validated by comparing model predictions to data for NMP in plasma (Fig. 1). 100% bioavailability was assumed	
	Human	Calibration	Payan et al. (2003)	Dermal (<i>in vitro</i>)	Neat NMP	Dermal absorption ($K_p = 4.3 \times 10^{-3} \text{ cm/h}$) was validated by comparison to <i>in vitro</i> results, which are in close agreement ($2\text{--}7.7 \times 10^{-3} \text{ cm/h}$)
			Bader and van Thiel, 2006	Inhalation	2.5, 10, 20 ppm (10, 40, and 80 mg/m^3)	Metabolic rate constants ($V_{maxC} = 44 \text{ mg/h/kg} \cdot 0.75$, $K_m = 68 \text{ mg/L}$; $V_{maxC2} = 4.5 \text{ mg/h/kg} \cdot 0.75$, $K_{m2} = 61 \text{ mg/L}$) were optimized to match the behavior of NMP and 5HNMP in plasma (Figs. 2, A1-4)
			Akesson and Paulsson (1997)	Inhalation + Dermal (vapor)	2.5, 6, 13 ppm (10, 24, 53 mg/m^3)	Dermal vapor absorption (Vapor $K_p^b = 22 \text{ cm/h}$) was optimized to match NMP the behavior of NMP in plasma following dermal and inhalation exposure (Fig. A1-5)
		Validation	Akesson et al. (2004)	Dermal (liquid)	300 mg (neat, 50% solution)	Dermal liquid absorption (Liquid $K_p^b = 2.0 \times 10^{-3} \text{ cm/h}$) was optimized to match plasma 5HNMP following exposures to either neat NMP or NMP diluted in aqueous solution (50%) (Fig. A1-6)
Xiaofei et al. (2000)			Inhalation + dermal (vapor)	0.14–0.42 ppm (from dosimeter)	Occupational exposure, Dosimeter data and presumptive NMP splatter was used to fit the worker and observer data (Fig. A1-7)	
HLS (2002)			Dermal (<i>in vitro</i>)	Neat NMP	Dermal absorption (K_p) was validated by comparison to <i>in vitro</i> results, which are in close agreement ($2.7 \times 10^{-3} \text{ cm/h}$ vs. $2.2 \times 10^{-3} \text{ cm/h}$)	

^a Values provided reflect those derived in Appendix A1. Alternative values for some parameters are also provided in Appendix A2 (see text for discussion).

^b K_p is the permeability coefficient for NMP. K_p from different phases (i.e., liquid or vapor) are related. For simplicity, different K_p were optimized from the different phases, but a single K_p could be used with a modifying factor to account for air/water partitioning.

Summary of key data sets for the developmental effects of NMP.

Reference	Exposure regimen (gestation period coverage)	Administered dose (mg/kg-day) or concentration (ppm)	Internal dose ^a		Fetal/pup weight		Skeletal malformation	
			Average daily AUC, GD13-20 (h*mg/L)	Average peak, GD7-20 (mg/L)	n	Mean ± SD (g)	Litter incidence	Fetal incidence
Saillenfait historical controls	NA	NA	0	0	604	5.65 ± 0.35	NC	NC
Saillenfait et al. (2002)	Gavage (GD6-20)	0	0	0	21	5.73 ± 0.50	0/20	0/133
		125	1290	106	21	5.59 ± 0.22	0/21	0/138
		250	2820	219	24	5.18 ± 0.35	0/24	0/152
		500	6430	461	25	4.02 ± 0.21	12/25	14/155
Saillenfait et al. (2003)	Inhalation, 6 h/d (GD6-20)	750	10,500	717	8	3.01 ± 0.39	3/5	5/9
		0	0	0	24	5.67 ± 0.37	0/24	0/167
		30	158	15.0	20	5.62 ± 0.36	0/19	0/126
		60	322	30.3	19	5.47 ± 0.25	0/19	0/133
Becci et al. (1982)	Dermal, 8 h/d (GD6-15)	120	668	61.9	25	5.39 ± 0.45	0/25	0/150
		0	0	0	24	3.45 ± 0.20	0/24	0/187
		75	340	68	22	3.49 ± 0.24	0/22	0/108
		237	1230	187	23	3.54 ± 0.29	0/23	0/192
Solomon et al. (1995)	Inhalation, 6 h/d (GD1-20)	750	3910	470	22	2.83 ± 0.39	11/22	14/156
		0	0	0	39	7.48 ± 0.71	1/14	1/205
		10	52	4.9	16	7.03 ± 0.70	NA	NA
		51	272	25	15	7.14 ± 0.69	NA	NA
Thornton (1999)	Feed (GD1-20)	116	643	85	22	6.66 ± 0.62	0/13	0/185
		0	0	0	25	6.7 ± 0.65	NA	NA
		50	591	NC	25	6.9 ± 0.39	NA	NA
		160	1960	NC	24	6.8 ± 0.77	NA	NA
		500	7920	NC	22	5.3 ± 0.78	NA	NA

N = number of live litters; NC = not calculated; NA = not assessed.

^a The PBPK model for NMP, as modified in Appendix A, was used to predict rat internal doses for NMP in blood over the window of susceptibility as determined in Appendix B. Values rounded to 3 significant figures.

As per Poet et al, these data were used to estimate short-term and chronic occupational exposures limits of 86 and 24 ppm, respectively. Notably, the model included only inhalation data in rat following a single 6-hour nose only exposure and cited a study completed by Dupont in 1995. The study was submitted to the NDA and reviewed below.

The Dupont 1995b reference does have data testing a single 6-hour nose only inhalation exposure of radiolabeled NMP in rats testing either 10 ppm or 100 ppm. The results are depicted below from the report:

Table 49. PK Data Following 6-hour Nose-only Inhalation of NMP in Rats (DuPont, 1995b; aka Ghantous, 1995).

Dose	Sex	T _{max} (h)	C _{max} (ppm)	t _{1/2} (h)	AUC (mcg•h/mL)
10 ppm	M	6.0	3.5 ± 0.2	2.1 ± 0.2	25.8 ± 2.1
10 ppm	F	6.5	5.4 ± 0.5	3.4 ± 0.9	42.5 ± 4.4
100 ppm	M	6.25	72.2 ± 12.2	2.0 ± 0.4	534.4 ± 93.0
100 ppm	F	6.5	82.5 ± 12.1	3.0 ± 0.3	696.6 ± 33.8

Poet et al plotted the actual 100 ppm data from the Dupont study (aka Ghantous 1995) and depicted the results and their PBPK model in the figure below reproduced from the publication.

Figure 4. Plasma NMP vs. Time Curve Following Inhalation of 100 ppm NMP in Rat (Poet et al., 2016)

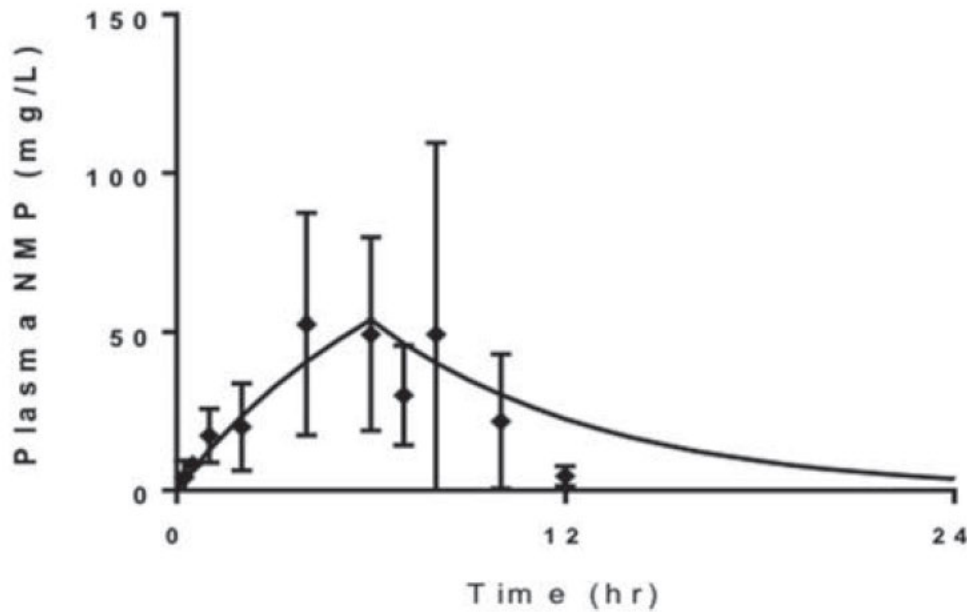
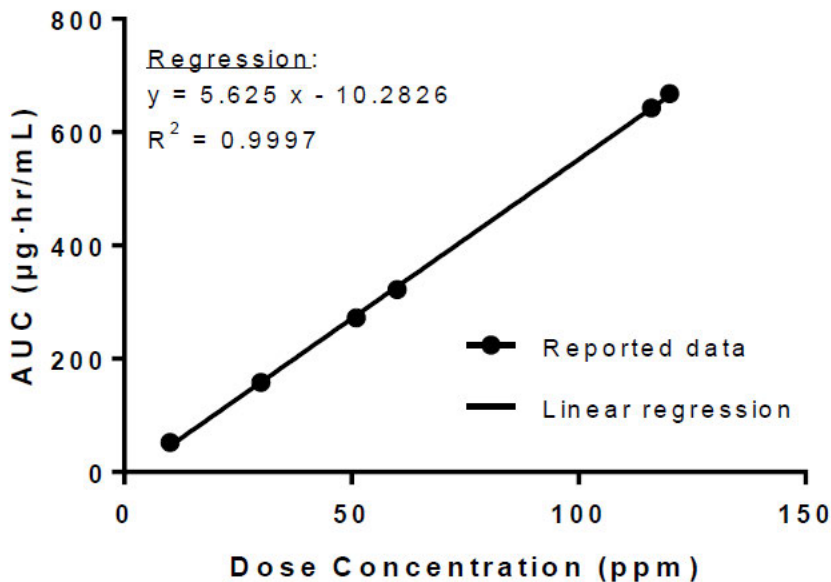


Fig. 1. Plasma NMP following inhalation exposure of rats to NMP: diamonds = arithmetic mean (100 ppm, Ghantous, 1995), error bars = standard deviation, solid line = PBPK model predictions.

The Applicant also contracted with the consulting company ^{(b) (4)} to estimate the plasma exposures that were likely obtained in the reproductive and developmental studies following inhalation exposures based on the predictions outlined in Poet et al. The modeled inhalation data were plotted and a linear model was estimated as per the figure below from the ^{(b) (4)} report.

Figure 5. (b) (4) Linear Model of NMP Exposure Based on Poet et al. PBPK Model
Relationship between NMP AUC and Dose Based on a Physiologically-Based Pharmacokinetic Model after Inhalation Dosing



Based on data from Table 1

AUC: area under the plasma concentration vs. time curve; NMP: N-methyl-2-pyrrolidone; ppm: parts per million

The above equation was used to estimate exposures in the published reproductive and developmental toxicology studies.

ADME Conclusions

Collectively, the above data do support the Applicants statement that, in contrast to buprenorphine, the NMP derived from CAM2038 q4w is cleared from the plasma relatively quickly. Based on their data, the NMP in the dosage form is eliminated from plasma within 24 hours.

For the purposes of this second cycle review, the worst-case MDD of NMP will be (b) (4) mg/day via the 128 mg q4w product. A dose of (b) (4) mg/60 kg person = (b) (4) mg/m² on a body surface area basis. At the time of the original submission, this daily dose was greater than any of the other two FDA-approved drug products making this use novel in terms of dose, duration, and risk-benefit compared to the FDA-approved drug products listed in the IID (accessed 12/12/2017). Further, (b) (4) (b) (4) this dose is also (b) (4) times higher than the ICH Q3C(R6) PDE of 5.3 mg/day.

Finally, although the PK data presented suggest similar T_{max} and $t_{1/2}$ values, the argument that all routes of administration will result in similar risk assessments in terms of toxicity was not convincing given the limited data provided.

In the second cycle submission, the Applicant conducted new oral PK bridging studies in pregnant rabbits and rats. These studies are reviewed and the data used to estimate exposure margins for the oral reproductive and developmental studies discussed below. They also proposed a PBPK model to estimate the exposures via the inhalation route. These data are still estimates and not considered definitive. Nonetheless, the values predicted were used to estimate the exposures that likely occurred in the published inhalation studies discussed below in the reproductive and developmental toxicology section.

TOXICOLOGICAL RISK ASSESSMENT

The Applicant has provided a review of the literature in support the safety of NMP. Although there are multiple toxicology studies in the literature with NMP, none of the studies use the subcutaneous route of administration. Bridging studies with appropriate TK have not been provided. Therefore, exposure margins will be based on body surface area comparisons.

General Toxicology

A 9-month general toxicology study in the dog testing CAM2038 q4w was conducted in support of this NDA. This study provides an adequate safety assessment of the NMP in the drug product formulation in the nonrodent species. The reader is referred to the body of this review for further details.

In addition, the Applicant summarized acute and repeat-dose general toxicology data from the published literature. This appendix will focus only on the published subchronic and toxicology studies submitted (Becci et al., 1983, Lee et al., 1987, Malek et al., 1997, Malley et al., 1999). The primary literature references and the NOAELs identified by the Applicant are depicted in the Applicant's table below:

Species	Route of Administration	Study Duration	Doses*	Reference
Mouse/ B6C3F1	Oral (diet)	28 Days	0, 500, <u>2500</u> , 7500, 10,000 ppm	Malek et al, 1997
Mouse/ B6C3F1	Oral (diet)	90 Days	0, <u>1000</u> , 2500, 7500 ppm	Malley et al., 1999
Rat/ CD	Oral (diet)	28 Days	0, 2000, <u>6000</u> †, 18,000‡, 30,000 ppm	Malek et al, 1997
Rat/ CD	Oral (diet)	90 Days	0, <u>3000</u> , 7500, 18000 ppm	Malley et al., 1999
Dog/ Beagle	Oral (diet)	90 Days	0, 25, 79, <u>250</u> mg/kg	Becci P, et al 1983
Rat/ CD	Inhalation	6 hr/day, 5 days/week for 4	0, 0.1, <u>0.5</u> and 10 mg/L	Lee et al, 1987

*The NOAEL is underlined; † NOAEL males; ‡ NOAEL females

Subchronic Toxicity

As noted in the table above, there are 90-day oral rat, mouse, and dog toxicology studies.

In the 90-day rat study, rats were administered 3000, 7500, or 18000 ppm NMP via the diet. Based on food consumption, male doses were 169, 433, and 1057 mg/kg and female doses were 217, 565, and 1344 mg/kg at the low-, mid-, and high-dose groups (LD, MD, and HD), respectively (Malley et al., 1999). The LD was considered the NOAEL for both sexes. There were no effects of NMP on mortality. The HD group demonstrated reduced body weights (~25% decrease compared to controls). The MD and HD male animals demonstrated increased foot splay values, low arousal, and palpebral closure in the neurological assessments, consistent with a general sedative effect of the compound. HD animals demonstrated increased relative liver, kidney and lung weights and decreased relative brain and testes weights. Microscopically, the high-dose group female demonstrated hepatocellular hypertrophy which was considered an adaptive response. An increase in hemosiderin deposits in spleen was also noted in both HD males and females. There was no adverse histopathology in the CNS. The NOAEL was the low dose. These doses provide a safety margin of 14.6x and 18.7x for males and females, respectively for the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

In the 90-day mouse study, mice were fed diets containing 1000, 2500, or 7500 ppm NMP which resulted in mean daily doses of 277, 619, and 1931 mg/kg for both sexes based on food consumption data (Malley et al., 1999). There was no effect on mortality. There was a dose related increase in liver weights. Evidence of centrilobular hepatocellular hypertrophy was found in the HD animals, which was attributed to an adaptive response. The LD was considered the NOAEL/LOAEL based on liver weight increases. This dose provides a safety margin of 11.9x for the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

In the 90-day dog study, beagles were administered NMP via the diet resulting in daily doses of 25, 79, and 250 mg/kg (Becci et al., 1983). There was no impact on survival. HD animals demonstrated reduced body weight gain compared to controls ($\downarrow$ 53% males, $\downarrow$ 37% females). Decreased cholesterol, serum albumin, and total protein levels in MD and HD male dogs was noted; however, there were no histological correlates to explain these findings. Platelet counts increased with dose at Week 8, but levels were subsequently decreasing by Week 12. Based on these unexplained changes, the HD was deemed the NOAEL which provides a 72x safety margin for NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

Chronic Toxicity

In a 2-year inhalation toxicology study in male rats, Lee et al report that 0.04 or 0.4 mg/L for 6 hours a day NMP, 5 days a week did not result in any clear treatment-related histopathology (Lee et al., 1987). This concentration in air, assuming 290 L/day (12 L/h) and 0.5 kg body weight, would result in 58 mg/kg dose (5x the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area).

Malley et al. treated rats with oral 1600, 5000, or 15000 ppm NMP via the diet for 2-years resulting in estimated daily intakes of 66.4, 207, and 678 mg/kg in males and 87.8, 283, and 939 mg/kg in females (Malley et al., 2001). NMP reduced body weights in HD males and females by 25 and 35% relative to controls which corresponded to lower food consumption and food efficiency. HD males demonstrated reduced survival which was associated with an increased incidence of chronic progressive nephropathy/uremia. Decreased survival in MD males was associated with an increase in pituitary and skin tumors which were not dose dependent and therefore, not likely treatment-related. As noted in the table below, NMP increased the incidence of chronic progressive nephropathy, increased pigment containing macrophages in the spleen, produced hepatocellular fat accumulation, and generated testicular, bone and kidney damage in males. In females, the only findings were increased pigmented macrophages in the spleen and lymphoid depletion. The NOAEL was 5000 ppm (207 and 283 mg/kg in males and females, respectively) which suggests a safety margin of 18x and 24x for males and females, respectively, for the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

Figure 6. Non-neoplastic Lesions in 2-year Oral Rat Toxicology Study (Malley et al., 2001)

Lesion Concentration (ppm)	0	1600	5000	15 000
Male rats				
Severe chronic progressive nephropathy	7/62	10/62	12/62	25/62 [#]
Moderate accumulation of pigment containing macrophage, spleen	18/62	14/62	16/62	28/62 [#]
Hepatic centrilobular fatty change	4/62	8/62	4/62	19/62 [#]
Thrombus in renal vein of kidney	0/62	4/62	4/62	11/62 [#]
Polyarteritis, cecum	0/62	1/31	0/49	5/62 [#]
Lymphoid depletion, mesenteric lymph node	10/62	10/30	9/48	28/60*
Hypertrophy/cystic degeneration, adrenal cortex	18/62	10/34	22/52	28/62*
Polyarteritis, testes	10/62	16/62	11/62	31/62 [#]
Bilateral degeneration/atrophy, seminiferous tubules	15/62	17/62	15/62	35/62 [#]
Bilateral oligospermia/germ cell debris in epididymides	11/62	14/62	12/62	35/62 [#]
Fibrous osteodystrophy, femur/knee	3/62	5/32	5/49	13/62*
Fibrous osteodystrophy, sternum	2/60	5/32	5/49	11/62*
Polyarteritis, mesenteric lymph node	0/60	0/30	0/48	5/62*
Female rats				
Moderate accumulation of pigment containing macrophage, spleen	38/62	35/62	36/62	51/62 [#]
Lymphoid depletion, mesenteric lymph node	15/58	18/47	11/41	29/60*

[#]Statistically significant by Cochran Armitage test for trend, $p < 0.05$.

*Statistically significant by Fisher's exact test, $p < 0.05$.

Malley et al. also treated mice for 18 months with oral NMP via the diet (600, 1200, or 7200 ppm) resulting in daily doses of 89, 173, or 1089 mg/kg in males and 115, 221, or 1399 mg/kg in females based on food consumption (Malley et al., 2001). There were no clear effects of NMP on body weight or food consumption; however, the NMP appeared to stick to the food hopper, so the sporadic changes in BW are difficult to interpret. There was no increased mortality in males or females. NMP significantly increased liver weights, likely the result of the increased incidence of liver tumors and hepatocellular hypertrophy in this study (see carcinogenicity section below). In males, absolute kidney weights were decreased in all dose groups although there were no histopathological correlates to explain this finding and the effects were not clearly dose dependent. Absolute testes weight was increased in the HD males; relative weights were increased in all treatment groups without histopathological correlates. The same can be said of the increased relative adrenal weight in HD males and lower ovarian weights in MD and HD females. In females, absolute brain weight increases in all treatment group without histopathological changes noted.

Table 50. Organ Weights 18-month Oral Mouse Study (Malley et al., 2001)

Dosage (ppm)	0	600	1200	7200
Male mouse				
Absolute liver weight (mg)	1437.3 (407.8)	1308.7 (150.2)	1455.8 (388.3)	1776.9** (467.9)
Relative liver weight (% of body weight)	3.8 (1.0)	3.6 (0.3)	4.0* (1.5)	5.0** (1.1)
Absolute kidney weight (mg)	684.4 (70.1)	633.4** (54.5)	633.6** (68.2)	635.8** (58.3)
Relative kidney weight (% of body weight)	1.8 (0.2)	1.7 ** (0.2)	1.7** (0.2)	1.8 (0.2)
Absolute testes weight (mg)	228.8 (15.4)	235.0 (16.5)	233.8 (14.8)	238.2** (18.4)
Relative testes weight (% of body weight)	0.62 (0.06)	0.65* (0.07)	0.65* (0.08)	0.67** (0.08)
Absolute adrenal weight (mg)	5.4 (1.2)	5.5 (1.4)	5.2 (1.4)	5.6 (0.9)
Relative adrenal weight (% of body weight)	0.014 (0.003)	0.015 (0.004)	0.014 (0.004)	0.016* (0.003)
Female mouse				
Absolute liver weight (mg)	1273.6 (181.2)	1241.3 (159.9)	1293.8 (189.4)	1423.1 (691.8)
Relative liver weight (% of body weight)	3.9 (0.6)	4.0 (0.4)	4.0 (0.6)	4.6** (1.9)
Absolute brain weight (mg)	498.2 (24.5)	515.8** (24.2)	518.2** (26.6)	508.8* (24.5)
Relative brain weight (% of body weight)	1.6 (0.3)	1.7 (0.3)	1.6 (0.3)	1.7 (0.2)
Absolute ovarian weight (mg)	36.7 (44.4)	64.5 (263.8)	22.9* (28.2)	26.6* (25.9)
Relative ovarian weight (% of body weight)	0.111 (0.103)	0.203 (0.824)	0.074 (0.108)	0.085 (0.078)

*Statistically significant difference by one-way analysis of variance and Dunnett's tests, $p < 0.05$.

**Statistically significant difference by one-way analysis of variance and Dunnett's tests, $p < 0.01$.

Table 51. Summary of Test Substance-related Non-neoplastic Lesions in 18-month Oral Mouse Study (Malley et al., 2001)

Lesion	Concentrations (ppm)			
	0	600	1200	7200
Male mice				
Hepatocellular clear cell foci	0/50	1/50	1/50	6/50*
Hepatocellular basophilic foci	4/50	2/50	3/50	5/50
Hepatocellular eosinophilic foci	1/50	2/50	2/50	19/50*
Hepatocellular centrilobular hypertrophy	0/50	0/50	3/50	43/50*
Cellular alterations in liver	5/50	5/50	6/50	25/50*
Female mice				
Hepatocellular clear cell foci	0/50	0/50	0/50	1/50
Hepatocellular basophilic foci	2/50	2/50	1/50	10/50*
Hepatocellular eosinophilic foci	1/50	0/50	0/50	6/50*
Hepatocellular centrilobular hypertrophy	0/50	1/50	0/50	0/50
Cellular alterations in liver	3/50	2/50	1/50	17/50*

*Statistically significant by Cochran Armitage test for trend, $p < 0.05$.

As per the authors, the NOAEL was reported to be LD in males and the MD in females, the former apparently driven primarily by the liver effects in males at 1200 ppm. These NOAELs suggest exposure margins of 3.8x and 9.5x for the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

As discussed with the Applicant, this study could serve as the rodent chronic toxicology study requirements for NMP. Upon review, a dedicated rodent chronic toxicology study is not necessary.

REPRODUCTION AND DEVELOPMENTAL TOXICITY

The Applicant submitted a literature review to address the reproductive and developmental toxicology requirements for NMP in their formulation. In their review, the Applicant did not specifically delineate why they felt any given study addressed all standard endpoints of modern reproductive and developmental toxicology studies. The multigenerational studies below do contain some fertility and early embryonic study endpoints.

Male and Female Fertility and Early Embryonic Development

As per ICH S5(R2), fertility and early embryonic development studies are intended to test for toxic effects of a compound resulting from treatment prior to mating (males and females) through mating and implantation. For females, the studies should characterize effects on the estrus cycle, tubal transport, implantation, and development of preimplantation states of the embryo as well as mating behavior and fertility assessments. The acceptable dosing regimen for females is to dose them at least 2 weeks prior to mating through implantation in females (for rats implantation is generally by Gestation Day 6-7 of pregnancy). For males, the studies should evaluate the impact of a drug on functional effects (e.g., libido, epididymal sperm maturation) that may not be detected via histopathological examination of the male reproductive tissues. Although spermatogenesis in the rat lasts 63 days, acceptable dosing regimens require that the rats be tested for at least 28 days prior to mating.

Study Title: Multi-Generation Rat Reproduction Study with N-Methylpyrrolidone (Exxon, 1991; Project Number: 236535)

Exxon Biomedical Sciences (1991) conducted a study in which male and female rats were administered NMP via the diet with target dosing intended to administer 50, 160, and 500 mg/kg/day. The study contains some fertility and early embryonic development endpoints and is therefore discussed here in that context. Both male and females were dosed daily for 10 weeks prior to mating and throughout the mating period. Females were also dosed during gestation, lactation, and through weaning to Post-Partum Day (PPD) 21. Second generation pups (F1) were dosed directly starting on PND 21 for an additional 10 weeks and through gestation, lactation, and to weaning of the F2 pups. Paternal toxicity was noted in the high-dose (HD) animals as demonstrated by decreased body weights and food consumption. The HD group also demonstrated reduced fertility indices in both males and females and reduced survival of the F1 offspring in the HD group. Key summary tables from the report are reproduced below:

DOSE (MG/KG)	Male		Female		Gestational Index	Mean Days Gestation	Mean Litter Size	Mean		Percent	
	Mating Index	Fertility Index	Fertility Index	Fecundity Index				Live	Dead	Live	Dead
0	93.1	82.8	93.3	89.3	96.0	22.7	12.6	12.5	0.2	98.7	1.3
50	100.0	83.3	100.0	83.3	100.0	22.4	14.5	14.4	0.2	98.9	1.1
160	96.6	75.9	96.7	79.3	100.0	22.4	13.6	13.0	0.5	96.2	3.8
500	86.7	80.0	86.7	92.3	95.8	22.5	12.9	11.2	1.7	86.1	13.3

INDICES:

Male Mating Index (%) =	$\frac{\text{Number of males for which mating confirmed}}{\text{Number of males used for mating}} \times 100$
Male Fertility Index (%) =	$\frac{\text{Number of males impregnating females}}{\text{Number of males used for mating}} \times 100$
Female Fertility Index (%) =	$\frac{\text{Number of females for which mating confirmed}}{\text{Number of females paired}} \times 100$
Female Fecundity Index (%) =	$\frac{\text{Number of females pregnant}}{\text{Number of females sperm positive}} \times 100$
Gestational Index (%) =	$\frac{\text{Number of females with live litters}}{\text{Number of females pregnant}} \times 100$

Although not exactly dose dependent, the above data suggest that the doses of 160 and 500 mg/kg are associated with a slight reduction in male fertility index and the 500 mg/kg HD reduced fertility index in females. The authors of the study suggest that this change is not likely biologically significant and considered them non-adverse. However, male rodents are very fertile compared to humans and evidence of changes in rodent fertility index does suggest some concern. There were no reported histopathological correlates in male testes in the study report. Toxicokinetic data or palatability data were not reported. As this study was conducted by dietary administration, the actual exposures in the MD and HD animals are estimates only and it is not known if there are palatability issues which impacted the actual dose consumed. This confounds the ability to make dose response conclusions with confidence.

	D	D	D	D	D	D	D	D	D	D	D	D
	A	A	A	A	A	A	A	A	A	A	A	A
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	0	1	2	3	4	5	6	7	8	9	10	11
SURVIVORS												
0 MG/KG/DAY	312	311	311	311	310	221	221	221	221	221	221	221
50 MG/KG/DAY	359	355	353	353	352	240	240	240	240	240	240	240
160 MG/KG/DAY	300	299	298	298	298	209	209	209	209	209	209	209
500 MG/KG/DAY	267	209	200	196	192	155	153	152	151	151	151	150
GENERAL OBSERVATION												
NO OBSERVABLE ABNORMALITIES												
0 MG/KG/DAY	303	308	310	309	309	219	219	217	215	218	218	219
50 MG/KG/DAY	352	354	352	352	351	239	239	239	239	239	239	239
160 MG/KG/DAY	295	291	289	289	296	208	208	208	207	207	207	207
500 MG/KG/DAY	261	204	195	187	183	148	145	145	145	145	144	146
FOUND DEAD												
0 MG/KG/DAY	3	0	0	0	0	0	0	0	0	0	0	0
50 MG/KG/DAY	6	1	2	0	1	0	0	0	0	0	0	0
160 MG/KG/DAY	11	1	0	0	0	0	0	0	0	0	0	0
500 MG/KG/DAY	45	19	1	4	0	1	1	1	0	0	1	0
WITHOUT MILK												
0 MG/KG/DAY	8	0	0	1	1	0	0	0	2	0	0	0
50 MG/KG/DAY	0	1	4	1	2	0	0	0	0	0	0	0
160 MG/KG/DAY	2	7	2	7	8	0	3	0	0	0	0	0
500 MG/KG/DAY	22	3	0	3	4	2	3	1	2	0	0	0
PARTIALLY CANNIBALIZED (INCLUDED IN FOUND DEAD TOTALS)												
0 MG/KG/DAY	1	0	0	0	0	0	0	0	0	0	0	0
50 MG/KG/DAY	0	0	0	0	0	0	0	0	0	0	0	0
160 MG/KG/DAY	1	0	0	0	0	0	1	0	0	0	0	0
500 MG/KG/DAY	2	11	1	6	0	0	0	0	0	0	0	0
MISSING/PRESUMED CANNIBALIZED												
0 MG/KG/DAY	2	0	0	0	1	0	0	0	0	0	0	0
50 MG/KG/DAY	0	1	0	0	0	0	0	0	0	0	0	0
160 MG/KG/DAY	1	0	0	0	0	0	0	0	0	0	0	0
500 MG/KG/DAY	3	25	7	1	3	1	1	0	1	0	0	0

DOSE (MG/KG)	Live Birth Index %	Day 4 Survival Index %	Day 7 Survival Index %	Day 14 Survival Index %	Day 21 Survival Index %	Lactation Index %
0	98.7	99.4	100.0	100.0	100.0	100.0
50	98.9	98.1	100.0	100.0	100.0	100.0
160	96.2	99.3	100.0	100.0	95.2	95.2
500	86.7	71.9	96.8	97.4	98.6	93.0

INDICES:

Live Birth Index =	$\frac{\text{Number of live pups at birth}}{\text{Number of pups born}} \times 100$	Day 14 survival Index =	$\frac{\text{Number of live pups at day 14}}{\text{Number of live pups at day 7}} \times 100$
Day 4 survival Index =	$\frac{\text{Number of live pups at day 4 (precul}}{\text{Number of live pups at birth}} \times 100$	Day 21 survival Index =	$\frac{\text{Number of live pups at day 21}}{\text{Number of live pups at day 14}} \times 100$
Day 7 survival Index =	$\frac{\text{Number of live pups at day 7}}{\text{Number of live pups at day 4 (postcul}} \times 100$	Lactation Index =	$\frac{\text{Number of live pups at day 21}}{\text{Number of live pups at day 4 (postcul}} \times 100$

The NOAEL for parental and reproductive and developmental effects was reported by the study to be the MD of 160 mg/kg (960 mg/m²) which is 13.8x the maximum daily dose of NMP of (b) (4) mg via the 128 mg q4w drug product based on body surface area. However, the data above suggests a potential adverse effect of NMP on the MD pup survival as well even if not statistically significant. The LD of 50 mg/kg provides a 4.3x exposure margin based on body surface area. The study appears to be consistent with typical multigenerational study protocols from the time period and the study was

conducted after GLPs were instituted. Although there is a GLP statement, the statement is not signed, and the report is clearly marked as "Draft Not Subjected to QA Audit." There were no assessments of sperm counts and sperm morphology. There were no specific evaluation of bone malformations using specialized staining. Further, it is worth noting that the dietary route of administration would be expected to result in lower C_{max} values than the SC route. As such, this is not a complete fertility and early embryonic development study by current standards or an ideal embryo-fetal development (EFD) study based on current models (See Exxon, 1992 for EFD data).

Male Fertility

Solomon et al. evaluated male fertility as part of a two-generation study in rat. Males were exposed to whole body inhalation of 10, 51, or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day, 7 day/week from age 34 days to end of mating period (100 days total) (Solomon et al., 1995). No effects on male fertility were observed but limited endpoints were assessed. Male mating and fertility indices were assessed and both were based only on reproductive performance (i.e., number of rats copulating or litters sired). Testes were weighed and reproductive organs were examined for gross lesions. No evaluation of spermatogenesis or histopathology of reproductive organs was performed. The NOEL for male reproductive performance is the highest dose tested, 68 mg/kg, which is 5.9 times the maximum daily dose of NMP of (b) (4) mg via the 128 mg q4w drug product based on body surface area (BSA).

Sitarek and Stetkiewicz administered NMP to male rats orally at doses of 100, 300 and 1000 mg/kg for 10 weeks prior to mating (Sitarek and Stetkiewicz, 2008). Males were mated with untreated females then necropsied and epididymis and testis were fixed for histopathological evaluation. The individual stages of spermatogenesis were assessed. Viability and development of offspring were observed up to 28 days. The doses of 100 and 300 mg/kg did not significantly alter fertility or spermatogenesis. However, significantly lower viability of the offspring in the first four days of life was seen at 300 mg/kg and above. Infertility and extensive damage to the seminiferous epithelium and the seminal tubule of the testis were observed at 1000 mg/kg (86x the MDD based on BSA). The NOEL for male fertility is 300 mg/kg (26-fold the human dose based on BSA) although the NOEL for paternal effects of prenatal development of the progeny is 100 mg/kg which is 8.6 times the HDD based on BSA. This study appears to contain all the primary endpoints of a male fertility study. Exposure data are not available and oral dosing is expected to result in lower bioavailability compared to SC administration as such, the safety margin of 8.6 likely is an overestimate. The apparent male-mediated adverse impact on subsequent offspring are not endpoints tested in standard fertility studies nor are they addressed via pre- and postnatal development (PPD) studies because PPD study designs do not include paternal dosing. In terms of labeling, Section 13.1 and 8.1 could state:

Section 13.1

Adverse effects on testes and male fertility were noted in a published study in which rats were treated for 10 weeks with daily oral doses of NMP, an excipient in BRANDNAME ONCE MONTHLY at 86 times the MDD and resulted in male-mediated adverse effects on offspring (decreased pup weight and survival) at daily doses 26 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. No adverse effects were noted at oral doses 8.6 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY.

Section 8.1

Male-mediated adverse effects on offspring (decreased pup weight and survival) were noted in a published study in which male rats were treated orally for 10 weeks with NMP, an excipient in BRANDNAME ONCE MONTHLY at daily doses 26 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. No adverse effects were noted at oral doses 8.6 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY.

The proposed above statements do not include information from the Exxon 1991 study as the results of that study are difficult to interpret given the route of administration and uncertainty of the actual doses administered.

In the second cycle, the Applicant completed an oral rat PK study to estimate exposures via the oral route (Study PK-18-611); which was summarized in the ADME section of this review. The estimated exposure margins compared to the estimated human AUC are depicted below.

Table 52. Estimated Exposure Margins for Oral Rat Fertility and Early Embryonic Development Data

Rat Dose (mg/kg)	Rat AUC _(0-last) (mcg•h/mL)	Human AUC _(0-last) (mcg•h/mL)	Exposure Margin
Males			
100	716	6.61	108
300	3070	6.61	464
1000	10400	6.61	1573
Females			
125	1050	6.61	159
250	2570	6.61	389
500	7010	6.61	1061

Taking into consideration the AUC data, the originally proposed labeling would then read as follows:

In terms of labeling, the previously proposed Section 13.1 and 8.1 would then state:

Section 13.1

Adverse effects on testes and male fertility were noted in a published study in which rats were treated for 10 weeks with daily oral doses of NMP, an excipient in BRANDNAME ONCE MONTHLY at 1573 times the MDD and resulted in male-mediated adverse effects on offspring (decreased pup weight and survival) at daily doses 464 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. No adverse effects were noted at oral doses 108 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY.

Section 8.1

Male-mediated adverse effects on offspring (decreased pup weight and survival) were noted in a published study in which male rats were treated orally for 10 weeks with NMP, an excipient in BRANDNAME ONCE MONTHLY at daily doses 464 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. No adverse effects were noted at oral doses 108 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY.

However, given these exposure margins, there is no need to include these data in the labeling.

Female Fertility and Early Embryonic Development

Solomon et al. evaluated female fertility as part of their two-generation study in rat (Solomon et al., 1995). Females were exposed to whole body inhalation of 10, 51 or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to weaning with interruption between GD 20 to Day 4 post-partum (134 days total). Treated males were mated with treated females. Female mating and fertility indices were assessed, and both were based only on reproductive performance (i.e., number of rats copulating or bearing litters). The uterus was weighed, and reproductive organs were examined for gross lesions. Live and dead fetuses, resorptions, corpora lutea, and fetal weights were evaluated. The female fertility parameters that were evaluated appeared to be adequate in this study and no NMP-related effects were observed. The NOEL for female fertility is the highest dose tested, 68 mg/kg, which is 5.9-fold the human dose of (b) (4) mg NMP via the 128 mg q4w drug product based on BSA. PK data were not provided and there are no whole-body inhalation data to permit clear extrapolation of exposures from this study. If one were to assume the nose only inhalation single-dose data were adequate to estimate exposures and using the equation generated by (b) (4) based on the Poet et al (2016) PBPK model, the exposure of 116 ppm would correlate with an AUC of 642.2 mcg•h/mL which would be 97x the human AUC of 6.61).

Sitarek et al. treated female rats with oral NMP 150, 450, or 1000 mg/kg for 5 days/week two weeks before mating, during mating, and through gestation and lactation (Sitarek et al., 2012). The fertility index was significantly lower at 450 and 1000 mg/kg (38.7 and 86 times the MDD based on BSA). No standard early embryonic endpoints were examined (only fertility index = percentage of pregnant females in mating female

group). However, examination of the HD females that did not get pregnant revealed evidence of early resorptions indicating intrauterine mortality of the embryos. The NOEL for fertility in this study is 150 mg/kg and is 12.9-fold the human dose of (b) (4) mg NMP based on BSA. The authors note that the 150 mg/kg dose “slightly toxic” (body weight changes) to the dams. It should be noted that although there was no effect on female fertility parameters at 150 mg/kg, pup survival was decreased at this dose. Both the index of viability (% of pups born alive that survived to 4 days) and index of lactation (% of pups alive at 4 days that survived to 21 days) were significantly lower than control for all treated groups, as noted in the table below from the publication.

Table 53. Effect of NMP on Reproductive Performance in Female Rats and their Progeny (Sitarek et al., 2012)

	Effect of <i>N</i> -Methyl-2-Pyrrolidone on the Reproductive Performance of Female Rats and Viability of Their Progeny			
	Daily dose of NMP (mg/kg)			
	0	150	450	1000
Number of				
Mating females with males	24	26	28	22
Pregnant females	22	24	20	15
Died females*	0	0	0	2
Number of				
Live pups per litter	11.5 ± 3.5 ^a	10.4 ± 2.6	10.5 ± 3.4	0.33 ± 0.82 ^b
Dead pups per litter	0.18 ± 0.85	0	0.13 ± 0.34	0.80 ± 1.1 ^b
Sex ratio (F : M)	132 : 125	112:137	105:107	5:3
Indices				
Fertility	91.7	92.3	71.4 ^b	68.2 ^b
Viability	94.0	86.4 ^b	71.6 ^b	0
Lactation	96.1	78.2 ^b	43.4 ^b	0
Body weight gain of mothers from 0 to 20 GD (percentage of control)	100	87.7	75.6	40.8

*Two nonpregnant females died in the 30th and in the 32nd day of experiment, respectively.

^aMean ± SD.

^bSignificantly different ($p < 0.05$) from control value.

F, female; M, male; GD, gestation day.

Index of fertility—percentage of pregnant females in mating females group.

Index of viability—percentage of pups born alive that survived to 4 days.

Index of lactation—percentage of pups alive at 4 days that survived to 21 days.

The NOEL for female fertility is 150 mg/kg which is 12.9-fold the human dose of (b) (4) mg based on BSA. No NOAEL for pup effects can be reported (<12.9x the MDD). Given the limited endpoints, this study is technically not an adequate full assessment of early embryonic development. Given the lack of exposure data, the lack of adequate early embryonic development endpoints in these studies, and the likelihood that oral bioavailability is not 100%, a definitive SC EFD study should be completed.

In terms of labeling, for an impact on offspring, labeling (Section 8.1) could state (based on Sitarek et al., 2012):

Section 8.1

Decreased pup survival was noted following oral treatment of female rats prior to and during gestation with NMP, an excipient in BRANDNAME ONCE MONTHLY at doses 12.9 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. A NOAEL was not demonstrated.

Taken together, the literature reports provide an adequate assessment of the effects on male fertility for NMP and limited data on female fertility and early embryonic development. Definitive TK data are lacking for the inhalational studies. However, adverse effects in the offspring of both treated males and females are observed at doses of NMP that have no effect on fertility index. The key results of these studies are summarized below. Estimated AUC based exposure margins are included from the second cycle data submitted.

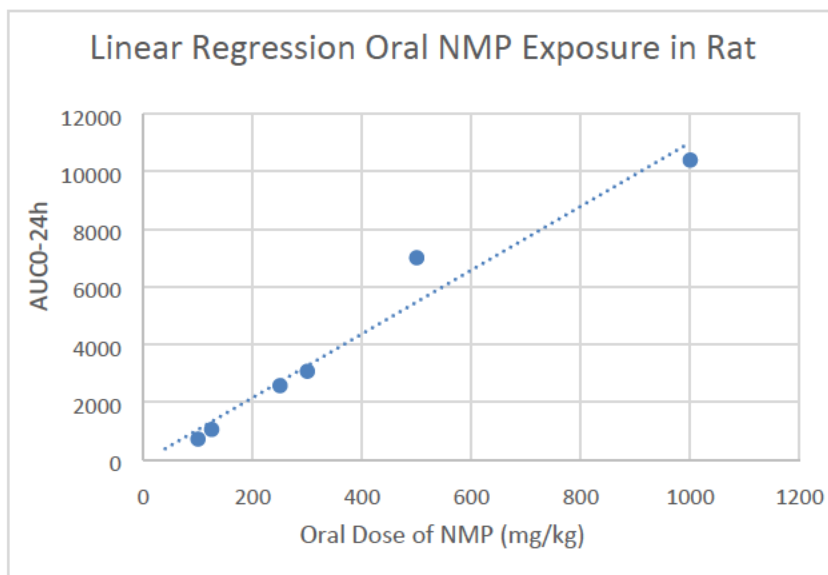
Table 54. Summary Table of Effects of NMP on Male and Female Fertility and Early Embryonic Development

Effect Reported	Species, Route	Treatment Period	Dose	Estimated Exposure Margin Based on BSA	Exposure Margin Based on Estimated AUC	Citation	Comment on Adequacy
Reduced Fertility Index, Reduced pup survival	Rat, Dietary both males and females treated	Treated 10 weeks prior to mating and through gestation	500 mg/kg	43x	1060x	(Exxon, 1991)	No sperm morphology to counts in males Draft unaudited report
			Reduced pup survival 160 mg/kg	13.8x	259x		
			50 mg/kg NOAEL	4.3x	74x		
Female Fertility							
Fertility decreased at 450 and 1000 mg/kg	Rat, oral	F: 5 days/wk 2 wks prior to mating, during mating, gestation and lactation	NOEL F fert: 150 mg/kg (12.9x)	38.7x	242x	(Sitarek et al., 2012)	No early embryonic development endpoints, only a fertility index determined.
~5% decrease in fetal weight in F1 offspring when dams along treated with HD of 68 mg/kg	Rat, whole body inhalation	M: 100 days total (from age 34 days to end of mating period) F: 134 days total (from age 34 d days to weaning with	68 mg/kg NOAEL = 30 mg/kg (2.6x)	5.9x 2.6x	56x 24x	(Solomon et al., 1995)	No clear adverse effect on female fertility. Males and females both dosed via whole body inhalation, so hard to interpret NOAEL for female fertility and early embryonic

		interruption between GD 20- Day 4 post-partum)					effects.
Male Fertility							
Male infertility and damage to seminiferous epithelium and tubules (at 1000 mg/kg)	Rat, oral	Treated from age 7 weeks 5 days/wk for 10 weeks and for 1 wk during mating, mated with untreated F	NOEL for M fertility: 300 mg/kg	26x	464x	(Sitarek and Stetkiewicz, 2008)	Endpoints are acceptable to assess male fertility
Survival rate of pups lower than controls at 300 mg/kg treated M untreated F			NOEL for effects on offspring of treated M: 100 mg/kg	8.6x	108x		Endpoint not examined in typical studies but suggests male mediated adverse effects on offspring.

The above exposure margins were estimated from the collective rat PK data using linear regression as follows:

Dose	AUC	Estimated value
40		380
100	716	
125	1050	
250	2570	
300	3070	
500	7010	
1000	10400	
400		4367
150		1598.5
160		1709
50		491
68		690



If the AUC estimates are correct, the revised proposed labeling would read:

In terms of labeling, for an impact on offspring, labeling (Section 8.1) could state (based on Sitarek et al., 2012):

Section 8.1

Decreased pup survival was noted following oral treatment of female rats prior to and during gestation with NMP, an excipient in BRANDNAME ONCE MONTHLY at doses 242 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. A NOAEL was not demonstrated.

No NOAEL was demonstrated in the study by Sitarek. Given the revised exposures margins, the findings were noted at a very high exposure margin. As such, the utility for labeling is questionable. Nonetheless, until a NOAEL is defined, the findings should be included in labeling and a definitive pre- and post-natal study should be completed as a PMR.

Embryofetal Development (EFD)

The Applicant's submitted a literature search as well as obtaining several unpublished studies to address the impact of NMP on embryofetal development. As per ICH S5(R2), embryo-fetal development studies are intended to detect adverse effects on the pregnant female and development of the embryo and fetus consequent to test article exposure of the female implantation to closure of the hard palate. The studies should characterize the toxicity profile of the test article compared to nonpregnant females as well as assess embryofetal death, altered growth and structural changes (including soft tissue and skeletal examination). The acceptable dosing regimen is to dose pregnant females from implantation to the closure of the hard palate (GD 6 to 18 for mice and rats; GD 6-20 for rabbits) and then sacrifice females one day prior to parturition (GD 18 mice; GD 20-21 rats; GD 29 rabbits). Fetuses should be examined for soft tissue and skeletal changes. At terminal examination, the following endpoints are recorded: macroscopic examination of all adults (histological evaluation), corpora lutea number, number of live and dead implantations, individual fetal body weight, fetal abnormalities, and gross evaluation of placenta.

Embryofetal Development in Rat

Reports from the literature in rat are available for dermal, oral, and inhalation routes of administration. It has been shown that NMP causes embryo- and fetotoxicity as well as malformations. NMP also causes maternal toxicity. Based on body surface area comparisons, the data in the literature are inconsistent with regard to the dose at which various effects are observed. These inconsistencies are most likely due to differential exposure via the routes of administration tested and the lack of toxicokinetic data needed to more accurately compare exposure.

Becci et al. examined the teratogenic potential of NMP in rats following dermal administration of 75, 237, or 750 mg/kg NMP from GD 6-15 (6.5, 20.4, or 64.6x the

MDD) (Becci et al., 1982). Maternal toxicity was noted at 750 mg/kg as decreased body weight gain. Reduced number of viable fetuses, increased resorptions, and skeletal abnormalities (missing sternabra extra or fused ribs, incomplete ossification of vertebra, incomplete closing of skull, fused atlas) were noted at 750 mg/kg group. There was no evidence of teratogenicity or maternal toxicity noted in the 75 or 237 mg/kg groups. The NOAEL for EFD provides a safety margin of 20.4x based on BSA. No PK data exist to estimate exposure based on AUC.

Lee et al. treated pregnant rats via inhalation with 0.1 or 0.36 mg/L (15 or 52 mg/kg) for 6 hours per day from GD 6-15 (Lee et al., 1987). Evidence of maternal toxicity was limited to sporadic lethargy and irregular respiration. Evaluation of the fetuses at GD 15 revealed no evidence of teratogenicity or other adverse fetal effects. The HD was the NOAEL for EFD and predicts an exposure margin of 4.5x based on BSA (0.36 mg/L = 0.36 ppm; does not fit PBPK model as AUC estimated to be -8).

Study Title: Developmental Toxicity Study in Rat with N-Methylpyrrolidone (Exxon, 1992; Project Number: 236534)

Exxon Biomedical Sciences (1992) conducted an embryofetal development study in which pregnant female rats were administered NMP via oral gavage at doses of 40, 125, and 400 mg/kg/day from Gestation Day 6 to 15. On Gestation Day 21, animals were euthanized and evaluated via standard EFD study endpoints including visceral and bone malformations of the fetuses for potential teratogenicity. There was no clear evidence of maternal toxicity at any dose although the HD group did have reduced uterine weights which indirectly impacted overall body weight gain in that group. Fetal body weights were reduced in HD animals by approximately 10-11% compared to controls. MD body weights were reduced by 3%. There was also an increase in the number of "stunted fetuses" in the HD group compared to controls and reduced ossification in the hindlimb proximal phalanges in the HD group as well. The NOAEL for maternal and fetal effects was reported to be the MD of 125 mg/kg (750 mg/m²) which is 10.8 times maximum daily dose of NMP of ^{(b) (4)} mg via the 128 mg q4w drug product based on BSA. A conservative evaluation given the slight decrease in fetal body weights in the MD would declare the LD the NOAEL which would result in a safety margin of 3.4x based on BSA (159x based on AUC).

Parameter	0 mg/kg	40 mg/kg	125 mg/kg	400 mg/kg
Uterine weights		-3.9%	-1.7%	-10.9%*
BW Change GD 6-9	14 g	13 g	14.4 g	7.9 g*
BW Change GD 9-12	18.4 g	20.5 g	18.8 g	15.2 g*
Mean Fetal Body Weights: Males	5.46	5.35 (-2%)	5.32 (-2.6%)	4.90* (10%)
Mean Fetal Body Weights: Females	5.19	5.13 (-1%)	5.01* (-3.5%)	4.63* (-11%)
Stunted Fetuses	1/340	1/393	2/395	12/397*

	1/21 litters	1/25 litters	2/24 litters	6/25 litters*
Sternebrae: Asymmetric	7	6	11	11
Sternebrae Unossified	0	1	3	4
Ossification Sites, Distal Phalanges	4.1	4.0	4.0	3.7*

* statistically significant

The study appears to be consistent with current protocols and was conducted after GLPs were instituted; however, although there is a GLP statement, the statement is not signed, the report is not QA'ed and is clearly marked as "Draft Not Subjected to QA Audit." As such, it cannot be considered an adequate study to support approval of the drug product.

Hass et al. treated rats 0 or 165 ppm NMP via whole body inhalation on GD 4-20 (Hass et al., 1995) for 6 hours per day. Increased pre-implantation loss, delayed ossification of the skeleton and decreased fetal body weight was observed in the absence of maternal toxicity. No NOAEL can be established in this study. The dose of 165 ppm (97 mg/kg) is approximately 8.4 times the human dose of (b) (4) mg NMP based on BSA. As this study did dose slightly earlier than a standard EFD study, implantation sites were assessed which could be used to inform embryonic development endpoints. Assuming the PBPK model is reasonably accurate, the exposure to 165 ppm would correlate with an AUC of 918 mcg•h/mL (139x the estimated human AUC).

Solomon et al. evaluated embryofetal development as part of a two-generation study in rat (Solomon et al., 1995). Females were exposed to whole body inhalation of 10, 51 or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to weaning with interruption between GD 20 to Day 4 post-partum (134 days total). Males were exposed to whole body inhalation of 10, 51, or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to end of mating period (100 days total). Treated males were mated with treated females. Gestation and viability indices were similar between control and exposed groups. No malformations or variations were attributed to exposure of NMP. The highest dose provides an exposure margin of 5.9x for the proposed human dose based on BSA. Assuming the PBPK model is reasonably accurate, the exposure to 116 ppm NMP would correlate with an AUC of 642 mcg•h/mL (97x the estimated human AUC). The only finding was reduction of body weight of F1 offspring when both parents were exposed to 116 ppm NMP. Therefore, the NOEL for embryofetal development is the mid dose, 51 ppm (30 mg/kg), which is 2.9x the human dose of (b) (4) mg based on BSA (42x based on PBPK model).

Saillenfait et al. orally administered 125, 250, 500, or 750 mg/kg NMP to pregnant rats from GD 6-20 (Saillenfait et al., 2002). Maternal toxicity (decreased body weight gain and food consumption) was evident at 500 and 750 mg/kg. NMP increased resorptions at these same doses. Fetal malformations (imperforate anus, absence of tail, anasarca, malformations of the great vessels and cervical arches) were noted at 500 and 750 as

well and one fetus in the 250 mg/kg group showed similar evidence of teratogenicity. Decreased fetal weights were evident at 250 mg/kg and above. The maternal NOAEL was 250 mg/kg (21.5x the MDD based on BSA; 388x based on estimated AUC); however the developmental NOAEL was 125 mg/kg (10.8x the human dose of (b) (4) mg based on BSA; 159x based on estimated AUC).

Saillenfait et al. exposed pregnant rats to NMP via whole body inhalation at 0, 30, 60 and 120 ppm (18, 35, or 71 mg/kg) 6 h/day from GD 6-20 (Saillenfait et al., 2003). Maternal toxicity was evident as transient body weight gain decreases at 60 and 120 ppm. Reduced fetal body weights were observed at 120 ppm. No effects were noted on the number of corpora lutea, implantation sites, dead or live fetuses. No NMP-related visceral or skeletal variations or malformations were observed. The NOEL for EFD effects is 60 ppm (35 mg/kg) is 3-fold the human dose of (b) (4) mg based on BSA (50x based on estimated AUC). Transient maternal toxicity was observed at this dose as a 17% decrease in body weight GD 6-13.

Table 55. NOAEL and Adverse Effects in Published Rat EFD Studies

Study	Route	NOAEL for EFD	Exposure Margin NOAEL (BSA)	Dose for AEs	Adverse Fetal Effects	Exposure Margin AE (based on BSA)	Exposure Margin AE based on AUC*	Comment on Adequacy
(Becci et al., 1982)	Dermal	237 mg/kg	20.4x	750 mg/kg	Fetal lethality Increased resorptions Skeletal malformations	64.6x	Unknown	No exposure data No maternal toxicity
(Lee et al., 1987)	Inhalation	52 mg/kg	4.5x		None	4.5x	Does not fit model based on equation proposed by (b) (4)	No maternal toxicity No exposure data
Exxon, 1992	Oral	40 mg/kg	3.4x	125 mg/kg 400 mg/kg	Reduced ossification and Decreased Fetal body weight	10.8x	159x	Unaudited draft report No exposure data No maternal toxicity
(Solomon et al., 1995)	Inhalation	30 mg/kg	2.6		No malformations. Reduced body weight of F1 offspring at HD of 68 mg	2.6x 5.9x	42x 97x	Inadequate dosing regimen to provide coverage for clinical. Modeling only for PK data
(Hass et al., 1995)	Inhalation	<97 mg/kg	<8.4x	97 mg/kg	Preimplantation loss Delayed ossification Decreased fetal body weight	8.4x	139x	Single dose only. No exposure data in study. No NOAEL

(Saillenfait et al., 2002)	Oral	125 mg/kg	10.8x BSA (159x based on AUC)	250 mg/kg	Increased resorptions Fetal malformations	21.5x	388x	No maternal toxicity. No exposure data SM could be lower with SC
(Saillenfait et al., 2003)	Inhalation	35 mg/kg	3x		None	3x	50x	Maternal toxicity noted at this dose, unclear significance

*AUC based on linear equation predicted by (b) (4)

Collectively, the potential embryofetal effects in rat with NMP has been tested in rats via oral, dermal, and inhalation routes of administration. No embryo-fetal development studies with the SC route have been completed and there were no exposure data available in the original submission. Based on body surface area, the existing data suggest adverse effects on fetal development at exposures that 8.4 to 64.5 times the MDD of NMP via BRANDNAME ONCE MONTHLY. As these adverse effects appear to result in exposure margins that are smaller than the exposure margins for buprenorphine-related adverse effects as per the referenced data, the findings were recommended to be included in the product labeling. Recommended language for the label was as follows:

Preimplantation losses, delayed ossification and reduced fetal body weights were reported in published studies following treatment of pregnant rats during organogenesis with NMP, an excipient in BRANDNAME ONCE MONTHLY, via inhalation at 8.4 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY. Reduced ossification and decreased fetal body weights at 10.8 times the MDD of NMP and fetal malformations and resorptions have also been reported following oral administration of 21.5 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY at the MDD based on a body surface area comparison.

In the original submission, it was not clear how the inhalation and oral routes of administration compare the proposed SC route of administration. As oral delivery is not likely 100% bioavailable and malformations may have a smaller safety margin than predicted by these data, a definitive SC EFD study in the rat was recommended to be completed. As reviewed in the ADME section, in the second cycle, the Applicant completed an oral rat PK study to estimate exposures via the oral route (Study PK-18-611) and attempted to estimate inhalation exposure using a PBPK model. Given the recently submitted oral PK bridging data in the rat and estimated inhalation data, the above proposed labeling would read as follows:

Preimplantation losses, delayed ossification and reduced fetal body weights were reported in published studies following treatment of pregnant rats during

organogenesis with NMP, an excipient in BRANDNAME ONCE MONTHLY, via inhalation at 139 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY. Reduced ossification and decreased fetal body weights at 159 times the MDD of NMP and fetal malformations and resorptions have also been reported following oral administration of 388 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY at the MDD based on a body surface area comparison.

Based on these estimated exposure margins, the data do not appear to be necessary for labeling. Although the cited studies are technically not GLP, collectively they provide replicative data to suggest that the adverse effects of NMP occur at very high exposures compared to the levels that appear to occur with the CAM2038 q4w drug product. No further rat EFD studies are necessary given these data.

Embryofetal Development in Rabbit

The Applicant submitted a 1991 study report by the International Research and Development Corporation¹ of a rabbit study which tested 0, 55, 175, and 540 mg/kg oral NMP daily from GD 6-18. Maternal toxicity (statistically significant decreases in body weights, reduced food consumption) was observed at 175 and 540 mg/kg. Post-implantation loss, increased incidence of cardiovascular malformations and skull malformations were observed at 540 mg/kg (93x the MDD based on body surface area). The NOEL for developmental toxicity in this study is 175 mg/kg which is 30-fold the human dose of (b) (4) mg based on BSA. Maternal toxicity was present at this dose. The study was conducted in accordance with GLPs and appeared to evaluate the appropriate embryofetal development endpoints and the line listings of the data were provided. However, it is not clear how the oral route compares to the SC route. As such, the above exposures margins provided are only estimates for the proposed drug product.

In the second cycle submission, as noted above, the Applicant submitted an oral PK study in pregnant rabbits (Study PK-18-610). Based on these data, the revised estimated exposure margins based on AUC are depicted below.

Table 56. Exposure Margins for Rabbit NMP EFD Study Based on AUC Data

Rabbit Dose (mg/kg)	Rabbit AUC_(0-last) (mcg•h/mL)	Human AUC_(0-last) (mcg•h/mL)	Exposure Margin
55	124	6.61	19
175	775	6.61	117
540	3860	6.61	584

In terms of the impact on labeling, suggesting language is as follows:

¹ International Research and Development Corporation (IRDC). 1991. NMP (N-methyl-2-pyrrolidone): Developmental Toxicity Study in New Zealand White Rabbits.

Post-implantation loss and increased cardiovascular and skull malformations were demonstrated in pregnant rabbits administered oral NMP, an excipient in BRANDNAME ONCE MONTHLY, at doses 584 times the human MDD of NMP via BRANDNAME ONCE MONTHLY in the absence of maternal toxicity. No adverse effects were reported at an oral dose 117 times to the MDD via BRANDNAME ONCE MONTHLY based on a body surface area comparison.

However, given the large exposure margins, these data need not be included in labeling. A new rabbit EFD study need not be completed to further qualify the safety of NMP given the adequate bridging oral PK data.

Embryofetal Development in the Mouse

Although not specifically identified or discussed by the Applicant, Becci et al cite a report from Schmidt in 1976² that apparently tested IP NMP in the mouse model (Becci et al., 1982). According to Becci:

Schmitt (1976) found that N-methylpyrrolidone caused dose-dependent embryotoxic and teratogenic effects in AJ JENA and C57BL mice when given in single or repeated i.p. doses on various days of gestation. The most pronounced embryotoxic effect of N-methylpyrrolidone was noted after a single i.p. administration of 165 mg/kg was given on the 7th day postconception. Twenty-three percent of all implanted fetuses died. The same dose level of N-methylpyrrolidone given on the 9th day caused the highest rate of fetal malformations, 18.6%.

A dose of 165 mg/kg in the mouse is approximately 7x the MDD in humans via BRANDNAME ONCE MONTHLY based on body surface area.

The Schmidt data were also summarized by the WHO as follows (World Health Organization, 2001):

NMP doses of 14–166 mg/kg body weight singly or repeatedly intraperitoneally administered to mice during various phases of pregnancy caused increased post-implantation loss and a reduced body weight of the fetuses. Morphological defects such as exencephaly, open eyelids, microphthalmia, cleft palate, oligodactyly, shortened or kinked tails, fusions and curvature of neck and chest vertebrae, and fusion of sternbrae and ribs were observed. The LOAEL for repeated doses was 74 mg/kg body weight administered on days 7–11 of gestation. No information on maternal toxicity is given in this study; thus, evaluation of the results is difficult (Schmidt, 1976).

² Schmidt, R. (1976). Tierexperimentelle Untersuchungen zur embryotoxischen und teratogenen Wirkung von N-Methyl-Pyrrolidon (NMP). Biol. Rundsch. 14:38-41.

The LOAEL dose of 74 mg/kg in the mouse is 3x the MDD in humans via BRANDNAME ONCE MONTHLY based on body surface area.

The Applicant submitted summaries of reproductive and developmental studies in mice conducted by BASF. These studies reportedly tested either 1/5th the LD₅₀ or 1/2 the LD₅₀ and employed the IP and PO route. The studies suggest significant adverse effects; however, the study summaries lacked adequate detail to inform risk and labeling for this product.

Study Title: Report on Testing of N-Methylpyrrolidone for Possible Mouse Teratogenicity (BASF 1970a; Study XIX/422)

NMP was administered to pregnant NMRI mice once daily on Gestation Day 11 to 15 at a dose that was reportedly one half the LD₅₀ via the IP and PO routes of administration. Doses were expressed as mm³/kg (= mL/kg). The high dose tested (1/2 LD₅₀ = 1525 mL/kg or 1570 mg/kg via the IP route and 2565 mL/kg or 2641 mg/kg via the PO route, were reported to cause an increase in cleft palate. At the low dose (1/5 the LD₅₀ = 610 mL/kg IP or 628 mg/kg and 1026 mL/kg PO or 1057 mg/kg), NMP was report to have no effect on resorptions, incidence of runts, or fetal weights. To covert mL/kg to mg/kg we used the density of NMP of 1.03 grams/mL. The NOAEL in this study for the IP injection was 45.5 times the human dose of (b) (4) mg NMP via this product based on body surface area. The conclusions of the study are limited in that the full period of organogenesis was not examined and given the sparsity of the details provided in these summary documents.

Study Title: Report on Testing of Pyrrolidone for any Teratogenic Effect in the Rat after Oral Administration (BASF 1970b; Study XIX/421)

NMP was administered NMP orally to pregnant Sprague-Dawley rat once daily on Gestation Day 6 to 15 at a dose 1700 mm³/kg (= mL/kg). This dose was presumably 1/5th the LD₅₀ in this species. The dose tested equals 1751 mg/kg. To covert mL/kg to mg/kg we used the density of NMP of 1.03 grams/mL. There was no maternal toxicity reported. There were no reported changes in percentages of resorptions compared to the control group. There were no effects on fetal weights or evidence of adverse effects on the fetus. The NOAEL in this study was 150 times the human dose of (b) (4) mg NMP via this product based on body surface area. The conclusions of the study are limited given the sparsity of the data presented in the summary document provided.

Peri- and Post-Natal Development (PPND)

As per ICH S5, PPND studies are intended to characterize the effects of a drug on the pregnant/lactating female and on development of the conceptus and the offspring after

exposure of the female from implantation through weaning. Endpoints in the maternal animals should include clinical signs and mortalities, food intake and body weight changes, duration of pregnancy, and partition. Endpoints in the offspring include macroscopic examinations at necropsy, abnormalities, number of live/dead offspring, body weight at birth, pre- and post-weaning survival and growth, maturation and fertility of the offspring, physical development, sensory function and reflexes, and behavior. Specific functional testing methods is not outlined by ICH S5; however, studies that assess sensory function, motor activity, and learning and memory are recommended.

Hass et al. treated pregnant rats via whole body inhalation to 0 or 150 ppm (88 mg/kg = 7.6x the MDD based on BSA of NMP via BRANDNAME ONCE MONTHLY) NMP for 6 h/day on GD 7-20 (Hass et al., 1994). The authors state that this dose level was chosen so as not to cause maternal toxicity or decrease viability of the offspring. Although the study did not include exposures during lactation, the offspring were allowed to be born and were evaluated via functional assessments, similar to a PPND study. Exposed offspring showed decreases in body weight and had delayed physical development. Performance in tasks including the reversal procedure (retesting with a new platform location) in the Morris Water Maze and operant delayed spatial alteration (Skinner boxes) was impaired in exposed offspring. No differences between NMP-exposed animals and control animals were seen in motor function (rotarod), activity level (open field) and learning tasks of low complexity. No NOAEL can be established for PPD parameters and the exposure at the dose employed (88 mg/kg) is approximately 7.6 times the dose of (b) (4) mg NMP in human based on BSA). Assuming the PBPK modeling is reasonably accurate, the exposure to 150 ppm for 6 hours per day is estimated to result in an AUC of 833 mcg•h/mL which is 126x the MDD of NMP via CAM2038 q4w.

Sitarek et al. treated female rats with oral NMP 150, 450, or 1000 mg/kg for 5 days/week two weeks before mating, during mating, and through gestation and lactation (Sitarek et al., 2012). The authors considered the 150 mg/kg dose “slightly toxic” (body weight changes) to the dams. The incidence of stillbirths was increased at 1000 mg/kg. At 150 mg/kg there was no effect on fertility, but pup survival was decreased. As previously noted in the fertility section of this appendix, both the index of viability (% of pups born alive that survived to 4 days) and index of lactation (% of pups alive at 4 days that survived to 21 days) were significantly lower than control for all groups, as illustrated in the table below again reproduced from the publication.

Effect of *N*-Methyl-2-Pyrrolidone on the Reproductive Performance of Female Rats and Viability of Their Progeny

	Daily dose of NMP (mg/kg)			
	0	150	450	1000
Number of				
Mating females with males	24	26	28	22
Pregnant females	22	24	20	15
Died females*	0	0	0	2
Number of				
Live pups per litter	11.5 ± 3.5 ^a	10.4 ± 2.6	10.5 ± 3.4	0.33 ± 0.82 ^b
Dead pups per litter	0.18 ± 0.85	0	0.13 ± 0.34	0.80 ± 1.1 ^b
Sex ratio (F : M)	132 : 125	112:137	105:107	5:3
Indices				
Fertility	91.7	92.3	71.4 ^b	68.2 ^b
Viability	94.0	86.4 ^b	71.6 ^b	0
Lactation	96.1	78.2 ^b	43.4 ^b	0
Body weight gain of mothers from 0 to 20 GD (percentage of control)	100	87.7	75.6	40.8

*Two nonpregnant females died in the 30th and in the 32nd day of experiment, respectively.

^aMean ± SD.

^bSignificantly different ($p < 0.05$) from control value.

F, female; M, male; GD, gestation day.

Index of fertility—percentage of pregnant females in mating females group.

Index of viability—percentage of pups born alive that survived to 4 days.

Index of lactation—percentage of pups alive at 4 days that survived to 21 days.

In terms of typical PPNP endpoints the offspring were only evaluated for general appearance, litter weight, mean pup weight, and mortality from birth to PND 21. As such, there are limited PPNP endpoints in this report. No NOAEL was established for this study for these PPNP endpoints. The low dose of 150 mg/kg is 12.9-fold the human dose of (b) (4) mg based on BSA (242x the AUC based on the PK bridging data). However, no NOAEL was noted in this study.

In terms of labeling, based on these two published studies, the following was originally recommended to be included in Section 8.1:

Decreased pup survival was noted following oral treatment of pregnant rats prior to and during gestation and lactation with NMP, an excipient in BRANDNAME ONCE MONTHLY at doses 12.9 times the MDD. Developmental delays and impaired cognitive function were reported in pups born to pregnant rats treated with NMP via inhalation during gestation at doses 7.6 times the MDD of NMP via BRANDNAME ONCE MONTHLY based on a body surface area comparison.

In the original submission, the reports reviewed in the literature did not provide adequate exposure margins or complete methodology to accurately assess effects of NMP on PPNP endpoints. The submitted oral PK bridging data assist in the interpretation of the published studies and suggest a lower level or risk than when BSA was used. Based on the PK data we now have, the above language would read:

Decreased pup survival was noted following oral treatment of pregnant rats prior to and during gestation and lactation with NMP, an excipient in BRANDNAME ONCE MONTHLY at doses 242 times the MDD. Developmental delays and impaired cognitive function were reported in pups born to pregnant rats treated

with NMP via inhalation during gestation at doses 126 times the MDD of NMP via BRANDNAME ONCE MONTHLY based on a body surface area comparison. A NOAEL was not identified.

Given the lack of a NOAEL in the Sitarek study, a definitive PPND study the rat should still be completed. Given the new PK data suggesting reduced risk, the indication, and potential public health benefit of this drug product, the study can be completed as a Postmarketing Requirement (PMR) with the above labeling if the application can be approved this cycle.

Overall Reproduction and Developmental Conclusions

The published literature on NMP clearly suggest the potential for adverse effects on development at doses that are typically about 7 times higher than the levels that would occur via this drug product based on body surface area comparisons. However, the published studies are limited as they employ different routes of administration which likely have different bioavailabilities. To address this issue, the Applicant completed rat and rabbit oral PK bridging studies. These data, in general, suggest a significantly greater margin of safety than the BSA comparisons predicted. Given the new data, male fertility, rat EFD, and rabbit EFD studies need not be completed. However, a female fertility and early embryonic development study and a PPND study should be completed via the SC route of administration. Given the estimated exposure margins, these definitive studies may be completed as post marketing requirements should this application be approved this cycle.

In their second cycle submission, the Applicant acknowledges that the Agency may still require final audited reports from definitive studies as PMRs. As per the submission, they have initiated a female fertility study and a PPND study for NMP with the following timelines.

Female Fertility: Final Audited Report: Mid to Late October 2018

PPND: Final Audited Report: Late February 2019

A female fertility study has not been received as of the date of this review.

GENETIC TOXICOLOGY

The Applicant provided a literature review of published genetic toxicology studies, as summarized their table below.

Genotoxicity Assays with N-Methyl Pyrrolidone			
Test method	Test Organism/Species	Results	References
Salmonella microsome assay	TA97, TA98, TA100, TA2638 ^a , UTH8413, UTH8414	negative	Wells <i>et al.</i> 1988

	TA102, TA104 ^a	equivocal*	
	TA98, TA104 ^b	negative	
Ames Assay	TA1535, TA1537, TA 1538, TA98, TA100	Negative	(b) (4)
Aneuploidy induction	<i>Saccharomyces cerevisiae</i> Strain D61.M	positive	Mayer and Goin 1988
	<i>S. Cerevisiae</i> Strain D61.M	positive	
	<i>S. Cerevisiae</i> Strain D61.M	positive	Zimmermann <i>et al.</i> 1988
Bone marrow chromosome analysis	Chinese hamsters	negative	Engelhardt and Fleig 1993
CHO/HGPRT forward mutation assay	Chinese hamsters	negative	Hazelton Laboratories 1988a
Unscheduled DNA synthesis	Rat hepatocytes	negative	Hazelton Laboratories 1988b
Micronucleus test	NMRI mice	negative	Engelhardt and Fleig 1993

* The authors indicated that the increase was less than 2-fold over background without a clear linear dose-response relationship. No increases were observed in 6 other strains of *Salmonella typhimurium*.

a - Standard plate incorporation assay

b - Preincubation assay

NMP was tested in the Ames Assay using strains TA1535, TA1537, TA1538, TA98, TA100, TA102, and TA104 in the presence and absence of metabolic activation (Mortelmans et al., 1986, Wells et al., 1988). In strains TA102 and TA104, small increases in revertants above control were observed. However, the increases were less than two-fold above control values and therefore do not meet the criteria for a positive response per the OECD protocol. NMP was negative in the Ames Assay in all other strains tested.

NMP was found to be negative in the mouse micronucleus assay and Chinese hamster bone marrow assay (Engelhardt and Fleig, 1993).

In studies conducted by Hazelton Laboratories, NMP did not induce forward mutations *in vitro* in a CHO/HGPRT assay or in an unscheduled DNA synthesis (UDS) assay in rat primary hepatocyte cultures (HLA, 1988a; HLA, 1988b) at the limit dose of 5000 mcg/mL. In the CHO/HGPRT assay, NMP did not induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovaries (CHO) with and without metabolic activation. Of the six doses tested, only one with metabolic activation reached statistical significance for mutation but was within the background level for mutation. In the UDS assay, no significant increases in UDS were observed in hepatocytes from Fischer 344 rats. The UDS and CHO/GRPT studies were conducted according to OECD guidelines and by GLP. NMP was also negative in the Ames assay (b) (4)

NMP was found to be induce aneuploidy in yeast (Mayer et al., 1988, Zimmermann et al., 1988). As discussed in Engelhardt and Fleig (1993), a cold shock treatment condition that destroys the spindles and has questionable relevance in intact mammals was used in these studies. Further work by Basler showed that various organic

solvents that were known to be potent inducers of aneuploidy in yeast did not increase the frequency of micronucleated polychromatic erythrocytes in bone marrow cells of hamsters (Basler, 1986). Further, the weight of evidence suggests that the genotoxic potential for NMP is minimal.

CARCINOGENICITY

Two studies in rats and one in mice evaluating the carcinogenicity of NMP were identified in the literature.

Lee and colleagues exposed rats to 0, 0.04 or 0.4 mg/L (0.4 ppm) NMP for 6 h/day 5 days/week via whole body inhalation for two years (Lee et al., 1987). No treatment-related tumors were identified in either males or females. The NOEL for carcinogenic effects of NMP is the high dose, 0.4 mg/L (58 mg/kg/day) yields and exposure margin of 5-fold the human dose of (b) (4) mg based on BSA. The linear equation proposed by the (b) (4) model for this study to estimate AUC predicts -8 mcg•h/mL and therefore is not considered reliable for this study.

Malley et al. assessed the carcinogenic effect of NMP in rats and mice (Malley et al., 2001). The administration of NMP was in the diet and food consumption was measured. Although not a GLP study, an adequate assessment of organs and tissues were conducted and it appears that methods are acceptable. In rats, NMP was administered in the diet at 0, 1600, 5000, or 15000 ppm for approximately two years. No carcinogenic effects of NMP were observed at any dose. The mean daily consumption at 15000 ppm, the NOEL for oncogenic effects was 678 mg/kg in males and 939 mg/kg in females. Therefore, the exposure margins at the NOEL for oncogenic effects is 58-fold and 81-fold in males and females, respectively, as compared to the human dose of (b) (4) mg based on BSA. PK data are not available.

In mice, NMP was administered in the diet at 0, 600, 1200, or 7200 ppm for approximately 18 months. Increases in hepatocellular adenoma and carcinoma were observed in both males and females at the highest dose tested. In females, the hepatocellular carcinoma was within the historical control range. The mean daily consumption of NMP at 1200 ppm, the NOEL for oncogenic effects was 173 mg/kg in males and 221 mg/kg in females (Malley et al., 2001). Therefore, the exposure margins at the NOEL for oncogenic effects is 7.4-fold and 9.5-fold in males and females, respectively. The dose at which a statistically significant increase in hepatocellular adenomas and carcinomas were noted (7200 ppm) was estimated to result in daily exposures of 1089 mg/kg/day in male mice and 1399 mg/kg/day in female mice. Based on a body surface area basis, these doses were ~47 and 60 times the maximum human daily dose via the drug product. PK data are not available.

Table 57. Summary of Neoplastic Lesions in Male and Female Mice

Lesion	Concentration (ppm)			
	0	600	1200	7200
Male mice				
Hepatocellular adenoma	5/50	2/50	4/50	12/50*
Hepatocellular carcinoma	4/50	1/50	3/50	13/50*
Pulmonary adenoma	1/50	2/50	3/50	5/50
Pulmonary adenocarcinoma	1/50	4/50	6/50	2/50
Total animals with benign tumors	15/50	5/50	8/50	18/50
Total animals with malignant tumors	9/50	7/50	11/50	14/50
Female mice				
Hepatocellular adenoma	2/50	2/50	1/50	7/50*
Hepatocellular carcinoma	0/50	0/50	0/50	3/50*
Total animals with benign tumors	10/50	6/50	5/50	12/50
Total animals with malignant tumors	12/50	5/50	9/50	10/50

*Statistically significant differences at $p < 0.05$ by the Cochran Armitage test for trend.

Table 58. Summary of NMP-related Non-neoplastic Lesions in Male and Female Mice

Lesion	Concentrations (ppm)			
	0	600	1200	7200
Male mice				
Hepatocellular clear cell foci	0/50	1/50	1/50	6/50*
Hepatocellular basophilic foci	4/50	2/50	3/50	5/50
Hepatocellular eosinophilic foci	1/50	2/50	2/50	19/50*
Hepatocellular centrilobular hypertrophy	0/50	0/50	3/50	43/50*
Cellular alterations in liver	5/50	5/50	6/50	25/50*
Female mice				
Hepatocellular clear cell foci	0/50	0/50	0/50	1/50
Hepatocellular basophilic foci	2/50	2/50	1/50	10/50*
Hepatocellular eosinophilic foci	1/50	0/50	0/50	6/50*
Hepatocellular centrilobular hypertrophy	0/50	1/50	0/50	0/50
Cellular alterations in liver	3/50	2/50	1/50	17/50*

*Statistically significant by Cochran Armitage test for trend, $p < 0.05$.

According to Malley et al., Van Esch and Kroes included an NMP solvent control arm in a toxicology studies in Swiss mice (Van Esch and Kroes, 1972). Mice presumably received 25 mg (~25 g body weight = 1000 mg/kg or 3000 mg/m²) NMP a total of 9 times over the 18-month assay (Day 14, Month 1.5, 3, 4.5, 6, 10, 14, and 17). No increased tumors were noted; however, a saline/water control arm was also not included in this study. One incidence of hepatocellular carcinoma was noted in the control group and one in one of the test arms. Although of limited utility given the study

limitations, the dose tested was approximately 43 times the MDD and the dosing interval was punctuated, similar to that which will occur via the proposed dosing regimen.

In the original submission, the Applicant did not provide a mode of action (MOA) assessment to address the hepatic tumors in the mice. Although rodent liver tumors are frequently not believed to be relevant to humans, a MOA assessment is generally completed to put these findings into perspective (Holsapple et al., 2006). For example, a MOA assessment for phenobarbital-induced liver tumors in mice and rats has been undertaken and the data suggest that the increased cellular proliferation, decreased apoptosis, formation of altered hepatic foci, and ultimate liver tumors in rodents did not appear to translate to human risk (Elcombe et al., 2014). This conclusion was primarily driven by data that demonstrated that, unlike rodent hepatocytes, human hepatocytes did not proliferate in vitro in response to phenobarbital. That coupled with a lack of an epidemiological signal for increased tumors following extensive human exposure to phenobarbital support the conclusion that the risk of liver tumors in humans appear to be low for phenobarbital.

Like phenobarbital, repeat-dose toxicology studies with NMP demonstrate increased hepatocellular hypertrophy, suggesting that these liver tumors in mice may not correlate with increased human risk. However, as a similar MOA assessment has not been undertaken for NMP, we recommended that the product labeling include the following:

NMP, an excipient in BRANDNAME ONCE MONTHLY, produced an increase in hepatocellular adenomas and carcinomas in male and female mice at 47 and 60 times the maximum daily dose (MDD) of NMP via BRANDNAME ONCE MONTHLY. No tumors were noted at 7.4 and 9.5 times the MDD. The clinical significance of these findings is unclear. In 2-year inhalation and dietary studies in rats, NMP did not result in evidence of carcinogenicity.

Further, as this risk may not be relevant and could impact prescription practices, we recommended that, if approved in the first cycle, a post-marketing requirement be instituted to conduct a MOA for NMP induced liver tumors, which could support removal of the above labeling statement. Because the NDA was not approved in the first cycle, this was listed as a deficiency.

In the second cycle submission, the Applicant attempted to address the clinical relevance of the mouse hepatocellular adenoma and carcinoma findings. This assessment is reviewed below.

Mode of Action Assessment for NMP in Rodent Tumor Findings

The Applicant put forth a justification for the mode of action underlying the hepatocellular tumors observed in the 18-month mouse study with NMP. Based on the available information delineated below, the Applicant believes that the NMP-induced tumor response observed in the B6C3F1 mice administered NMP at the high-dose of 7200 mg/kg/day is underlied by an adaptive proliferative response of the liver that likely

occurs through a peroxisome proliferator-activated receptor-alpha (PPAR α) mechanism, which is a known pathway for nongenotoxic tumorigenesis in rodents for some compounds.

1. NMP was not genotoxic in a battery of genetic toxicology studies

As noted above, the Applicant provided published studies that demonstrated NMP was negative in a host of genetic toxicology studies, including several in vitro bacterial mutation assays, a forward mutation assay, unscheduled DNA synthesis assay, and in vivo mouse micronucleus assay and Chinese hamster bone marrow assay. Based on these data, the Applicant concluded that the mode of action was through a non-genotoxic mechanism.

2. NMP causes an adaptive response at high doses in the liver of B6C3F1 mice

The Applicant argued that the dose-dependent increases in liver weight and hepatocellular hypertrophy that were observed in the published 90-day and 18-month NMP studies in B6C3F1 mice by Malley et al., 1999 and Malley et al., 2001, respectively, could be considered adaptive changes, similar to those that have been reported to lead to tumorigenesis for other liver toxicants through non-genotoxic mechanisms (Holsapple et al., 2006, Cattley and Cullen, 2013, Vacca et al., 2013). For example, Holsapple et al (2006) discuss how an expert working group concluded that some porphyrinogenic compounds, including those that produce iron overload, have a definable MOA for rodent hepatic cancer that involves a threshold and a chronic, dose-dependent, persistent hepatocellular injury with resulting compensatory hyperplasia. Phenobarbital (PB), considered to be a prototypical rodent hepatocarcinogen, has also been characterized to have an MOA to induce tumors by a non-genotoxic mechanism and involves liver hyperplasia (Elcombe et al., 2014).

To this end, the Applicant emphasized that in an 18-month NMP feeding study (MAK Commission, 2011), males exhibited dose-dependent increases in hepatocellular hypertrophy and relative liver weights at ≥ 1200 ppm, and increased hepatocellular adenomas, carcinomas and cellular foci at 7200 ppm. In contrast, females only exhibited increases in relative liver weights at 7200 ppm with no increase in hypertrophy and only a modest increase in adenomas over historical controls and no increase in carcinomas at this dose level.

In another study, hepatocellular proliferation was measured in B6C3F1 mice fed a diet containing NMP for 1 or 4 weeks (MAK Commission, 2011). After one week of exposure, cell proliferation in the liver, measured by bromodeoxyuridine uptake, increased by 6.9-fold in male mice and 3.3-fold in female mice relative to untreated controls. Centrilobular hypertrophy of the liver was also observed. After treatment for 4 weeks, the incidence of cell proliferation was increased by 2.1-fold in male mice and by 1.7-fold in female mice. The number of apoptotic cells also increased in male mice. Minimal to slight centrilobular hypertrophy was observed with higher incidence in males

than females, and reduction in fat deposition was observed in both male and female mice.

3. NMP may work through a nuclear receptor-mediate process

The MOAs of well-characterized rodent carcinogens involve activation of specific nuclear receptors (e.g., constitutive androstane receptor (CAR), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptors (PPARs)). For example, activation of the constitutive androstane receptor (CAR) by PB is critical to produce the tumorigenic response in rodents (Elcombe et al., 2014). In addition, a MOA was developed for liver tumors produced by peroxisome proliferating chemicals, which also occur via a nongenotoxic mechanism, and it was determined that PPAR α activation is an obligatory event (Klaunig et al., 2003). Experts have concluded that the animal MOAs for PB and PPAR α are not to be likely in humans (see Discussion below).

The Applicant cited a published study (MAK Commission, 2011) where B6C3F1 mice were administered 7200 ppm NMP in the diet, which was the dose that produced tumors, for 2 weeks and pentoxoresorufin-o-deethylase (PROD) and ethoxoresorufin-o-deethylase (EROD) activity and cyanidine-sensitive palmitoyl-CoA (PALCoA) oxidation were measured, which are indicative of CAR, AhR, and PPAR activation, respectively. NMP did not induce CYP2B1 or CYP1A1, which are related to CAR and AhR activation, respectively. However, NMP treatment caused a slight increase in PALCoA, suggesting PPAR activation, in male animals relative to untreated controls. There was also a slight increase in the level of peroxisomes observed by electron microscope in 2 of 5 male animals. Based on these data, the Applicant suggested that NMP may activate PPAR and this could be involved in the mechanism of NMP-induced tumorigenesis in mice.

4. Lack of tumorigenicity for NMP in two rat studies

The Applicant argued that the available data support a MOA for NMP-induced liver tumors in mice that does not occur in rats. They noted that there was no evidence of tumorigenicity observed in rat (CrI:CD (SD) BR) studies where NMP was administered via diet at up to 15000 ppm (approximate mean dose of 834 mg/kg/day between males and females) for 2 years (Malley et al., 2001) and via whole body inhalation at up to 400 mg/m³ (approximate dose of 68 mg/kg/day for 425 g rat) for 2 years (Lee et al., 1987). There was also no evidence of test article-related adaptive liver changes in these studies. The human equivalent dose (HED) of the highest dietary dose is 135 mg/kg/day, or 71-times the potential NMP exposure at the MRHD of Brixadi based on a body surface area (BSA) comparison. The HED of the highest inhalation dose is 11 mg/kg/day, or 6 times the NMP exposure at the MRHD based on BSA comparison. Based on these data, the Applicant argued that the MOA is mouse specific, and unlikely to occur in humans.

5. High background incidence of liver tumors in B6C3F1 mice

B6C3F1 mice have a high background incidence of spontaneously forming hepatic tumors compared to other strains (see figure below) (King-Herbert and Thayer, 2006). The Applicant has proposed that the higher incidence of hepatic tumors observed in the high-dose group of the 18-month mouse study could be attributed to an exacerbation of effects underlying the high background rate of hepatic tumors in this mouse strain by the adaptive changes caused by high concentrations of NMP.

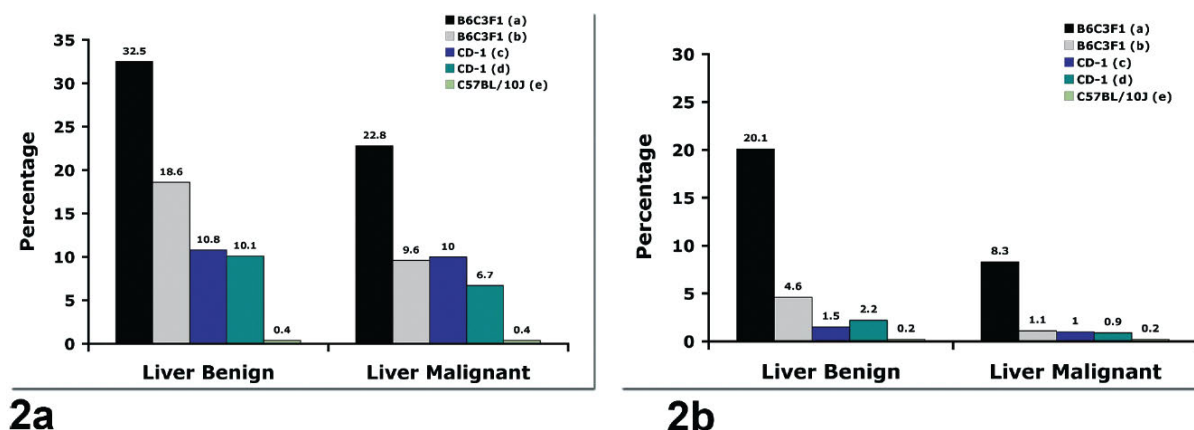


FIGURE 2.—Incidence of benign and malignant hepatocellular liver tumors in (a) male and (b) female B6C3F1/N mice and comparisons with other mice. Data presented at the NTP workshop (<http://ntp.niehs.nih.gov>) (see “Meetings and Workshop”). (a) NTP B6C3F1, (b) NCTR B6C3F1, (c) CD-1 proprietary data, (d) CD-1 proprietary data, and (e) C57BL/10J proprietary data.

Discussion of Applicant’s Mode of Action (MOA) Assessment

In summary, the Applicant proposed that the MOA for mouse hepatic tumors observed in the 18-month study using B6C3F1 mice was underlied by a non-genotoxic mechanism in which high concentrations of NMP caused adaptive proliferative responses, including hepatocellular hypertrophy and hyperplasia that preceded development of tumors through a mechanism likely involving PPAR activation, but not CAR or AhR activation. They added that the MOA is mouse specific because it did not apply to rats and only occurred at such high concentrations of NMP that it would not pose a risk for tumorigenesis in humans.

The Applicant referred to MOA principles described by Holsapple et al (2006) that represented discussions between members of the International Life Sciences Institute (ILSI), the Health and Environmental Sciences Institute (HESI), and the U.S. Environmental Protection Agency (EPA). As noted in the figure below (from Holsapple et al., 2006), the questions to be considered to determine human risk included the following:

1. Is the weight of evidence sufficient to establish a MOA in animals?
2. Is the animal MOA plausible in humans?
3. Taking into account kinetic and dynamic factors, in the animal MOA plausible in humans?”

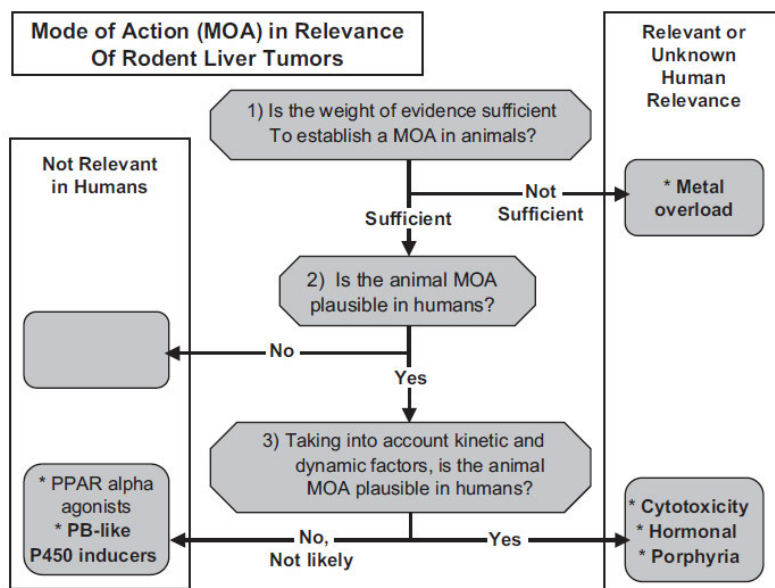


FIG. 1. Summary of conclusions for various modes of action (MOA) for rodent liver tumors (RLT) using the human relevance framework (HRF). The workshop considered the following MOA: metal overload, phenobarbital-like P450 inducers, cytotoxicity, hormonal toxicity, and porphyrogenicity. The peroxisome proliferator activated receptor alpha (PPAR α) agonists are included for comparative purposes.

As to the first question regarding whether an adequate weight of evidence has been established in animals, the Applicant does not appear to have adequately demonstrated that NMP-induced tumorigenesis is underlied by a PPAR-related mechanism. Klaunig et al (2003) developed an MOA to understand and explain the human relevance of PPAR α agonist-induced rodent tumors. The authors note that studies in rodent liver have identified several “key event” including three “causal events”, which include activation of PPAR α , perturbation of cell proliferation and apoptosis, and selective clonal expansion. Associative events include increased expression and activity of peroxisomal enzymes including acyl CoA oxidase, alteration of expression of genes that mediate cell cycle progression in response to PPAR α activation, alteration in expression and activities of nonperoxisomal lipid-metabolizing enzymes in response to PPAR α activation, increased peroxisome proliferation, hepatocyte oxidative stress, and Kupffer-cell-mediated events.

1. To support that the NMP MOA may occur through a PPAR α mechanism, the Applicant cited data demonstrating that NMP treatment caused a slight increase in PALCoA oxidation in treated male animals relative to untreated controls and that there was a slight increase in the level of peroxisomes observed by electron microscope in 2 of 5 treated male animals. These data appear to be consistent with some of the associative events that are described by Klaunig et al (2003). The authors do note that induction of acyl CoA oxidase requires PPAR α and generally does not occur with other nongenotoxic carcinogens; however, they do emphasize that all PPAR α agonists that cause high-level sustained induction of

acyl CoA oxidase also cause increases in mouse and rat liver tumors. The evidence available for NMP demonstrated that only slight increases in oxidation occurred. The authors also note that the most common method used to demonstrate that a compound is a PPAR α agonist is through the use of in vitro reporter assays and go so far as to state that these data are highly specific to a PPAR α MOA and should be considered essential data.

2. The Applicant does appear to provide adequate evidence that NMP causes a proliferative response via several methods (i.e., histopathology, BrdU labeling) and these changes, including hypertrophy and hyperplasia, correlated with tumorigenesis at high NMP doses. However, the Applicant did not address whether NMP causes perturbations in apoptosis (via TUNEL assay, caspase staining). Perturbations in these processes are considered to be hallmarks in the carcinogenic process and therefore additional data to address the latter issue would bolster the weight of evidence for an MOA assessment.
3. The Applicant did not address whether NMP causes selective clonal expansion. However, Klaunig et al (2003) state the following regarding this causal event, and therefore no additional information may be needed in this regard.

This process is believed to lie at the heart of the early neoplastic process and has been demonstrated convincingly to be a chronic response to PPAR α agonist treatment. However, its demonstration can be a problematic and labor-intensive endeavor. In addition, it is completely non-specific occurring irrespective of the carcinogenic modality and is therefore not particularly useful as evidence for demonstration of a PPAR α agonist-related MOA.

With respect to the second question regarding whether the animal MOA is plausible in humans, the Applicant proposes that if it were confirmed that NMP causes hepatocellular tumorigenesis through PPAR α activation, the concern for human risk would be low because it has been demonstrated that humans are less susceptible to this mechanism than rodents (Vacca et al., 2013). This reviewer would agree that the human relevance would be low if it were adequately demonstrated that the NMP MOA were aligned with a PPAR mechanism. However, the MOA for NMP has not been adequately established as noted above.

With respect to the third question regarding whether the MOA is plausible in humans after taking into consideration other factors, the Applicant emphasized that the NMP-induced tumorigenesis observed in B6C3F1 mice exhibits a threshold and noted that the no-effect level (NOEL) for tumorigenicity at 1200 ppm (197 mg/kg/day) provides a large safety margin when compared to the potential human exposure associated with the MRHD of Brixadi. They propose that these large margins would further reduce concern for human risk since animal tumorigenesis that occurs at doses greater than 25-fold anticipated human doses is unlikely to be of clinical concern. However, it is

important to note that the tumor NOEL only provides a safety margin of approximately 8-fold the potential human exposure from the MRHD of Brixadi.

Table 5: Safety Margins for Hepatocellular Tumorigenicity in Mice and Rats

	Dose (mg/kg/day)	Human Equivalent Dose (HED) (mg/kg/day) ^a	Safety Margin ^b	Safety Margin (corrected for monthly administration) ^c
Mouse				
Tumorigenic LOEL ^d	1244	101	53	1488
Tumorigenic NOEL ^d	197	16	8.4	235
Rat				
Tumorigenic NOEL ^e	834	135	71	1988

^a Based on body surface area conversion factors (km) in the mouse of 12.3 and in the rat of 6.2, from: *FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*, July 2005.

^b Calculated by dividing the animal HED by the highest anticipated human NMP dose of (b) (4) mg, or (b) (4) mg/kg for a 60-kg subject.

^c Corrected for the once every 28-day administration of NMP in the CAM2038 q4w formulation

^d From Malley et al., 2001.

^e Highest dose tested. From Malley et al., 2001.

The Applicant argues that the safety margin would be much higher if considering the intermittent exposure to NMP through clinical use of the Brixadi monthly formulation. This is a valid hypothesis given that the mice that developed tumors with NMP were dosed daily and given that the proliferative changes that preceded tumor formation appeared to be an adaptive response to toxicity associated with high concentrations of NMP. Intermittent exposure may allow recovery from NMP-induced hepatic insult and reduce the potential for tumor development even at the doses associated with mouse tumorigenesis. However, we do not have data to support this to date. Therefore, although we agree that the risk for NMP-induced cancer development should be minimal in humans based on the available data, we cannot provide definitive data to eliminate the potential human relevance and the safety margin is less than 10 based on existing data.

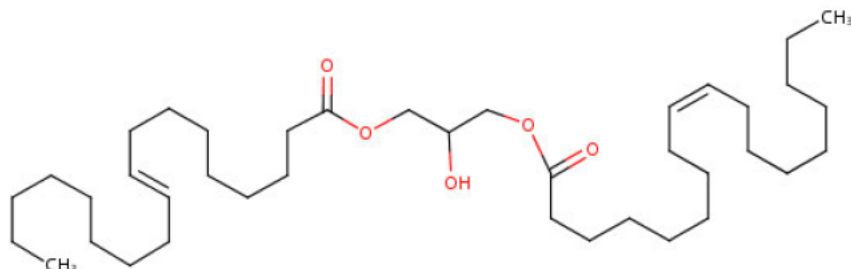
Conclusions regarding MOA assessment

Although an MOA has not been adequately established for NMP-induced tumorigenesis in B6C3F1 mice, we expect the human risk to be minimal due to the safety margin based on the available mouse data taking into consideration the decreased adaptive changes that would be expected with monthly dosing as would be recommended for the relevant Brixadi monthly formulation. Therefore, although further studies are not necessary, we continue to recommend that the label includes information on NMP-related liver tumors in mice.

Appendix 2: Glycerol Dioleate (GDO) Toxicological Risk Assessment

Glycerol Dioleate (GDO; CAS 25637-84-7):

Glycerol dioleate (aka Diolein, or glyceryl dioleate) is a diglyceride with two oleic acid molecules attached to glycerol in the 1,3 positions via an ester linkage.



GDO is included in both the CAM2038 q1w and q4w formulations. The highest proposed level is (b) (4) mg/32 mg dose via the weekly dosed product. The proposed concentration is (b) (4) mg/mL or (b) (4) % w/v. GDO is not listed in the FDA IID and is therefore considered a novel excipient that must be qualified for safety per the guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*. Diglycerides, like GDO, are endogenously formed via the hydrolysis of triglycerides. Diglycerides are metabolized by lipases to the free fatty acids and glycerol. The fatty acids are further metabolized via beta-oxidation or utilized as substrates for cellular metabolism.

PK studies in animals have demonstrated that after SC administration of CAM2038 vehicle that included (b) (4) % by weight GDO, levels of GDO were within the endogenous levels in rats. Metabolite levels were not examined.

The toxicological safety data submitted by the Applicant for GDO include the following studies:

Study Type and Duration	Route of Administration	Species	Test Article	GLP Compliance
Glycerol dioleate (GDO) (excipient)				
Safety Pharmacology				
CNS/FOB endpoints (within the 26-week study)	SC	Rat	GDO	GLP
Pharmacokinetic and Toxicology				
Single dose PK	SC	Rat	GDO	Non-GLP
Repeat dose TK (within the PPND study)	SC	Rat	GDO	Non-GLP (Bioanalysis only)
Single dose (acute) toxicity, including local tolerance 8 days 6 weeks	SC	Rat	GDO	GLP
Repeat dose toxicity, including local tolerance 28 days 26 weeks	SC	Rat	GDO	GLP
Genotoxicity in vitro bacterial reverse mutation in vitro mouse lymphoma in vivo micronucleus	In vitro In vitro In vivo	S. typhimurium Mouse lymphoma L5178Y Rat	GDO	GLP
Reproduction and Developmental Toxicity	PPND study for GDO. Reference to literature data for Diglyceride oil (DAG) for other reproduction and developmental endpoints			
Pre- and postnatal development, including TK	SC	Rat	GDO	GLP
Carcinogenicity	No studies – Reference to literature data for Diglyceride oil (DAG)			

BPN – buprenorphine; CNS – central nervous system; CVS – cardiovascular system; FC – FluidCrystal (placebo); CHO – Chinese hamster ovary; GDO – diacyl glycerol; GLP – Good Laboratory Practice; HEK293 cells – human embryonic kidney 293 cells; hERG - human ether-a-go-go related gene; IM – intramuscular; NBPN – norbuprenorphine; NTX – naltrexone; PK – pharmacokinetic; PPND – pre- and post-natal development; SC – subcutaneous; TK – toxicokinetic

As noted in the table above, the Applicant also references published studies with diacyl glycerol oils (DAG) as a surrogate molecule for GDO. According to the Applicant, DAG oils are largely GDO but also contain linoleic acids, and other fatty acids attached to glycerol.

The composition of the DAG oils in the referenced articles summarized below are depicted in the Applicant's table below:

Figure 7. Comparison of Composition of GDO and DAG oil Used in Studies Referenced by the Applicant

Study reference (see also Appendix I)	TO-10-405 / TO-12-446	TO-05-153 / TO-05-189 / TO-04-141 / TO-04-142 / TO-05-156 / TO-05-177	TO-04-146	Kasamatsu et al., 2005 (before heating / after heating)	Chengelis et al., 2006a / Chengelis et al., 2006b / Chengelis et al., 2006c	Morita et al., 2008b / Morita et al., 2008c	Soni et al., 2001	Morita et al., 2008a
Acylglycerol distribution (%)								
Monoacyl glycerol	(b) (4)			0.5 / 2.7	0.9-2.9	2.2	5 (indicated in text)	No detailed composition provided in the study.
Diacyl glycerol				85.5 / 80.3	82.9-83.9	86.1	75 (indicated in text)	
Triacyl glycerol				14.0 / 17.1	12.8-15.2	9.9	20 (indicated in text)	
Fatty acid composition (%)								
C16:0 (palmitic)				3.1 / 3.2	2.9-3.0	3.1	No detailed composition provided for the indicated study. The DAG oil prepared from high C18:1, C18:2 and C18:3 content raw materials.	No detailed composition provided for the indicated study. The DAG oil prepared from high C18:1, C18:2 and C18:3 content raw materials (soybean and rapeseed oil).
C18:0 (stearic)				1.3 / 1.3	1.1-1.2	1.1		
C18:1 (oleic)				41.1 / 42.3	35.6-37.6	39.5		
C18:2 (linoleic)				45.6 / 44.8	47.7-48.6	46.4		
C18:3 (linolenic)				8.7 / 8.1	6.3-6.5	6.2		
C20:0 (arachidic)				0.3 / 0.3	-	-		

In the first cycle, the Applicant did not provide an adequate scientific bridge to the referenced published studies testing diacylglycerol oil (DAG) as a surrogate chemical solution for glycerol dioleate (GDO). The Agency raised concerns that although the levels of oleic acid in the materials tested provided some information, they did not distinguish the content of glycerol dioleate specifically. In the second cycle, the Applicant addresses this deficiency by quantitating the amount of GDO in DAG oil and provide a justification that the DAG oil tested in the published studies is representative of the material that was tested in the published toxicology studies. The published studies used to support the safety of GDO for the NDA all used DAG purchased from (b) (4). The identification and quantitation by NMR of the components of the DAG oil (b) (4) was described in a publication by Hatzakis, et al. 2011³. Glycerol dioleate is a glycerol molecule with two oleic acids attached either at the 1,2 position or the 1,3 position. Hatzakis purchased six samples of DAG oil (ENOVA) from U.S. supermarkets and analyzed them for mono-, di-, and tri-acyl glycerol content as well as fatty acid substitutions. They reported that 88.9% of the materials were diacylglycerols, the ratio of 1,2 DAG to 1,3 DAG was 1:2, and 40.5% of the total fatty acids were oleic acid residues. These are summarized in the table below, reproduced from the Applicant's summary report (TO-18-625).

³ Hatzakis E, Agiomyrgianaki A, Kostidis S, Dais P (2011) High-Resolution NMR Spectroscopy: An Alternative Fast Tool for Qualitative and Quantitative Analysis of Diacylglycerol (DAG) Oil. J Am Oil Chem Soc 88(11):1695-1708.

Table 59. Compositional Frequency of Diacylglycerol Isomers in DAG Oil (as per Hatzakis, 2011)

Frequency of 1,2-DAG Present in DAG Oil (%) ^b	Frequency of 1,3-DAG Present in DAG Oil (%) ^b	Total Frequency of DAGs in DAG Oil (%) ^c	Relative DAG Isomer Ratios Used in GDO Calculations (1,2:1,3) ^d
29.2	59.8	88.9	0.33:0.67

^aValues averaged from six replicates.

^bValue is fraction of total acylglycerol composition from Table 3, ³¹P-NMR.¹

^cCalculated by summing the frequencies of 1,2-DAG and 1,3-DAG.

^dCalculated from the relative frequencies of 1,2-DAG:1,3-DAG.

Hatzakis also determined the positional and compositional distribution of oleic acid at the 1-, 2- and 3- positions which are reproduced in the table below also from the Applicant's analysis (Report TO-18-625):

Table 60. Positional Frequencies of Oleic Acid in DAG Oil (Report TO-18-625)

Total Oleic Acid Content (%) ^b	1,3-Diacylglycerols		1,2-Diacylglycerols	
	Frequency of Oleic Acid at 1-position (%) ^c	Frequency of Oleic Acid at 3-position (%) ^c	Frequency of Oleic Acid at 1-position (%) ^c	Frequency of Oleic Acid at 2-position (%) ^c
40.5	40.0	40.0	40.2	35.5
[1.00]	[0.99] ^e	[0.99] ^e	[0.99] ^e	[0.88] ^e

^aValues averaged from six replicates.

^bValue from Table 4, ¹H-NMR.¹

^cValue from Table 4, ¹³C-NMR, carbonyl carbons.¹

^eBracketed values are the rate of oleic acid incorporation relative to the total oleic acid content.

The Applicant then calculated the probability that oleic acid residues would be present at either Position 1 and 2 or Position 1 and 3 in order to estimate the total amount of GDO in the DAG oil tested in the published toxicology studies using the equation provided by the Applicant below.

$$\text{Dose GDO (mg/kg)} = \text{Dose DAG oil (mg/kg)} * [\% \text{DAG}] * \left(\begin{array}{l} \text{Accounts for 1,3-DAG fraction} \\ \left[0.67 * (\% \text{oleic acid at 1-position}) + (\% \text{oleic acid at 3-position}) \right] \end{array} \right) + \left(\begin{array}{l} \text{Accounts for 1,2-DAG fraction} \\ \left[0.33 * (\% \text{oleic acid at 1-position}) + (\% \text{oleic acid at 2-position}) \right] \end{array} \right)$$

Equation 1. Calculation of GDO Doses from DAG Oil Doses in Literature Toxicology Studies Using the Positional and Compositional Frequencies Determined in Hatzakis 2011.

The calculations provided by the Applicant for the GDO equivalent in the DAG oil have been reviewed by Dr. Steven Kinsley of ONDP, OPQ. To estimate safety margins for GDO based on the probability of GDO being present in DAG oil tested in the published

literature, the Applicant employed a worst-case scenario based on the lowest level of oleic acid residues and lowest level of DAGs present in the six samples tested by Hatzakis. Given OPQ agreement that the estimated levels of GDO in DAG oil are justified scientifically by the data provided we consider this approach to be a reasonable estimate of GDO dose. As these amounts were used to calculate the amount of GDO tested and the safety margins for GDO for each of the publications reviewed to support this novel excipient. These data are presented in the table below and discussed under the relevant study reviews later in this appendix.

Table 61. Estimated GDO Content and Exposure Margins Based on Probability

Reference	Species and Study	DAG Oil Dose (mg/kg)	DAG Content	DAG Dose (mg/kg)	% 1,3	Sub Freq 1,3	% 1,2	Sub freq 1,2	Intermediate calculation	GDO Dose (mg/kg)	HED mg for 60 kg person	Exposure Margin based on mean of 37.14 mg/day	Exposure Margin based on total of 260 mg/day
Chengelis 2006a	Rat 2 Year	1946	0.829	1613	0.67	0.127	0.33	0.127	0.127	204	1979	53	8
Chengelis 2006b	Mouse 2 year	1792	0.829	1486	0.67	0.127	0.33	0.127	0.127	188	918	25	4
Morita 2008a	2-Gen Rat	1160	0.829	962	0.67	0.127	0.33	0.127	0.127	122	1179	32	5
Morita 2008b	Rat EFD	4630	0.829	3838	0.67	0.127	0.33	0.127	0.127	486	4708	127	18

Intermediate calculation = $(0.67 \times \text{probability of oleic acid at 1 and 3}) + 0.33 \times \text{probability of oleic acid at 1 and 2}$

Values for DAG content and probability of substitution frequency for oleic acid used the worst-case data from Hatzakis (2011) of 82.9% and 35.6%, respectively.

SINGLE-DOSE PK

Study Title: A single dose study in rat evaluating circulating plasma glycerol dioleate levels following s.c. administration of FluidCrystal® Injection depot placebo formulation or glycerol dioleate. Study PK-15-539 (dated 12/21/2015)

In this non-GLP study, plasma levels of GDO were measured in rats following either a SC injection of GDO or of the proposed FluidCrystal (FC) delivery system containing GDO. Rats were treated with either 940 mg/kg of GDO or 432 mg/kg of FC placebo via SC injection. Plasma samples were collected at 6 hours, 2, 14, 42, 56, 63, and 72 days. A total of 10 rats per group were employed (three groups: Saline, GDO, or FC).

Maximum Plasma Concentrations of GDO (mcg/mL) for each dose group are below:

Treatment Group	Saline	GDO	FC
Highest Mean	3.5	3.8	3.4
Highest Maximum	5.0	6.9	4.7

The highest levels in the table above were based on any time points examined. The levels of GDO were slightly higher (38%) compared to saline; however, the levels via the FC vehicle dose were not different than controls.

The mean results are also plotted below, reproduced from the study report:

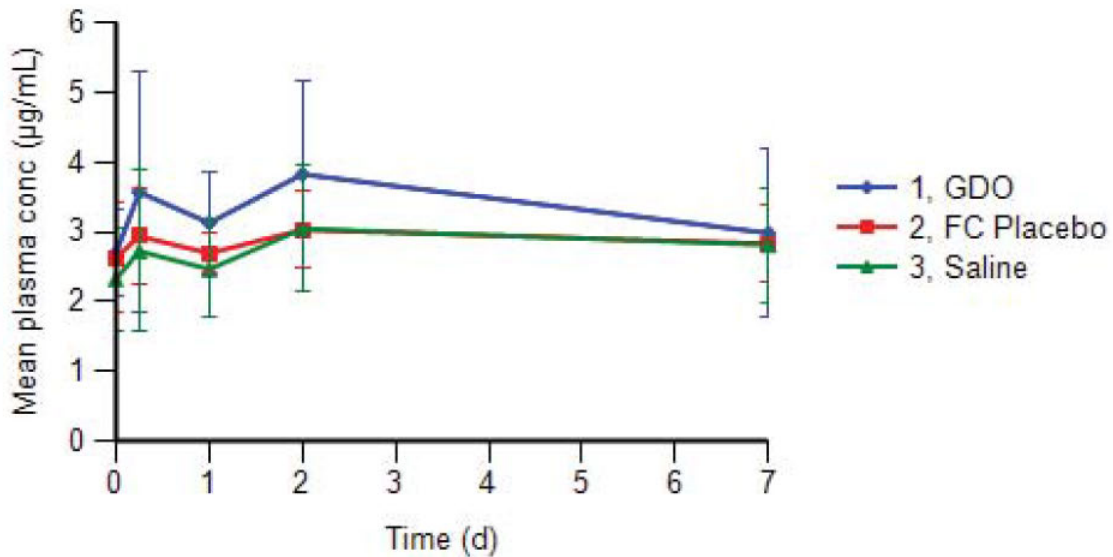


Figure 7. Group mean plasma concentrations of GDO; detailed on day 0-7 (linear scale)

Change from baseline are also depicted.

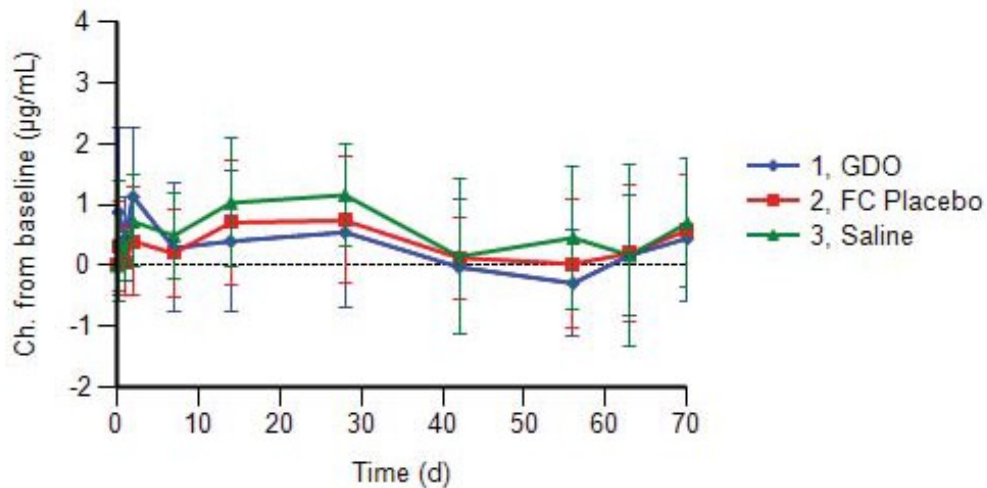


Figure 11. Group mean plasma concentrations of GDO, as absolute change from baseline; day 0-70 (linear scale)

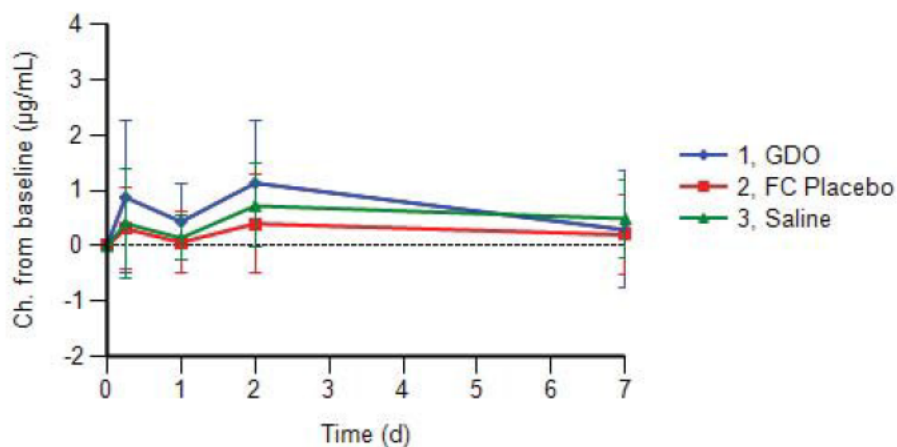


Figure 12. Group mean plasma concentrations of GDO, as absolute change from baseline; detailed on day 0-7 (linear scale)

In addition, the local tissue effect was evaluated via histopathology at Day 71. The report notes that for both animals dosed with GDO and FC, some remains of the test article were still observed at the injection site even 71 days after injection.

REPEAT-DOSE TOXICOLOGY

The Applicant conducted a 28-day and 26-week repeat-dose toxicology study for GDO. In addition, they have conducted a 9-month repeat-dose toxicology study with the drug product containing GDO (reviewed in the body of this review) to address the requirement for two species toxicity data for a novel excipient.

The review of the 26-week repeat-dose toxicology study with GDO and the drug product vehicle below was completed by Dr. Gary Bond.

Study title: A 26 Week Toxicity Study of Glycerol Dioleate and FluidCrystal® Vehicle by Subcutaneous Injection in Rats

Study no.:	TO-10-405 (Sponsor); 519932 (b) (4)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 12, 2011
GLP compliance:	Yes
QA statement:	Yes, signed

Drug, lot #, and % purity: Glycerol dioleate (GDO): sterile, Batch 3002413 (supplied packaged), purity NR (requested during the IND). The Applicant amended the report to include a CoA of the product, however, purity information was not provided.

2022IAS-I (CAM2038 q1w vehicle: SPC (b) (4)%, GDO (b) (4)%, EtOH 10%, (b) (4) (b) (4)%): sterile, Batch 3002396, purity NR (requested during IND). The Applicant amended the report to include a CoA of the product, however, purity information was not provided.

0.9% sodium chloride (negative control), Batch (10L09G61 and 11C09G61), purity NR

Key Study Findings

- The objective of this study was to determine the potential toxicity of glycerol dioleate (GDO) when given by subcutaneous injection (SC) once daily for 26 weeks to rats and to provide data to support the use of GDO in humans. The secondary objective was to determine the potential toxicity of a FluidCrystal® vehicle (FCV; Code Name 2022IAS-I), specifically the EtOH-based q1w FCV, when given by subcutaneous injections once weekly for 26 weeks to rats and to provide data to support its use in humans.

Group Number	Number of Animals		Test Item	Dose Level (mg/kg)	Dosage Volume (mL/kg)	Dosing Frequency
	Males	Females				
1	15	15	Control (0.9% sodium chloride w/v)	0	1.06	Daily
2	15	15	Glycerol dioleate	100	0.11	Daily
3	15	15	Glycerol dioleate	350	0.37	Daily
4	15	15	Glycerol dioleate	1000	1.06	Daily
5	15	15	2022IAS-I	1000	1.06	Weekly

- Dosing was rotated among 6 sites on the back resulting in ~30 injections per site. For the q1w FCV, both the incidence and severity of cystic granulomatous inflammation were lower at Site 3 (42 days after the last injection) compared with Site 2 (7 days after the last injection), suggestive of recovery.
- The subcutaneous administration to rats during 26 weeks of up to the limit dose

of 1000 mg/kg/day GDO was associated with no evidence of systemic effects, indicating the low order of systemic toxicity of GDO. In addition, subcutaneous administration of the dose 1000 mg/kg/week of the FluidCrystal® vehicle (FCV) formulation was not associated with any indication of systemic toxicity indicating low order of systemic toxicity for the FCV.

- Treatment-associated findings for GDO as well as q1w FCV directly attributed to the test items were localized and confined to the subcutaneous injection sites. There was no evidence of similar such reactions in any other tissue. The inflammation of the injection sites, for both GDO and the q1w FCV, had features of the not unexpected typical foreign body reaction. Injection site findings for GDO were primarily subcutaneous cystic granulomatous inflammation with sporadic mononuclear cell inflammation at all dose levels. The primary finding was generally dose-related and reached a severity usually no higher than marked (1 instance of severe in 1 high dose GDO female). Additional but less frequent GDO observations were mixed inflammation at 1000 mg/kg/day, fibrosis/fibroplasia, and granulation tissue that were confined to 350 or 1000 mg/kg/day. Additional, less frequent observations associated with q1w FCV were pigmented/foamy macrophages, mixed/mononuclear cell inflammation, fibrosis/fibroplasias, and granulation tissue.
- Also not unexpected and secondary to the inflammation at the injection sites was an inflammatory response were effects on hematology (increased white blood cells and fibrinogen).
- The NOAEL for GDO systemic toxicity is the highest dose tested of 1000 mg/kg/day (6000 mg/m²). This dose is (b) (4) times the single maximum dose of (b) (4) mg/kg/day via the q1w product. An acceptable LOAEL for GDO local toxicity is the low dose of 100 mg/kg/day based on cystic granulomatous inflammation that appeared reversible with longer recovery periods.
- In summary, the adverse local effects noted in this study are anticipated findings following repeated subcutaneous injections of an insoluble material. There was no evidence of systemic toxicity.

Methods

Doses:

Group Number	Number of Animals		Test Item	Dose Level (mg/kg)	Dosage Volume (mL/kg)	Dosing Frequency
	Males	Females				
1	15	15	Control (0.9% sodium chloride w/v)	0	1.06	Daily
2	15	15	Glycerol dioleate	100	0.11	Daily
3	15	15	Glycerol dioleate	350	0.37	Daily
4	15	15	Glycerol dioleate	1000	1.06	Daily
5	15	15	2022IAS-I	1000	1.06	Weekly

Frequency of dosing: GDO – daily; 202IAS-I - q1w FluidCrystal vehicle (FCV)

Route of administration: Subcutaneous (SC)

Dose volume: See table

Formulation/Vehicle: FVC (ethanol-based q1w placebo)

Species/Strain: Rat/Han Wistar (CrL:WI Han)

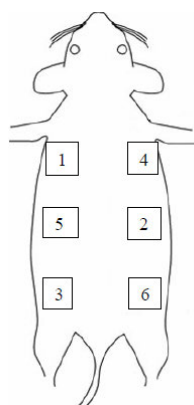
Number/Sex/Group: 15

Age: 6 weeks at shipping

Weight: 144-183 g (males) and 100-149 g (females)

Satellite groups: none

Unique study design: Dosing - The test and control items were administered by subcutaneous injection at volumes and frequencies indicated in the table. Six injection sites on the back of the rats (as defined in the diagram below) were used and the use of these sites were rotated in ascending numerical order throughout the study. For the controls and animals receiving GDO (Groups 1-4), each injection site typically received 30 injections (29-32 based on rotation of sites). For the animals receiving FCV (Group 5), Dose Sites 1 and 2 received 5 injections and the remaining dose sites received 4 injections.



Dose Site 1: Left shoulder
 Dose Site 2: Right flank
 Dose Site 3: Left rump
 Dose Site 4: Right shoulder
 Dose Site 5: Left flank
 Dose Site 6: Right rump

Deviation from study protocol: Nothing considered significant to outcome of study. Calcium, phosphorus, sodium, potassium, chloride and creatinine were not measured in urine, which

was a deviation from the protocol (see results).

Observations and Results

Mortality

All animals were checked for viability early morning and again as late as possible on each day.

There were six unscheduled deaths, although there were none later than Week 16. Four of these were sacrificed as moribund, due to treatment-related skin lesions associated with the injection sites (e.g., ulcerative dermatitis). The remaining two animals were found dead (one control animal related to the anesthesia necessary to obtain blood samples on Week 26 and one HD GDO animal related to nephroblastoma). The early sacrifices were related to dermal inflammatory reactions, unrelated to the SC foreign body response and considered relating to mechanical dosing and possible self-trauma/scratching. Starting at Week 20, any animal that was noted with such dermal lesions was single-housed until the lesion was considered sufficiently healed and the animal, where possible, was returned to its original cage without incident.

Unscheduled deaths are considered treatment-related and occurred only in HD GDO (1 animal) and FCV (3 animals) groups which were injected with the highest dose volume (1.06 mL/kg).

Clinical Signs

All animals received a detailed clinical examination weekly. All animals receiving daily injections were examined for reaction to treatment at frequent daily intervals. The onset, intensity and duration of any signs were recorded, starting immediately post dose and at least 1 h post dose. All animals receiving FCV were, up until Day 134 (Week 20), examined for reaction to treatment on the day of the weekly dosing and the following day only (i.e., 2 days per week). From Day 135 (Week 20), these animals were examined twice daily. From Week 20 onwards, any animal that was noted with a 'wet' lesion of the injection site was single housed until the lesion was considered sufficiently healed and the animal, where possible, could be returned to its original multi-animal cage. This was initiated, following the unscheduled deaths of 4 animals on the study, as a measure to improve the potential for recovery from the lesions and to lessen the likelihood of additional unscheduled deaths.

Observations were observed in cage (alertness), outside the cage in a standardized arena (e.g., alertness, diarrhea, tremors, respiration, aggression, other), during handling (e.g., body temperature, pupils, salivation, tail flick pain response, other).

No apparent systemic toxicity was observed in any group administered either test item (GDO or FCV).

Injection Site observations

Sites of control and GDO animals were examined for reaction to treatment at least once daily. Animals receiving FCV, up until Day 134 (Week 20), were examined at least once daily on the day of the weekly dosing and the following day only. From Day 135 (Week 20), these animals were examined for reaction to treatment at least twice daily.

Adverse signs of treatment were observed locally at the injection sites and included swellings, staining, and scabbing. Not unexpected staining of fur on the dorsal surface or neck was noted in any group throughout the dosing period. Sparse hair or baldness was observed in a dose volume-related manner with most high-volume animals (control and HD GDO) being effected and few LD GDO being effected (lowest dose volume). Greasy hair was also observed in GDO-treated groups. Greasy coats were also observed in HD GDO animals assumed related to the volume of GDO-based treatment.

Swellings (soft or firm) were noted at injection sites for MD and HD GDO groups and for the FCV group. Swellings were dose-dependent with respect to the number of animals affected, the number of dose sites with swellings, and size of the swellings increasing with dosage with essentially no swelling observed in LD GDO and control animals. Swellings were generally observed at earlier time points with increasing dosage. The swellings were consistent with the presence of the GDO at the injection sites, since GDO is viscous, insoluble oil that will slowly be cleared from the injection site. Swelling was also observed in some FCV animals. Lesions described as dry, wet, or scabbed were observed in some animals and were apparently related to scratching after dosing.

Body Weights

For controls and GDO animals, body weights were recorded once during the pre-dosing period and daily during the dosing period and before necropsy. For FCV animals, body weights were recorded weekly and at necropsy.

Body weight and body weight gains were not affected by treatment in any groups.

Food Consumption

Food consumption was measured once during the pre-dosing period and weekly thereafter.

Food consumption was not affected by treatment in any groups.

Ophthalmoscopy

An ophthalmic examination was performed on all animals pretrial and on all control and high dose (GDO and FVC) animals (GDO and FVC) during Weeks 13 and 26.

No ophthalmic effects were affected by treatment in any groups.

Modified Irwin Test

The examinations were conducted on 10 animals/sex/group for control and GDO dose groups (not FCV animals) 1 hour post dose on Day 1 and 1 day during Week 13 and Week 26. Evaluations included pain response, body temperature, and locomotor activity.

There were no behavioral or functional differences noted between groups for the Modified Irwin test.

ECG – not evaluated

Clinical Pathology Examinations

Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Haematology	Coagulation	Clinical Chemistry	Urinalysis
1-5	Week 13	X	X	X	X
1-5	Week 26	X	X	X	X

X = Sample collected

Hematology

Haematology Parameters

Red blood cell count Haemoglobin Haematocrit Mean cell volume Mean cell haemoglobin concentration Mean cell haemoglobin Reticulocytes Reticulocyte count (absolute) Red blood cell distribution width	Platelets Blood Smear ^a White blood cell count Neutrophils Lymphocytes Monocytes Eosinophils Basophils Large unstained cells

^a A blood smear was prepared from each hematology specimen and labelled, stained, stored and archived. The blood smears were not evaluated as it was considered that examination would not yield any further information.

Statistically significant (SS) increases in the WBC population were observed after treatment with GDO at 1000 mg/kg at Weeks 13 and 26. WBC counts in females and the mean number of specific WBC cell populations in male and female animals receiving 1000 mg/kg/day when compared with controls. These indices were also increased in the FCV groups but statistical analysis was not conducted compared to controls.

GDO treatment - At 13 weeks in HD GDO males, neutrophils and monocytes were statistically significantly (SS) increased 54 and 89%, respectively. Lesser but not SS increases were observed at 100 mg/kg (19% and 32%, respectively) and 350 mg/kg (22 and 33%, respectively). At 26 weeks in HD GDO males, neutrophils and monocytes were increased 122 and 78%, respectively. Lesser but not SS increases were observed

at 100 mg/kg (12 and 21%, respectively) and 350 mg/kg (30 and 36%, respectively). As will be discussed regarding these WBC/WBC cell population changes for GDO and FCV treated groups, such changes are consistent with the inflammation observed because of the SC dosing and resultant foreign body reaction.

Differences in White Blood Cells: Males

Group/Dosage (mg/kg/day)	Summary Information	Week 13		Week 26	
		Neut $\times 10^9/L$	Mono $\times 10^9/L$	Neut $\times 10^9/L$	Mono $\times 10^9/L$
1 (0)	Mean	0.78	0.09	0.76	0.14
	SD	0.24	0.03	0.19	0.05
	N	15	15	15	15
4 (1000)	Mean	1.20	0.17	1.69	0.25
	SD	0.35	0.07	1.06	0.12
	N	15	15	12	12
	P value	< 0.01	<0.001	<0.001	<0.001

At 13 weeks in HD GDO females, WBCs, neutrophils, lymphocytes, and monocytes were SS increased 51, 73, 44, and 140%, respectively. Lesser but not SS increases except for monocytes at 350 mg/kg, were observed at 100 mg/kg (8, 9, 1, and 25%, respectively) and 350 mg/kg (20, 17, 21, and 38%, respectively). At 26 weeks in HD GDO females, WBCs, neutrophils, lymphocytes, and monocytes were SS increased 57, 150, 33, and 160%, respectively. Lesser but not SS increases were observed at 100 mg/kg (8, 24, 4 and 43%, respectively) and 350 mg/kg (13, 38, 11 and 29%, respectively). As will be discussed regarding these WBC/WBC cell population changes for GDO and FCV treated groups, such changes are consistent with the inflammation observed because of the SC dosing and resultant foreign body reaction.

Differences in White Blood Cells: Females

Group/Dosage (mg/kg/day)	Summary Information	Week 13				Week 26			
		WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$	WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$
1 (0)	Mean	3.92	0.64	3.09	0.08	2.91	0.50	2.25	0.07
	SD	1.00	0.27	1.04	0.05	0.53	0.29	0.60	0.03
	N	14	14	14	14	14	14	14	14
4 (1000)	Mean	5.91	1.11	4.44	0.19	4.56	1.25	2.99	0.18
	SD	1.72	0.69	1.09	0.09	0.95	0.43	0.69	0.07
	N	13	13	13	13	13	13	13	13
	P value	<0.001	< 0.01	< 0.01	<0.001	<0.001	<0.001	< 0.01	<0.001

FVC treatment - For FCV males, there were increases in the WBC counts and number of specific WBC cell populations at 13 and 26 weeks compared to control. Most notable were a 206 and 161% increase in neutrophils at 13 and 26 weeks, respectively, and a 167 and 64% increase in neutrophils at 13 and 26 weeks, respectively (see table for all changes). These results are consistent with the observed inflammation at the injection site.

Differences in White Blood Cells: Males

Group/Dosage (mg/kg)	Summary Information	Week 13				Week 26			
		WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$	WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$
1 (0/day)	Mean	5.69	0.78	4.69	0.09	5.12	0.76	4.08	0.14
	SD	0.97	0.24	0.87	0.03	1.13	0.19	1.04	0.05
	N	15	15	15	15	15	15	15	15
5 (1000/week)	Mean	8.60	2.39	5.76	0.24	7.01	1.98	4.60	0.23
	SD	1.64	0.83	0.89	0.08	1.73	1.02	0.87	0.07
	N	15	15	15	15	14	14	14	14
	% difference from control	51	206	23	167	37	161	13	64

% difference from control added to table as no statistical analysis performed

For FCV females, there were increases in the WBC counts and number of specific WBC cell populations at 13 and 26 weeks compared to controls. Most notable were a 91 and 120% increase in neutrophils at 13 and 26 weeks, respectively, and a 75 and 129% increase in monocytes at 13 and 26 weeks (see table for all changes). These results are consistent with the observed inflammation at the injection site.

Differences in White Blood Cells: Females

Group/Dosage (mg/kg)	Summary Information	Week 13				Week 26			
		WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$	WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$
1 (0/day)	Mean	3.92	0.64	3.09	0.08	2.91	0.50	2.25	0.07
	SD	1.00	0.27	1.04	0.05	0.53	0.29	0.60	0.03
	N	14	14	14	14	14	14	14	14
5 (1000/week)	Mean	5.73	1.22	4.23	0.14	4.28	1.10	2.91	0.16
	SD	1.51	0.43	1.22	0.07	0.97	0.44	0.76	0.06
	N	14	14	14	14	13	13	13	13
	% difference from control	46	91	37	75	47	120	29	129

% difference from control added to table as no statistical analysis performed

Coagulation

Coagulation Parameters

Activated partial thromboplastin time Fibrinogen	Prothrombin time

Statistically significant (SS) increases in fibrinogen were observed after treatment with GDO and FCV at 1000 mg/kg at Weeks 13 and 26.

At 13 and 26 weeks for GDO, increases in fibrinogen concentrations were 22 and 37% for males and 18 and 48% for females compared to controls. Other dose level values were lower. This occurrence can be related to the inflammation observed as a result of the SC dosing and resultant foreign body reaction.

Fibrinogen Concentration

Group/Dosage (mg/kg/day)	Summary Information	Week 13		Week 26	
		Males mg/dL	Females mg/dL	Males mg/dL	Females mg/dL
1 (0)	Mean	236	171	238	158
	SD	31	22	15	24
	N	15	14	14	13
4 (1000)	Mean	288	202	327	234
	SD	53	30	74	71
	N	15	14	13	14
	P value	<0.01	<0.001	<0.001	<0.001

At 13 and 26 weeks for FCV, increases in fibrinogen concentrations were 55 and 34% for males and 46 and 44% for females compared to controls. This occurrence can also be related to the inflammation observed because of the SC dosing and resultant foreign body reaction. Larger values for fibrinogen were noted than for GDO at the same dose.

Fibrinogen Concentration

Group/Dosage (mg/kg)	Summary Information	Week 13		Week 26	
		Males mg/dL	Females mg/dL	Males mg/dL	Females mg/dL
1 (0/day)	Mean	236	171	238	158
	SD	31	22	15	24
	N	15	14	14	13
5 (1000/week)	Mean	365	249	319	227
	SD	50	29	35	44
	N	13	13	14	12
	% difference from control	55	46	34	44

% difference from control added to table as no statistical analysis performed

Clinical Chemistry

Clinical Chemistry Parameters

Urea	Total protein
Glucose	Albumin
Aspartate aminotransferase	Globulin
Alanine aminotransferase	Albumin/globulin ratio
Alkaline phosphatase	Cholesterol
Creatine phosphokinase	Creatinine
Lactate dehydrogenase	Total bilirubin
Sodium	Calcium
Potassium	Inorganic phosphate
Chloride	Triglycerides

No treatment-related effects were observed for clinical chemistry when comparing GDO or FCV to control.

Urinalysis

Urinalysis Parameters

Microscopic evaluation of spun deposit Colour Turbidity Specific gravity Volume pH Protein	Glucose Bilirubin Ketones Leukocytes Blood Urobilinogen

No treatment-related effects were observed for urinalysis when comparing GDO or FCV to control. While calcium, phosphorus, sodium, potassium, chloride, and creatinine were not measured, which was a deviation from the protocol, this deviation was considered not to impact on the outcome of the study by the Sponsor. This reviewer agrees as values for these parameters were measured in the plasma with no treatment-related effects when comparing treated animals with controls. In addition, there were no treatment-related histological findings in the kidney which supports that these urinary indices were not affected by treatment.

Terminal Procedures and Evaluations

Terminal Procedures

Group No.	No. of Animals		Scheduled Euthanasia Day	Necropsy Procedures			Histology	Histopathology
	M	F		Necropsy	Tissue Collection	Organ Weights		
1	15	15	183	X	X	X	Full Tissue	Full Tissue ^a
2	15	15					Injection Sites	Injection Sites
3	15	15					Gross Lesions	Gross Lesions
4	15	15					Injection Sites	Injection Sites
5	15	15					Gross Lesions	Gross Lesions
Unscheduled deaths				X	X	-	Full Tissue	Full Tissue

X = Procedure to be conducted; - = Not applicable

^a See Tissue Collection and Preservation table for listing of tissues

Gross Pathology

After sacrifice, all animals were subjected to a complete necropsy examination. This included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal and pelvic cavities with their associated organs and tissues.

Externally visible injection site effects (e.g., swellings) were previously discussed in the clinical observations section. Most notable observations during gross necropsy compared to controls were injection site cysts. Nothing different in animals was observed for those sacrificed as moribund (1 HD GDO, 3 FCV animals due to skin

morbidity) or found dead (1 control, 1 HD – not GDO treatment-related) than reported in the following descriptions.

GDO group injection sites - Cysts, usually described as clear or pale, were the most notable feature at the sites dosed with GDO at all dose levels. The content of the cysts was likely GDO at the injection sites. This was expected since GDO is a slightly viscous oil, slowly cleared from the injection site. Cysts were most frequently observed in the animals that received 1000 mg GDO/kg/day, and a clear dose and volume related trend was apparent throughout the groups. Considering all 6 injection sites, the highest incidence at any given site in animals (not all sites affected) was 0/15 0/14 for controls, 2/15 and 1/15 (LD GDO), 10/15 and 8/15 (MD GDO), and 14/14 and 13/14 (HD GDO), in males and females, respectively. "Thickening" at sites was observed for 5/14 HD GDO males at one site with no more than 1 animal/site being affected from the other groups including control. This observation was of low occurrence (number of sites and animals) and its significance is unknown.

"Raw area" was noted only at one site and 1 male and 1 female at 1000 mg/kg/day. Scabs were observed at a very low incidence across all dose levels, most frequently at 1000 mg/kg/day where the highest incidence for 1 site was 6/14 males with no scabs in HD females. Scab formation/raw areas tended to correlate with ulcerative dermatitis at histopathology, and were considered secondary to trauma rather than an extension of the subcutaneous changes.

FCV group injection sites - Swelling and raised areas were recorded in animals that received 1000 mg FCV/kg/week at different sites with the highest incidence of 7/14 males and 6/13 females. The swellings likely represented test item at the injection sites. This was expected as the FCV is a formulation that when injected into the subcutaneous tissue self-assembles into a gel-like, firm, slowly degradable matrix. Almost one third of the sites had swellings and fewer had raised areas. There was generally no obvious trend for the distribution of the findings in relation to the various sites. Cysts were a less frequent observation with no more than 2/14 and 7/14 males and females being affected, respectively for a single site. Discoloration was treatment-related, but was not related clearly to the time since the administration of the last injection of FCV.

Scab formation was noted with the highest incidence for any site at 6/15 males and 2/13 females. Raw areas were noted infrequently with the highest incidence of 1/14 males and 1/13 females. These findings tended to correlate with ulcerative dermatitis at histopathology.

Other - Secondary to the injection site reactions, findings in the axillary/inguinal lymph nodes included enlargement of occasional nodes in 4 of 27 rats that received 1000 mg FCV/week, compared with none in controls. Lung discoloration was also noted in treated groups and not controls at a low incidence and not in a dose-responsive manner. Nothing else different was observed in early deaths (sacrificed as moribund or found dead).

Organ Weights

Organs were weighed at necropsy.

Organs Weighed at Necropsy

Brain	Liver
Epididymis ^a	Lung
Gland, adrenal	Ovary ^a
Gland, pituitary	Spleen
Gland, prostate	Testis ^a
Gland, thyroid	Thymus
Heart	Uterus
Kidney ^a	

^a – paired organs were not weighed together, but weights were combined for organ weight evaluation.

No treatment-related effects were observed for organ weights when comparing GDO or FCV to control.

Histopathology - Representative samples of the tissues listed the table below were collected from all animals, and preserved in 10% neutral buffered formalin, unless otherwise noted. Hematoxylin and eosin-stained issues from different groups were evaluated as listed in the terminal evaluation included previously above.

Adequate Battery – Yes.

Peer Review – Yes, by internal pathologist.

Tissue Collection and Preservation

Administration site	Liver
Animal identification	Lung
Artery, aorta	Lymph node, axillary
Bone marrow smear	Lymph node, inguinal
Bone marrow, femur	Lymph node, mandibular ^c
Bone marrow, sternum	Lymph node, mesenteric
Bone, femur with articulating surface	Muscle, skeletal
Bone, sternum	Nerve, optic ^a
Brain	Nerve, sciatic ^c
Cervix	Oesophagus
Epididymides	Ovaries
Eyes ^a	Oviducts ^c
Gland, adrenal	Pancreas
Gland, harderian ^a	Skin
Gland, lacrimal ^c	Small intestine, duodenum
Gland, mammary	Small intestine, ileum
Gland, parathyroid	Small intestine, jejunum
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary ^c	Stomach
Gland, seminal vesicle	Testes ^b
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Ureter ^c
Kidney	Urinary bladder
Large intestine, caecum	Uterus
Large intestine, colon	Vagina
Large intestine, rectum	

^a Preserved in Davidson's fixative

^b Preserved in Modified Davidson's fixative

^c Only one examined

Histological Findings - Treatment associated findings that were directly attributed to the test items were localized and confined to the subcutaneous injection sites. There was no evidence of reactions similar to these seen at the injection sites in any other tissue.

At the injection site, the region affected was the subcutis, with findings sometimes involving the panniculus muscle and occasionally the dermis. The microscopic features ranged from chronic active to chronic inflammation, a usual, expected foreign body reaction. Individual site information is provided in the tables after text.

Cystic granulomatous inflammation was the primary treatment-related finding for GDO and FCV with an incidence for number of animals and severity as listed in the following tables.

Incidence of Subcutaneous Cystic Granulomatous Inflammation at the 6 Injection Sites after SC dosing Daily with Glycerol Dioleate (GDO) or Weekly with FluidCrystal Vehicle (FCV) for 26 Weeks in Rats														
	# of animals/group		# of animals/group with inflammation at that site											
			Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Dose GDO (mg/kg)	M*	F*	M	F	M	F	M	F	M	F	M	F	M	F
0 (q1w placebo)	15	14	0	0	0	0	0	0	0	0	0	0	0	0
100	15	15	6	9	10	6	8	6	8	6	9	5	7	6
350	15	15	12	10	13	7	14	12	12	13	11	14	13	
1000	14	14	14	14	14	14	13	14	13	14	14	13	13	13
Dose FCV (mg/kg)														
1000	14	13	11	8	10	11	5	3	6	10	10	10	4	8

* - M (male), F (female)

Histopathological Severity* of Subcutaneous Cystic Granulomatous Inflammation at the 6 Injection Sites after SC dosing Daily with Glycerol Dioleate (GDO) or Weekly with FluidCrystal Vehicle (FCV) for 26 Weeks in Rats												
	Severity range (highest incidence severity)											
	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Dose GDO (mg/kg)	M	F	M	F	M	F	M	F	M	F	M	F
0 (q1w placebo)	0	0	0	0	0	0	0	0	0	0	0	0
100	1-2 (2)	1-2 (2)	1-3 (2)	1-2 (1)	1-2 (1-2)	1-2 (2)	1-2 (1)	1-2 (1-2)	1-2 (1)	1-2 (1)	1-2 (2)	1-2 (1)
350	1-3 (2)	1-3 (2)	1-4 (2)	1-2 (2)	1-3 (3)	1-3 (1)	1-3 (1-3)	1-3 (1)	1-3 (2)	1-3 (2)	2-4 (2)	1-3 (2)
1000	2-4 (2)	1-3 (3)	1-3 (3)	1-4 (3)	2-4 (3)	1-3 (3)	2-4 (3)	1-5 (4)	1-4 (3)	1-4 (2)	2-4 (3-4)	2-4 (2)
Dose FCV (mg/kg)												
1000	2-4 (3)	2-4 (4)	2-4 (3)	1-4 (3)	1-4 (4)	1-3 (1-3)	3-4 (3)	2-4 (3)	2-4 (3)	2-4 (2)	2-3 (2)	2-4 (2)

* - Grade 1-5 (minimal, mild, moderate, marked, severe)

** - M (male), F (female)

Based on the tables, the following text describes SC cystic granulomatous inflammation and other observed occurrences in GDO and FCV groups.

GDO injection sites – SC cystic granulomatous inflammation was the primary treatment-related finding. This was seen at all dose levels and correlated with the cysts observed at necropsy. There was a general dose-response for the cystic granulomatous response with respect to severity and incidence. Mononuclear cell inflammation was recorded at a relatively low incidence across the groups, with greater severity noted in animals that received 1000 mg/kg/day. Mixed inflammation was noted at 1000 mg/kg/day at both a low severity and incidence. Low incidences of fibrosis/fibroplasia and granulation tissue were confined to 350 or 1000 mg/kg/day, occasionally correlated with thickening.

Occasional ulcerative dermatitis was considered to be procedurally related, probably associated with mechanical trauma and scratching. This occurred at very low incidences at all dose levels of GDO, and correlated with necropsy findings including raw area and/or discoloration.

FCV injection sites - SC cystic granulomatous inflammation was the primary treatment-related finding. This correlated with the swelling/raised areas and less frequently cysts observed at necropsy in this particular group.

Pigmented macrophages (and less often foamy macrophages) were recorded at the majority of injection sites. Pigmented macrophages related to the necropsy observation of dark/discolored. Mixed/mononuclear cell inflammation, fibrosis/fibroplasia and granulation tissue were other features of the inflammatory reaction, sometimes correlating with raised area/swelling.

Reactions at the six injection sites in animals receiving 1000 mg/kg/week were similar overall with no consistent differences. However, both the incidence and severity of cystic granulomatous inflammation were lower at Site 3 (42 days after the last injection) compared with Site 2 (7 days after the last injection). Although this was suggestive of recovery, this tendency was not consistently demonstrated at all time points following the last injection.

Ulcerative dermatitis, epidermal hyperplasia, dermal fibrosis/inflammation and scab were noted at higher incidences compared with the incidence in control. This was possibly mechanically related (dosing) and due to self-trauma/scratching, rather than being directly attributed to treatment. This effect was not noted in animals that were individually housed, suggesting that the effect was due to external mechanical trauma from their littermates. Relatively few sites were affected overall, although the necropsy correlations, particularly scab, were more frequent.

Group	:	1	2	3	4	5
Test Item	:	Control (Saline)	-----	Glycerol dioleate	-----	2022IAS-I
Dosage (mg/kg)	:	0	100	350	1000	1000

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 1		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		15	8**	3***	0***	1	14	6***	5***	0***	4
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	1	3	0	0	0	2	3	1	0
mild		0	5*	6*	3	2	0	7**	4	3	1
moderate		0	0	3	6**	5	0	0	3	7**	3
marked		0	0	0	5*	4	0	0	0	3	4
Total Incidence		0	6*	12***	14***	11	0	9***	10***	14***	8
Inflammation, subcutaneous, mixed											
mild		0	0	0	1	1	0	0	0	1	2
moderate		0	0	0	0	1	0	0	0	2	2
Total Incidence		0	0	0	1	2	0	0	0	3	4
Inflammation, subcutaneous, mononuclear cell											
minimal		0	1	0	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	1	0	0	1	0	0	0	1	0
Fibrosis/fibroplasia, subcutaneous											
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	1	0	2	0	0	0	1	0
moderate		0	0	0	1	0	0	0	0	1	0
Total Incidence		0	0	1	1	3	0	0	0	2	0
Granulation tissue, subcutaneous											
mild		0	0	0	1	0	0	0	0	0	0
Total Incidence		0	0	0	1	0	0	0	0	0	0
Foamy macrophages, subcutaneous											
mild		0	0	0	0	1	0	0	0	1	2

moderate	0	0	0	0	0	0	0	0	0	3
Total Incidence	0	0	0	0	1	0	0	0	1	5
Pigmented macrophages, subcutaneous										
minimal	0	0	0	1	0	0	0	0	0	0
mild	0	0	0	0	2	0	0	0	0	0
Total Incidence	0	0	0	1	2	0	0	0	0	0
Haemorrhage, subcutaneous										
minimal	0	1	2	1	0	0	0	0	0	0
mild	0	0	0	1	1	0	0	0	1	0
Total Incidence	0	1	2	2	1	0	0	0	1	0
Dermatitis, ulcerative										
mild	0	0	0	0	1	0	0	0	0	1
moderate	0	0	0	0	1	0	0	0	0	2
Total Incidence	0	0	0	0	2	0	0	0	0	3
Epidermal hyperplasia										
mild	0	0	0	0	1	0	0	0	0	0
Total Incidence	0	0	0	0	1	0	0	0	0	0
Scab	0	0	0	0	4	0	0	0	0	0

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 2		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		15	3***	2***	0***	3	13	8*	8*	0***	2
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	2	3	0	0	4	1	2	1	
mild		0	7**	7**	5*	4	0	2	6*	4	
moderate		0	1	2	9***	5	0	0	5*	4	
marked		0	0	1	0	1	0	0	3	2	
Total Incidence		0	10***	13***	14***	10	0	6*	7**	14***	
Inflammation, mixed, subcutaneous											
minimal		0	0	0	0	0	0	0	1	0	
mild		0	0	0	0	0	0	0	1	0	
moderate		0	0	0	0	2	0	0	4	1	
Total Incidence		0	0	0	0	2	0	0	6*	1	
Inflammation, mononuclear cell, subcutaneous/muscle											
minimal		0	2	0	0	0	1	0	0	0	
mild		0	0	0	0	0	0	1	0	0	
moderate		0	0	0	0	0	0	0	2	0	
Total Incidence		0	2	0	0	0	1	1	2	0	
Fibrosis/fibropiasia, subcutaneous											
mild		0	0	1	1	2	0	0	1	0	
moderate		0	0	0	1	2	0	0	1	0	
Total Incidence		0	0	1	2	4	0	0	1	5	
Granulation tissue, subcutaneous											
mild		0	0	0	0	0	0	0	0	1	
Total Incidence		0	0	0	0	0	0	0	0	1	
Foamy macrophages, subcutaneous											
mild		0	0	0	0	0	0	0	0	1	
moderate		0	0	0	0	0	0	0	0	1	
Total Incidence		0	0	0	0	0	0	0	0	2	
Pigmented macrophages, subcutaneous											
minimal		0	0	0	0	0	0	0	1	0	
mild		0	0	0	0	0	0	0	0	5	
moderate		0	0	0	0	1	0	0	0	1	
Total Incidence		0	0	0	0	1	0	0	1	6	
Haemorrhage, subcutaneous											
minimal		0	0	0	0	2	0	0	1	1	
mild		0	0	0	1	3	0	0	0	1	
Total Incidence		0	0	0	1	5	0	0	1	2	
Fibrosis, dermal											
mild		0	0	0	0	1	0	0	0	0	
Total Incidence		0	0	0	0	1	0	0	0	0	

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 3		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		14	6**	1***	1***	4	14	9*	3***	0***	3
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	4	3	0	1	0	2	7**	2	1
mild		0	4	4	1	1	0	4	4	3	1
moderate		0	0	7**	9***	1	0	0	1	9***	1
marked		0	0	0	3	2	0	0	0	0	0
Total Incidence		0	8**	14***	13***	5	0	6*	12***	14***	3
Inflammation, mixed, subcutaneous											
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	1
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	3	0	0	0	0	1
Fibrosis/fibroplasia, subcutaneous											
mild		0	0	0	0	2	0	0	0	1	3
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	3	0	0	0	1	3
Foamy macrophages, subcutaneous											
mild		0	0	0	0	0	0	0	0	0	1
Total Incidence		0	0	0	0	0	0	0	0	0	1
Pigmented macrophages, subcutaneous											
minimal		0	0	0	0	0	0	0	0	0	1
mild		0	0	0	0	5	0	0	0	0	8
moderate		0	0	0	0	2	0	0	0	0	0
Total Incidence		0	0	0	0	7	0	0	0	0	9
Haemorrhage, subcutaneous											
minimal		0	1	0	0	0	0	0	0	1	0
mild		1	0	0	0	0	0	0	0	0	0
Total Incidence		1	1	0	0	0	0	0	0	1	0
Dermatitis, ulcerative											
mild		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0
Epidermal hyperplasia											
mild		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0
Fibrosis, dermal											
mild		0	0	0	0	2	0	0	0	0	1
Total Incidence		0	0	0	0	2	0	0	0	0	1

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 4		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		14	6**	2***	1***	6	13	8*	2***	0***	2
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	3	4	0	0	0	3	6*	1	0
mild		0	5*	2	1	0	0	3	2	0	4
moderate		0	0	4	7**	4	0	0	4	5*	5
marked		0	0	2	5*	2	0	0	0	7**	1
severe		0	0	0	0	0	0	0	0	1	0
Total Incidence		0	8**	12***	13***	6	0	6*	12***	14***	10
Inflammation, mixed, subcutaneous											
minimal		0	1	0	0	0	0	0	1	0	0
mild		0	0	0	0	1	0	0	0	0	0
moderate		0	0	0	1	0	0	0	0	4	0
Total Incidence		0	1	0	1	1	0	0	1	4	0
Inflammation, mononuclear cell, subcutaneous											
mild		0	0	0	2	1	0	0	0	0	2
Total Incidence		0	0	0	2	1	0	0	0	0	2
Fibrosis/fibroplasia, subcutaneous											
mild		0	0	0	1	0	0	0	1	1	0
moderate		0	0	0	1	2	0	0	0	1	0
Total Incidence		0	0	0	2	2	0	0	1	2	0
Granulation tissue, subcutaneous											
mild		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0
Foamy macrophages, subcutaneous											
mild		0	0	0	0	0	0	0	0	1	0
moderate		0	0	0	0	2	0	0	0	0	1
Total Incidence		0	0	0	0	2	0	0	0	1	1
Pigmented macrophages, subcutaneous											
minimal		0	0	0	0	0	0	0	0	0	2
mild		0	0	0	0	5	0	0	0	0	4
moderate		0	0	0	0	1	0	0	0	0	3
Total Incidence		0	0	0	0	6	0	0	0	0	9
Haemorrhage, subcutaneous											
minimal		0	1	1	0	0	0	0	0	0	0
mild		1	0	0	1	0	1	0	0	0	0
Total Incidence		1	1	1	1	0	1	0	0	0	0
Dermatitis, ulcerative											
minimal		0	0	0	0	0	0	0	1	0	0
mild		0	0	0	0	0	0	1	0	0	0
Total Incidence		0	0	0	0	0	0	1	1	0	0

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 5		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		15	4***	2***	0***	1	14	10*	2***	1***	1
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	5*	1	1	0	0	3	3	1	0
mild		0	4	9***	5*	3	0	2	7**	7**	5
moderate		0	0	3	6**	4	0	0	1	4	3
marked		0	0	0	2	3	0	0	0	1	2
Total Incidence		0	9***	13***	14***	10	0	5*	11***	13***	10
Inflammation, mixed, subcutaneous											
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	1
moderate		0	0	0	0	1	0	0	0	0	1
Total Incidence		0	0	0	0	3	0	0	0	0	2
Inflammation, subcutaneous, mononuclear cell											
minimal		0	1	0	0	0	0	0	1	0	0
mild		0	0	1	0	1	0	0	0	0	0
Total Incidence		0	1	1	0	1	0	0	1	0	0
Fibrosis/fibroplasia, subcutaneous											
minimal		0	0	0	0	0	0	0	1	0	0
mild		0	0	1	0	2	0	0	0	0	1
moderate		0	0	0	2	4	0	0	0	0	0
Total Incidence		0	0	1	2	6	0	0	1	0	1
Granulation tissue, subcutaneous											
mild		0	0	0	1	0	0	0	0	0	0
moderate		0	0	0	0	0	0	0	0	0	1
Total Incidence		0	0	0	1	0	0	0	0	0	1
Foamy macrophages, subcutaneous											
mild		0	0	0	0	1	0	0	0	0	2
moderate		0	0	0	0	1	0	0	0	0	4
Total Incidence		0	0	0	0	2	0	0	0	0	6
Pigmented macrophages, subcutaneous/muscle											
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	7	0	0	0	0	4
moderate		0	0	0	0	1	0	0	0	0	1
Total Incidence		0	0	0	0	9	0	0	0	0	5
Haemorrhage, subcutaneous											
minimal		0	1	0	0	0	0	0	0	1	2
mild		0	0	0	1	1	0	0	0	1	0
Total Incidence		0	1	0	1	1	0	0	0	2	2
Dermatitis, ulcerative											
marked		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 6		(15)	(15)	(15)	(14)	(13)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		15	8**	1***	0***	3	11	8	2***	1***	1
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	1	0	0	0	0	5*	2	0	0
mild		0	6*	11***	1	3	0	1	10***	5*	4
moderate		0	0	2	6**	1	0	0	1	4	3
marked		0	0	1	6**	0	0	0	0	4	1
Total Incidence		0	7**	14***	13***	4	0	6*	13***	13***	8
Inflammation, subcutaneous, mixed											
minimal		0	0	0	0	0	0	1	0	0	0
moderate		0	0	0	1	0	0	0	0	0	3
Total Incidence		0	0	0	1	0	0	1	0	0	3
Fibrosis/fibroplasia, subcutaneous											
mild		0	0	1	1	0	0	0	1	1	2
moderate		0	0	0	0	3	0	0	0	0	0
Total Incidence		0	0	1	1	3	0	0	1	1	2
Foamy macrophages, subcutaneous											
mild		0	0	0	1	1	0	0	0	0	2
moderate		0	0	0	0	0	0	0	0	0	1
Total Incidence		0	0	0	1	1	0	0	0	0	3
Pigmented macrophages, subcutaneous/muscle											
mild		0	0	0	0	3	0	0	0	0	7
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	4	0	0	0	0	7
Haemorrhage, subcutaneous											
minimal		0	0	1	0	1	1	1	1	0	0
mild		0	0	1	0	0	0	2	1	2	2
Total Incidence		0	0	2	0	1	1	3	2	2	2
Dermatitis, ulcerative											
mild		0	0	0	1	1	0	0	0	0	1
moderate		0	0	0	0	1	0	0	0	0	1
Total Incidence		0	0	0	1	2	0	0	0	0	2
Epidermal hyperplasia											
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0
Fibrosis/inflammation, dermal											
mild		0	0	0	0	2	0	0	0	0	0
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	3	0	0	0	0	0

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

Special Evaluation – None other than already listed.

Toxicokinetics – None conducted.

Dosing Solution Analysis - Test drug and CAM2038 q1w placebo (FCV, 202IAS-1) were used within expiration dates. The analysis was conducted under a separate protocol which was not found/referenced, which is not considered a critical issue considering the nature of the test materials.

GENETIC TOXICOLOGY

The Applicant complete a full standard battery of genetic toxicity studies with GDO (see table). All studies demonstrated a negative result under the conditions tested.

Study number/description	Doses	Results
TO-04-141: GLP <i>in vitro</i> reverse mutation in bacteria (Ames)	Glycerol dioleate (95.3 %): 0, 50, 160, 500, 1600, 5000 mcg/plate ± S9	Negative
TO-04-146: GLP <i>in vitro</i> reverse mutation in bacteria (Ames)	Glycerol dioleate (92.1 %): 0, 50, 160, 500, 1600, 5000 mcg/plate ± S9	Negative
TO-04-142: GLP <i>in vitro</i> gene mutation assay in mouse lymphoma cells (L5178Y TK ^{+/+})	Glycerol dioleate (95.3 %): 0, 156, 313, 625, 1250, 2500 and 5000 mcg/mL. - S9: 4 and 21 hours, +S9: 3 hours.	Negative
TO-12-446: GLP <i>in vivo</i> rat micronucleus assay	Glycerol dioleate (not reported*): 0, 500, 1000 and 2000 mg/kg SC in male and female rats (2 doses, 24 hours apart, n = 5/sex/group). Animals sacrificed 48 hours after first dose.	Negative

* - same batch as used in 26-week SC study in rats

NOTE: Genetic Toxicology studies were reviewed by Dr. Nikunj Patel of the Division of Anesthesia, Analgesia, and Addiction Products.

***In Vitro* Reverse Mutation Assays in Bacterial Cells (Ames)**

Study title: (b) (4) (Glycerol dioleate, (b) (4) %) - AMES TEST

Study no.: TO-04-141
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Not stated
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4) (Glycerol dioleate, (b) (4) %), Lot 2292/132, purity 95.3%

Key Study Findings

- All criteria were met for a valid assay.
- Glycerol dioleate concentrations of up to 5000 mcg/plate were negative for mutagenic potential in the bacterial reverse mutation assay under the conditions tested.

Methods

Strains: *Salmonella typhimurium* strains TA102, TA100, TA98, TA1537, and TA1535.
 Concentrations in definitive study: 50, 160, 500, 1600, and 5000 mcg/plate.
 Basis of concentration selection: A preliminary dose-range finding toxicity study conducted using the plate-incorporation method in bacterial strain TA98 with and without S9 showed no

toxicity at glycerol dioleate doses of up to 5000 mcg/plate (no marked reductions in number of revertants or reduced growth of background lawn of non-revertants). Based on these results, 5000 mcg/plate was chosen as the highest dose in the definitive study.

Negative control: DMSO (100 mcL/plate)

Positive control: See table below

Formulation/Vehicle: DMSO

Incubation & sampling time: The plate-incorporation and pre-incubation methods were used for this assay. In each test, the plates were incubated at 37°C for approximately 72 hours, and the number of revertant colonies on each plate was counted.

Plate incorporation: A series of test tubes was prepared containing test item formulation (100 mcL), vehicle (100 mcL), or positive control solution (50 or 100 mcL), S-9 mix or 0.02 M phosphate buffer pH 7.4 (500 mcL), bacterial suspension (100 mcL, approximately 10⁹ bacteria/mL), and top agar (2 mL). After vortex mixing, the mixtures were spread on selective agar plates.

Pre-incubation: A series of test tubes was prepared containing test item formulation (100 mcL), vehicle (100 mcL), or positive control solution (25, 50, or 100 mcL), S-9 mix or 0.02 M phosphate buffer pH 7.4 (500 mcL), and bacterial suspension (100 mcL, approximately 10⁹ bacterial mL). Each test tube was incubated for 1 hour at 37°C with gentle shaking. Top agar (2 mL) was added, and after vortexing, the mixtures were spread on selective agar plates.

Positive Controls for the *in vitro* bacterial reverse mutation assays

Tester Strain(s)	S9	Positive Control	Concentration (mcg/plate)
TA102	-	Cumene hydroperoxide	Plate incorporation: 100 Pre-incubation: 25
TA100, TA1535	-	Sodium azide	1
TA98	-	2-nitrofluorene	1
TA1537	-	N-aminoacridine	80

TA100, TA98, TA1537, TA1535	+	2-aminoanthracene	2
TA102	+	2-aminoanthracene	4

Study Validity

The definitive studies utilized the plate incorporation and pre-incubation methods to examine the mutagenicity induction potential of glycerol dioleate on five strains of *S. typhimurium* (TA102, TA100, TA98, TA1537, and TA1535). The Sponsor examined an adequate number and type of strains required for a full bacterial reverse mutation battery. The concentrations of glycerol dioleate used were adequate to have at least three nontoxic doses for each strain tested with and without metabolic activation (no toxicity was observed at any of the doses tested). Each glycerol dioleate concentration, positive, and negative controls were tested in triplicate, in the presence or absence of S9 mix. Plates were examined to assess the quality of the background lawn and colonies. Revertant colonies were counted and compared to the negative controls for determination of statistically significant differences using ANOVA test, followed by Dunnett's test if statistically significant differences were shown with ANOVA. No precipitation of glycerol dioleate was observed at any concentration tested up to the 5000 mcg/plate maximal dose (limit dose per the ICH S2 (R1) Guidance). The assays were all considered valid based on the following criteria: the negative control counts fell within the normal ranges of laboratory historical controls, and all positive controls produced increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain. For all tester strains, the test article was considered to be positive for inducing mutations if the number of revertants showed a dose-dependent, reproducible, and statistically significant increase to at least 1.5-fold the concurrent negative controls. All criteria for a valid assay were met.

Results

No reductions in revertant colony counts occurred following exposure of any bacterial strain in the absence or presence of S9, indicating that glycerol dioleate was not toxic to any of the bacterial strains up to the maximum dose of 5000 mcg/plate. No precipitation of the test article was observed in the assay. All positive and negative controls were within acceptable ranges. In strain TA1537, glycerol dioleate caused a statistically significant increase in revertant colonies at 5000 mcg/plate with S9 in the plate incorporation test, however this increase was only 1.29-fold higher than the corresponding negative control value and therefore not considered to be positive for mutagenic activity. Glycerol dioleate did not increase mutant frequency by 1.5-fold over negative control values in any tester strain, with or without metabolic activation. Therefore, glycerol dioleate was not found to be mutagenic in this *in vitro* bacterial reverse mutagenicity assay.

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Study title: (b) (4) (Glycerol dioleate, (b) (4) %) – AMES TEST

Study no.: TO-04-146

Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Not stated
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4) (Glycerol dioleate, (b) (4) %), Lot 2292/124-4, purity 92.1%

Key Study Findings

- All criteria were met for a valid assay.
- Glycerol dioleate concentrations of up to 5000 mcg/plate were negative for mutagenic potential in the bacterial reverse mutation assay under the conditions tested.

Methods

Strains: *Salmonella typhimurium* strains TA102, TA100, TA98, TA1537, and TA1535.

Concentrations in definitive study: 50, 160, 500, 1600, and 5000 mcg/plate.

Basis of concentration selection: A preliminary dose-range finding toxicity study conducted using the plate-incorporation method in bacterial strain TA98 with and without S9 showed no toxicity at glycerol dioleate doses of up to 5000 mcg/plate (no marked reductions in number of revertants or reduced growth of background lawn of non-revertants). Based on these results, 5000 mcg/plate was chosen as the highest dose in the definitive study.

Negative control: DMSO (100 mcL/plate)

Positive control: See table below

Formulation/Vehicle: DMSO

Incubation & sampling time: The plate-incorporation and pre-incubation methods were used for this assay. In each test, the plates were incubated at 37°C for approximately 72 hours, and the number of revertant colonies on each plate were counted.

Plate incorporation: A series of test tubes was prepared containing test item formulation (100 mcL), vehicle (100 mcL), or positive control solution (50 or 100 mcL),

S-9 mix or 0.02 M phosphate buffer pH 7.4 (500 mL), bacterial suspension (100 mL, approximately 10^9 bacteria/mL), and top agar (2 mL). After vortex mixing, the mixtures were spread on selective agar plates.

Pre-incubation: A series of test tubes was prepared containing test item formulation (100 mL), vehicle (100 mL), or positive control solution (25, 50, or 100 mL), S-9 mix or 0.02 M phosphate buffer pH 7.4 (500 mL), and bacterial suspension (100 mL, approximately 10^9 bacterial mL). Each test tube was incubated for 1 hour at 37°C with gentle shaking. Top agar (2 mL) was added, and after vortexing, the mixtures were spread on selective agar plates.

Positive Controls for the *in vitro* bacterial reverse mutation assays

Tester Strain(s)	S9	Positive Control	Concentration (mcg/plate)
TA102	-	Cumene hydroperoxide	Plate incorporation: 100 Pre-incubation: 25
TA100, TA1535	-	Sodium azide	1
TA98	-	2-nitrofluorene	1
TA1537	-	N-aminoacridine	80
TA100, TA98, TA1537, TA1535	+	2-aminoanthracene	2
TA102	+	2-aminoanthracene	4

Study Validity

The definitive studies utilized the plate incorporation and pre-incubation methods to examine the mutagenicity induction potential of glycerol dioleate on five strains of *S. typhimurium* (TA102, TA100, TA98, TA1537, and TA1535). The Sponsor examined an adequate number and type of strains required for a full bacterial reverse mutation battery. The concentrations of glycerol dioleate used were adequate to have at least three nontoxic doses for each strain tested with and without metabolic activation (no toxicity was observed at any of the doses tested). Each glycerol dioleate concentration, positive, and negative controls were tested in triplicate, in the presence or absence of S9 mix. Plates were examined to assess the quality of the background lawn and colonies. Revertant colonies were counted and compared to the negative controls for

determination of statistically significant differences using the ANOVA test, followed by Dunnett's test if statistically significant differences were shown. No precipitation of glycerol dioleate was observed at any concentration tested up to the 5000 mcg/plate maximal dose (limit dose per the ICH S2 (R1) Guidance). The assays were all considered valid based on the following criteria: the negative control counts fell within the normal ranges of laboratory historical controls, and all positive controls produced increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain. For all tester strains, the test article was considered positive for inducing mutations if the number of revertants showed a dose-dependent, reproducible, and statistically significant increase to at least 1.5-fold the concurrent negative controls. All criteria for a valid assay were met.

Results

No reductions in revertant colony counts occurred following exposure of any bacterial strain in the absence or presence of S9, indicating that glycerol dioleate was not toxic to any of the bacterial strains up to the maximum dose of 5000 mcg/plate. No precipitation of the test article was observed in the assay. All positive and negative controls were within acceptable ranges. Glycerol dioleate not increase mutant frequency by 1.5-fold of negative control values in any tester strain, with or without metabolic activation. Therefore, glycerol dioleate was not found to be mutagenic in this *in vitro* bacterial reverse mutagenicity assay.

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In Vitro Assay in Mammalian Cells

Study title: (b) (4) (Glycerol dioleate, (b) (4) %) - *IN VITRO*
MAMMALIAN CELL GENE MUTATION TEST PERFORMED WITH
MOUSE LYMPHOMA L5178Y CELLS

Study no.:	TO-04-142
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not stated
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) (Glycerol dioleate, (b) (4) %), Lot 2292/132, purity 95.3 %

Key Study Findings

- All criteria were met for a valid assay
- Glycerol dioleate concentrations of up to 5000 mcg/mL were negative for mutagenic potential

Methods

Cell line: Mouse lymphoma cell line L5178Y TK^{+/-}

Concentrations in definitive study: – S9: 4 and 21 hours, +S9; 3 hours: 156, 313, 625, 1250, 2500 and 5000 mcg/mL.
Exception: the 156 mcg/mL dose was omitted from the second duplicate with S9.

Basis of concentration selection: A preliminary dose-range finding study was not indicated in the study report. The Sponsor indicated that the highest concentration of test-article used (5000 mcg/mL) is the maximum required by the OECD and ICH Guidelines and that the test article concentrations were separated by factors of 2.

Negative control: DMSO

Positive control: – S9; 4 and 21 hours: N-ethyl-N-nitrosourea (50 mcg/mL)
+ S9; 3 hours: 7,12 -dimethyl-1 ,2-benzanthracene (3.3 mcg/mL)

Formulation/Vehicle: DMSO

Incubation & sampling time: Duplicate cultures were used for each treatment. For cell cultures tested in the absence of S9 mix, cells (4.5×10^6) were suspended in an appropriate volume of normal medium and the vehicle (100 mL), test article solution (100 mL), or positive control solution (50 mL) were added. The cultures were incubated at 37°C with shaking for 4 or 24 hours in a final volume of 10 mL.

For cell cultures tested in the presence of S9 mix, cells (4.5×10^6) were suspended in an appropriate volume of normal medium and the vehicle (50 mL), test article solution (50 mL), or positive control solution (17 mL), and S9 mix (1 mL) were added. The cultures were incubated at 37°C with shaking for 3 hours in a final volume of 5 mL.

After the treatment periods, cultures were centrifuged and the supernatant replaced with fresh complete medium (15 mL per culture). Resuspended cultures were transferred to flasks and incubated for

a period of 3 days (counted from the start of the experiment). Cell densities were adjusted daily to a density of 3×10^5 cells/mL. After the 3-day incubation, aliquots of cultures were plated in medium containing trifluorothymidine for determination of mutant counts and cloning efficiency. Plates were incubated for 10 days and the mutant colonies were counted to determine mutant frequency. Large and small colonies were counted separately since large colonies have small changes to DNA (e.g., point mutations) whereas small colonies have larger defects such as chromosome aberrations. Large colonies were categorized as being diffuse and covering >50 % of the base of the culture well, whereas small colonies were more dense and covered < 25% of the well.

Study Validity

The study is valid based upon the following criteria: 1) the mutation frequencies of the negative and positive controls were within the range of the historical control data, 2) appropriate positive controls were used, which induced significant increases in mutation frequencies compared to the concurrent negative controls, 3) an appropriate rodent cell line was used (L5178Y), 4) the highest concentration of test article used in the study (5000 mcg/mL) is the maximum required by OECD and ICH guidelines, and 5) at least 3 analyzable concentrations were used.

According to the protocol, the test article would be considered to have shown mutagenic activity if the following criteria were met: statistically significant, dose-dependent and reproducible increases in mutant frequency in treated cultures compared to negative controls, at least 10% relative growth of the treated group at the end of the treatment period, and mean mutation frequency of at least 125 mutants/10⁶ cells higher in treated groups as compared to the negative control. The criteria for evaluation of mutagenic activity are considered valid.

Results

Glycerol dioleate was not toxic in the absence or presence of S9 (no reductions in relative total growth). Positive control cultures exhibited clear increases in mutation frequency. Mutation frequencies of negative and positive control cultures were within or close to the historical control range from the previous 10 studies performed by the testing laboratory and are considered acceptable. The test-article did not induce statistically significant increases in mutation frequency under the assay conditions.

In conclusion, glycerol dioleate was negative for mutagenic activity in this *in vitro*

mammalian cell gene mutation assay with mouse L5178Y cells.

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In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Glycerol Dioleate: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow: 0 h and 24 h Subcutaneous Injection Dosing and 48 h Sampling

Study no:	TO-12-446
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/11/12
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Glycerol dioleate, Lot 3002413, purity NR

Key Study Findings

- The criteria for a valid assay were met. Animals were adequately dosed according to a limit dose of 2000 mg/kg.
- Glycerol dioleate was negative for inducing the formation of micronuclei in the bone marrow erythrocytes of male and female rats after 2 subcutaneous doses of up to 2000 mg/kg, 24 hours apart.

Methods

Doses in definitive study:	0 and 24 hours: 0, 500, 1000, and 2000 mg/kg
Frequency of dosing:	Twice (one dose at 0 hours and one dose at 24 hours)
Route of administration:	Subcutaneous injection
Dose volume:	0.53, 1.06, and 2.12 mL/kg for 500, 1000, and 2000 mg/kg doses, respectively and 2.12 and 10 mL/kg for negative and positive controls, respectively
Formulation/Vehicle:	Glycerol dioleate, sterile (GDO)
Species/Strain:	Wistar (Han) rats (CrI:WI(Han))
	Approximately 6 weeks of age at study start
	Males: 156.3-189.7 g, Females: 120.2-153.7 g
Number/Sex/Group:	Glycerol dioleate and negative control (saline) groups: 5/sex/group
Satellite groups:	Positive control group n = 3/sex
Basis of dose selection:	A preliminary dose-range finding study was conducted in which male and female rats (1/sex) were dosed subcutaneously with 2000 mg/kg

glycerol dioleate in a dose volume of 2.12 mL/kg, at 0 and 24 hours. Rats were observed for clinical signs and mortality for up to 3 days after the second dose. No signs of toxicity were observed and there were no mortalities during the 3-day observation period. Therefore, the Sponsor considered the maximum recommended dose of 2000 mg/kg acceptable for the micronucleus test based upon limit dose recommended in OECD guidelines.

Negative control: Saline, dosed at the same time point as the test article

Positive control: Cyclophosphamide 50 mg/kg, dosed at the same time points as the test article. N = 3/sex.

Study Validity

For the micronucleus test, Wistar rats (5/sex/group) were administered glycerol dioleate subcutaneously at doses of 0 (negative control, saline), 500, 1000, and 2000 mg/kg at 0 and 24 hours. A separate group of rats (3/sex) was dosed with the positive control (50 mg/kg cyclophosphamide) at the same time points. The doses of glycerol dioleate, positive, and negative controls were appropriate for the test to be considered valid. Bone marrow was collected from the femur of each rat 48 hours after the first dose and bone marrow smears were prepared for examination according to an established protocol. Bone marrow slides were examined by blinded operators. At least 2000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei and the frequency of micronucleated cells (MN-PCE). The PCE/NCE (normochromatic) ratio was determined by counting a minimum total of 1000 erythrocytes (PCE + NCE) per marrow preparation to assess potential systemic toxicity. The assay was considered valid based upon the following criteria: 1) administration of the test-article at the limit dose twice (24 hours apart) with sample collection occurring 24 hours after the second dose, and administration of negative and positive controls at the same time points, 2) the species (male and female Wistar rats) and the number of animals per group for the test-article (n = 5/sex), 3) a total of 2000 PCE per animal were examined for presence of micronuclei, 4) mean values of MN-PCE for negative controls were within the expected historical control range for the Wistar rats in the testing laboratory, and 5) the positive control (cyclophosphamide) exhibited an appropriate response in significantly increasing the number of MN-PCE. All criteria for a valid assay were met.

The test item was judged positive if an increase in the number of micronucleated polychromatic erythrocytes (MN-PCE) was obtained for one or more of the test item-treated dose groups (an increase greater than 10% over the expected historical control ranges for a group of animals). A negative result was indicated if no evident increases in the number MN-PCE relative to historical control frequencies.

Results

The results of the *in vivo* micronucleus test are summarized in the table below excerpted from the Sponsor's submission). The mean % MN-PCE in the negative control group was within the ranges observed in historical control animals of the same strain from the testing laboratory. The positive control induced strong and clear increased in % MN-PCE which was accompanied by some bone marrow toxicity, as shown by a suppression of the PCE/NCE ratios as compared to the negative control group.

Animals treated with glycerol dioleate did not show any significant increases in the % MN-PCE and individual and mean values for % MN-PCE were close to or within the historical range for control animals. Some indication of bone marrow toxicity was observed in the high-dose male group, as noted by a suppression of the PCE/NCE ratio, although a large variation within the group precluded a definitive determination of toxicity.

In conclusion, glycerol dioleate administered subcutaneously in rats did not induce genotoxic activity in this *in vivo* rat bone marrow micronucleus test when tested up to a limit dose of 2000 mg/kg.

Summary of results from the *in vivo* rat micronucleus assay (table excerpted from Sponsor's submission)

Treatment	Dose (h)	Sex	No. of Rats Scored	Erythrocytes				
				Normochromatic Cells (NCE)	Polychromatic Cells (PCE)			PCE/NCE Mean ± S.D.
					No. of MN-NCE	PCE Analysed	No. of MN-PCE	
Saline (2.12 mL/kg/day)	0 + 24	M	5	2	10002	5	0.05	0.81 ± 0.12
		F	5	1	10005	4	0.04	0.84 ± 0.14
		M/F	10	3	20007	9	0.04	0.83 ± 0.12
Glycerol Dioleate (500 mg/kg/day)	0 + 24	M	5	0	10005	5	0.05	0.83 ± 0.08
		F	5	4	10002	2	0.02	0.86 ± 0.07
		M/F	10	4	20007	7	0.03	0.84 ± 0.07
Glycerol Dioleate (1000 mg/kg/day)	0 + 24	M	5	0	10006	1	0.01	0.80 ± 0.13
		F	5	1	10002	1	0.01	0.83 ± 0.11
		M/F	10	1	20008	2	0.01	0.81 ± 0.11
Glycerol Dioleate (2000 mg/kg/day)	0 + 24	M	5	4	10000	4	0.04	0.66 ± 0.16
		F	5	0	10002	3	0.03	0.81 ± 0.15
		M/F	10	4	20002	7	0.03	0.74 ± 0.16
Cyclophosphamide (50 mg/kg/day)	0 + 24	M	3	8	6005	98	1.63 Φ	0.66 ± 0.03
		F	3	16 α	6004	102	1.70 Φ	0.61 ± 0.03
		M/F	6	24	12009	200	1.67 Φ	0.63 ± 0.04

PCE = Polychromatic erythrocytes
MN-PCE = Micronucleated PCE

NCE = Normochromatic erythrocytes
MN-NCE = Micronucleated NCE
Φ = Positive response in PCE
α = Evident response in NCE

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CARCINOGENICITY

The Applicant did not conduct carcinogenicity studies for GDO. Rather, they referenced two published studies with DAG in mice and rats (Chengelis et al., 2006b, a). These publications were reviewed by R. Daniel Mellon, PhD.

Study Title: Chengelis CP, Kirkpatrick JB, Bruner RH, Freshwater L, Morita O, Tamaki Y, Suzuki H (2006a) A 24-month dietary carcinogenicity study of DAG (diacylglycerol) in rats. Food Chem Toxicol 44:98-121.

In the published GLP rat study intended primarily to compare the effects of dietary triglycerides (TG) to diacylglycerides (DAG) on carcinogenicity, rats were treated with diets supplemented with TG and DAG for 2 years as per the following table from the publication:

Figure 8. Treatment Groups; 2-year Rat Carcinogenicity Study DAG (Chengelis et al., 2006a)

Experimental design of a 24-month dietary carcinogenicity study of DAG in rats

Group	Treatment	Dietary fat/oil level			Number of animals	
		Standard (%) ^a	TG (%) ^b	DAG (%) ^b	Male	Female
<i>Dietary optimization groups</i>						
1	Control-1	4.5	–	–	50	50
2	Control-2	–	5.5	0	50	50
3	DAG-low	–	4.5	1	50	50
4	DAG-mid	–	2.75	2.75	50	50
5	DAG-high	–	0	5.5	50	50
<i>Ad libitum groups</i>						
6	Control-3	–	5.5	0	65	65
7	DAG-high	–	0	5.5	65	65

^a Diet was standard basal diet (PMI Certified Rodent Diet 5002) with 4.5% fat content.

^b Diet was WIL PMI Certified Rodent Diet 7247/1004740 with 5.5% fat content.

The composition of the oils tested are depicted below from the publication. As noted below, both the DAG and the TG groups contained comparable levels of oleic acid unsaturated fatty acids.

Figure 9. Fat Composition; 2-year Rat Carcinogenicity Study DAG (Chengelis et al., 2006a)

Composition of DAG and TG oils used on a dietary 24-month carcinogenicity study of DAG in rats

	DAG	TG
Monoglycerides (%)	0.90–2.90	0–1.08
Diglycerides (%)	82.85–83.94	0.57–3.16
Triglycerides (%)	12.77–15.18	95.32–98.35
Saturated fatty acids (%)		
Palmitic C16:0	2.89–3.04	5.23–5.62
Stearic C18:0	1.07–1.16	2.03–2.15
Unsaturated fatty acids (%)		
Oleic C18:1	35.62–37.60	32.77–34.11
Linoleic C18:2	47.71–48.62	47.72–49.12
Linolenic C18:3	6.31–6.49	6.98–8.26

Based on the composition of the DAG and TG oils used and food consumption, as reported in the publication, the dose of DAG in this study was determined, as depicted in the table below:

Figure 10. Mean DAG Consumption; 2-year Rat Carcinogenicity Study (Chengelis et al., 2006a)

Average test article consumption during a 24-month dietary carcinogenicity study of DAG in rats

Group	Regimen	Dietary fat/oil level		Average calculated DAG consumption (mg/kg/day)	
		TG (%)	DAG (%)	Males	Females
3	Restricted	4.5	1	356.2	477.5
4	Restricted	2.75	2.75	984.8	1326.2
5	Restricted	0	5.5	1982.4	2645.1
7	Ad libitum	0	5.5	1946.3	2507.2

Animals that were dosed with DAG/TG diets demonstrated lower survival, higher body weights and organ weights, changes in clinical chemistry, and increased incidence pituitary and mammary gland tumors compared to controls. All of these findings are consistent with a high fat diet. The authors conclude that DAG was no different than triglycerides with respect to tumor risk. The incidence of tumors in the study are depicted in the table below from the publication. The authors report that none of the neoplastic findings were statistically significant for common tumors. They employed statistical methods in accordance with published FDA guidelines and analyzed combined tumor types in accordance with McConnell (McConnell et al., 1986), consistent with current Agency practice on combining tumor incidence.

Figure 11. Tumor Incidence; 2-year Rat Carcinogenicity Study DAG (Chengelis et al., 2006a)

Incidence of primary neoplastic microscopic findings in a 24-month dietary carcinogenicity study of DAG in rats: all necropsies combined

	Group													
	0% DAG/ 0% TG ^a		0% DAG/ 5.5% TG ^b		1% DAG/ 4.5% TG ^b		2.75% DAG/ 2.75% TG ^b		5.5% DAG/0% TG ^b		5.5% TG ad lib ^b		5.5% DAG ad lib ^b	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
<i>Mammary gland</i>														
Number examined	36	47	26	48	18	47	29	47	26	47	24	60	31	63
Adenoma	0	0	0	1	0	1	0	3	0	1	0	4	0	2
Adenoma, multiple	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fibroadenoma	0	18	0	14	0	15	0	7	1	13	1	15	1	13
Fibroadenoma, multiple	0	4	0	3	0	4	0	5	0	4	0	4	0	2
Adenocarcinoma	1	6	0	10	0	9	0	12	1	14	3	13	0	8
Adenocarcinoma, multiple	0	4	0	6	0	6	0	8	0	8	0	4	0	6
Adenoacanthoma	0	0	0	1	0	2	0	2	0	0	0	0	0	0
Adenoacanthoma, multiple	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Overall mammary tumor burden	1	32	0	35	0	39	0	37	2	40	4	41	1	32
Percent affected (%)	3	68	0	73	0	83	0	79	8	85	17	68	3	51
<i>Pituitary gland</i>														
Number examined	48	49	50	50	42	48	41	48	50	50	64	64	64	65
Adenoma	27	37	37	37	26	40	32	39	33	37	49	53	45	56
Carcinoma	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Percent affected (%)	56	76	74	74	62	83	78	83	66	76	77	83	70	86

N = number of rats/sex/group; M = males; F = females.

^a Control group fed standard basal diet with 4.5% fat content on restricted feeding regimen.

^b Diet was WIL Rodent Diet 7247/1004740 with 5.5% fat content, Groups 2–5 on restricted feeding regimen, Groups 6–7 ad libitum-fed.

With respect to the relevance of these findings to the safety of CAM2038, it was not clear in the first cycle how the exposure to GDO in humans compared to the estimated doses of GDO via DAG in the rat study above. However, if the q1w formulation were used chronically, and assuming the GDO were to be released ~equally from the injection site to the systemic circulation on any given day, the human would be exposed to (b) (4) mg/m² GDO per day ((b) (4) mg/week = (b) (4) mg/60 kg/day x (b) (4) = (b) (4) mg/m²/day). The low dose of DAG in males ((b) (4) mg/kg) is (b) (4) mg/m² based on body surface area comparisons. Even if we adjust this dose based on 35.62% oleic acid fatty acid content in the dietary study material tested, that would be (b) (4) mg/m² oleic acid, which is still roughly (b) (4) times the estimated mean daily dose of GDO in humans.

In the second cycle, the Applicant provided an assessment of the GDO present in the DAG oil material tested based on a probably estimate using data from Hatzakis. The material tested in this dietary study was obtained (b) (4)

Based on the levels of oleic acid reported in this paper and using the probability estimate of GDO content in the DAG oil tested based on food consumption in the males (1946 mg/kg/day DAG oil) would be ~204 mg/kg of GDO (HED of 1978 mg based on BSA) which is ~53x the estimated daily human dose of 37.14 mg. Although not definitive, these values suggest that the study is reasonably adequate to support this requirement for a novel excipient in this drug product.

Study Title: Chengelis CP, Kirkpatrick JB, Bruner RH, Freshwater L, Morita O, Tamaki Y, Suzuki H (2006b) A 24-month dietary carcinogenicity study of DAG in mice. Food Chem Toxicol 44:122-137.

In a companion study to compare the carcinogenic potential of DAG to TG, mice were treated via the diet with up to 6% DAG oil for 2 years. Triglycerides (triacylglycerol oil) was used as the control article for fat intake. A separate control group was given the standard rodent diet (fat content of 4.5%).

Figure 12. Treatment Groups; 2-year Mouse Carcinogenicity Study DAG (Chengelis et al., 2006b)

Experimental design of a 24-month dietary carcinogenicity study of DAG in mice

Group	Treatment	Dietary fat/oil level			Number of animals	
		Standard (%)	TG (%)	DAG (%)	Males	Females
1	Control-1 ^a	4.5	0	0	50	50
2	Control-2 ^b	0	6.0	0	50	50
3	DAG-low ^b	0	4.5	1.5	50	50
4	DAG-mid ^b	0	3.0	3.0	50	50
5	DAG-high ^b	0	0	6.0	50	50

^a Diet was standard basal diet (PMI Certified Rodent Diet 5002) with 4.5% fat content.

^b Diet was WIL PMI Certified Rodent Diet 7247/1004740 with 6.0% fat content.

Figure 13. DAG Composition; 2-year Mouse Carcinogenicity Study DAG (Chengelis et al., 2006b)

Composition of DAG and TG oils used on a 24-month dietary carcinogenicity study of DAG in mice

	DAG	TG
Monoglycerides (%)	0.90–2.90	0–1.08
Diglycerides (%)	82.85–83.94	0.57–3.16
Triglycerides (%)	12.77–15.18	95.32–98.35
Saturated fatty acids (%)		
Palmitic C16:0	2.89–3.04	5.23–5.62
Stearic C18:0	1.07–1.16	2.03–2.15
Unsaturated fatty acids (%)		
Oleic C18:1	35.62–37.60	32.77–34.11
Linoleic C18:2	47.71–48.62	47.72–49.12
Linolenic C18:3	6.31–6.49	6.98–8.26

Figure 14. DAG Consumption; 2-year Mouse Carcinogenicity Study (Chengelis et al., 2006b)

Average DAG consumption during a 24-month dietary carcinogenicity study of DAG in mice

Group	Dietary fat/oil level		Average calculated DAG consumption (mg/kg/day)	
	TG (%)	DAG (%)	Males	Females
3	4.5	1.5	1792	2509
4	3.0	3.0	3773	5286
5	0	6.0	7412	9796

The results of the study report that the effects of the DAG diet were not substantially different than the effects of the TG diet. Both higher fat diets decreased survival, increased body weights, lowered food consumption, and increased macroscopic and microscopic findings consistent with a high fat diet. As noted in the table below, there was an increased incidence of liver tumors in male mice; however, the authors attributed this to the increased lipid intake rather than specifically to DAG. It is not clear from the publication if these changes were statistically significant or not. The publication reports that the historical control incidence in the laboratory for male hepatocellular adenomas and carcinomas is 8% and 3%, respectively.

Figure 15. Tumor Incidence; 2-year Mouse Carcinogenicity Study DAG (Chengelis et al., 2006b)

Incidence of neoplasms in mice given diets containing 6.0% fat (DAG and/or TG) or the standard diet (4.5% fat) for 24 months

Finding	Group									
	0% DAG/0% TG ^a		0% DAG/6.0% TG ^b		1.5% DAG/4.5% TG ^b		3.0% DAG/3.0% TG ^b		6.0% DAG/0% TG ^b	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Liver										
Number examined	50	50	50	50	42	41	38	41	50	50
Hepatocellular adenoma	0	1	3	1	4	4	1	0	6	2
Hepatocellular carcinoma	2	1	8	0	11	2	6	0	4	0
Systemic										
Number examined	50	50	50	50	50	50	50	50	50	50
Histiocytic sarcoma	1	7	0	0	0	2	0	7	0	4

^a Diet was standard basal diet (PMI Certified Rodent Diet 5002) with 4.5% fat content.

^b Diet was WIL PMI Certified Rodent Diet 7247/1004740 with 6.0% fat content.

With respect to the relevance of these findings to the safety of CAM2038, it was not clear in the first cycle how the exposure to GDO in humans compared to the estimated doses of GDO via DAG in the mouse study above. However, if the q1w formulation were used chronically (worst-case exposure), and assuming the GDO were to be released ~equally from the injection site to the systemic circulation on any given day,

the human would be exposed to (b) (4) mg/m² GDO per day ((b) (4) mg/week = (b) (4) mg/60 kg/day x (b) (4) = (b) (4) mg/m²/day). The low dose of DAG in males ((b) (4) mg/kg) is (b) (4) mg/m² based on body surface area comparisons. Even if we adjust this dose based on 35.62% oleic acid fatty acid content in the diet, that would be (b) (4) mg/m² oleic acid, which is still roughly (b) (4) times the estimated mean daily dose of GDO in humans.

In the second cycle, the Applicant provided an assessment of the GDO present in the DAG oil material tested based on a probably estimate using data from Hatzakis. The material tested in this dietary study was obtained (b) (4)

(b) (4) Based on the levels of oleic acid reported in this paper and using the probability estimate of GDO content in the DAG oil tested based on food consumption in the males ((b) (4) mg/kg/day DAG oil) would be ~ (b) (4) mg/kg of GDO (HED of (b) (4) mg based on BSA) which is (b) (4) x the estimated daily human dose of (b) (4) mg. Although not definitive, these values suggest that the study is reasonably adequate to support this requirement for a novel excipient in this drug product.

Conclusions of the Carcinogenicity Data. Although no carcinogenicity studies for GDO have been completed, it is a diglyceride and should be metabolically cleared via typical fat metabolic pathways. The data with DAG suggest the potential for increases in tumors consistent with a high fat diet and do not suggest anything unexpected. The DAG oil contains high levels of oleic acid fatty acids making it a reasonable surrogate for GDO. DAG and GDO would likely be cleared in a similar manner. Given the relatively low daily levels and rotating sites of administration compared to the dietary levels the animals were exposed to, the risk of any tumor from the GDO appears minimal to none. Given these data, dedicated carcinogenicity studies, consistent with the parameters outlined in the FDA guidance to industry on novel excipients are not warranted (i.e., negative genetic toxicology data, limited systemic exposure, absence of accumulation, negative histopathology data from chronic toxicology studies, and knowledge of other excipients in the same class).

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

The Applicant conducted a GLP embryofetal development study in rabbit and a GLP pre- and post-natal developmental toxicity study in rat for GDO. To address the remaining portions of the standard battery of reproductive and developmental toxicity studies for GDO, they are relying upon publications for the surrogate DAG oil (Morita et al., 2008a, Morita et al., 2008b).

Note: This published literature study is a combined Fertility and Early Embryonic Development study and Pre- and Post-Natal Development study

Study title: Morita O, Knapp JF, Tamaki Y, Nemec MD, Varsho BJ, Stump DG (2008a) Safety assessment of dietary diacylglycerol oil: a two-generation

reproductive toxicity study in rats. Food Chem Toxicol 46:3059-3068.

Study report location:	N/A
Conducting laboratory and location:	Global R&D Safety Science, Kao Corporation, Japan WIL Research Laboratories, LLC, Ashland, OH, United States
Date of study initiation:	Unknown
GLP compliance:	Yes
QA statement:	Not reported
Drug, lot #, and % purity:	The article states: DAG oil was prepared by esterifying glycerol with free fatty acids from rapeseed oil and soybean oil using the method reported by Huge-Jensen et al. (1988). The fatty acid composition of DAG oil is described in the table below. For dose calculation purposes, the test material was considered to be 100% pure, and 0.9260 g/mL was used as the specific gravity. The test material was stored at room temperature and protected from light.

Composition of DAG and TAG oil used on a two-generation reproductive toxicity study in rats

	DAG oil	TAG oil	Corn oil ^a
Monoglycerides (%)	2.16	0.17	–
Diglycerides (%)	86.06	6.13	2.8
Triglycerides (%)	9.91	91.09	95.8
Saturated fatty acids (%)			
Palmitic C16:0	3.05	5.60	9.2–16.5
Stearic C18:0	1.13	2.19	0–3.3
Unsaturated fatty acids (%)			
Oleic C18:1	39.53	35.19	20–42.2
Linoleic C18:2	46.36	47.50	39.4–65.6
Linolenic C18:3	6.21	7.07	0.5–1.5

^a D'alanzo et al. (1982) and Firestone (2006).

Key Study Findings

- The objective of this fertility/two-generation study was to evaluate potential adverse effects of DAG oil on reproductive processes. DAG oil was administered via gavage to rats (30/sex/group) for at least 70 days prior to mating, at dose levels of 0, 1.25, 2.5 or 5.0 mL/kg/day (0, 1160, 2320 and 4630 mg/kg/day). The rats were treated throughout the mating, gestation and lactation periods.

- Administration of DAG oil did not reveal any toxicologically significant effects on reproductive performance (mating, fertility and copulation/conception indices). DAG oil did not affect mean gestation lengths, the process of parturition, spermatogenic parameters, organ weights, histopathologic findings, mean numbers of pups born, implantation sites, and unaccounted sites. F2 pup viability, live litter sizes, body weights, mean age of attainment of balanopreputial separation and vaginal patency were similar to those in the control group. F1 pup viability may have been reduced from PND 0-1 but was within control range and unaffected at the low dose DAG group and in all F2 pups.
- Based on the results of this study, a dose level of 1.25 mL/kg/day (1160 mg/kg/day, HED of 186 mg/kg/day) was considered as the no-observed-adverse-effect level for fertility and early embryonic development and pre- and post-natal development with the only possible effect as reduced F1 pup viability for PND0-1. At 40% oleic acid, the component of glycerol dioleate (GDO), the dose of GDO could be at most 464 mg/kg/day (2784 mg/m²). However, the actual levels are not known and the diacylglycerol composition could be heterogenous.
- In the second cycle, the Applicant provided an assessment of the GDO present in the DAG oil material tested based on a probably estimate using data from Hatzakis. The material tested in this dietary study was obtained (b) (4)
 Based on the levels of oleic acid reported in this paper and using the probability estimate of GDO content in the DAG oil tested (1160 mg/kg/day DAG oil) would be ~122 mg/kg of GDO (HED of 1179 mg based on BSA) which is ~32x the estimated daily human dose of 37.14 mg. Although not definitive, these values suggest that the study is reasonably adequate to support this requirement for a novel excipient in this drug product.
- **NOTE:** A considerable amount of data was not included in this literature study, but what was reported supports the high dose NOAEL for DAG oil which likely contains some GDO for fertility and embryo-fetal development and for pre- and post-natal development.

Methods

Doses:

0, 1.25, 2.5, or 5.0 mL/kg/day (0, 1160, 2320 and 4630 mg/kg/day) with total volume made to 5 mL/kg/day with triacylglycerol (corn) oil.

Frequency of dosing:

- F0 animals were doses 70 days prior to pairing and continuing throughout the mating, gestation and lactation periods until euthanasia.
 - The offspring of the F0 generation (F1 litters) were potentially exposed in utero,

through nursing during lactation (postnatal days [PND] 0–21) and orally by gavage following weaning (beginning on PND 22).

- The F1 pups selected for breeding (30 rats/sex/group) were dosed for a minimum of 70 days prior to pairing and throughout mating, gestation and lactation as described for the F0 generation.

- The offspring of the F1 generation (F2 litters) were potentially exposed in utero and through nursing during lactation (PND 0–21).

Dose volume:

5 mL/kg/day

Route of administration:

Oral gavage

Formulation/Vehicle:

Corn oil

Species/Strain:

Sprague-Dawley Crl:CD (SD)IGS BR rats

Number/Sex/Group:

F0 rats to each treatment group (n = 30 rats/sex/group)

Satellite groups:

None

Study design:

Combined Fertility and Early Embryonic Development study and Pre-and Post-Natal development study

Deviation from typical study review:

As this was a literature study, review of supporting data sets are not available for review. Summary tables were reviewed to confirm report assessments and conclusions.

Observations and Results

Mortality

One F0 male was found dead and one F0 male in the 50% DAG oil group (i.e., 2.5 mL/kg/day) was euthanized in extremis. Two, one, and one F1 males in the 0% DAG oil, 50% DAG oil and 100% TAG oil groups were found dead, respectively. One and two F1 females in the 0% and 25% DAG oil groups were euthanized in extremis. No relationship to treatment was reported evident for the rats found dead or euthanized, and no deaths were observed in either sex at both generations in the 100% DAG group (data not shown).

Clinical Signs

No treatment-related clinical signs were detected in males and females in either generation that survived to scheduled euthanasia (data not shown).

Body Weight (F0 and F1 females and males)

During the pre-mating treatment interval, F0 and F1 body weight was measured weekly. Following evidence of mating, female body weight was recorded on Gestation Days (GD) 0, 4, 7, 11, 14, and 20 and on Lactation Days (LD) 1, 4, 7, 14, and 21.

No DAG oil related effects on mean F0 or F1 weekly body weights, body weight gains and cumulative body weight gains were noted for either sex in the 25%, 50% and 100% DAG oil groups for the pre-mating period F0 (Fig. 1) and F1 (Fig. 3) generation females or F0 (Fig. 2) and F1 (Fig. 4) generation males.

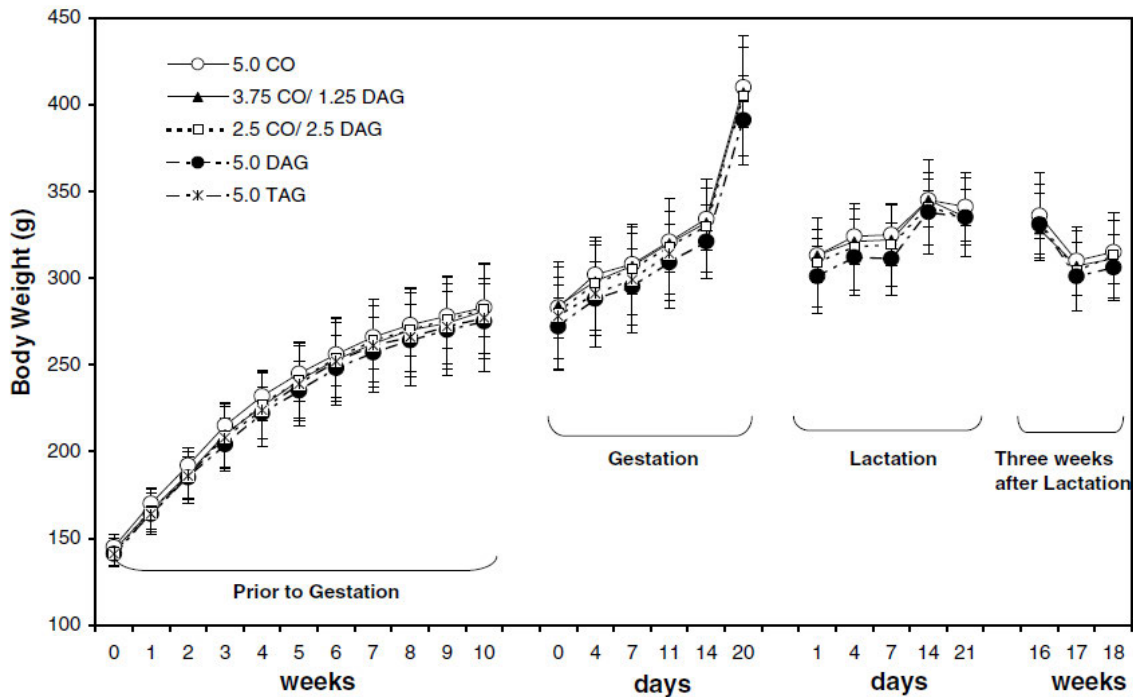


Fig. 1. Mean F0 female body weights prior to gestation, during gestation and lactation, and three weeks after lactation.

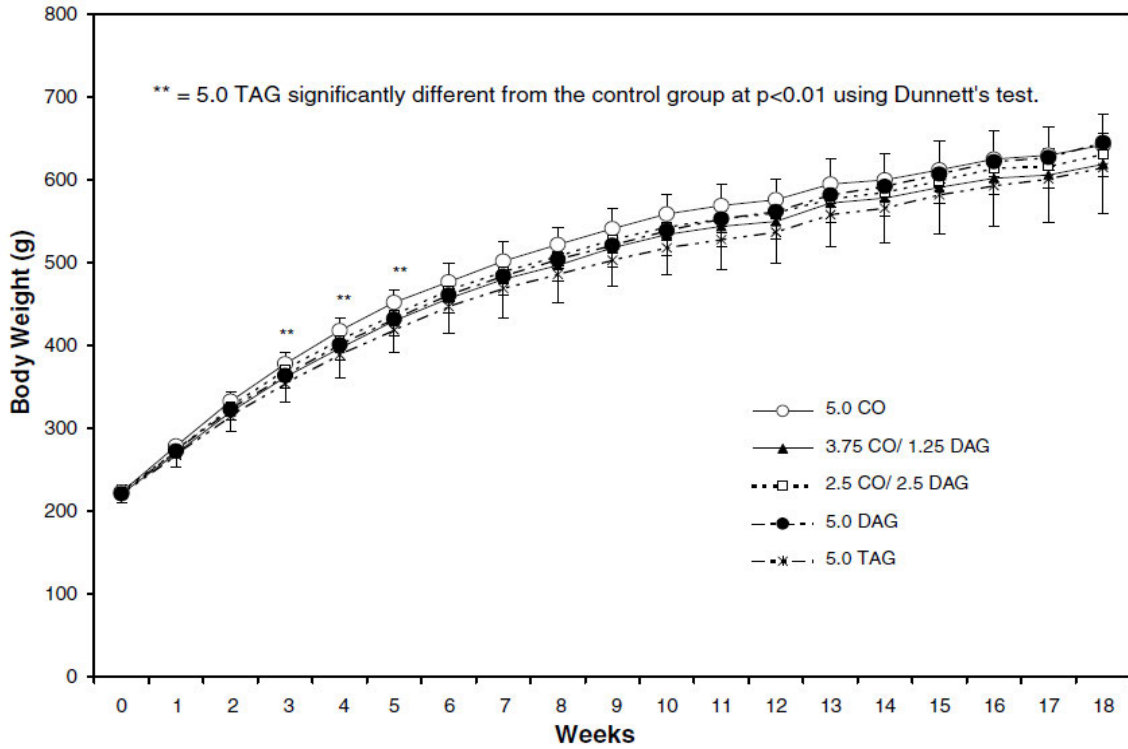


Fig. 2. Mean F₀ male body weights on a two-generation reproductive toxicity study of DAG oil in rats.

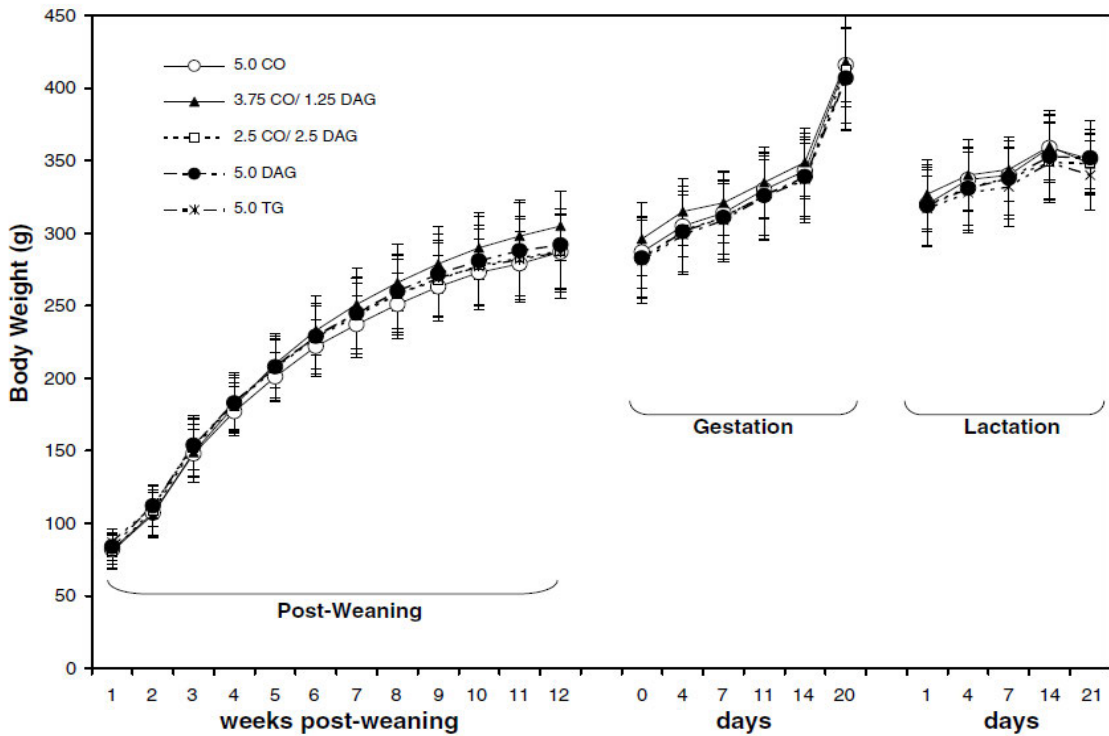


Fig. 3. Mean F₁ female body weights prior to mating, during gestation and lactation.

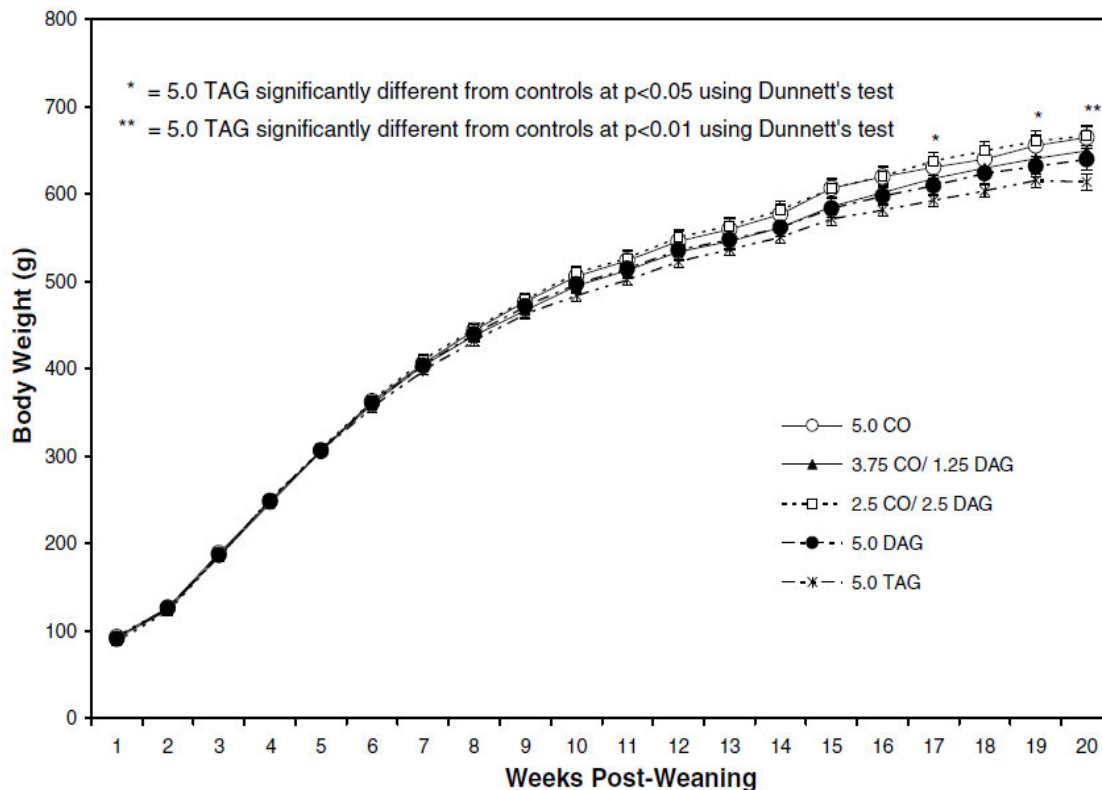


Fig. 4. Mean F₁ male body weights on a two-generation reproductive toxicity study of DAG oil in rats.

Food Consumption (F0 and F1 females and males)

During the pre-mating treatment interval, F0 and F1 food consumption was measured weekly. Following evidence of mating, female food consumption was recorded on Gestation Days (GD) 0, 4, 7, 11, 14, and 20 and on lactation days (LD) 1, 4, 7, 14, and 21.

Mean F0 and F1 weekly food consumption and food efficiency in the DAG oil-treated males and females were reported to be unaffected by test article administration (data not shown).

Toxicokinetics – none

Dosing Solution Analysis – not reported

Necropsy (Gross and Histopathology)

All surviving F0 and F1 parental animals were euthanized following weaning of the F1 and F2 litters, respectively. All surviving unselected F1 pups and all F2 pups were euthanized by carbon dioxide inhalation on PND 21. A complete necropsy was done on all parental animals (F0 and F1) dying spontaneously, euthanized in extremis or surviving until scheduled euthanasia. The necropsy included macroscopic examination

of external surfaces, all orifices, the cranial cavity, external surfaces of the brain and spinal cord, and the thoracic, abdominal and pelvic cavities including viscera. The following tissues were preserved from all F0 and F1 animals and examined microscopically from all animals that were found dead or euthanized in extremis and from 10 rats/sex/group in the control (0% DAG oil group) and high dose group (100% DAG oil group) that survived to the scheduled euthanasia: adrenal glands, brain, cervix, coagulating glands, epididymis (right), kidneys, liver, ovaries, oviducts, pituitary gland, prostate, seminal vesicles, spleen, testis (right), thymus, uterus, vagina, vas deferens and all gross lesions. A quantitative histopathologic evaluation of five sections from the inner third of each ovary from 10 control and high-dose group F0 and F1 females was performed to enumerate the total number of primordial follicles and corpora lutea.

No DAG or TAG oil-related macroscopic or microscopic findings were observed for the parental animals in either generation.

Study-Specific Observations

Upon completion of parturition, litters were sexed, pups were examined for gross abnormalities, and the numbers of live and dead pups were recorded. All pups were identified using special markings on PND 0. Litters were culled to eight pups (four/sex, when possible) on PND 4. Pups were examined daily for survival. Each pup was weighed and received a detailed physical examination on PND 1, 4, 7, 14, and 21. Intact pups dying from PND 0 to 4 were necropsied using a fresh dissection technique. A detailed gross necropsy was performed on all pups dying between PND 5 and weaning.

At weaning on PND 21, 30 pups/sex/group (minimum of one pup/sex/litter) were randomly selected to comprise the F1 generation. These pups were administered DAG oil/corn oil formulations or TAG oil beginning on PND 22. Each selected male pup was observed for balanopreputial separation beginning on PND 35 (separation of the prepuce from the glans penis). Each selected female pup was observed for vaginal patency beginning on PND 25. Examinations for both the males and females continued daily until a positive finding was recorded. The body weight of the weanling was recorded on the day of attainment of the developmental landmark.

All surviving F0 and F1 males were euthanized following the weaning of the F1 and F2 pups, respectively. Immediately upon euthanasia, the reproductive tract of each F0 and F1 male used for breeding was exposed by a ventral mid-line incision. The right epididymis was excised and weighed, and an incision was made in the distal right cauda epididymis. The right cauda epididymis was then placed in Dulbecco's phosphate buffered saline (maintained at approximately 37°C) with 10 mg/mL bovine serum albumin. After a 10-min incubation, sperm motility was determined. Where possible, at least 200 spermatozoa (motile and non-motile) from each male were analyzed. Sperm morphology was evaluated for all F0 and F1 males by light microscopy. Abnormal forms of sperm (double heads, double tails, microcephalic, or megacephalic, etc.) from a differential count of 200 spermatozoa per animal were

recorded, if possible. The left testis and epididymis from all F0 and F1 males were weighed, homogenized and evaluated for sperm numbers.

F0 and F1 males

In the F0 males, several statistically significant differences were observed in mean absolute and/or relative left epididymis, right testis, and seminal vesicle/coagulating gland weights compared to the 0% DAG oil group males (Table 2 from publication below). These changes were not considered DAG or TAG oil-related because of the lack of dose relationship, lack of consistency between left and right organs and lack of correlating macroscopic and microscopic changes in these organs.

Table 2
Organ weights for F₀ males on a two-generation reproductive toxicity study of DAG oil in rats

Organ	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
<i>Absolute (g)</i>					
Brain	2.20 ± 0.075	2.15 ± 0.083	2.16 ± 0.097	2.14 ± 0.127	2.12 ± 0.099**
Pituitary gland	0.0154 ± 0.00217	0.0146 ± 0.00212	0.0149 ± 0.00189	0.0141 ± 0.00178	0.0147 ± 0.00258
Liver	23.76 ± 3.344	22.37 ± 2.532	23.20 ± 3.363	24.79 ± 4.950	23.17 ± 3.583
Kidneys	4.52 ± 0.422	4.33 ± 0.400	4.49 ± 0.391	4.48 ± 0.548	4.24 ± 0.412
Seminal vesicles/coagulating gland	2.42 ± 0.373	2.19 ± 0.310*	2.33 ± 0.378	2.17 ± 0.297**	2.26 ± 0.217
Prostate	1.00 ± 0.195	1.10 ± 0.206	1.07 ± 0.184	1.05 ± 0.158	1.06 ± 0.243
Left testis	1.8 ± 0.157	1.87 ± 0.164	1.85 ± 0.163	1.81 ± 0.139	1.83 ± 0.171
Right Testis	1.71 ± 0.320	1.86 ± 0.175	1.82 ± 0.136	1.74 ± 0.250	1.82 ± 0.154
Left epididymis	0.69 ± 0.064	0.72 ± 0.080	0.74 ± 0.055*	0.73 ± 0.067	0.73 ± 0.077 [†]
Right epididymis	0.71 ± 0.074	0.72 ± 0.066	0.74 ± 0.069	0.71 ± 0.079	0.73 ± 0.064
<i>Relative to final body weight (g/100 g)</i>					
Final body weight (g)	642 ± 68.0	621 ± 61.4	632 ± 59.8	648 ± 103.9	617 ± 60.6
Brain	0.346 ± 0.0352	0.349 ± 0.0346	0.344 ± 0.0312	0.337 ± 0.0483	0.346 ± 0.0309
Pituitary gland	0.002 ± 0.0005	0.002 ± 0.0005	0.002 ± 0.0005	0.002 ± 0.0003	0.002 ± 0.0006
Liver	3.694 ± 0.2320	3.603 ± 0.2509	3.658 ± 0.2506	3.815 ± 0.3169	3.745 ± 0.3171
Kidneys	0.707 ± 0.0473	0.700 ± 0.0604	0.713 ± 0.0567	0.698 ± 0.0765	0.689 ± 0.0545
Seminal vesicles/coagulating gland	0.382 ± 0.0752	0.356 ± 0.0633	0.370 ± 0.0659	0.343 ± 0.0652	0.369 ± 0.0415
Prostate	0.157 ± 0.0315	0.178 ± 0.0386	0.171 ± 0.0322	0.166 ± 0.0376	0.173 ± 0.0413
Left testis	0.282 ± 0.0298	0.304 ± 0.0339	0.294 ± 0.0317	0.285 ± 0.0395	0.298 ± 0.0343
Right testis	0.269 ± 0.0553	0.301 ± 0.0341*	0.290 ± 0.0284	0.275 ± 0.0547	0.297 ± 0.0315*
Left epididymis	0.108 ± 0.0125	0.117 ± 0.0166	0.118 ± 0.0126*	0.115 ± 0.0201	0.120 ± 0.0131*
Right epididymis	0.112 ± 0.0142	0.117 ± 0.0129	0.117 ± 0.0103	0.111 ± 0.0202	0.118 ± 0.0108

* Significantly different from the control group at 0.05 using Dunnett's test.

** Significantly different from the control group at 0.01 using Dunnett's test.

Spermatogenic assessment parameters in the F0 and F1 males were unaffected by DAG or TAG oil exposure (Table 4).

Table 4
Spermatogenic evaluation of male rats on a two-generation reproductive toxicity study of DAG oil

Parameter	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
<i>F₀ males</i>					
No. rats examined	30	30	28	29	30
Sperm concentration					
Left testis ($\times 10^6/g$)	78.0 $\pm$ 10.62	80.6 $\pm$ 14.55	76.5 $\pm$ 13.22	82.4 $\pm$ 9.90	83.5 $\pm$ 12.70
Left epididymis ($\times 10^6/g$)	434.7 $\pm$ 88.15	450.7 $\pm$ 89.28	468.6 $\pm$ 94.50	453.5 $\pm$ 85.57	480.8 $\pm$ 109.46
Motility (%)	88.0 $\pm$ 7.3	90.0 $\pm$ 5.7	92.0 $\pm$ 2.8	89.0 $\pm$ 6.3	90.0 $\pm$ 4.0
Morphology (% normal)	99.2 $\pm$ 3.19	99.7 $\pm$ 0.52	99.8 $\pm$ 0.42	99.4 $\pm$ 1.51	99.5 $\pm$ 0.67
<i>F₁ males</i>					
No. rats examined	28	30	28	29	28
Sperm concentration					
Left testis ($\times 10^6/g$)	68.2 $\pm$ 13.50	73.5 $\pm$ 11.83	72.5 $\pm$ 11.67	74.8 $\pm$ 11.45	74.1 $\pm$ 12.47
Left epididymis ($\times 10^6/g$)	462.6 $\pm$ 111.61	483.5 $\pm$ 91.53	457.2 $\pm$ 123.26	458.6 $\pm$ 111.27	444.0 $\pm$ 77.46
Motility (%)	82.0 $\pm$ 16.9	84.0 $\pm$ 5.1	85.0 $\pm$ 4.8	85.0 $\pm$ 5.3	85.0 $\pm$ 5.0
Morphology (% normal)	99.9 $\pm$ 0.33	99.9 $\pm$ 0.26	99.9 $\pm$ 0.28	99.8 $\pm$ 0.34	99.4 $\pm$ 0.95

None significantly different from the control group.

Fertility Parameters

Reproductive performance parameters for the F₀ generation were unaffected by DAG and TAG oil administration at any dose level (Table 5). Parameters evaluated included mating and fertility indices, estrous cycle lengths, pre-coital intervals, gestation lengths, sex ratios, live litter size, postnatal survival and offspring weights. The only statistically significant differences were slightly lower F₀ mean litter proportions of litter viability during PND 0-1 in the 100% DAG oil group and the 100% TAG oil group (98.8% and 97.2% per litter, respectively) compared to the 0% DAG oil group. The differences were slight, were comparable to the historical control mean (99.0 $\pm$ 1.24%) and were higher than the mean minimum value (94.3% per litter) in the historical control data. In addition, only 5 of 372 pups in the 100% DAG oil group and 10 of 370 pups in the 100% TAG oil group died during PND 0–1 and survival in these groups were not lower than the control group from birth to PND 4. On this basis, the low dose DAG group (1.25 mL/kg) was considered to be unaffected.

Table 5
Reproductive parameters and litter data for F₀ generation on a two-generation reproductive toxicity study of DAG oil in rats

Parameter	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
No. mating pairs	30	30	30	30	30
Precoital interval (days)	2.6 $\pm$ 2.18	2.6 $\pm$ 1.81	2.8 $\pm$ 2.00	2.7 $\pm$ 1.96	3.1 $\pm$ 2.07
Mating index (%)	100.0	96.7	96.7	100.0	93.3
Fertility index (%)	86.7	80.0	93.3	90.0	90.0
Gestation length (days)	22.0 $\pm$ 0.45	22.0 $\pm$ 0.47	21.9 $\pm$ 0.26	21.9 $\pm$ 0.27	22.1 $\pm$ 0.55
Total no. pups/litter	14.1 $\pm$ 2.84	14.3 $\pm$ 2.32	14.4 $\pm$ 1.87	13.9 $\pm$ 2.01	13.9 $\pm$ 1.34
No. live pups/litter	13.8 $\pm$ 2.99	14.3 $\pm$ 2.42	14.1 $\pm$ 1.79	13.8 $\pm$ 1.95	13.7 $\pm$ 1.49
Sex ratio (% per litter)	53.4 $\pm$ 15.48	49.5 $\pm$ 11.37	53.5 $\pm$ 9.61	49.3 $\pm$ 13.14	49.6 $\pm$ 14.17
Pup survival (% per litter)					
PND 0	98.0 $\pm$ 4.55	99.3 $\pm$ 2.38	98.3 $\pm$ 6.48	99.5 $\pm$ 1.68	98.6 $\pm$ 3.53
PND 0–1	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	99.3 $\pm$ 2.20	98.8 $\pm$ 2.63 [*]	97.2 $\pm$ 5.66 ^{**}
PND 1–4 (precul)	99.4 $\pm$ 2.11	100.0 $\pm$ 0.00	98.1 $\pm$ 4.42	99.1 $\pm$ 2.88	99.5 $\pm$ 1.84
PND 0–4 (precul)	97.4 $\pm$ 5.21	99.3 $\pm$ 2.38	95.7 $\pm$ 7.73	97.4 $\pm$ 4.57	95.4 $\pm$ 7.50
PND 4 (postcul)–21	98.3 $\pm$ 5.04	99.5 $\pm$ 2.55	97.8 $\pm$ 6.85	97.7 $\pm$ 7.78	97.7 $\pm$ 9.83

^{*} Significantly different from the control group at 0.05 using Dunnett's test.

^{**} Significantly different from the control group at 0.01 using Dunnett's test.

Reproductive performance parameters for the F₁ generation was unaffected by DAG and TAG oil administration at all dose levels (Table 6).

Table 6Reproductive parameters and litter data for F₁ generation on a two-generation reproductive toxicity study of DAG oil in rats

Parameter	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
No. mating pairs	30	30	30	30	30
Precoital interval (days)	3.5 ± 2.01	3.5 ± 2.81	2.6 ± 1.05	3.5 ± 2.56	3.6 ± 2.53
Mating index (%)	100.0	93.3	96.7	93.3	96.7
Fertility index (%)	89.7	86.7	90.0	90.0	86.7
Gestation length (days)	22.1 ± 0.32	22.2 ± 0.49	22.2 ± 0.37	22.0 ± 0.44	22.0 ± 0.29
Total no. pups/litter	14.3 ± 2.83	13.3 ± 3.45	13.2 ± 3.04	12.5 ± 2.98	13.9 ± 2.01
No. live pups/litter	14.1 ± 2.89	13.0 ± 3.35	12.6 ± 2.94	12.3 ± 3.03	13.7 ± 1.95
Sex ratio (% per litter)	51.6 ± 17.46	46.4 ± 18.52	50.7 ± 13.63	51.2 ± 13.51	53.6 ± 15.47
Pup survival (% per litter)					
PND 0	98.7 ± 4.64	98.3 ± 5.35	96.0 ± 8.23	98.5 ± 4.03	99.0 ± 3.62
PND 0-1	98.9 ± 3.13	99.7 ± 1.31	98.4 ± 6.58	99.7 ± 1.60	95.1 ± 19.96
PND 1-4 (precul)	96.5 ± 14.16	99.3 ± 2.73	95.0 ± 19.47	96.0 ± 19.24	98.8 ± 3.78
PND 0-4 (precul)	94.8 ± 15.58	97.3 ± 5.81	90.8 ± 20.74	94.2 ± 19.31	93.4 ± 20.03
PND 4 (postcul)-21	93.1 ± 22.29	99.0 ± 3.46	99.5 ± 2.45	99.5 ± 2.45	98.4 ± 5.61

None significantly different from the control group.

F₀ Dams

Survival: No treatment-related changes (discussed previously)

Clinical signs: No treatment-related changes (discussed previously)

Body weight: No treatment-related changes (see Table 2)

Food

consumption: No treatment-related changes (see Table 3) were observed.

Organ weights:

In the 100% DAG oil group F₀ females, mean absolute and relative (to final body weight) uterus weights were increased compared to the 0% DAG oil group (Table 3). This increase was attributed to the slightly increased number of females in this group that were in estrus or proestrus at the time of necropsy. Therefore, this change was not considered DAG oil-related.

weights for F₀ females on a two-generation reproductive toxicity study of DAG oil in rats

	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
(g)	2.00 ± 0.102	2.00 ± 0.097	2.02 ± 0.092	1.98 ± 0.091	2.01 ± 0.090
gland	0.0192 ± 0.00360	0.0179 ± 0.00297	0.0191 ± 0.00357	0.0187 ± 0.00349	0.0181 ± 0.00317
	12.85 ± 1.416	12.49 ± 1.410	12.78 ± 1.519	12.22 ± 1.562	12.60 ± 1.701
	2.39 ± 0.167	2.42 ± 0.322	2.42 ± 0.217	2.40 ± 0.227	2.34 ± 0.214
	0.1629 ± 0.03185	0.1590 ± 0.03256	0.1512 ± 0.02285	0.1535 ± 0.02847	0.1584 ± 0.01751
	0.68 ± 0.182	0.67 ± 0.165	0.78 ± 0.239	0.82 ± 0.270	0.73 ± 0.246
to final body weight (g/100 g)					
dy weight (g)	317 ± 16.8	314 ± 21.2	316 ± 22.7	310 ± 18.8	314 ± 28.4
	0.634 ± 0.0345	0.641 ± 0.0562	0.641 ± 0.0529	0.643 ± 0.0444	0.644 ± 0.0630
gland	0.006 ± 0.0010	0.006 ± 0.0010	0.006 ± 0.0012	0.006 ± 0.0011	0.006 ± 0.0010
	4.055 ± 0.3833	3.979 ± 0.3169	4.044 ± 0.3843	3.941 ± 0.3585	4.010 ± 0.3597
	0.755 ± 0.0527	0.771 ± 0.0763	0.768 ± 0.0740	0.777 ± 0.0697	0.749 ± 0.0639
	0.051 ± 0.0095	0.051 ± 0.0098	0.048 ± 0.0081	0.050 ± 0.0094	0.051 ± 0.0078
	0.214 ± 0.0589	0.214 ± 0.0575	0.247 ± 0.0752	0.265 ± 0.0852	0.233 ± 0.0800

cantly different from the control group at 0.05 using Dunnett's test.

Uterine content:

Conducted, with no report of adverse effects.

Necropsy

observation:

Discussed previously.

Toxicokinetics:

None.

F₁ Generation

Survival:

Conducted, with no report of adverse effects (no data).

Clinical signs:

Conducted, with no report of adverse effects (no data).

Body weight:

No apparent effects on pup weight were observed based on Figure 5.

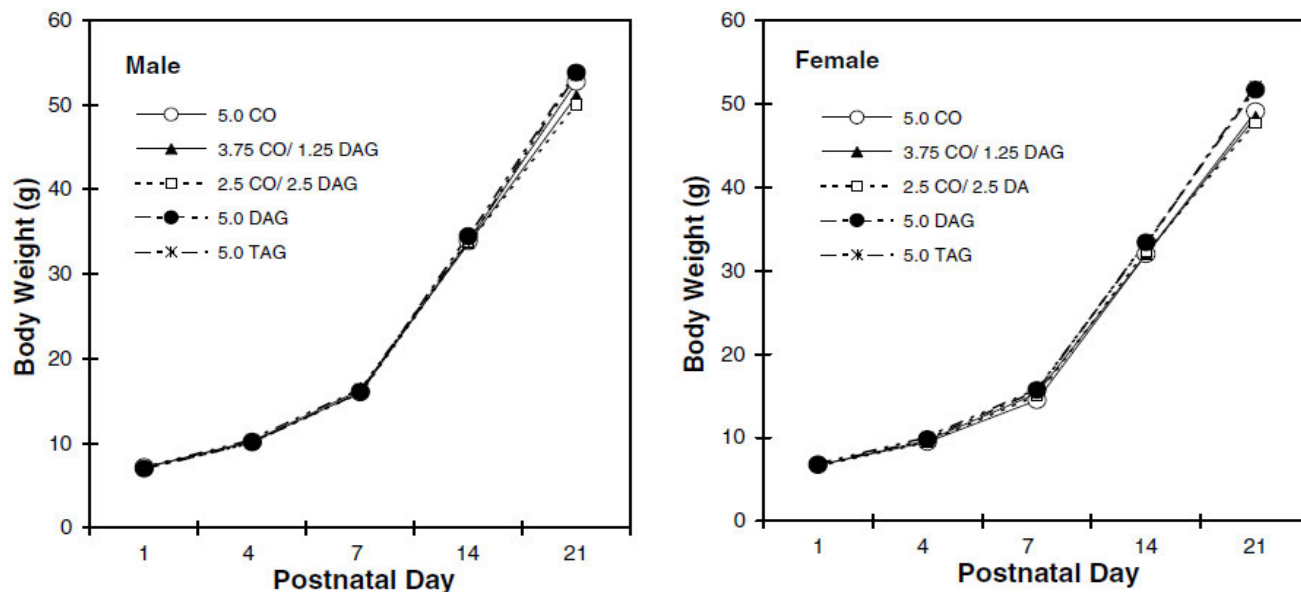


Fig. 5. Male and female F₁ pup body weights on a two-generation reproductive toxicity study of DAG oil in rats.

Food

consumption: Reported previously.

Organ weights: No DAG or TAG oil-related effects on organ weights (absolute and relative to final body weight) in animals of the F₁ generation were observed when the DAG and TAG oil-treated animals were compared to the 0% DAG oil group (data not shown).

Sexual

Development: In F₁ male weanlings, the mean age of attainment of balanopreputial separation and mean body weights on the day of attainment were unaffected by DAG and TAG oil administration (Table 7). In the F₁ female weanlings, the mean age of attainment of vaginal patency and mean body weights on the day of attainment were similar between the control group and the treated groups (Table 7).

Table 7

Developmental landmarks for F₁ male and female rats on a two-generation reproductive toxicity study of DAG oil

Balanoepreputial separation	Male				
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
Day of attainment	44.3 ± 2.70	44.1 ± 2.23	44.5 ± 3.05	43.8 ± 2.05	43.4 ± 2.22
Body weight (g)	228.2 ± 25.92	226.6 ± 19.02	229.1 ± 17.61	224.1 ± 13.87	227.0 ± 17.60
No. rats examined	29	30	30	30	30
Vaginal patency	Female				
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
Day of attainment	34.4 ± 2.28	34.9 ± 2.33	35.4 ± 1.96	34.3 ± 1.80	35.0 ± 2.01
Body weight (g)	123.2 ± 15.20	126.1 ± 18.08	128.3 ± 14.70	124.9 ± 18.62	133.1 ± 16.35
No. rats examined	30	30	30	30	30

None significantly different from the control group.

Neurological

assessment: Not reported (presumably not conducted).

Reproduction: No treatment-related effect. See fertility parameters above.

F₂ Generation

Survival: Conducted, with no report of adverse effects (no data).

Body weight: Statistically significant increases in male and female F₂ pup body weight gains were noted in all DAG oil-treated groups during PND 1–4 compared to the 0% DAG oil group values (data not shown). However, no dose-related trends were apparent. During PND 4–7, mean male and female pup body weight gains in the 25% DAG oil group were statistically significantly increased compared to the 0% DAG oil group. Mean body weight gains in the DAG oil-treated groups were similar to those in the control group during PND 7–14 and 14–21. The increases in mean body weight gain in the DAG oil-treated groups resulted in higher (often statistically significant) mean F₂ male and female pup body weights compared to the 0% DAG oil group during the pre-weaning period. The greatest differences were reported to be observed on PND 4 (Fig. 6). Mean male pup weights in the 100% DAG oil group were 6.0–12.1%.

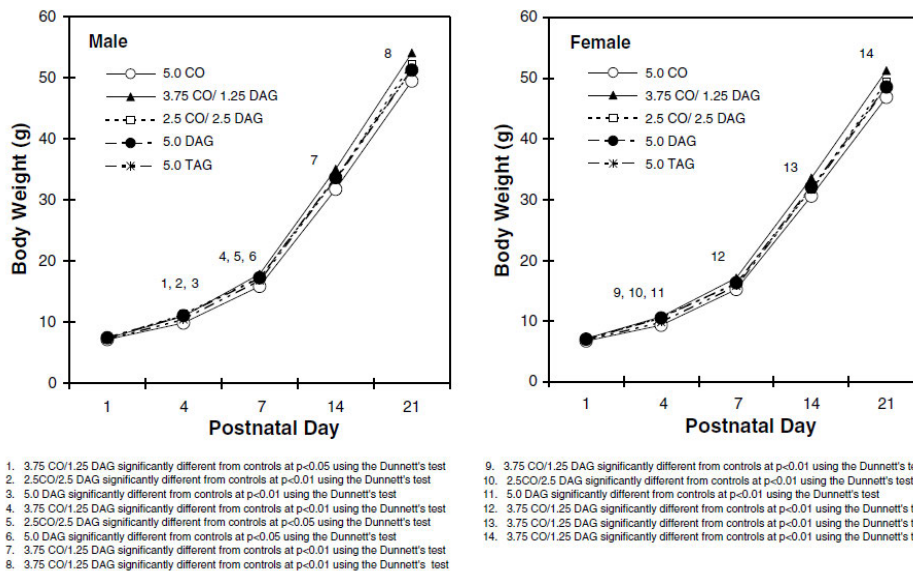


Fig. 6. Male and female F₂ pup body weights on a two-generation reproductive toxicity study of DAG oil in rats.

External evaluation: Conducted, with no report of adverse effects (no data).
 Male/Female ratio: No treatment-related effects (see fertility parameters section above)
 Other: No.

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9.2 Embryonic Fetal Development

The Applicant submitted a published rat embryofetal development study for DAG oil in lieu of testing GDO.

NOTE: This study from the published literature was reviewed by Dr. Jay Chang.

Study title: Morita O, Knapp JF, Tamaki Y, Varsho BJ, Stump DG, Nemeč MD (2008b) Effects of dietary diacylglycerol oil on embryo/fetal development in rats. Food Chem Toxicol 46:2510-2516.

Study no.: N/A
Study report location: N/A
Conducting laboratory and location: Global R&D Safety Science, Kao Corporation, Japan
 WIL Research Laboratories, LLC, Ashland, OH, United States
Date of study initiation: Unknown
GLP compliance: Yes
QA statement: Not reported
Drug, lot #, and % purity: The article states: DAG oil was prepared by

esterifying glycerol with free fatty acids from rapeseed oil and soybean oil using the method reported by Huge-Jensen et al. (1988). The fatty acid composition of DAG oil is described in the table below. For dose calculation purposes, the test material was considered to be 100% pure, and 0.9260 g/mL was used as the specific gravity. The test material was stored at room temperature and protected from light. DAG oil is reported to contain 40% oleic fatty acid. If the glycerol contains two oleic acid residues it would be glycerol dioleate (GDO).

It is not clear how much GDO is actually in this DAG product as the diacylglycerides may contain any combination of the above fatty acid moieties.

Composition of DAG and TAG oil used on a two-generation reproductive toxicity study in rats

	DAG oil	TAG oil	Corn oil ^a
Monoglycerides (%)	2.16	0.17	–
Diglycerides (%)	86.06	6.13	2.8
Triglycerides (%)	9.91	91.09	95.8
Saturated fatty acids (%)			
Palmitic C16:0	3.05	5.60	9.2–16.5
Stearic C18:0	1.13	2.19	0–3.3
Unsaturated fatty acids (%)			
Oleic C18:1	39.53	35.19	20–42.2
Linoleic C18:2	46.36	47.50	39.4–65.6
Linolenic C18:3	6.21	7.07	0.5–1.5

^a D'alanzo et al. (1982) and Firestone (2006).

Key Study Findings

Pregnant rats were treated with DAG from GD 7-16 with the following key findings:

- No mortality or treatment-related clinical or internal findings were noted in any of the groups. Compared to animals in control group, mean maternal body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights, and food consumption were not affected by DAG oil administration. Intrauterine growth and survival were not affected by DAG oil administration.

- No DAG oil-related fetal malformations or developmental variations were noted. A maternal maximum tolerated dose for DAG oil was not achieved in this study.
- Based on the results of this study, a dose level of 5.0 mL/kg (4630 mg/kg/day) was considered as the NOAEL for both maternal and developmental toxicity. This dose correlates to 27780 mg/m².
- In the second cycle, the Applicant provided an assessment of the GDO present in the DAG oil material tested based on a probably estimate using data from Hatzakis. The material tested in this dietary study was obtained (b) (4)
 (b) (4)
 Based on the levels of oleic acid reported in this paper and using the probability estimate of GDO content in the DAG oil tested (4630 mg/kg/day DAG oil) would be ~ (b) (4) mg/kg of GDO (HED of 4707 mg based on BSA) which is ~ (b) (4) x the estimated daily human dose of (b) (4) mg. Although not definitive, these values suggest that the study is reasonably adequate to support this requirement for a novel excipient in this drug product.

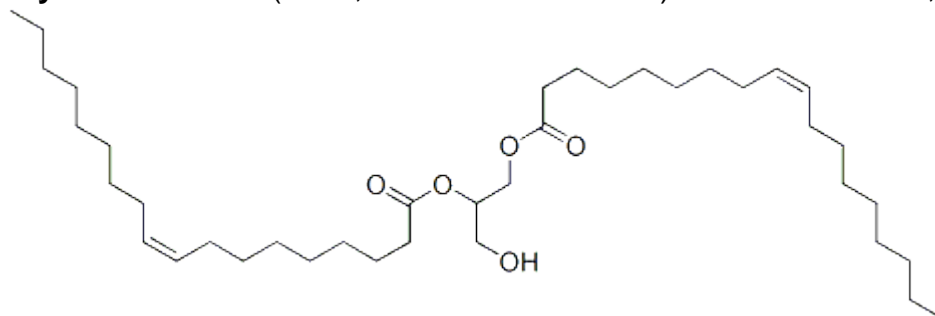
Methods

Doses:	0, 1.25, 2.5 or 5.0 mL/kg/day (0, 1160, 2320 and 4630 mg/kg/day) with total volume made to 5 ml/kg/day with triacylglycerol (corn) oil.
Frequency of dosing:	Once daily on Days 6-17 of gestation
Dose volume:	5 mL/kg/day
Route of administration:	Oral gavage
Formulation/Vehicle:	Triacylglycerol (corn) oil
Species/Strain:	sexually mature Sprague–Dawley CrI:CD®(SD)IGS BR rats
Number/Sex/Group:	25 mated females/group
Satellite groups:	none
Study design:	Standard embryo-fetal protocol
Deviation from typical study review:	As this was a literature study, review of supporting data sets did not occur. Summary tables were reviewed to confirm report assessments and conclusions.

Table 2.6.7.10-2 Comparison of compositions of GDO and the DAG oil used in the studies referenced as supportive for GDO safety

Study Reference	TO-10-405 / TO-12-446	TO-05-153 / TO-05-189 / TO-04-141 / TO-04-142 / TO-05-156 / TO-05-177	TO-04-146	Kasamatsu et al., 2005 (before heating / after heating)	Chengelis et al., 2006a / Chengelis et al., 2006b / Chengelis et al., 2006c	Morita et al., 2008b / Morita et al., 2008c	Soni et al., 2001	Morita et al., 2008a
Acylglycerol distribution (%)								
Monoacyl glycerol	0.2	0.5	4.2	0.5 / 2.7	0.9-2.9	2.2	5 (indicated in text)	No detailed composition provided in the study.
Diacyl glycerol	96.0	95.3	92.1	85.5 / 80.3	82.9-83.9	86.1	75 (indicated in text)	
Triacyl glycerol	4.0	4.0	3.5	14.0 / 17.1	12.8-15.2	9.9	20 (indicated in text)	
Fatty acid composition (%)								
C16:0 (palmitic)	0.3	0.3	0.3	3.1 / 3.2	2.9-3.0	3.1	No detailed composition provided for the indicated study. The DAG oil prepared from high C18:1, C18:2 and C18:3 content raw materials.	No detailed composition provided for the indicated study. The DAG oil prepared from high C18:1, C18:2 and C18:3 content raw materials (soybean and rapeseed oil).
C18:0 (stearic)	2.4	2.4	2.0	1.3 / 1.3	1.1-1.2	1.1		
C18:1 (oleic)	90.0	91.5	91.8	41.1 / 42.3	35.6-37.6	39.5		
C18:2 (linoleic)	5.5	3.5	3.6	45.6 / 44.8	47.7-48.6	46.4		
C18:3 (linolenic)	0.7	0.7	0.3	8.7 / 8.1	6.3-6.5	6.2		
C20:0 (arachidic)	0.0	1.1	0.1	0.3 / 0.3	-	-		

Glycerol dioleate (GDO; CAS No.25637-84-7): also known as 1,3-DIOLEIN



Observations and Results

Mortality

Animals were observed twice daily throughout the study for mortality and morbidity.

All females survived to the scheduled necropsy on Gestation Day 20.

Clinical Signs

Individual clinical observations were recorded daily prior to dose administration and approximately 1 h following dosing. The observations included, but were not limited to, evaluations for changes in appearance of skin and fur, eyes, mucous membranes, respiratory and circulatory system, autonomic and central nervous systems, somatomotor activity, and behavior.

At the daily examination or 1 h following dose administration, no treatment-related clinical findings were noted in the animals treated with 1.25, 2.5, and 5.0 mL/kg of DAG. On Gestation Days 16 and 17, dried red material around the mouth was noted 1 h after dose administration in three females treated with the highest dose of DAG. This finding

was only noted once for each female and therefore was not attributed to DAG oil administration. Clinical findings observed in the DAG oil-treated groups occurred infrequently or in a manner that was not dose-related (data not shown).

Body Weight

Individual body weights were recorded on Gestation Days 0, 6–18 (daily), and 20. Gravid uterine weight was recorded and net body weight (the Day 20 body weight exclusive of the weight of the uterus and contents) and net body weight change (the Day 0–20 body weight change exclusive of the weight of the uterus and contents) were calculated and determined for each gravid female at the scheduled laparohysterectomy.

Mean maternal body weights, body weight gains, net body weights, and net body weight gains in the 3.75 CO/1.25 DAG, 2.5 CO/2.5 DAG and 5.0 DAG groups were unaffected by DAG oil administration (Fig. 1; Table 2).

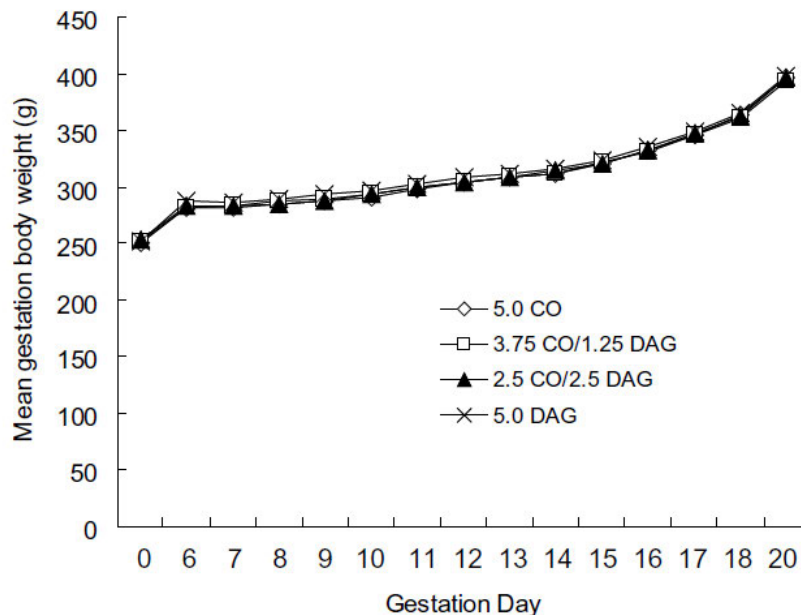


Fig. 1. Mean gestation body weights on an embryo/fetal developmental toxicity study of DAG oil in rats.

Table 2

Summary of maternal weight data (mean $\pm$ SD, g) on an embryo/fetal developmental toxicity study of DAG oil in rats

Parameter	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
Number of gravid females	24	24	24	25
Maternal absolute weight (GD 0)	251 $\pm$ 13.5	252 $\pm$ 13.0	253 $\pm$ 14.4	252 $\pm$ 13.3
Maternal absolute weight (GD 20)	397 $\pm$ 19.5	394 $\pm$ 20.2	396 $\pm$ 21.7	398 $\pm$ 22.3
Net body weight	314.3 $\pm$ 19.93	312.0 $\pm$ 16.96	312.5 $\pm$ 17.08	316.1 $\pm$ 16.02
Net body weight change	63.6 $\pm$ 11.42	60.0 $\pm$ 8.73	59.7 $\pm$ 9.39	63.9 $\pm$ 11.99
Gravid uterine weight	82.8 $\pm$ 13.69	82.1 $\pm$ 8.53	83.1 $\pm$ 12.83	81.4 $\pm$ 14.69

None significantly different from the control group.

Food Consumption

Food consumption, determined as g/animal/day and g/kg/day, in the 3.75 CO/1.25 DAG, 2.5 CO/2.5 DAG, and 5.0 DAG groups, was unaffected by DAG oil administration (Table 3).

Table 3

Summary of maternal food consumption (mean $\pm$ SD, g/animal/day) on an embryo/fetal developmental toxicity study of DAG oil in rats

Gestation day	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
GD 0–6	21 $\pm$ 1.7	21 $\pm$ 1.9	21 $\pm$ 2.1	22 $\pm$ 2.1
GD 6–9	19 $\pm$ 1.8	19 $\pm$ 1.6	19 $\pm$ 1.6	20 $\pm$ 2.1
GD 9–12	20 $\pm$ 1.9	20 $\pm$ 2.0	20 $\pm$ 1.8	21 $\pm$ 1.8
GD 12–18	22 $\pm$ 1.7	22 $\pm$ 1.8	22 $\pm$ 1.4	22 $\pm$ 1.3
GD 6–18	21 $\pm$ 1.5	21 $\pm$ 1.6	21 $\pm$ 1.2	21 $\pm$ 1.4
GD 18–20	26 $\pm$ 2.2	26 $\pm$ 1.5	26 $\pm$ 1.9	26 $\pm$ 1.4

None significantly different from the control group.

Toxicokinetics - none

Dosing Solution Analysis – not reported

Cesarean Section Data:

Euthanasia was performed by carbon dioxide inhalation on all females on Gestation Day 20. The thoracic and abdominopelvic cavities were opened and the contents were examined. The number of corpora lutea on each ovary was recorded. The uterus was weighed, and the number and location of all fetuses and early and late resorptions as well as the total number of implantation sites were recorded. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide for the detection of early implantation loss.

No DAG oil-related internal findings were noted in the maternal animals on Gestation Day 20 (data not shown). Intrauterine growth and survival were unaffected by DAG oil administration at all doses (Table 4). Parameters evaluated included mean numbers of corpora lutea, implantation sites and viable fetuses, mean fetal weights, and mean litter proportions of preimplantation loss, early resorptions, late resorptions, and fetal sex ratios.

Table 4Summary of maternal and fetal data (mean $\pm$ SD) on an embryo/fetal developmental toxicity study of DAG oil in rats

Parameter	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
Number of corpora lutea	17.5 $\pm$ 2.69	17.3 $\pm$ 2.54	17.9 $\pm$ 2.47	17.2 $\pm$ 2.40
Number of implantation sites	16.1 $\pm$ 2.55	16.1 $\pm$ 1.60	16.4 $\pm$ 2.59	15.8 $\pm$ 1.72
Number of viable fetuses	15.4 $\pm$ 2.45	15.4 $\pm$ 1.61	15.4 $\pm$ 2.47	14.8 $\pm$ 2.90
Preimplantation loss (% per litter)	7.6 $\pm$ 13.61	6.0 $\pm$ 7.30	8.2 $\pm$ 10.76	7.4 $\pm$ 9.13
Early resorptions (% per litter)	4.0 $\pm$ 4.41	4.6 $\pm$ 5.15	5.9 $\pm$ 6.96	6.8 $\pm$ 12.55
Late resorptions (% per litter)	0.3 $\pm$ 1.45	0 $\pm$ 0.0	0 $\pm$ 0.0	0.4 $\pm$ 1.82
Fetal weight (g)	3.5 $\pm$ 0.37	3.5 $\pm$ 0.22	3.6 $\pm$ 0.20	3.5 $\pm$ 0.23
Males (% per litter)	48.9 $\pm$ 13.99	49.2 $\pm$ 15.32	50.3 $\pm$ 17.49	46.4 $\pm$ 13.18

None statistically different from the control group.

Offspring (Malformations, Variations, etc.):

Live fetuses were examined for external morphological abnormalities, weighed, sexed, and euthanized by intrathoracic injection of sodium pentobarbital. The fetuses were then dissected for visceral examination using a fresh dissection technique, which included examination of the heart and major blood vessels. Heads from approximately one-half of the fetuses in each litter were placed in Bouin's fixative for subsequent soft-tissue examination by a free-hand section technique. The heads from the remaining fetuses were examined by mid-coronal slice. All fetal carcasses were eviscerated and fixed in 100% ethyl alcohol, macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson. Findings of anatomical departures from normal were recorded as either malformations (those structural anomalies that alter general body conformity, disrupt or interfere with body function, or may be incompatible with life) or developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity, representing slight deviations from normal).

Results listed in Table 4, 5, and 6 indicate that there were no untoward effects from treatment with DAG oil containing GDO.

Table 5
Summary of fetal malformations on an embryo/fetal developmental toxicity study of DAG oil in rats

Parameter	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
Number of fetuses (litters) examined	369 (24)	369 (24)	370 (24)	371 (25)
External malformations				
Cleft palate	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.3 ± 1.25
Vertebral agenesis	0 (0)	1 (1)	0 (0)	0 (0)
Mean litter % (±SD)	0	0.2 ± 1.20	0	0
Visceral malformations				
Kidney and ureter absent	1 (1)	0 (0)	0 (0)	0 (0)
Mean litter % (±SD)	0.2 ± 1.13	0	0	0
Skeletal malformations				
Vertebral anomaly	0 (0)	1 (1)	0 (0)	0 (0)
Mean litter % (±SD)	0	0.2 ± 1.20	0	0
Bent limb bones	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.3 ± 1.25
Total no. of malformations	1 (1)	2 (2)	0 (0)	1 (1)
% Per litter with malformations	0.2 ± 1.13	0.5 ± 1.66	0.0 ± 0.0	0.3 ± 1.25

% Per litter with malformations not statistically different from the control group.

Table 6
Summary of fetal developmental variations on an embryo/fetal developmental toxicity study of DAG oil in rats

Parameter	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
Number of fetuses (litters) examined	369 (24)	369 (24)	370 (24)	371 (25)
External variations	0 (0)	0 (0)	0 (0)	0 (0)
Visceral variations				
Major blood vessel variation	1 (1)	1 (1)	0 (0)	0 (0)
Mean litter % (±SD)	0.3 ± 1.46	0.2 ± 1.13	0	0
Renal papilla (e) not developed	0 (0)	1 (1)	0 (0)	2 (2)
Mean litter % (±SD)	0	0.3 ± 1.70	0	0.5 ± 1.86
Hemorrhagic ring around iris	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.2 ± 1.18
Skeletal variations				
Cervical centrum #1 ossified	101 (19)	117 (23)	84 (20)	112 (24)
Mean litter % (±SD)	26.4 ± 28.0	31.8 ± 22.6	22.1 ± 20.15	31.2 ± 21.55
14th Rudimentary rib	23 (10)	26 (13)	26 (12)	30 (13)
Mean litter % (±SD)	6.1 ± 8.59	7.2 ± 12.53	7.1 ± 9.66	8.0 ± 12.98
Pubis unossified	8 (2)	1 (1)	0 (0)	0 (0)
Mean litter % (±SD)	2.3 ± 10.22	0.3 ± 1.28	0	0
Sternebra(e) #5 and/or 6 unossified	15 (8)	7 (5)	9 (5)	10 (3)
Mean litter % (±SD)	3.8 ± 6.51	1.8 ± 3.98	2.3 ± 6.28	2.4 ± 7.8
Hyoid unossified	5 (2)	2 (2)	2 (1)	2 (1)
Mean litter % (±SD)	1.5 ± 5.93	0.5 ± 1.67	0.5 ± 2.40	0.5 ± 2.5
Bent rib	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.3 ± 1.25
25 Presacral vertebrae	1 (1)	0 (0)	0 (0)	0 (0)
Mean litter % (±SD)	0.3 ± 1.28	0	0	0
Reduced ossification of the 13th rib	1 (1)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0.2 ± 1.07	0	0	0.2 ± 1.18
7th Cervical rib	5 (4)	3 (3)	4 (4)	4 (4)
Mean litter % (±SD)	1.5 ± 3.7	0.8 ± 2.16	1.2 ± 2.76	1.0 ± 2.37
Sternebra(e) malaligned (slight or moderate)	0 (0)	1 (1)	0 (0)	2 (2)
Mean litter % (±SD)	0	0.3 ± 1.28	0	0.5 ± 1.73
Sternebra(e) #1, 2, 3 and/or 4 unossified	1 (1)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0.3 ± 1.28	0	0	0.3 ± 1.25
Reduced ossification of vertebral centra	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.3 ± 1.25
Ischium unossified	2 (1)	0 (0)	0 (0)	0 (0)
Mean litter % (±SD)	0.6 ± 2.92	0	0	0
Unco-ossified vertebral centra	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.2 ± 1.11
% Per litter with variations (mean ± SD)	37.3 ± 24.32	39.8 ± 20.82	29.7 ± 20.66	41.0 ± 21.31

% Per litter with variations not statistically different from the control group.

An embryofetal study in rabbit with the novel excipient, GDO, was not submitted in the first cycle of this NDA.

To address this data gap, the Applicant conducted an embryofetal development study with GDO in rabbit. The study below was reviewed by Elizabeth Bolan, PhD.

Study title: An Embryo-Fetal Development Study of Glycerol Dioleate (GDO) by Subcutaneous Injection in Rabbits

Study no.:	TO-18-607 (20146322)
Study report location:	EDR 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 23, 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GDO (glycerol dioleate), Lot PP1/972, 91%

Key Study Findings

- Maternal mortality occurred in two rabbits at the low dose, one rabbit in the mid dose and one high-dose rabbit was euthanized due to clinical signs. The deaths do not appear to be treatment-related and no other adverse findings were noted in rabbits at any dose. The NOAEL for maternal toxicity in this study is >1000 mg/kg.
- Test article-related injection site reactions were observed in several rabbits in all treated groups but were not considered adverse.
- The NOAEL for developmental toxicity in this study is 1000 mg/kg/day (12,000 mg/m²), the highest dose tested. This dose is (b) (4) times the human maximum daily dose of (b) (4) mg/60 kg x (b) (4) = (b) (4) mg/m², based on body surface area and (b) (4) times the mean daily dose of (b) (4) mg/60 kg person.

Methods

Doses:	See table below
Frequency of dosing:	Daily on Gestation Days (GD) 7-19
Dose volume:	See table below
Route of administration:	Subcutaneous injection
Formulation/Vehicle:	0.9% Sterile Saline
Species/Strain:	Rabbit, New Zealand

[Hra:(NZW)SPF]
Number/Sex/Group: 20 mated females/group
Satellite groups: None
Study design: See table below
Deviation from typical study review: None that compromise the integrity of the study.

Table 62. Experimental Design of EFD Rabbit Study with GDO (Reproduced From Submission)

Group No.	Test Material	Dose Level (mg/kg/day)	Concentration (mg/mL) ^a	Dose Volume (mL/kg)	No. of Animals
1	Control	0	0	1.08	20
2	GDO	100	930	0.11	20
3	GDO	300	930	0.32	20
4	GDO	1000	930	1.08	20

^a Based on density of 0.93 g/mL, not corrected for purity.

Observations and Results

Mortality: Four unscheduled deaths occurred in the study. Two occurred in the low-dose group (GD 11 and GD 21) and one in the mid-dose group (GD 22). One rabbit in the high-dose group was euthanized on GD 13 with adverse clinical signs.

The first rabbit in the low-dose group was found dead on GD 11. Clinical signs included decreased fecal output from GD 9 to 10 and liquid feces on GD 10. Approximately 9% of body weight was lost from GD 7 to 10 with concomitant decreases in food consumption. No abnormalities were detected at necropsy. The second rabbit in the low-dose group was found dead on GD 21. Clinical signs included decreased fecal output from GD 20 to 21, ungroomed fur from GD 19 to 21, and increased respiratory rate, and red liquid and clear mucoid material in the cage on GD 21. Approximately 11% of body weight was lost from GD 16 to 21 with concomitant decreases in food consumption. No abnormalities were detected at necropsy.

One rabbit in the mid-dose group was found dead on GD 22. Clinical signs included purple skin at injection sites on GD 11 to 13 and liquid feces and ungroomed fur on GD 21. Approximately 6% of body weight was lost from GD 18 to 21 with concomitant decreases in food consumption. No abnormalities were detected at necropsy.

One rabbit in the high-dose group was euthanized in extremis due to adverse clinical signs on GD 13 approximately 5 hours after dosing. Clinical signs included increased respiratory rate and decreased motor activity on GD 13. Dark sclera was observed in both eyes, splayed hindlimbs, and loss of righting reflex were seen at the veterinary visit on GD 13. Approximately 3% of body weight was lost from GD 7 to 13 with concomitant decreases in food consumption. No abnormalities were detected at necropsy.

None of the deaths appeared to be clearly treatment-related and the incidence was not dose dependent. No treatment-related changes in body weight or food consumption were observed in any rabbits that survived to scheduled termination. The only changes in respiratory rate were in the low-dose rabbit that was found dead on GD 21 and the high-dose rabbit that was euthanized on GD 13. None of the clinical signs observed in the high-dose rabbit (dark sclera, splayed hindlimbs, loss of righting reflex) were observed in any other rabbits in the study. The deaths will not be considered treatment-related.

Clinical Signs: Clinical signs were recorded daily. Injection site reactions occurred in some rabbits in all treated groups and included firm, swollen areas and red, purple or flaking skin. The reactions were considered test article-related, but not considered adverse.

Body Weight: Body weights were recorded daily during the dosing and post-dosing periods. No treatment-related changes in body weights were seen.

Food Consumption: Food consumption was recorded daily during the dosing and post-dosing periods. No treatment-related effects on food consumption were observed.

Toxicokinetics: Samples for TK analysis were collected at 0, 0.5, 1, 2, 6, 24 hours post-dose on GD 7 and GD 19. Exposure ($AUC_{(0-t)}$) was not dose-linear across doses. Accumulation was approximately 2-fold higher on GD 19. Glycerol dioleate is an endogenous diglyceride formed via hydrolysis of triglycerides. Various lipases metabolize diglycerides to free fatty acids and glycerol. The GDO measured in the control group likely represents endogenous GDO. Slow release from the subcutaneous injection site combined with local and systemic metabolism presumably account for the low levels measured in the TK analysis and the lack of dose-linear increases in exposure. The Applicant does not have systemic exposure data for GDO in humans, therefore, exposure comparisons will be based on body surface area. The NOAEL for developmental toxicity in this study is 1000 mg/kg/day (12,000 mg/m²). This dose is $\frac{(b)(4)}{(4)}$ times the human maximum daily dose of GDO of $\frac{(b)(4)}{(4)}$ mg/60 kg $\times \frac{(b)(4)}{(4)} = \frac{(b)(4)}{(4)}$ mg/m², based on body surface area and $\frac{(b)(4)}{(4)}$ times the mean daily dose of $\frac{(b)(4)}{(4)}$ mg/60 kg person based on BSA.

Table 63. Mean TK Parameters for GDO in Pregnant Rabbits (Reproduced From Submission)

GDO Dose (mg/kg/day):	0		100		300		1000	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Parameter (Units)					DG 7			
AUC(0-t) (µg·h/mL)	7.05	1.77	8.47	3.94	9.54	3.08	9.92	3.07
Cmax (µg/mL)	0.345	0.090	0.420	0.175	0.468	0.124	0.478	0.134
Tmax (h) (range)	0-24	NA	1-24	NA	6-24	NA	0-24	NA
					DG 19			
AUC(0-t) (µg·h/mL)	14.6	5.13	12.5† ³	3.86	15.8	5.85	13.4† ³	3.39
Cmax (µg/mL)	0.760	0.302	0.630† ³	0.166	0.740	0.325	0.707† ³	0.110
Tmax (h) (range)	2-6	NA	2-6† ³	NA	2-6	NA	0-2† ³	NA
Accumulation Ratio	2.1	0.33	1.6† ³	0.88	1.7	0.47	1.6† ³	0.15

N = 4, except where designated with †^N

NA = Not applicable

Dosing Solution Analysis:

Test-article formulations were stable and homogenous under the condition of use.

Cesarean Section Data:

Pregnancy occurred in 20 (100%), 20 (100%), 20 (100%), and 18 (80%) rabbits in the 0, 100, 300, and 1000 mg/kg/day dose groups, respectively. No differences from control in Cesarean-sectioning or litter parameters were observed at any dose tested. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable among the five dosage groups and did not significantly differ. All placentae appeared normal. Although there was reduced pregnancy rate in the high-dose rabbits, the ^{(b) (4)} historical control data for pregnancy index of New Zealand White Rabbits is 80 to 100% with a mean of 93.6%. Given the dosing period, the reduced pregnancy incidence in the high-dose group is not likely treatment-related.

Offspring (Malformations, Variations, etc.):

No GDO-mediated variations or malformations were observed in this study.

=====

Prenatal and Postnatal Development

As noted in the review above, the Morita et al. (2008a) combination Fertility and Early Embryonic Development study and Pre- and Post-Natal development study does not include any behavioral or cognitive testing of the F1 offspring and therefore is not considered an adequate pre- and post-natal development study by current standards.

To address this data gap, the Applicant conducted a dedicated pre- and post-natal developmental toxicology study with GDO. The study below was reviewed by R. Daniel Mellon, PhD.

Study title: Glycerol Dioleate (GDO): A Subcutaneous Pre- and Post-Natal Reproductive and Developmental Toxicity Study in Female Sprague-Dawley Rats

Study no.: TO-16-569
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: May 30, 2016
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: GDO, PP1/924, purity not specified, testing indicated (b) (4) % (b) (4) content, (b) (4) % (b) (4) content. Material from Camurus AB, Lund, Sweden

Key Study Findings

Female Sprague-Dawley Rats were treated daily from GD 6 through parturition and to post-prandial day 20 (PPD20) with 100, 350, or 1000 mg/kg GDO per day via subcutaneous injection with the following key findings:

1. Reduced pup viability and increased dead pups at birth were demonstrated in the HD females largely driven by effects in a single dam; however, a treatment-related effect cannot be ruled out because additional dams were impacted and the findings were outside of the historical control range for the laboratory.
2. There was a reduction in F1 reproductive performance (female fertility and conception rats) and an increase in the mean number of days to mating and reduced mating index in the LD and HD animals. These findings were not clearly dose-dependent and reportedly outside the normal control ranges for the laboratory. Due to the lack of dose dependency, the study director considered them to be "of questionable relationship to the test item".
3. Toxicokinetic data suggest that although there was an initial increase in systemic exposure to GDO on GD 6 in LD and MD animals compared to control levels, there were no differences via this route of administration at any other time point and the levels were basically within the levels of the saline treated control animals, suggesting minimal to no elevations over endogenous levels during the treatment period.
4. The study report concludes that the NOAEL for reproductive performance of the F0 group to be the MD of 350 mg/kg/day (2100 mg/m²). I (RDM) concur. This dose is (b) (4) times the human maximum daily dose of (b) (4) mg/60 kg x (b) (4) = (b) (4) mg/m², based on body surface area.

5. The study report concludes that the NOAEL for growth and development of the F1 offspring to be the MD as well. I (RDM) concur. This dose is $\frac{(b)(4)}{(b)(4)}$ times the human maximum daily dose of $\frac{(b)(4)}{(b)(4)}$ mg/60 kg $\times \frac{(b)(4)}{(b)(4)} = \frac{(b)(4)}{(b)(4)}$ mg/m², based on body surface area.
6. The study notes that "Findings on the reproductive performance of F1 females (fertility and conception rate indices) of questionable relationship to the test item were observed at dose levels > 100 mg/kg/day." I (RDM) concur that these are unusual findings but difficult to interpret given the lack of clear dose dependency.
7. Given the slow clearance of the GDO from the local injection site (as evident by the local tissue reactions) and the TK data, and the exposure margins noted, the adverse effects reported above are not likely to be clinically significant, particularly given the indication and the potential risks associated with continued illicit opioid use.

Methods

Doses: 100, 350, or 1000 mg/kg GDO per day
 Frequency of dosing: Daily
 Dose volume: 0.11 to 1.06 mL/kg
 Route of administration: SC
 Formulation/Vehicle: Controls received 0.9% saline injections
 Species/Strain: Sprague Dawley rats
 Number/Sex/Group: 25 Dams

Treatment Group ³	Targeted Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals		
			F ₀ Generation		
			Main		TK
			M ²	F	F
1. Control ¹	0	1.06	130	25	4
2. GDO	100	0.11		25	10
3. GDO	350	0.37		25	10
4. GDO	1000	1.06		25	10

¹Group 1 animals received the reference item alone (0.9 % Sodium Chloride for Injection USP (Saline)).

²Untreated sexually-mature males used to induce pregnancies only.

³Six injection sites on the back of the rats were used and were rotated throughout the study.

M: male; F: female; TK: toxicokinetic

Satellite groups: 10 per treatment group
 Study design: See above table. Males were not treated. Females were dosed from Gestation Day 6 to Post-partum Day 20

Deviation from study protocol: Based on test item density the doses of the material administered were slightly outside of the targeted doses (102, 344, and 986 mg/kg/day estimates). These differences do not compromise the study conclusions.

Observations and Results (Optional Table)

F₀ Dams

Survival: There was one mortality in the MD group on PPD16. Two unscheduled euthanasias were completed due to cannibalism of the pups by the dams, as noted below:

Group/Dose Level	Dam No.	Day of death	Fate	Reason for Euthanasia
2 – 100 mg/kg/day	2517	PPD1	Unscheduled euthanasia	Pups cannibalism
3 – 350 mg/kg/day	3512	PPD16	Found dead	-
4 – 1000 mg/kg/day	4514	PPD1	Unscheduled euthanasia	Pups cannibalism

Dam 3512 demonstrated evidence of deteriorating condition starting on PPD14 (decreased activity, decreased muscle tone, hunched back, dehydration, thinness, weakness, massive body weight loss from PPD 10 to 14. Lower body weights were noted in this dam's pups likely due to poor maternal health and behavior. Necropsy suggested cause of death related to lung edema and inflammation as well as inflammation in the kidney. Spleen and lymph nodes were enlarged, evidence of hemorrhage in the stomach lining, and decreased thymic weights were consistent with severe physiological stress. These findings were restricted to this animal and therefore not thought to be due to the GDO.

Clinical signs: Primarily restricted to injection site reactions. Described as slight to severe edema and erythema. The injection site demonstrated lump/firm swelling consistent with residual GDO at the site of injection. GDO did not appear to absorb systemically rapidly and was slowly cleared from the injection site.

One HD dam (4514) demonstrated signs of difficult parturition which correlated with poor maternal performance. She was terminated early due to cannibalism of the pups.

Modified Draize scoring for injection site reactions increased in a treatment dependent fashion in all treatment arms.

Body weight: A treatment-related trend in increased body weights was demonstrated in MD and HD animals starting from PPD7 to 21. However, body weight losses were noted in all groups from PPD17 to 21.

Food consumption: No treatment-related effects noted.

Uterine content: There was a higher number of pup deaths in the HD

group animals, largely driven by the seven dead pups from Animal 4514 discussed above. However, dead pups (2-5 per dam) were noted in four other dams as well.

Group/Dose Level	Post-implantation loss (%)	Live Birth Index (%)	Dead Pups
1 – 0 mg/kg/day	6.9	93	4
2 – 100 mg/kg/day	7.4	93	7
3 – 350 mg/kg/day	7.2	93	1
4 – 1000 mg/kg/day	12	88	19*

*Statistically significant when compared to Group 1: $p \leq 0.01$, Fisher

There was no reported effects on gestation indices, duration of parturition, corpora lutea counts, implantation sites, sex ratio, pre-implantation loss, or number of live or malformed pups noted.

There was an increased mean post-implantation loss percentage and decreased live birth index in the HD group as well, also largely driven by Dam 4514. If this dam were eliminated from the analysis, the Applicant notes that although the HD group was still numerically higher than the controls, the incidence rates were close to the historical control levels.

Necropsy observation: Macroscopic examination of the dams demonstrated local tissue injection site hemorrhage and presence of clear cysts/gelatinous material which is presumed to be the test item and related injection site damage.

There were two dams that did not litter. One demonstrated evidence of complete resorption (control animal), the other was not pregnant (MD).

Toxicokinetics: Levels (AUC) of GDO were higher in the LD and MD groups compared to the HD groups and controls on GD6, however, levels were comparable to controls on GD18 and PPD20. As the differences between these days all fluctuate within a factor of three and do appear to be clearly dose dependent or time dependent, these values were interpreted to be within the normal range.

Treatment group*	AUC _{0-24h} (µg·h/mL)		
	GD6	GD18	PPD20
1; Control (saline)	5.05	26.53	7.15
2; GDO 100 mg/kg/day	15.8	35.04	8.44
3; GDO 350 mg/kg/day	13.3	33.58	10.6
4; GDO 1000 mg/kg/day	9.63	25.24	7.59

* n=3/timepoint, refer to Text Table 9 in Section 7.16.

Dosing Solution Analysis: At the completion of the treatment period, a sample of the (b) (4) test item was returned to (b) (4) for analysis to confirm stability. The sample was within the prespecified ranges.

Other: None

F₁ Generation

Survival: Pup viability index was lower in the HD group on PND4.

Group/Dose Level	Day 4 Viability Index (%)	Day 7 Survival Index (%)	Day 14 Survival Index (%)	Day 21 Lactation Index (%)
1 – 0 mg/kg/day	97.9	100	100	100
2 – 100 mg/kg/day	93.6	100	100	99.5
3 – 350 mg/kg/day	95.1	100	100	100
4 – 1000 mg/kg/day	86.6	99.4	98.3	98.3

There were no effects on sex ratio, pup viability, survival or lactation indices between PPD7 and PPD21.

There were four post-weaning F₁ mortalities in the control animals, two in the LD group and one in the HD group. No clear treatment-related effects were demonstrated. Mortality/euthanasia was mostly due to euthanasia with deteriorating condition and evidence of hydrocephalus. Because the findings were more evidence in the control animals, the effects were not considered treatment-related.

Group/Dose Level (mg/kg/day)	F ₁ Animal No.	Day of death	Fate	Reason for Euthanasia
1 – 0	1017	PPD34	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
	1018	PPD26	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
	1020	PPD26	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
	1520	PPD40	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
2 – 100	2063	PPD66	Found dead	-
	2074	PPD23	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
4 – 1000	4082	PPD61	Unscheduled euthanasia	Deteriorating Condition

Clinical signs: No clear GDO related findings were reported. Clinical signs noted were distributed across the treatment groups.

Body weight: No clear GDO related effects reported.

Food consumption: Not recorded

Physical development: The following developmental landmarks were recorded:

<i>Physical Assessment</i>	<i>Reflexological Development</i>	<i>Sensory Development</i>
Pinna unfolding ¹ (PPD1 to PPD4)	Righting reflex ¹ (PPD2 to PPD4)	Pupillary closure †* (PPD21)
Eye opening ¹ (PPD12 to PPD20)	Negative geotaxis ¹ (PPD8 to PPD20)	Auricular startle response ¹ (PPD12 to PPD 20)
Preputial Separation† (PPD35 to PPD46)		Visual placing response †* (PPD21)
Tooth eruption ¹ (PPD7 to PPD20)		
Vaginal Opening† (PPD26 to PPD37)		

¹ Reported as the mean day of positive development per litter, by sex, and for combined sexes when applicable.

† Post weaning; on selected pups only (2 pups/sex/litter) and assessed until all pups in a given litter had a positive response.

†*Post weaning; on selected pups only (2 pups/sex/litter).

The report states there were no changes in any of these parameters.

There were no changes in vaginal opening or preputial separation reported.

Neurological assessment: The functional observational battery was assessed as per the following:

Open-field evaluation*	Involuntary motor movements (such as convulsion and tremors) Respiration Rearing Urination † Defecation † Gait Arousal Mobility Abnormal or stereotyped behavior
Manipulative evaluation	Ease of removal from the cage Handling reactivity Palpebral closure Eye observations (eg., lacrimation, exophthalmus) Salivation Piloerection Approach response † Auditory test (Click response) † Tail pinch response † Touch response † Pupil response to light Extensor thrust reflex Cornea reflex
Physiological evaluation	Body temperature
Neuromuscular evaluation**	Grip strength (forelimbs and hindlimbs) Landing foot splay***
Motor activity**	Figure 8 maze E-Water maze

* Animals were placed in the open-field area and observed for a period of at least 2 minutes.

** The neuromuscular evaluation and motor activity were performed following the completion of each series of manipulative evaluation, except E-water maze.

***Exceeded 5 evaluations when considered necessary.

†Was observed following the 2 minute interval while still in the open field.

No treatment-related changes in any FOB parameters were reported. There were no clear effects on motor activity or the E-Water Maze (learning and memory). Although this is not a complex learning task, it will be considered adequate given the low systemic exposures and endogenous nature of the test item.

Reproduction: There were no changes reported in the F1 offspring estrus cyclicity. F1 parenteral performance is summarized in the table below:

Group/Dose Level	Mean Day to Mating	Male Mating Index (%)	Female Fertility Index (%)	Conception Rate (%)
1 – 0 mg/kg/day	4.8	66.7	79.2	90.5
2 – 100 mg/kg/day	8.4	47.8	50.0	60.0
3 – 350 mg/kg/day	4.8	66.7	58.3	73.7
4 – 1000 mg/kg/day	7.7	52.2	56.5	68.4

The reduced indices in all treatment groups do not appear to be dose-dependent. The results are also outside of the historical control range for these animals. The clinical significance of these findings is not clear.

At Cesarean section of the F1 females on GD15, there were no changes in corpora lutea, number of implantation sites, or pre-implantation losses; however, there was an apparent increase in post-implantation losses and reduced live embryos in the LD and HD groups compared to controls. As these were within the range of the historical control values and not clearly dose dependent, the effects are not likely treatment-related.

Group/Dose Level	Post-Implantation Loss (%)	Live Embryos Index (%)
1 – 0 mg/kg/day	5.6	94
2 – 100 mg/kg/day	16	84
3 – 350 mg/kg/day	5.8	94
4 – 1000 mg/kg/day	11	89

Other: Toxicokinetic data were obtained from the TK animals on GD6, GD18, and PPD20 at 1, 2, 6, and 24 h after the injection per the following schedule.

No. of rats / timepoint		Pre-dose	Toxicokinetic timepoints (post-dose)			
			1 h	2 h	6 h	24 h
Group 1	3	√		√		√
Groups 2-4	3 (Subset 1)	√			√	
	3 (Subset 2)		√			√
	3 (Subset 3)			√		

F₂ Generation – no evaluations of the F₂ offspring were made.

Survival:

Body weight:

External evaluation:

Male/Female ratio:

Other:

Conclusions of Reproductive and Developmental Effects of GDO

During the development of this drug product (see EOP2 meeting minutes), the Division noted the following:

While your plan to rely on published literature may be acceptable, we note that this strategy may be risky as these articles typically do not include any underlying data and the designs of the studies may not have been conducted in the spirit of Good Laboratory Practices. We also note that if no specific information can be found for GDO, toxicity information for a "related" compound such as diacylglycerol (DAG), which contains GDO, may be supportive only if the levels of GDO tested in those studies can be clearly discerned and adequate levels of GDO were generated under the conditions tested, or if a persuasive argument can be made to support that the "related" compound would be predicted to have a similar or greater toxicity profile than GDO. We remind you that your safety risk assessment for GDO must address all of the standard toxicological endpoints per the FDA guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079250.pdf>. This includes evaluations for reproductive toxicology that include assessing: (1) the potential to affect fertility or early embryonic development to implantation; (2) teratology in both a rodent species and a mammalian nonrodent species; and (3) effects on prenatal and postnatal development, including maternal function.

It was noted in the first cycle that the existing safety assessment lacked embryo-fetal development data on GDO in rabbits. Further, although fertility and early embryonic development data and embryofetal development data in rats have been published with DAG, there was no clear understanding how much GDO is present in the DAG oil administered to the animals. The rabbit embryo-fetal development study with GDO (submitted in the second cycle) and the rat pre- and post-natal development study with GDO (submitted in the first cycle) demonstrated adequate safety margins. The amount of the GDO present in the DAG oil used in the published toxicology studies was calculated based on a probability estimate from the literature (discussed in detail above). With these data, the fertility and early embryonic development and rat embryo-fetal development studies from the literature were determined to provide adequate exposure margins for GDO. Taken together, the assessment of the developmental and reproductive toxicology of GDO is acceptable to support the safety of GDO as a novel excipient.

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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 REVIEW AND EVALUATION

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Indication: Opioid use disorder
Applicant: Braeburn Pharmaceuticals, Inc.
Review Division: Division of Anesthesia, Analgesia, and Addiction
Products
Reviewer: Elizabeth Bolan, PhD & Gary Bond, PhD
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Disclaimer

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1 Executive Summary

1.1 Introduction

Braeburn Pharmaceuticals submitted a 505(b)(2) NDA in support of their depot buprenorphine drug product referred to during development as CAM2038 for the treatment of opioid use disorder. The NDA relies upon an FDA-previous finding of safety for Subutex (buprenorphine sublingual) and literature. There are actually two drug product formulations in this NDA, a once weekly (q1w) and a once monthly (q4w) formulation. The formulations (CAM2038 q1w and CAM2038 q4w) primarily differ (b) (4). During development, the Division noted that as per any depot formulation development program, the fate of the injected material should be adequately characterized and any novel excipients should be adequately justified for safety as per the FDA guidance document on new excipients. In this case, the drug product contains two new excipients. First, the once monthly injection contains N-methyl-pyrrolidone (NMP) which during development was considered to be used in a new manner via this product (NMP was previously used in an FDA approved product for acute dental use and a product indicated for the treatment of advanced cancer). Both formulations contain glycerol dioleate (GDO), a diglyceride, which has also not previously been used in an FDA-approved drug product. Of note, the Agency recently approved a chronic depot injection product for a non-oncology indication that containing NMP at levels approximately (b) (4) times higher than this drug product with post-marketing requirements and labeling for potential adverse effects due to the NMP in that formulation. The review focuses on the safety of the CAM2038 formulations, and the two novel excipients (which are reviewed in Appendix 1 and 2, respectively).

1.2 Brief Discussion of Nonclinical Findings

There are no safety concerns with the drug substance or drug product specifications.

The components of the container closure system that contact the drug product primarily include a (b) (4) glass syringe with a (b) (4) rubber plunger stopper. Importantly, the drug products include (b) (4).

Although we provided detailed recommendations regarding the data needed to justify the safety of the container closure system, inadequate information was provided.

Some extractables and leachables data were submitted to characterize and justify the safety of potential leachables arising from the to-be-marketed container closure system and from manufacturing components that directly contact the drug product. However, working closely with the CMC review team, we have concluded that the extraction studies with the container closure system were not performed appropriately to give us confidence that the potential leachables profile of the container closure system was fully characterized. Specifically, the studies did not employ sufficiently exhaustive

conditions

Rather, the Applicant performed the studies

both q1w and q4w products contain the lipid-based excipient GDO, the studies should also have been performed with GDO and/or with additional based on our discussions with the CMC team. In addition, the Applicant employed an inappropriate analytical evaluation threshold, which determines whether an extractable compound should be monitored in definitive leachables studies and in some cases whether a toxicological risk assessment is warranted. As such, the Applicant only targeted two compounds in leachables testing and consequently may have missed other compounds that should have been monitored. The leachables studies were also deficient, in part because the extraction tests were insufficiently performed to inform the leachables studies as noted above, but also because they were not performed using at least three batches of each drug product with testing at multiple timepoints over the course of stability studies as we advised in Pre-NDA meeting discussions and in accordance with best practices. Rather, the Applicant submitted

We expressed our concerns regarding the extractables and leachables evaluations at the time of filing and the Applicant subsequently proposed to conduct new extractables and leachables studies and provide these data during the review cycle. New extraction data and leachables data from drug product on stability for 24 months were submitted on 12/14/2017. However, the additional extraction data and leachables data from interim stability samples has not yet been submitted, and some of these data are not expected until at least February of 2018. Therefore, the new data will not be reviewed and our deficiencies will be outlined in the complete response letter.

In terms of general toxicity, the Applicant is relying upon the Agency previous finding of safety of Subutex to address general toxicity of buprenorphine. As noted in the clinical pharmacology review, technically, the 128 mg proposed buprenorphine drug product q4w result in exposures to buprenorphine that exceed the referenced drug product Subutex. In general, this would require general toxicity studies in two species

The 9-month dog study with the clinical formulation discussed below does provide general toxicity data for buprenorphine in the nonrodent species. The applicant did not provide a chronic rat toxicology study for buprenorphine. Although the 128 mg q4w dose also exceeds the exposure from 24 mg dose of Subutex, the Applicant cited the fact that the referenced drug product Subutex contains long-term carcinogenicity data in mice and rats. The Division has concluded that there are adequate clinical data on buprenorphine such that general toxicity in two species are not necessary to support this proposed dose.

As with any depot formulation, the Applicant was required to address the fate of the injected materials. The Applicant evaluated the effects of q1w and q4w following SC injection in the dog model and q1w in the minipig model. In addition, local effects were also recorded in the 9-month dog study discussed below. The results indicate that following injection of either formulation primarily swelling at the injection site and tenderness was observed with little evidence of erythema or hemorrhage. Swelling lasted around 2-3 weeks in most cases, were resolving by around 30 days; however, longer duration reactions were also noted in some animals. Microscopically, the injection sites were characterized by mononuclear cell infiltration, cystic spaces at times surrounded by inflammatory cells, fibrosis, hemorrhage and eosinophilic material. Collectively the data suggest that the depot can last up to 30 days or longer and local tissue reactions are consistent with a foreign body reaction and the evidence that the body is attempting to clear the material from the injection site.

The Applicant conducted a 9-month subcutaneous repeat-dose toxicology study in the dog model testing both the q1w and the q4w formulations injected over rotating sites which provides adequate local and systemic safety justification for the proposed drug product in this model. From a local tissue perspective, CAM2038 q1w and q4w product local injection site produced swelling and thickening of the skin with both drug products and drug product vehicles. The swelling was the result of both the remaining depot material as well as the expected local tissue response to the depot (inflammatory cell infiltrates). Microscopically, the local injection site demonstrated minimal fibrosis and mononuclear cell infiltrates surrounding cystic spaces (likely residual injection depot matrix material) and evidence of chronic inflammation. In addition, there were changes in the axillary (draining) lymph nodes that included small cystic spaces (likely lipid) with "little to no" associated tissue response. The chronic tissue response at older injection sites consisted of cavitation of the tissue with mononuclear and multinuclear giant cells. Collectively these changes are consistent with a foreign body reaction to a depot injection.

To address the remaining requirements for the new excipient GDO, the Applicant submitted PK studies in the rat that demonstrated that SC injection of GDO resulted in no detectable levels of GDO in the plasma over background levels. They also submitted a chronic toxicology study in the rat testing GDO and leveraged the findings in the 9-month dog study with CAM2038 q4w to address chronic toxicity. The results suggest expected local tissue reactions and evidence of systemic inflammation, also not unexpected for a lipid. Adequate NOAELs were established to support safety. The Applicant conducted the full genetic toxicology battery of studies with GDO which indicated no risk for genotoxicity. To address the standard reproductive and developmental toxicology study requirements, they submitted published literature testing diacyl glycerol (DAG) oil containing significant levels of GDO to address fertility and early embryonic development and embryofetal development studies in the rat and conducted a pre- and post-natal study on GDO. No embryofetal development studies in the rabbit were completed or submitted. Adequate NOAELs were established to support safety. Finally, the Applicant cited published carcinogenicity studies with DAG oil to address the requirement for carcinogenicity of GDO. These data suggest that the

risk of carcinogenicity of DAG is no different than the risk of triglycerides. The Agency agreed that if the company can provide data to document that the DAG oil studies contained adequate levels of GDO in the material tested, and provided adequate coverage for the safety of the GDO administered, these data would be adequate to support the new excipient. The Applicant did not specifically address this requirement. The published articles do contain data indicating that the DAG oil tested did contain a significant amount (~25%) of oleic acid residues suggesting that some of the material tested would likely have two oleic acids attached to the glycerol moiety (hence GDO). However, no definitive data were provided. As the GDO does not appear to increase systemically in the rat following SC injection, the material is likely metabolized locally resulting in release of free oleic acid residues and glycerol. These should be cleared by normal metabolic processes. As such the risk is likely low for systemic exposure to GDO and therefore low for reproductive risk. Nonetheless, as it is not clear how much GDO is in DAG oil, and the Applicant did not provide justification as requested for the use of this surrogate, an adequate scientific bridge (justification) should be provided to definitively leverage those data. Further, the lack of any data for rabbit EFD is not consistent with the requirements outlined in the FDA guidance for new excipient safety testing. An EFD study in the rabbit should be provided unless the Applicant can provide adequate justification that systemic levels of GDO and metabolites are not elevated over baseline in humans.

In addition to the 9-month dog study that contained NMP in the formulation tested, the Applicant also submitted a literature review to support the safety of NMP in this drug product formulation. Based on PK studies in humans following administration of CAM2038 q4w, the NMP in this formulation appears to be cleared from plasma within 24 hours of drug product injection. NMP has been tested in several published subchronic toxicology studies using the oral or inhalation route of administration and although some adverse effects (mostly hepatocellular hypertrophy) were reported in these studies, NOAELs that established reasonable safety margins were demonstrated for this product suggesting limited concern. NMP was deemed overall negative in terms of genotoxic potential. Published carcinogenicity studies with NMP do suggest the potential for NMP-induced liver tumors in mice which may not be relevant to human risk assessment; however, a mode of action assessment was not provided to permit dismissal of the findings in the study as not relevant to human risk assessment. These findings should be included in labeling, unless demonstrated to be irrelevant to human risk via a mode of action assessment.

The Applicant submitted published and unpublished reproductive and developmental studies with NMP in lieu of new studies. The published studies employed the oral (gavage/dietary) or inhalation routes and did not test the clinical route of administration. These studies indicate that NMP can have adverse effects on male and female fertility and fetal development. The existing fertility studies suggest a conservative NOAEL of approximately 4.3x for male fertility effects via the dietary route; whereas published studies suggest no impact on male fertility at higher doses in the rat. Given the lack of clarity on the impact of NMP on fertility, the lack of studies via the clinical route, and the potential for adverse effects, definitive studies via the SC route are warranted.

The published and unpublished embryofetal development data for NMP submitted by the Applicant and identified by the Agency suggest the potential for adverse effects of NMP on embryofetal development in the rat that includes preimplantation losses, delayed ossification, and decreased fetal weight at doses 8.4 times the maximum daily dose (MDD) via this product. At higher doses, increased resorptions and fetal malformations were reported at 21.5x and fetal lethality and malformations have been reported at 64.6x the MDD based on body surface area. An audited draft report of an embryofetal development study in rabbits was also submitted. This report suggested greater safety margins in this species; however, the study was not a final report and it is not clear how the oral route exposures compare to SC exposures via this product. Collectively, although there are several publications in the literature on NMP, the Applicant did not provide an adequate scientific bridge to put these findings into perspective, and the unpublished draft report in the rabbit is not acceptable to qualify a new excipient. Finally, several published reports on the impact of NMP on pre- and postnatal development were reviewed in support of this application. The data suggest that NMP can result in decreased pup survival at doses 12.9 times the MDD and developmental delays at 7.6 times the MDD. Again, these are based on body surface area comparisons following oral administration and it is not clear how much SC dosing will impact these margins. Because the safety margins and potential adverse effects for buprenorphine in the current labeling overlap to some extent with the safety margins and adverse effects of NMP, given the data to date and inclusion of the findings in labeling, should this NDA be approved this cycle, definitive studies for the SC route could be completed as a post-marketing requirement. If the NDA is not approved this cycle, the Applicant should attempt to address these concerns in the resubmission.

If there are adequate data to support approval of this NDA from all other disciplines, given the potential for public benefit, the recommended reproductive and developmental study deficiencies and the mode of action assessment described below could be considered as post-marketing requirements. Based on the data submitted in the NDA, the lack of adequate data on the container closure system should be address to support approval.

There are limited data to inform what could occur should someone attempt to misuse the drug product intravenously. In a nonGLP rat intravenous toxicity study in 3 males, administration of the drug to the tail vein resulted in occlusion of the vessel to the base of the tail with no clear evidence of distribution to other tissues. Lungs, however, could not be evaluated as the animals were sacrificed via CO₂ which results in lung pathology itself. The data suggest that if the drug product were to be administered intravenously, it will either gel rapidly and potentially block the injected vessel as it apparently did in the rat tail vein, or if the injected vein is larger and the product does not gel quickly, it could likely result in a lung emboli or eventually be lodged in other small capillaries. The clinical significant of any blockade of blood vessels would depend upon the redundancy of vascular perfusion and degree of blockade, but it could result in tissue hypoxia and necrosis with an adverse impact on tissue functionality. Given the existing

evidence of prolonged tissue residency, the vascular blockade of any tissue would be expected to take considerable time to clear.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, there are inadequate data to support an approval recommendation at this time. Specifically, the Applicant has not provided an adequate extractable leachable evaluation of the container closure system.

The following deficiencies apply:

1. You have not provided adequate extractables studies to fully characterize the potential leachables profile of the proposed container closure system. Specifically, the studies submitted with the NDA did not employ sufficiently exhaustive condition (b) (4)

We acknowledge that your TTCs were based on concepts outlined in ICH M7, and this guidance notes that the acceptable daily intake for genotoxic impurities may be based on total number of dosing days over a lifetime. However, your product is intended to provide continuous exposure to the API for the duration of dosing. Due to the way in which the CAM2038 drug depot is intended to form in subcutaneous tissue after drug administration, leachables arising from the container closure system are likely to be incorporated into the depot (b) (4). Consequently, leachables may be exposed over a long duration just as the API is released over the course of at least 7 days for 1qw and 28 days for q4w. Moreover, treatment for opioid abuse disorder may exceed 10 years. Therefore, your AETs should be able to detect any compound that could be exposed at 1.5 mcg/day or greater. We acknowledge that you have initiated new extractables studies to address these concerns. However, these new data were not provided in a time frame in which they could be reviewed during this review cycle. With your resubmission, submit final study reports for extraction studies testing (b) (4) employing rigorous extraction techniques in accordance with *USP <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems* and ensure that your analytical methods are capable of detecting any compound over 1.5 mcg/day. These data should be used to inform which compounds should be monitored in the leachables studies.

2. You have not provided an adequate leachables evaluation to justify the safety of the proposed container closure system. Specifically, your leachables evaluation did not 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 as we advised at the Pre-NDA meeting held on 3/16/2017

and in accordance with best practices per *USP <1664>: Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems*. To address this deficiency, conduct a new leachables study that evaluates at least three batches of the to-be-marketed q1w and q4w drug products for leachables and include assessments at multiple timepoints over the course of your stability studies (beginning, middle, and end of proposed shelf-life) 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. 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 upstream manufacturing processes that suggest the potential for additional leachable compounds in the final drug product formulation. For all drug products, establish your AET to be able to detect any compound that could be present at 1.5 mcg/day or greater. If you cannot meet these thresholds, safety evaluations should be based on the limits of quantitation (LOQ). 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. Provide a toxicological risk assessment that qualifies any leachable detected at or greater than 1.5 mcg/day from a genotoxicity perspective, and any leachable detected at or greater than 5 mcg/day from a general toxicity perspective. Include copies of all referenced studies upon which a safety assessment is based. We acknowledge that you initiated new leachables studies during this review cycle; however, these new data were not provided in a time frame in which they could be reviewed during this review cycle. Submit final study reports for leachables studies that take into consideration the comments outlined above with your resubmission.

3. You have not provided adequate pharmacokinetic data to appropriately inform exposure margins for buprenorphine in your labeling. Although you have provided AUC_{0-24h} data in animals, you have only provided human AUC_{ss} data. The exposure margins based on AUC in the labeling should be based on the worst-case exposures in humans over the treatment period at steady state. To address this issue, provide mean partial AUC (24-hour interval) data in humans at steady state that represents the highest exposures over any given 24-hour period to inform labeling.
4. You have not provided adequate justification that the cited and published data on the reproductive and developmental effects of NMP adequately address all standard toxicological endpoints for reproductive and developmental toxicity studies. Further, you have not provided adequate data to bridge the published and unpublished studies which employed routes other than the proposed clinical route. Although the data suggest some degree of safety following the differential routes of administration, lack of adequate toxicokinetic data and inclusion of all standard endpoints in these studies preclude definitive conclusions regarding the

potential risk of NMP via your drug product on these endpoints. To address this deficiency, conduct definitive reproductive and developmental studies with NMP administered via the SC route. Alternatively, if you provide adequate PK bridging studies to the existing studies and clearly delineate how the referenced studies address all standard endpoints for fertility and early embryonic development, embryofetal development in two species, and pre- and postnatal development. Draft unpublished study reports are not acceptable as they may not reflect the final presentation of the data. Should you elect to conduct bridging studies, your resubmission must also include a discussion regarding the impact of the safety margins for reported effects of NMP given the known risks to these endpoints from buprenorphine.

5. You have not provided an adequate scientific bridge to the referenced published studies testing diacylglycerol oil (DAG) as a surrogate chemical solution for glycerol dioleate (GDO) for the effects of this novel excipient on rat fertility and early embryonic development, rat embryofetal development, rodent general toxicity, or rat and mouse carcinogenicity. As discussed in the EOP2 meeting held on 2/24/2015, provide data to support your conclusion that the DAG oil tested in these publications contains GDO at levels which provide an adequate characterization of the effects of GDO on these required studies.
6. You have not provided a rabbit embryo-fetal development study for the new excipient GDO. Either conduct an EFD study in the rabbit testing GDO or provide adequate justification why a study is not necessary.
7. You have not provided adequate justification for your conclusion that there is no carcinogenic potential of NMP even though your toxicological risk assessment of NMP noted hepatic tumors in mice. Conduct a mode of action assessment for N-methyl-pyrrolidone-induced mouse hepatocellular adenomas and carcinomas to inform the human risk assessment for NMP.

1.3.2 Additional Non Clinical Recommendations

1.3.3 Labeling

The labeling recommendations below have not been discussed with the team or the Applicant and may not reflect final labeling recommendations. For the final labeling, the reader is referred to the Action Letter.

<i>Applicant's proposed label language</i>	<i>Reviewer's recommended changes</i>	<i>Comments and rationale for recommended changes</i>
8.1 Pregnancy <u>Risk Summary</u>	8.1 Pregnancy <u>Risk Summary</u>	

(b) (4) Embryofetal death was observed in both rats and rabbits administered buprenorphine during the period of organogenesis at doses (b) (4) (b) (4)	(b) (4) Embryofetal death was observed in both rats and rabbits administered buprenorphine during the period of organogenesis at doses approximately 13 and 18 times, (b) (4) respectively, the mean daily dose of 4.6 mg buprenorphine per day as delivered by either the BRAND NAME WEEKLY or BRANDNAME MONTHLY products. (b) (4) Pre- and post-natal development studies in rats demonstrated increased neonatal deaths at doses approximately (b) (4) 2 times and (b) (4) above and dystocia at approximately 11 times the mean daily dose of 4.6 mg/ of buprenorphine. No clear teratogenic effects were seen when buprenorphine was administered during organogenesis with a range of doses 3 times and greater than the mean daily dose of 4.6 mg of buprenorphine. (b) (4) (b) (4)	Exposure margins have been updated. The human dose of 32 mg CAM2038 q1w was expressed as the mean daily dose. The human AUC data are from Study HS-15-549. At steady state, exposure is highest from about 12-36 h post dose. The Applicant will be asked to provide partial AUC values at the mean maximal exposure. This value will be used in the exposure margins for AUC and the mg dose will be adjusted accordingly for BSA comparisons. (b) (4) The Applicant omitted the description of the studies in red that appear in the risk summary of their referenced product, Subutex. They have been added back in with appropriate exposure margins.
However, increases in skeletal abnormalities were noted in rats and rabbits administered buprenorphine daily during organogenesis at doses (b) (4)	However, increases in skeletal abnormalities were noted in rats and rabbits administered buprenorphine daily during organogenesis at doses 2 and 4 times (b) (4) for the mean daily dose of 4.6 mg/ of	

(b) (4) In a few studies, some events such as acephalus and omphalocele were also observed but these findings were not clearly treatment-related.	buprenorphine (b) (4) In a few studies, some events such as acephalus and omphalocele were also observed but these findings were not clearly treatment-related. In published animal reproduction studies with NMP, an excipient in TRADENAME ONCE MONTHLY, preimplantation losses, delayed ossification, reduced fetal weight, developmental delays and reduced cognitive function were reported at doses 8-10 times the doses of NMP via TRADENAME ONCE MONTHLY. Decreased pup survival at 13 times the dose of NMP and malformation and postimplantation losses were reported at 21.5 times the dose of NMP via TRADENAME ONCE MONTHLY [see Data].	
<u>Data</u> <i>Animal Data</i> The exposure margins below are based on (b) (4) (b) (4)	<u>Data</u> <i>Animal Data</i> The exposure margins below are based on the mean daily dose (MDD) of 4.6 mg buprenorphine per day delivered by either 32 mg BRANDNAME WEEKLY or 128 mg BRANDNAME MONTHLY based on body surface area, unless otherwise noted.	Note: Applicant will be asked to provide partial AUC data for human exposures as steady state. This will likely result in an adjustment of the mean daily dose to the maximum daily dose over the course of the treatment period. This will be revised accordingly.
(b) (4)	No definitive drug-related teratogenic effects were observed in rats and rabbits at IM doses up to 30 mg/kg/day (estimated exposure approximately 64 times and 128 times, respectively, the MDD). Maternal toxicity resulting in mortality was noted in these studies in both rats and rabbits. Acephalus was observed in one rabbit fetus from the low-dose	Exposure margins were updated and the omitted text from the Subutex label that was omitted by the Applicant was added back in.

(b) (4)	group and omphalocele was observed in two rabbit fetuses from the same litter in the mid-dose group; no findings were observed in fetuses from the high-dose group. Maternal toxicity was seen in the high-dose group but not at the lower doses where the findings were observed. Following oral administration of buprenorphine to rats, dose-related post-implantation losses, evidenced by increases in the numbers of early resorptions with consequent reductions in the numbers of fetuses, were observed at doses of 10 mg/kg/day or greater (estimated exposure approximately 21 times the MDD). In the rabbit, increased post-implantation losses occurred at an oral dose of 40 mg/kg/day. Following IM administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 mg/kg/day.	
Maternal toxicity resulting in mortality was noted in studies in both rats and rabbits. Acephalous was observed in one rabbit fetus from the low-dose group (b) (4) and omphalocele was observed in two rabbit fetuses from the same litter in the mid-dose group (b) (4) no findings were observed in fetuses from the high-dose group. Maternal toxicity was seen in the high-dose group (b) (4) but not at the lower doses. Following oral administration of buprenorphine to rats, dose-related post-implantation losses, evidenced by increases in the numbers of early resorptions with consequent reductions in the numbers of fetuses, were observed at doses of (b) (4) mg/kg/day or greater (b) (4)	Buprenorphine was not teratogenic in rats or rabbits after IM or subcutaneous (SC) doses up to 5 mg/kg/day (estimated exposure was approximately 11 and 21 times, respectively, the MDD), after IV doses up to 0.8 mg/kg/day (estimated exposure was approximately 2 and 3 times, respectively, the MDD), or after oral doses up to 160 mg/kg/day in rats (estimated exposure was approximately 341 times the MDD) and 25 mg/kg/day in rabbits (estimated exposure was approximately 106 times the MDD). Significant increases in skeletal abnormalities (e.g., extra thoracic vertebra or thoraco-lumbar ribs) were noted in rats after SC administration of 1 mg/kg/day and up (estimated exposure was approximately 2 times the MDD), but were not	Exposure margins were updated and the omitted text from the Subutex label that was omitted by the Applicant was added back in.

(b) (4)	observed at oral doses up to 160 mg/kg/day. Increases in skeletal abnormalities in rabbits after IM administration of 5 mg/kg/day (estimated exposure was approximately 21 times the MDD) in the absence of maternal toxicity or oral administration of 1 mg/kg/day or greater (estimated exposure was approximately 4 times the MDD) were not statistically significant. In rabbits, buprenorphine produced statistically significant pre-implantation losses at oral doses of 1 mg/kg/day or greater and post-implantation losses that were statistically significant at IV doses of 0.2 mg/kg/day or greater (estimated exposure approximately equal to the MDD). No maternal toxicity was noted at doses causing post-implantation loss in this study.	
Fertility, pre-, and post-natal development studies with buprenorphine in rats indicated increases in neonatal mortality after oral doses of (b) (4) An apparent lack of milk production during these studies likely contributed to the decreased pup viability and lactation indices. Delays in the occurrence of righting reflex and startle response were noted in rat pups at an oral dose of 80 mg/kg/day (b) (4)	Dystocia was noted in pregnant rats treated intramuscularly with buprenorphine from Gestation Day 14 through Lactation Day 21 at 5 mg/kg/day (approximately 11 times the MDD). Fertility, and pre- and post-natal development studies with buprenorphine in rats indicated increases in neonatal mortality after oral doses of 0.8 mg/kg/day and up (approximately 2 times the MDD), after IM doses of 0.5 mg/kg/day and up (approximately equal to the MDD), and after SC doses of 0.1 mg/kg/day and up (approximately 0.2 times the MDD). An apparent lack of milk production during these studies likely contributed to the decreased pup viability and lactation indices. Delays in the occurrence of righting reflex and startle response were noted in rat pups at an oral dose of 80 mg/kg/day (approximately 170 times the MDD).	Exposure margins were updated and the omitted text from the Subutex label that was omitted by the Applicant was added back in.
	Preimplantation losses, delayed	See summary table in Appendix

	ossification and reduced fetal body weights were reported in published studies following treatment of pregnant rats during organogenesis with NMP, an excipient in BRANDNAME ONCE MONTHLY, via inhalation at 8.4 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY. Reduced ossification and decreased fetal body weights at 10.8 times the MDD of NMP and fetal malformations and resorptions have also been reported following oral administration at 21.5 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY at the MDD based on a body surface area comparison.	1
	Post-implantation loss and increased cardiovascular and skull malformations were demonstrated in pregnant rabbits administered oral NMP, an excipient in BRANDNAME ONCE MONTHLY, at doses 93 times the human MDD of NMP via BRANDNAME ONCE MONTHLY in the absence of maternal toxicity. No adverse effects were reported at an oral dose 30 times the MDD via BRANDNAME ONCE MONTHLY based on a body surface area comparison.	For now, these data are from the IRDC draft report.
	Decreased pup survival was noted following oral treatment of pregnant rats prior to and during gestation and lactation with NMP, an excipient in BRANDNAME ONCE MONTHLY at doses 13 times the MDD. A NOAEL was not demonstrated. Developmental delays and impaired cognitive function were reported in pups born to pregnant rats treated with NMP via inhalation during gestation at doses 7.6 times the MDD of NMP via BRANDNAME ONCE MONTHLY based on a	Data source: (Sitarek et al., 2012)

	body surface area comparison.	
	Male-mediated adverse effects on offspring (decreased pup weight and survival) were noted in a published study in which male rats were treated orally for 10 weeks with NMP, an excipient in BRANDNAME ONCE MONTHLY at daily doses 26 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. No adverse effects were noted at oral doses 8.6 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY.	NMP data from (Sitarek and Stetkiewicz, 2008)
8.3 Females and Males of Reproductive Potential <u>Infertility</u> Chronic use of opioids may cause reduced fertility in females and males of reproductive potential. It is not known whether these effects on fertility are reversible [see <i>Adverse Reactions (6.2), Clinical Pharmacology (12.2), Nonclinical Toxicology (13.1)</i>].	8.3 Females and Males of Reproductive Potential <u>Infertility</u> Chronic use of opioids may cause reduced fertility in females and males of reproductive potential. It is not known whether these effects on fertility are reversible [see <i>Adverse Reactions (6.2), Clinical Pharmacology (12.2), Nonclinical Toxicology (13.1)</i>].	No changes were made to this section.
(b) (4)		
13 NONCLINICAL TOXICOLOGY 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenicity</u> Carcinogenicity studies of buprenorphine were conducted in Sprague-Dawley rats and CD-1 mice. Buprenorphine was administered in the diet to rats	13 NONCLINICAL TOXICOLOGY 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenicity</u> Carcinogenicity studies of buprenorphine were conducted in Sprague-Dawley rats and CD-1 mice. Buprenorphine was administered in the diet to rats at doses of 0.6, 5.5, and 56 mg/kg/day (estimated exposure was approximately 0.1, 1 and 12 times the mean maximum daily	Exposure margins were updated based on AUC data. Omissions were added back in.

(b) (4) increase in Leydig cell tumors occurred (b) (4) In CD-1 mice, buprenorphine was not carcinogenic at dietary doses up to 100 mg/kg/day (b) (4)	dose [MDD] of 4.6 mg buprenorphine per day as delivered by either 32 mg BRAND NAME WEEKLY or 128 mg BRANDNAME MONTHLY on an AUC basis) for 27 months. As in the buprenorphine/naloxone carcinogenicity study in rat, statistically significant dose-related increases in Leydig cell tumors occurred. In an 86-week study in CD-1 mice, buprenorphine was not carcinogenic at dietary doses up to 100 mg/kg/day (estimated exposure was approximately 10 times the MDD on an AUC basis). NMP, an excipient in BRANDNAME ONCE MONTHLY, produced an increase in hepatocellular adenomas and carcinomas in male and female mice at 47 and 60 times the maximum daily dose (MDD) of NMP via BRANDNAME ONCE MONTHLY. No tumors were noted at 7.4 and 9.5 times the MDD. The clinical significance of these findings is unclear. In 2-year inhalation and dietary studies in rats, NMP did not result in evidence of carcinogenicity.	
Mutagenicity Buprenorphine was studied in a series of tests utilizing gene, chromosome, and DNA interactions in both prokaryotic and eukaryotic systems. Results were negative in yeast (<i>S. cerevisiae</i>) for recombinant, gene convertant, or forward mutations; negative in <i>Bacillus subtilis</i> "rec" assay; negative for clastogenicity in CHO cells, Chinese hamster bone marrow and spermatogonia cells, and negative in the mouse lymphoma L5178Y assay. Results were equivocal in the Ames test: negative in studies in	Mutagenicity Buprenorphine was studied in a series of tests utilizing gene, chromosome, and DNA interactions in both prokaryotic and eukaryotic systems. Results were negative in yeast (<i>S. cerevisiae</i>) for recombinant, gene convertant, or forward mutations; negative in <i>Bacillus subtilis</i> "rec" assay, negative for clastogenicity in CHO cells, Chinese hamster bone marrow and spermatogonia cells, and negative in the mouse lymphoma L5178Y assay. Results were equivocal in the Ames test: negative in studies in two laboratories, but positive for	No changes were made to this section.

two laboratories, but positive for frame shift mutation at a high dose (5 mg/plate) in a third study. Results were positive in the Green-Tweets (<i>E. coli</i>) survival test, positive in a DNA synthesis inhibition (DSI) test with testicular tissue from mice, for both in vivo and in vitro incorporation of [³H] thymidine, and positive in unscheduled DNA synthesis (UDS) test using testicular cells from mice.	frame shift mutation at a high dose (5 mg/plate) in a third study. Results were positive in the Green-Tweets (<i>E. coli</i>) survival test, positive in a DNA synthesis inhibition (DSI) test with testicular tissue from mice, for both in vivo and in vitro incorporation of [³H]thymidine, and positive in unscheduled DNA synthesis (UDS) test using testicular cells from mice.	
<u>Impairment of Fertility</u> Reproduction studies of buprenorphine in rats demonstrated no evidence of impaired fertility at daily oral doses up to 80 mg/kg/day (estimated exposure was approximately [REDACTED] (b) (4) [REDACTED])	<u>Impairment of Fertility</u> Reproduction studies of buprenorphine in rats demonstrated no evidence of impaired fertility at daily oral doses up to 80 mg/kg/day (estimated exposure was approximately 170 times the mean maximum daily dose [MDD] of 4.6 mg buprenorphine per day as delivered by either 32 mg BRANDNAME WEEKLY or 128 mg BRANDNAME MONTHLY on a mg/m² basis) or up to 5 mg/kg/day IM or SC (estimated exposure was approximately 6 times the MDD). Adverse effects on testes and male fertility were noted in a published study in which rats were treated for 10 weeks with daily oral doses of NMP, an excipient in BRANDNAME ONCE MONTHLY at 86 times the MDD and resulted in male-mediated adverse effects on offspring (decreased pup weight and survival) at daily doses 26 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. No adverse effects were noted at oral doses 8.6 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY.	Exposure margins were updated. Data on NMP do suggest the potential for adverse effects (Sitarek and Stetkiewicz, 2008).

2 Drug Information

2.1 Drug

CAS Registry Number: 52485-79-7

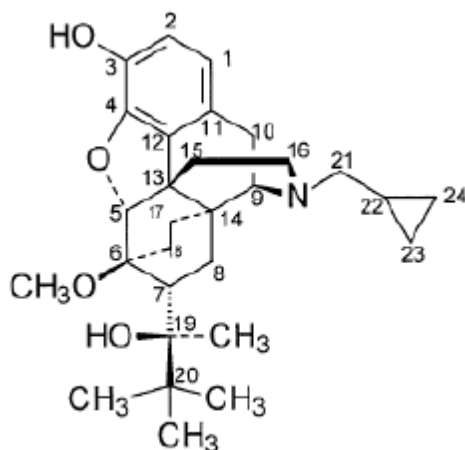
Generic Name: Buprenorphine base

Code Name: N/A

Chemical Name: (2S)-2-[-(-)-(5R,6R,7R,14S)-9 α -cyclopropylmethyl-4,5-epoxy-6,14-ethano-3-hydroxy-6-methoxymorphinan-7-yl]-3,3-dimethylbutan-2-ol

Molecular Formula/Molecular Weight: C₂₉H₄₁NO₄

Structure:



Pharmacologic Class: Partial opioid agonist (FDA Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

<i>NDA</i>	<i>Name</i>	<i>Division</i>	<i>Strength (route)</i>	<i>Marketing Status</i>	<i>Approval Date</i>	<i>Indication</i>	<i>Applicant</i>
20732	Subutex	DAAAP	SL	Approved	10/8/2002	Opioid Use Disorder	Indivior

<i>IND</i>	<i>Drug</i>	<i>Status</i>	<i>Division</i>	<i>Indication</i>	<i>Stamp Date</i>	<i>Sponsor</i>
114082	CAM2038	Active	DAAAP	Opioid dependence	5/9/2015	Braeburn

<i>DMF</i>	<i>Subject</i>	<i>Holder</i>	<i>Status</i>	<i>Reviewer's Comments</i>
			(b) (4) Active since	Referenced in

(b) (4)	4/13/2010	multiple FDA-approved drug products
	Active since 3/25/1992	Not previously reviewed formally Not referenced via other FDA-approved applications
	Active since 4/24/2007	
	Active since 10/4/2013	
	Active since 12/14/2015	Referenced by multiple FDA-approved drug products
	Active since 8/9/20047	Referenced by other FDA-approved products
	Active since 1/30/2015	Not formally reviewed to date.

2.3 Drug Formulation

The CAM2038 q1w and CAM2038 q4w formulations are a once-weekly and once-monthly extended-release lipid-based subcutaneous depots containing 50 mg/mL buprenorphine base. The proposed strengths of the single-use prefilled syringes for the q1w product are 8 mg, 16 mg, 24 mg, and 32 mg and the strengths for the q4w product are 64 mg, 96 mg, 128 mg (b) (4). The injection depot platform contains soybean phosphatidylcholine (SPC), glycerol dioleate (GDO) and a (b) (4) and is referred to by the Applicant as “FluidCrystal” technology. The two formulations (b) (4) (b) (4) with the q1w product containing ethanol and the q4w formulation containing N-methyl-2-pyrrolidone (NMP). According to the Applicant, “once injected, CAM2038 spontaneously transforms from a low-viscous solution to a highly viscous crystalline gel that encapsulates the BUP and releases it at a steady rate as the depot degrades.” The quantitative composition of each strength for the two products are outlined in the tables below.

Table 1. Composition of CAM2038 q1w Drug Product

Component	Standard	Function	Composition (mg) per dose			
			8 mg/ 0.16 mL	16 mg/ 0.32 mL	24 mg/ 0.48 mL	32 mg/ 0.64 mL
Buprenorphine Base (BUP)	Ph. Eur.	Active ingredient	8	16	24	32
Ethanol Anhydrous (Dehydrated alcohol) (EtOH)	USP	(b) (4)	(b) (4)			
Soybean Phosphatidylcholine (SPC)	In-house	(b) (4)	(b) (4)			
Glycerol Dioleate (GDO)	In-house	(b) (4)	(b) (4)			
Total weight (mg) for all components per dose	Not Applicable	Not Applicable	(b) (4)			

Table 2. Composition of CAM2038 q4w Drug Product

Component	Standard	Function	Composition (mg) per dose		
			64 mg/ 0.18 mL	96 mg/ 0.27 mL	128 mg/ 0.36 mL
Buprenorphine Base (BUP)	Ph. Eur.	Active ingredient	64	96	128
N-Methyl-2-Pyrrolidone (NMP)	USP	(b) (4)	(b) (4)		
Soybean Phosphatidylcholine (SPC)	In-house	(b) (4)	(b) (4)		
Glycerol Dioleate (GDO)	In-house	(b) (4)	(b) (4)		
Total weight (mg) for all components per dose	Not Applicable	Not Applicable	(b) (4)		

2.4 Comments on Novel Excipients

There are two novel excipients in the drug product formulations covered by this NDA.

N-methyl-2-pyrrolidone (NMP) - Only the CAM2038 q4w contains NMP (b) (4). As discussed during development, the use of NMP in this drug product formulation is technically novel by the SC route of administration. According to the Inactive Ingredients Database (IID, December 12, 2017), NMP is currently used as an excipient in two FDA-approved drug products, one of which is approved for the periodontal route for an acute indication and the other for the subcutaneous route for the palliative treatment of prostate cancer.

The novelty of NMP for a chronic non-oncology indication was discussed with the Applicant over the course of development and the Agency noted that the Applicant would have to address the safety for this indication. Literature could be used to support the standard requirements for a novel excipient; however, the adequacy of the literature could only be determined during the NDA review. At the preNDA meeting in 2017, the Division noted that there was technically no chronic rodent toxicology study with NMP; however, we acknowledged that the carcinogenicity studies with NMP may be adequate to address this requirement for any novel chronic excipient.

Refer to Appendix 1 in Section 12 for more information on NMP and to Section 1.3 Recommendation for proposed regulatory activity regarding NMP and this NDA submission.

Glycerol dioleate (GDO) - Glycerol dioleate (GDO) is included in both the CAM2038 q1w and q4w formulations. The highest proposed level is (b) (4) mg/32 mg dose via the weekly dosed product. The proposed concentration is (b) (4) mg/mL or (b) (4) % w/v. GDO is not listed in the FDA IID and is therefore considered a novel excipient that must be qualified for safety per the guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*. Diglycerides, like GDO, are endogenously formed via the hydrolysis of triglycerides. Diglycerides are metabolized by lipases to the free fatty acids and glycerol. The fatty acids are further metabolized via beta-oxidation or utilized as substrates for cellular metabolism.

Toxicological safety data was submitted by the Applicant for GDO (see Appendix 2). The Applicant also references published studies with diacyl glycerol oils (DAG) as a surrogate molecule for GDO. According to the Applicant, DAG oils are largely GDO but also contain linoleic acids, and other fatty acids attached to glycerol.

Refer to Appendix 2 in Section 12 for more information on GDO and to Section 1.3 Recommendation for proposed regulatory activity regarding GDO and this NDA submission.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Specifications

The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substance for a maximum daily dose (MDD) of ≤ 2 g/day is 0.15% or 1 mg/day intake, whichever is lower. The Applicant has set the specifications for impurities in the buprenorphine drug substance obtained from (b) (4) (DMF (b) (4)) at NMT (b) (4) % and no qualification will be necessary. It is noted that several additional impurity specifications are listed in (b) (4) DMF (b) (4). The CMC team will determine whether additional drug substance specifications are necessary for buprenorphine in this NDA.

Table 3. Drug Substance Impurities for Buprenorphine in CAM2038 q1w and CAM2038 q4w

<i>Impurity</i>	<i>Proposed Specification</i>	<i>ICH Qualification Thresholds or Limits</i>	<i>Comments</i>
(b) (4)	< (b) (4) %	0.15% or 1 mg/day	Acceptable

Drug Product Specifications

For the CAM2038 q1w product (highest strength 32 mg BUP), the qualification threshold according to the ICH Q3B(R2) guidelines for impurities/degradants in the drug product for an MDD between 10 mg and 100 mg of BUP administered per day is 0.5% or 200 mcg TDI, whichever is lower. For the CAM2038 q4w product (highest strength (b) (4) BUP), the qualification threshold according to the ICH Q3B(R2) guidelines for impurities/degradants in the drug product for an MDD between >100 mg- 2 g of BUP administered per day is 0.2% or 3mg TDI, whichever is lower. (b) (4)

The (b) (4) specification (b) (4) in the CAM products is considered acceptable.

Table 4. Drug Product Degradants for CAM2038 q1w

<i>Degradant</i>	<i>Proposed Specification</i>	<i>ICH Qualification Thresholds or Limits</i>	<i>Comments</i>
(b) (4)	NMT (b) (4) %	0.5% or 200 mcg	Acceptable

Table 5. Drug Product Degradants for CAM2038 q4w

<i>Degradant</i>	<i>Proposed Specification</i>	<i>ICH Qualification Thresholds or Limits</i>	<i>Comments</i>
(b) (4)	NMT (b) (4) %	0.2% or 3 mg/day	Acceptable

(b) (4)

Elemental Impurities

The Applicant analyzed the CAM2038 q1w and q4w drug products for elemental impurities as per ICH Q3D. CMC has concurred with the analysis submitted by the Applicant. No elemental impurities exceed the permitted daily exposures for a parenteral drug product, therefore no specifications are required to be set. Tables detailing the elements analyzed in the CAM2038 q1w and CAM2038 q4w products are below. Refer to the review by the chemist Dr. Valerie Amspacher for details.

Table 6. Permitted daily exposure and actual elemental impurities in the CAM2038

q1w drug product, batch no. 23505.04: 6-month storage at 40°C/75% RH

Element	Permitted daily exposure		Found amount
	(µg/day)	(ppm, injection volume ^{(b) (4)} mL)	(ppm)



(b) (4)

(b) (4)

Table 7 Permitted Daily Exposure and Actual Elemental Impurities in CAM2038 q4W Drug Product Batch 23518.03 (Accelerated Conditions)

Element	Permitted daily exposure		Found amount
	(µg/day)	(ppm, injection volume (b) (4) mL)	(ppm)
(b) (4)			

Container Closure System

The CAM2038 q1w and CAM2038 q4w drug products are presented as pre-filled syringes (PFS). (b) (4) syringes and (b) (4) (b) (4) plunger stoppers are the primary container closure. The drug product-filled syringe is inserted in a safety device assembly for post injection needle stick prevention. The individual syringe system components and the safety device assembly are shown in the figure below.



CAM2038 q1w and CAM2038 q4w drug products are packaged into a (b) (4) 1 mL (b) (4) glass syringe sealed with a sterile (b) (4) rubber plunger stopper. The individual components selected for, and in direct contact with, the drug product are detailed in the table below.

Component	Description	Specification	Manufacturer
(b) (4)	Syringe, staked-in needle, 1 mL long with 23G ½" 3B TW, with RNS inserted (b) (4) Grey		(b) (4)
Syringe	1 mL long Glass (b) (4)		
Needle	Staked 23G thin wall (TW) ½" Stainless steel		
Needle shield	Rigid needle shield (RNS) Rubber formulation (b) (4)		
Plunger stopper	(b) (4) elastomer		

The labeled (drug product filled) syringe is inserted into the safety device assembly. The assembly consists of the safety device body and finger flange. The individual components selected for the combination product are detailed in the table below.

Component	Description	Specification	Manufacturer
CAM2038 q1w or CAM2038 q4w	Drug Product -Filled Syringe		(b) (4)
Safety Device (Body)	(b) (4)		
Finger Flange			

The product kit includes the product, pre-printed carton with (b) (4) tray, plunger rod, Medication Guide, Prescribing Information (PI), Instructions for Use (IFU) and (b) (4). All the components are packaged as a single "Combination Product Kit". An example of a CAM2038 product kit is illustrated in the figure below.



CAM2038 q1w and CAM2038 q4w drug product filled syringes

(b) (4)

(b) (4)

An example of the drug product label is shown below.



Extractables/Leachables Evaluation

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

- Module 3.2.P.2.4 Container Closure System (CAM2038 q1w and CAM2038 q4w)
- Extractables and leachables study on (b) (4) PFS (23G (b) (4) inch TW needle) including (b) (4) rigid needle shield, (b) (4) needle shield and (b) (4) (b) (4) stopper (Report (b) (4)-2015-0B6-01)
- Determination (b) (4) in CAM2038 q1w placebo formulation stored in prefilled syringes (Report AM-922)

- A Discussion of the Available Extractables and Leachables Data for CAM2038 Drug Products and Packaging System (Pre-filled Syringe) (Report (b) (4)-17-009)
- CAM2038 (buprenorphine FluidCrystal® Injection depot): Extractables Study of (b) (4) Prefilled Syringe (Report S.52.REP.S279_01)
- Extractables study of (b) (4) needle shield (Report S.52.REP.S310_01)

Extractables Testing on Container Closure System

Title: Extractables and leachables study on (b) (4) PFS (23G (b) (4) inch TW needle) including (b) (4) rigid needle shield, (b) (4) needle shield and (b) (4) stopper

Study Number: (b) (4)-2015-0B6-01

Conducting Lab: (b) (4)

Components tested:

Part tested	Manufacturer and Part number	Photos	Batches tested
(b) (4)	(b) (4)	(b) (4)	
Glass Syringes	(b) (4) Syringes (b) (4) 1 mL long glass syringe with staked 23G 1/2 inch TW needle	(b) (4)	lot no: 1000141922
Needle sheath	(b) (4) grey rubber	(b) (4)	lot no: 1000141922
Rubber Stopper (1.0 mL)	(b) (4)	(b) (4)	lot no: D000024261

Solvents and Extraction Conditions:

(b) (4)

(b) (4)



To address these review issues and potential approval issues, the Applicant consequently scheduled a teleconference (held 10/20/2017) with the Division to propose new extractables and leachables studies. After some discussion, the Applicant committed to perform new extraction studies with the rubber stopper (b) (4). (b) (4) The Applicant also committed to provide new leachables data from both q1w and q4w products on stability at several time points, including interim time points and projected end of shelf-life. The Applicant proposed the following timelines to provide these new data. Note that some of these dates are after the GRMP goal date for primary reviews and some are past the PDUFA date for the NDA.

14 DECEMBER 2017 (no change from 30 November 2017 email)

(b) (4)



29 DECEMBER 2017 (new)

(b) (4)



10 JANUARY 2018 (new)

(b) (4)



2 FEBRUARY 2017 (revised to remove extractable data)

(b) (4)



The Applicant submitted the following new data on 12/14/2017 (SDN 67), which included the following studies.

(b) (4)



These data were not reviewed during this NDA review cycle and will not lead to review clock extension as there are deficiencies from other disciplines that cannot be addressed within this cycle even with an extension of the clock.

2.6 Proposed Clinical Population and Dosing Regimen

CAM2038 q1w and CAM2038 q4w are indicated for (b) (4) treatment of opioid use disorder in adults and should be administered once-weekly and once-monthly, respectively. The depot formulation should ensure medication adherence, ultimately improving treatment effectiveness.

2.7 Regulatory Background

Pre-IND meeting were held for IND 114082 on April 12, 2012 and March 31, 2015. The original IND was submitted April 9, 2015. This IND was given fast-track designation on July 9, 2015. A Pre-NDA meeting was held on March 16, 2017. NDA 210136 was submitted July 9, 2017 and priority review was granted.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study Number	Location
Buprenorphine 8-Week Subcutaneous Toxicity Study in Beagle Dogs (CAM2038 q1w and q4w)	TK-12-448	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tk-12-448\tk-12-448.pdf
A 4-Weeks Pharmacokinetic Study in Minipigs (not GLP)	PK-08-313	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4222-absorp\pk-08-313\pk-08-313-fr.pdf
Intramuscular Local Tolerability of CAM2038 Drug Products in Dogs	TO-15-537	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4231-single-dose-tox\to-15-537\to-15-537.pdf
Non-GLP Evaluation of the Effects of Buprenorphine, Norbuprenorphine, and Naltrexone on Cloned HERG Channels Expressed in Human Embryonic Kidney (HEK293) Cells	TO-17-589	\\cdsesub1\evsprod\nda210136\0002\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\to-17-589\to-17-589.pdf
Effects of Naltrexone HCl and Norbuprenorphine HCl on Cloned hNAV1.5 Sodium Peak and hNAV1.5 Sodium Late Current, hCAV1.2 Calcium, and hKvLQT1/hminK Potassium Channels Expressed in Mammalian Cells	TO-17-594	\\cdsesub1\evsprod\nda210136\0002\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\to-17-594\to-17-594.pdf
9-Month Subcutaneous Toxicity Study of CAM2038 Drug Products in Dogs	TO-15-531	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\to-15-531\to-15-531-2335012.pdf
Intravenous Administration of CAM2038 Q1W in rats (non-GLP)	P-17-598	IND 114082
A 12 to 14-Day	2335-003	\\cdsesub1\evsprod\nda210136\0001\m4\42-

Subcutaneous Pharmacokinetic Study of Buprenorphine in Gravid and Non-Gravid Sprague-Dawley Rats		stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\2335-003\2335-003.pdf
A 12-Day Subcutaneous Pharmacokinetic Study of Buprenorphine in Gravid New Zealand White Rabbits	2335-004	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\2335-004\2335-004.pdf
A 28 Day Dietary Pharmacokinetic Study of Buprenorphine in Sprague-Dawley Rats	2335-001	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud\2335-001\2335-001.pdf
A 28-Day Dietary Pharmacokinetic Study of Buprenorphine in CD1 Mice	2335-002	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud\2335-002\2335-002.pdf
A 26 Week Toxicity Study of Glycerol dioleate and FluidCrystal® Vehicle by Subcutaneous Injection in Rats	TO-10-405	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-10-405\to-10-405.pdf
Glycerol Dioleate: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow: 0 h and 24 h Subcutaneous Injection Dosing and 48 h Sampling	TO-12-446	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-12-446\to-12-446.pdf
(b) (4) (Glycerol dioleate, (w) (4)%) Ames Test	TO-04-141	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-04-141\to-04-141.pdf
(b) (4) (Glycerol dioleate, (w) (4)%) Ames Test	TO-04-146	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-04-146\to-04-146.pdf
(b) (4) (Glycerol dioleate, (w) (4)%) In Vitro Mammalian Cell Gene Mutation Test Performed with Mouse Lymphoma L5178Y Cells	TO-04-142	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-04-142\to-04-142.pdf
Glycerol Dioleate (GDO): A Subcutaneous Pre- and Post-Natal Reproductive and Developmental Toxicity Study in Female Sprague-Dawley Rats	TO-16-569	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-16-569\to-16-569.pdf
A single dose study in rat evaluating circulating plasma glycerol dioleate levels following s.c. administration of FluidCrystal® Injection depot placebo formulation or glycerol dioleate	PK-15-539	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4227-other-pk-stud\pk-15-539\pk-15-539-fr2.pdf
Exploratory evaluation of	PK-15-551	\\cdsesub1\evsprod\nda210136\0001\m4\42-

NMP (N-methyl-2-pyrrolidinone) levels in plasma from CAM2038 q4w treated healthy volunteers from Study HS-13-487		stud-rep\422-pk\4227-other-pk-stud\pk-15-551\pk-15-551-fr.pdf
NMP (N-methyl-2-pyrrolidinone) Toxicokinetic Study in Rats	TO-15-540	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4227-other-pk-stud\to-15-540\to-15-540-77246-fr.pdf
NMP (N-methyl-2-pyrrolidinone) Toxicokinetic Study in Rabbits	TO-15-541	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\422-pk\4227-other-pk-stud\to-15-541\to-15-541-77247-fr.pdf
Multi-Generation Rat Reproductive Study with N-Methylpyrrolidone	Exxon (1991) Project Number 236535 Draft Report	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\exxon-1991.pdf
Developmental Toxicity Study in Rat with N-Methylpyrrolidone	Exxon (1992) Project Number 236534 Draft Report	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\exxon-1992.pdf
Report on Testing of N-Methylpyrrolidone for Possible Mouse Teratogenicity	BASF (1970a) Study XIX-422	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\basf-1970a.pdf
Report on Testing of Pyrrolidone for any Teratogenic Effect in the Mouse and Report on the Testing of Pyrrolidone for any Teratogenic Effect in the Rat after Oral Administration	BASF (1970b) Study XIX-421	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\basf-1970b.pdf
Mutagenicity Test on N-Methylpyrrolidone in the CHO/HGPRT Forward Mutation Assay	Hazelton Laboratories (1988a)	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\hazelton-1988a.pdf
Mutagenicity Test on N-Methylpyrrolidone in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay	Hazelton Laboratories (1988b)	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\hazelton-1988b.pdf
Evaluation of Three Compounds for their Mutagenic Potential	(b) (4)	\\cdsesub1\evsprod\nda210136\0001\m4\43-lit-ref\ (b) (4) .pdf
Toxicological Review and Risk Analysis of N-Methyl-2-Pyrrolidone	TO-16-570	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-16-570\to-16-570.pdf
Toxicological Review and Risk Analysis of Glycerol Dioleate	TO-16-581	\\cdsesub1\evsprod\nda210136\0002\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-16-581\to-16-581.pdf

In addition, the Applicant submitted an extensive literature search to justify the safety of NMP and GDO. These studies are reviewed and included in the literature cited sections of Appendix 1 and 2.

3.2 Studies Not Reviewed (Formally)

The studies listed in the table below were not formally reviewed because there were longer term studies that served as the pivotal supporting data.

Study Title	Study Number	Location
7 Month Repeat Dose Toxicity Study including Toxicokinetics and Local Tolerance of Long-acting Release FluidCrystal® Injection Depot Product of Leuprolide in Beagle Dogs	TO-13-490	\\cdsesub1\evsprod\nda210136\0002\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-13-490\to-13-490-526468-fr.pdf
Buprenorphine A Toxicology and Toxicokinetics Study on Single and Repeat Dosing in Dogs	TO-08-323	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\to-08-323\to-08-323-68434-fr.pdf
CAM2038: 18 Week Subcutaneous Administration Repeat Toxicity Study including Toxicokinetics and Local Tolerance of Longacting Release FluidCrystal® Injection Depot Products of Buprenorphine in Beagle Dogs	TO-13-489	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\to-13-489\to-13-489-8293608-fr.pdf
(b) (4) (Glycerol dioleate, 95%). Acute Single Dose Study in Rats	TO-05-153	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-05-153\to-05-153.pdf
(b) (4) (Glycerol dioleate, (w) (4) %). A Repeat Dose Subcutaneous Study in Rats	TO-05-156	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-05-156\to-05-156.pdf
(b) (4) (glycerol dioleate (w) (4) %). A 28 Days Toxicity Study in Rats	TO-05-177	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-05-177\to-05-177.pdf
(b) (4) (Glycerol dioleate, (w) (4) %). A Single Dose Study in Rats with Recovery	TO-0-189	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-05-189\to-05-189.pdf
4050-K-Placebo Toxicity and Local tolerance of FluidCrystal® vehicle Injection depot formulation – Evaluation of vehicle treated dogs from an 8-Week Subcutaneous Toxicity Study in Dogs with an 8-Week Recovery Period	TO-10-395	\\cdsesub1\evsprod\nda210136\0001\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\to-10-395\to-10-395.pdf

3.3 Previous Reviews Referenced

No other reviews are referenced.

4 Pharmacology

4.1 Primary Pharmacology

No pharmacology studies were conducted for the CAM2038 products. Buprenorphine is a synthetic opioid agonist that is 10-20 times more potent than morphine with a very long duration of action. It acts as a partial mu opioid receptor agonist and a kappa opioid receptor antagonist. The referenced product labeling includes the following information:

12.1 Mechanism of Action

SUBUTEX sublingual tablet contains buprenorphine, a partial agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor.

4.2 Secondary Pharmacology

The secondary pharmacologic effects of a mu opioid agonist such as BUP include dysphoria, euphoria, and sedation. These as well as primary pharmacodynamic effects are summarized in the referenced product labeling under 12.2 as follows:

12.2 Pharmacodynamics

Subjective Effects

Comparisons of buprenorphine to full opioid agonists such as methadone and hydromorphone suggest that sublingual buprenorphine produces typical opioid agonist effects which are limited by a ceiling effect. Opioid agonist ceiling-effects were also observed in a double-blind, parallel group, dose-ranging comparison of single doses of buprenorphine sublingual solution (1, 2, 4, 8, 16, or 32 mg), placebo and a full agonist control at various doses. The treatments were given in ascending dose order at intervals of at least one week to 16 opioid-experienced subjects who were not physically dependent. Both active drugs produced typical opioid agonist effects. For all measures for which the drugs produced an effect, buprenorphine produced a dose-related response. However, in each case, there was a dose that produced no further effect. In contrast, the highest dose of the full agonist control always produced the greatest effects. Agonist objective rating scores remained elevated for the higher doses of buprenorphine (8-32 mg) longer than for the lower doses and did not return to baseline until 48 hours after drug administration. The onset of effects appeared more rapidly with buprenorphine than with the full agonist control, with most doses nearing peak effect after 100 minutes for buprenorphine compared to 150 minutes for the full agonist control.

Physiologic Effects

Buprenorphine in IV (2, 4, 8, 12 and 16 mg) and sublingual (12 mg) doses has been administered to opioid-experienced subjects who were not physically dependent to examine cardiovascular, respiratory and subjective effects at doses comparable to those used for treatment of

opioid dependence. Compared to placebo, there were no statistically significant differences among any of the treatment conditions for blood pressure, heart rate, respiratory rate, O₂ saturation, or skin temperature across time. Systolic BP was higher in the 8 mg group than placebo (3-hour AUC values). Minimum and maximum effects were similar across all treatments. Subjects remained responsive to low voice and responded to computer prompts. Some subjects showed irritability, but no other changes were observed. The respiratory effects of sublingual buprenorphine were compared with the effects of methadone in a double-blind, parallel group, dose ranging comparison of single doses of buprenorphine sublingual solution (1, 2, 4, 8, 16, or 32 mg) and oral methadone (15, 30, 45, or 60 mg) in non-dependent, opioid experienced volunteers. In this study, hypoventilation not requiring medical intervention was reported more frequently after buprenorphine doses of 4 mg and higher than after methadone. Both drugs decreased O₂ saturation to the same degree. Androgen Deficiency Chronic use of opioids may influence the hypothalamic-pituitary-gonadal axis, leading to androgen deficiency that may manifest as low libido, impotence, erectile dysfunction, amenorrhea, or infertility. The causal role of opioids in the clinical syndrome of hypogonadism is unknown because the various medical, physical, lifestyle, and psychological stressors that may influence gonadal hormone levels have not been adequately controlled for in studies conducted to date.

4.3 Safety Pharmacology

The effects of BUP on critical physiological systems are well-known and extensive clinical experience exists with BUP. Two new safety pharmacology studies were completed by the Applicant in response to questions from the Agency about the potential impact of buprenorphine on the QT prolongation. In addition, several cardiovascular parameters were built into the toxicology studies, which are also summarized below. These data are consistent with the known effects of buprenorphine and suggest minimal risk for an impact on the receptors examined as the responses were in the micromolar range. The reader is referred to the review by the QT IRT team for specific details regarding the potential impact of buprenorphine on the QT interval.

The summary of these studies, as provided by the Applicant, is reproduced below:

Table 2.6.3.4-1 Safety Pharmacology							
Test Article: CAM2038 q1w and CAM2038 q4w							
Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses	Gender/ No. per Group	Noteworthy Findings	GLP Compliance	Report No.
I _{Kr} potassium ion channel hERG	Stably expressed HEK293 cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 1, 3, 10, 30 μM NBPN: 3, 10, 30, 100 μM NTX: 3, 10, 30, 100 μM Cisapride (positive control) 0.1 μM	3-4 cells per dose	BPN IC ₅₀ 11 μM; IC ₂₀ 2.5 μM NBPN IC ₅₀ 70 μM; IC ₂₀ 11 μM NTX IC ₅₀ 76 μM; IC ₂₀ 11 μM Cisapride ~82% inhibition (laboratory mean historical control value is 79.89%)	No	TO-17-589
Peak hNav1.5 sodium ion channel	Stably expressed HEK293 cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 3, 10, 30, 100 μM NBPN: 1, 3, 10, 30, 100 μM NTX: 3, 10, 30, 100 μM Lidocaine (positive control) 2 mM	3 cells per dose	BPN IC ₅₀ 13.8 μM; IC ₂₀ 4.9 μM NBPN IC ₅₀ 7.9 μM; IC ₂₀ 2.5 μM NTX IC ₅₀ >100 μM; IC ₂₀ <100 μM Lidocaine 91.6% inhibition	No	TO-17-594
Late hNav1.5 sodium ion channel	Stably expressed HEK293 cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 1, 3, 10, 30 μM NBPN: 1, 3, 10, 30 μM NTX: 3, 100 μM Ranolazine (positive control) 100 μM	3 or 5 cells per dose	BPN IC ₅₀ 7.3 μM; IC ₂₀ 2.1 μM NBPN IC ₅₀ 8.7 μM; IC ₂₀ 1.8 μM NTX IC ₅₀ >100 μM; IC ₂₀ <100 μM Ranolazine 74% inhibition	No	TO-17-594
hCav1.2 (L-type) calcium ion channel	Stably expressed CHO cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 1, 3, 10, 30 μM NBPN: 1, 3, 10, 30 μM NTX: 30, 100 μM Nifedipine (positive control) 0.1 μM	3 cells per dose	BPN IC ₅₀ 8.6 μM; IC ₂₀ 2.6 μM NBPN IC ₅₀ 29.7 μM; IC ₂₀ 10.5 μM NTX IC ₅₀ >100 μM; IC ₂₀ <100 μM Nifedipine 94.7% inhibition	No	TO-17-594

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses	Gender/ No. per Group	Noteworthy Findings	GLP Compliance	Report No.
hKvLQT1/hmin K (I _{Ks}) potassium ion channel	Stably expressed HEK293 cells	In Vitro in HEPES buffered saline (pH 7.4) and 0.3% DMSO	BPN: 3, 10, 30, 100 µM NBPB: 3, 10, 30, 100 µM NTX: 30, 100 µM Chromanol (positive control) 30 µM	3-5 cells per dose	BPN IC ₅₀ 34.8 µM; IC ₂₀ 5.9 µM NBPB IC ₅₀ 29.7 µM; IC ₂₀ 10.5 µM NTX IC ₅₀ >100 µM; IC ₂₀ >100 µM Chromanol 68.8% inhibition	No	TO-17-594
Cardiovascular system	Dog/Beagle	SC	Saline (weekly dosing) CAM2038 q1w – 32 mg, CAM2038 q1w Placebo CAM2038 q4w – 128, 160, 192 mg, CAM2038 q4w Placebo	MF/4	Repeat dose toxicity for 9 months (once-weekly CAM2038 q1w, once-monthly CAM2038 q4w); ECG was measured after the first (Day 1) and last dose. Day 1 post-dose interval consisting of a mild slowing of the heart rate and moderate lengthening of the QT and QTc intervals for both drug products compared to the respective placebo and saline control. Changes not observed at termination of the study.	Yes	TO-15-531
Cardiovascular system	Dog/Beagle	SC	CAM2038 q1w 32 mg (0.64 mL), FC Placebo 0.36 mL CAM2038 q4w 64, 128 mg (0.18, 0.36 mL)	MF/3	Repeated administration of CAM2038 q4w at 64 or 128 mg monthly, or weekly injections of CAM2038 q1w at 32 mg had no effect on ECGs at Days 71, 92 or 120 in males and females compared to the vehicle-treated group	Yes	TO-13-489

BPN – buprenorphine; CHO – Chinese hamster ovary; HEK293 – human embryonic kidney; NBPB – norbuprenorphine; NTX- naltrexone; SC – subcutaneous

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The Applicant conducted PK assessments following single dosing and repeat dosing in rats, dogs, and minipigs testing various formulations of the drug product. These data are not reviewed formally.

No new nonclinical studies examining the distribution, protein binding, metabolism, excretion, or drug interactions were conducted or required.

5.2 Toxicokinetics

See results under general toxicology.

In addition, the Applicant conducted two studies to obtain exposure data in pregnant rats and rabbits to attempt to bridge the referenced Subutex reproductive and developmental data in the literature to a prolonged depot injection. The results of these studies are summarized in Section 9 of this review.

The Applicant also conducted two 28-day repeat-dose PK studies in mice and rats to bridge to the referenced carcinogenicity studies. These data are summarized in Section 8 of this review.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: A 4-weeks Pharmacokinetic Study in Minipigs

Study Number: PK-08-313

Study Date: August 12, 2008

Regulatory Status: No GLP statement, presumed nonGLP and not audited.

Test Article: CAM2038 q1w

Methods: One male and one female minipig was injected subcutaneously with 20 mg (0.5 mL) dose of CAM2038 q1w on Day 1. At separate sites q1w vehicle was injected on Day 1, 15, and 22. Animals were euthanized on Day 29 and local tissue injection sites were evaluated macroscopically and microscopically. Blood samples were obtained for PK data.

Injection sites were evaluated for hemorrhage, erythema, swelling and tenderness and scores as per the following scale:

		<u>Score</u>
Not present	-	0
Minimal	-	1
Slight	-	2
Moderate	-	3
Marked	-	4

Microscopic evaluations graded the response on a 5-point system as follows:

Grade 1	-	Minimal/Very few/Very small
Grade 2	-	Slight/Few/Small
Grade 3	-	Moderate/Moderate number/Moderate size
Grade 4	-	Marked/Many/Large
Grade 5	-	Massive/Extensive number/Extensive size
Present	-	Finding present/Severity not scored

Results: There were no clinical signs reported.

At the injection site, swelling was noted with both drug formulation and vehicle. No clear evidence of hemorrhage or erythema was reported. In the Male at the q1w injection site, swelling was scored as slight on Days 1-5, moderate from Days 6 to 16, slight on Days 17-22, and minimal out through Day 30. Similar findings were reported at the vehicle injections sites but they appeared to reach minimal grading earlier (generally by Day 15).

Microscopically, cystic spaces, presumably drug matrix depot, was noted as well as subcutaneous fibrosis and chronic granulomatous inflammation. Findings were more severe on Day 1 than Day 15. Day 29 CAM2038 q1w local findings were similar to Day 22 vehicle findings suggesting that the buprenorphine was contributing to the prolonged local effects.

TK data are summarized below:

Table 2.6.6.2-1: Mean Toxicokinetic Parameters for Buprenorphine in Minipigs Treated with a Single Dose of a CAM2038 q1w Formulation

Dose (mg) (Dose volume)	T _{max} (days) Mean	C _{max} (ng/mL) Mean	AUC _{inf} (ng*d/mL) Mean	t _{1/2} (days) Mean
20 (0.5 mL)	1.0	100	490	2.7

From Study [PK-08-313](#). In the study report, AUC, T_{max} and T_{1/2} are expressed in hours but the values were converted to days by dividing by 24. The CAM2038 q1w formulation was 2038BUP-I (see [Table 2.6.1.1-3](#)).

Study title: Buprenorphine 8-Week Subcutaneous Toxicity Study in Beagle Dogs

Study Number: TK-12-488

Study Date: April 25, 2014

Regulatory Status: GLP

Test Article: 2038BUP-BJ = CAM2038 q4w and 21038BUP-G = CAM2038 q1w

Test item	Batch No	BUP free base	SPC	GDO	EtOH	NMP
2038BUP-BJ	B2038BUP(0)-1209-95				-	30.00
2038BUP-G	B2038BUP(0)-1209-94				10.00	-

BUP = Buprenorphine, SPC = Soy phosphatidylcholine, GDO = Glycerol dioleate, EtOH = Ethanol, and NMP = N-methyl-2-pyrrolidone

Methods: Male and Female beagle dogs were treated subcutaneously with a single dose of CAM2038 q4w or four weekly doses of CAM2038 q1w (Days 1, 8, 15, and 22) at four different injection sites as per the following groupings:

Group	Test item	Dose BUP base* (mg)	Concentration BUP base (mg/mL)	Dose volume test item (mL)	Animal Nos	
					Male	Female
1	2038BUP-BJ	1 x 60	368	1 x 0.16	1-3	4-6
2	2038BUP-G	4 x 15	50	4 x 0.30	7-9	10-12
3	2038BUP-G	4 x 30	50	4 x 0.60	13**-15	16-18

*Material as supplied

**Animal No 13 was only dosed on Day 1.

Animals were euthanized and necropsies on Day 57 (35 Days after the last injection).

Clinical signs were monitored. Local injection site reactions (hemorrhage, erythema, swelling, and tenderness) were scored as per the following:

		<u>Score</u>
Not present	-	0
Minimal	-	1
Slight	-	2
Moderate	-	3
Marked	-	4

Body weight, food consumption, clinical chemistry, hematology, urinalysis, organ weights, macroscopic and microscopic findings and TK were evaluated as per standard procedures. Microscopic evaluations were graded as follows:

Histological alterations were graded on a 5-grade system:

- Grade 1 - Minimal/Very few/Very small
- Grade 2 - Slight/Few/Small
- Grade 3 - Moderate/Moderate number/Moderate size
- Grade 4 - Marked/Many/Large
- Grade 5 - Massive/Extensive number/Extensive size
- Present - Finding present/Severity not scored

Results: Clinical signs consistent with an opioid were observed after injection (e.g., reduced activity, salivation, redness of eyes) which lasted several days in most cases. Other clinical signs included GI effects (soft feces, no feces, vomiting) over the first week.

Hemorrhage was not observed in any group. Erythema was only observed only in a few animals treated with q1w. Tenderness was observed in q4w animals which lasted 2-7

days. In q1w animals, tenderness and swelling was observed in all injection sites and scored minimal to slight. Duration of effects were highly variable with some lasting around 20-35 days.

The size of the depot in q4w animals was generally between 10 and 15 mm in diameter, started to gradually reduce in size after 2-3 weeks and lasted approximately 31 and 37 days (although one was still noted at Day 57). For q1w treated animals, the size of the reaction site was between 15 and 20 mm in diameter, started to reduce in size after about 3 weeks and the last reactions were noted on Days 42-43 but some animals continued to display evidence of the local depot out to Day 57.

At necropsy, subcutaneous nodules were noted at most injection sites in the q1w groups. No macroscopic changes were noted in q4w sites at Day 57.

Microscopically, evidence of mononuclear cell infiltrates, granulomatous inflammation including giant cells and yellow material, cystic spaces, fibrosis, hemorrhage, and eosinophilic materials were noted in the q4w injection sites as per the following table, reproduced from the study report.

Microscopic findings in the subcutaneous injection sites (Group 1)

Dose group	Group 1 2038 BUP-BJ 60 mg BUP Base/0.16 mL	
Injection site (pooled examination of two slides)	No 1	
Days from injection to necropsy	56	
Sex	M	F
Number of animals examined	3	3
Infiltration of mononuclear cells, s.c.		
Total	1	0
Grade 1	1	-
Inflammation, s.c., granulomatous, incl. giant cells & yellow pigment		
Total	2	3
Grade 2	2	3
Cystic spaces, s.c.		
Total	2	3
Grade 1	1	-
Grade 2	1	3
Fibrosis s.c.		
Total	2	3
Grade 2	2	3
Haemorrhage, focal, s.c.		
Total	1	0
Grade 1	1	-
Eosinophilic material, focal, s.c.		
Total	2	3
Grade 1	2	3

Similar microscopic changes were also noted in the q1w treated animals, as per the table below. As expected, the severity of the findings was most notable at Site 4, which had the least amount of recovery time.

Table 7 Microscopic findings in the subcutaneous injection sites (Group 2)

Dose group	Group 2, 2038 BUP-G, 50 mg BUP Base, 0.3 mL/injection							
Injection Site (pooled examination of two slides)	Site No 1		Site No 2		Site No 3		Site No 4	
Days from injection to necropsy	56		49		42		35	
Sex	M	F	M	F	M	F	M	F
Number of animals examined	3	3	3	3	3	3	3	3
Infiltration of mononuclear cells, s.c.								
Total	0	0	0	0	0	0	1*	0
Grade 1	-	-	-	-	-	-	1	-
Inflammation, s.c., pyogranulomatous, occasionally incl. necrosis & yellow pigment								
Total	3	3	3	3	3	3	2	3
Grade 1	3	2	-	-	1	-	-	-
Grade 2	-	1	1	2	2	2	-	1
Grade 3	-	-	2	1	-	1	2	2
Cystic spaces, s.c.								
Total	0	2	2	3	2	3	2	3
Grade 1	-	1	-	-	1	-	-	-
Grade 2	-	1	1	2	1	2	2	1
Grade 3	-	-	1	1	-	1	-	2
Fibrosis s.c.								
Total	3	3	3	3	3	3	2	3
Grade 1	2	1	-	-	-	-	-	-
Grade 2	1	2	2	2	3	3	-	2
Grade 3	-	-	1	1	-	-	1	1
Grade 4	-	-	-	-	-	-	1	-
Haemorrhage, focal, s.c.								
Total	0	1	1	0	2	0	1	0
Grade 1	-	1	1	-	2	-	1	-
Basophilic material, focal, s.c.								
Total	0	0	0	1	0	1	0	1
Grade 1	-	-	-	1	-	-	-	-
Grade 2	-	-	-	-	-	1	-	1

*Considered to represent a mis-sampling of the injection site, unsuccessful attempts to locate the centre of the lesion were made

Study title: Intramuscular Local Tolerability of CAM2038 Drug Products in Dogs.

Study no.: TO-15-537
Study report location: EDR 4.2.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: June 3, 2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CAM2038 q1w – Lot 23506.002A, 50 mg buprenorphine/0.64 mL syringe, 101.6%
CAM2038 q4w – Lot 14PII0324-070, 356 mg buprenorphine in 0.54 mL, 99%
CAM2038 q1w placebo – Lot 23513.001A, buprenorphine free
CAM2038 q4w placebo – Batch B2038IAS-1504-119, buprenorphine free

Key Study Findings

- The objective of this study was to evaluate local injection site tolerability following single dose of intramuscular (IM) of CAM2038 q1w or CAM2038 q4w and their respective placebos to Beagle dogs, a potential inadvertent route of dosing.
- Placebo and drug were administered to the same dog.
- For Group 1, 2/sex were sacrificed on days 2 and 29 after dosing (q1w and q1w placebo). For Group 2, 2/sex/group sacrificed on days 2 and 113 after dosing (q4w and q4w placebo).
- There were no definitive buprenorphine-related effects on the local site.
- All observations were related to the vehicle and/or procedure, and were similar for both vehicles on study. In general, injection site microscopic observations were present in both groups and in both the test article and the placebo injection sites, and included one or more of the following: mild cavitation, minimal to moderate myofiber degeneration/necrosis, and minimal to mild mixed leukocyte infiltration, which occasionally extended to mild subacute/chronic inflammation. Cavitation was characterized by variable large clear spaces between myofibers, which were considered to represent test article and placebo depots. In males, there was a higher incidence and severity of myofiber degeneration in sites receiving the test article (Injection Site 2) when compared to placebo (Injection Site 1). However, the injection site for both placebo and test article-treated females had the same finding

and as such, this observation was considered to be likely procedural or vehicle related.

- In conclusion, administration of a single intramuscular dose of CAM2038 q1w at 32mg (0.64 mL) and CAM2038 q4w at 192 mg (0.54 mL) produced expected opioid-like effects on clinical observations, body weight, and food consumption. There were no grossly observed abnormal injection site reactions. No other grossly observed abnormal tissues were observed. Microscopic evaluation was performed on injection sites only. Microscopically, the incidence and/or severity of injection site observations on Day 2 (cavitation, myofiber degeneration/necrosis, and/or mixed leukocyte infiltration) were generally similar between Groups and between placebo and test article injection sites (Injection Sites 1 and 2, respectively).
- Compared with Day 2, microscopic changes were decreased on Day 29 (Group 1) and absent on Day 113 (Group 2), indicating progressing or full recovery over a period of approximately 1 month for q1w and 4 months for q4w.

Methods

Doses:

Group Number	Treatment Identity	Dose Level (mg/dose)	Dose concentration (mg/mL)	Dose volume (mL/dose)	Number of Animals	
					Male	Female
1	CAM2038 q1w	32	50	0.64	4 ^a	4 ^a
	Placebo for CAM2038 q1w	NA	NA	0.64		
2	CAM2038 q4w	192	355.6	0.54	4 ^b	4 ^b
	Placebo for CAM2038 q4w	NA	NA	0.54		

^aTwo animals/sex were euthanized on Day 2 (at least 24 hours postdose). Two animals/sex were maintained for a 4-week (28-day) recovery period.
^bTwo animals/sex were euthanized on Day 2 (at least 24 hours postdose). Two animals/sex were maintained for a 16-week (112-day) recovery period.
 NA – Not Applicable

The dose levels were selected by the Sponsor, or in consultation with the Sponsor, on the basis of available data from previous studies.

Frequency of dosing: Single dose
 Route of administration: Intramuscular
 Dose volume: See table
 Formulation/Vehicle: CAM2038 1w placebo and CAM2038 q4w placebo
 Species/Strain: Beagle dog
 Number/Sex/Group: 4
 Age: 7-8 months
 Weight: 10.90 to 11.70 kg (males) and 9.05 to 10.35 kg (females)

Satellite groups: Group 1 - 2/sex sacrificed on Days 2 and 29 after dosing
Group 2 – 2/sex/group sacrificed on Days 2 and 113 after dosing
Unique study design: No
Deviation from study protocol: Nothing remarkable

Observations and Results

Mortality

All animals were observed for mortality twice daily.

There were no test article-related deaths. All animals survived to the scheduled necropsies.

Clinical Signs

All animals were observed for morbidity and injury twice daily. A detailed clinical examination of each animal was performed on the day of receipt or transfer, 6 hours post-dose on Day 1, and weekly during the recovery period, at the same time of day ± 90 minutes as the Day 1 observation.

There were no test article-related macroscopic incidences of local tolerability effects on the Day 2, 29, or 113 intervals. Because each animal served as its own control with test article administration occurring in the muscle tissue of the right hind limb and the corresponding placebo administered into the tissue of the left hind limb, distinctions between the placebo and the test articles can be made only with respect to local tissue effects. Although the role of the placebo on systemic effects cannot be identified, clinical observations were generally consistent with the known opioid-like effects of the active component (buprenorphine) and consisted of decreased activity, ataxia, hunched posture, lateral recumbency (one Group 2 female, Animal 106, on Day 1) salivation, and inappetence. The clinical observations tended to be more pronounced for the CAM2038 q4w 192 mg group (Group 2) than for the CAM2038 q1w 32 mg group (Group 1). Except for two Group 2 females noted as thin, potential test article-related clinical observations resolved within approximately a week of dosing.

Dosing and Injection Site Observations

The placebos and test articles were administered once on Day 1 during the study via slow bolus intramuscular injection into the muscle tissue of the hindlimbs. The placebo was administered into the left hindlimb of each animal (designated

as Injection Site 1) and the corresponding test article was administered into the right hindlimb of each animal (designated as Injection Site 2). The injection sites of each animal were evaluated for gross signs of irritation and any other signs of local or systemic effect once pre-dose to 46 minutes post-dose. Additional evaluations were conducted once at 48 and 72 hours post-dose and once weekly thereafter for recovery animals.

No injection site abnormalities were observed. On Day 2, one Group 2 female had a small nodule at Injection Site 2 (CAM2038 q4w), which was likely due to deposition of the gel matrix containing the test article.

Body Weights

Body weights for all animals were measured and recorded on the day of receipt or transfer, prior to the measured-value randomization (Day -3) of animals assigned to study on June 30, 2015, on Day -1, and weekly during the study. The body weights recorded prior to Day -1 are not reported but are maintained in the study file.

Consistent with known opioid-like side effects, administration of CAM2038 q1w 32 mg and CAM2038 q4w 192 mg were associated with body weight loss in the first weeks of the study. Compared with pretest body weights, animals given 32 mg CAM2038 q1w lost up to -14.1% of their body weight 7 to 21 days after dosing. Similarly, animals given CAM2038 q4w 192 mg lost up to -13.7% of their body weight 14 to 35 days post dose. Following the indicated intervals, all animals gained weight. By the end of the recovery period (Days 28 and 112 for Groups 1 and 2, respectively), animals given 32 mg CAM2038 q1w or CAM2038 q4w 192 mg were within 8.8 and 3.5% of pretest body weights, respectively.

Food and Water Consumption

All animals were observed for the availability of food and water twice daily. Food consumption was measured and recorded daily during the study and reported weekly during the recovery period.

Consistent with body weight decreases, food consumption was generally decreased for 1 to 3 weeks following dosing with CAM2038 q1w 32 mg, and 3 to 4 weeks following dosing with CAM2038 q4w 192 mg. Mean Week 1 food consumption was reduced -64.4% to -86.6% compared with food consumption during the week prior to recovery necropsy (Weeks 4 and 16 for Groups 1 and 2, respectively).

Ophthalmoscopy - none

ECG - none**Hematology, Clinical Chemistry, and Urinalysis - none****Gross Pathology**

Postmortem study evaluations were performed on all surviving animals euthanized at the scheduled terminal necropsy (Day 2) and recovery necropsies (Days 29 and 113).

There were mild to moderate focus/foci (red/brown) within the lung on Day 2, and this correlated to foreign material and/or subacute/chronic inflammation microscopically. There were moderate focus/foci (red) in the rectum that correlated to mild hemorrhage microscopically. The role of the test article in these observations could not be delineated because all animals on study received the test article, and systemic toxicity was not a goal of the study.

Organ Weights - none**Histopathology**

Adequate Battery – Yes for local effects.

The following list constitutes the full complement of organs and tissues that were collected, preserved, and examined. Only gross lesions and injection sites were microscopically examined. However, as there is a repeat-dose tox study, this study is only being used to address local effects.

- Adrenal (2) - Aorta - Bone with marrow [femur] - Bone with marrow [sternum] - Bone marrow smear [2 collected]^a - Brain - Epididymis (2) - Eye including optic nerve (2) - Gallbladder - GALT [Gut Associated Lymphoid Tissue] - Gastrointestinal tract: - esophagus - stomach [cardia, fundus, and pylorus] - duodenum - jejunum - ileum - cecum - colon - rectum - Gonads: - ovary (2) with oviduct (2) - testis (2) - Gross lesions[#] - Heart - Injection site [1 and 2][#] - Joint, tibiofemoral	- Kidney (2) - Larynx - Liver [3 sections collected] - Lung with bronchi [collected whole] - Lymph nodes: mandibular [2 collected] and mesenteric - Mammary gland - Pancreas - Pituitary - Prostate - Salivary gland, mandibular [2 collected] - Salivary gland, parotid [2 collected] - Salivary gland, sublingual [2 collected] - Sciatic nerve - Skeletal muscle, biceps femoris - Skin - Spinal cord [cervical, thoracic, and lumbar] - Spleen - Thymus - Thyroid/parathyroid (2) - Tongue - Trachea - Ureter (2) - Urinary bladder - Uterus [both horns]/Cervix - Vagina

^aBone marrow smears were collected at the scheduled necropsies and held.

(2) Paired organ

[#]Microscopically examined

Peer Review – No.

Histological Findings

Day 2 (see table)

There were no definitive buprenorphine-related effects on the local site. All observations were considered to be related to the vehicle and/or procedure, and were similar for both vehicles on study. In general, injection site microscopic observations were present in both groups and in both the test article and the placebo injection sites, and included one or more of the following: mild cavitation, minimal to moderate myofiber degeneration/necrosis, and minimal to mild mixed leukocyte infiltration, which occasionally extended to mild subacute/chronic inflammation. Cavitation was characterized by variable large clear spaces between myofibers, which were considered to represent test article and placebo depots. In males, there was a higher incidence and severity of myofiber degeneration in sites receiving the test article (Injection Site 2) when compared to placebo (Injection Site 1). However, the injection site for both placebo and test article-treated females had the same finding and as such, this observation was considered to be likely procedural or vehicle related.

Injection Site Microscopic Observations – Day 2 Terminal					
Sex		Male		Female	
Dose Group		Group 1	Group 2	Group 1	Group 2
Number Examined		2	2	2	2
Injection site 1 (placebo)					
cavitation					
	-mild	0	0	2	2
degeneration/necrosis, myofiber		0	1	2	2
	-minimal	0	1	2	0
	-mild	0	0	0	2
infiltration, mixed leukocyte		0	1	1	1
	-minimal	0	1	1	0
	-mild	0	0	0	1
inflammation, subacute/chronic					
	-mild	0	0	0	1
Injection site 2 (test article)					
cavitation					
	-mild	2	2	2	2
degeneration/necrosis, myofiber		2	2	2	2
	-minimal	1	0	2	1
	-mild	1	1	0	1
	-moderate	0	1	0	0
infiltration, mixed leukocyte		2	2	1	1
	-minimal	1	0	1	0
	-mild	1	2	0	1
inflammation, subacute/chronic					
	-mild	0	0	0	1

The incidence and/or severity of procedure/vehicle-related findings were generally comparable in Injection Sites 1 (placebo) and 2 (test article) when male and female microscopic data are considered for both test articles.

Day 29 (see table)

There were no Buprenorphine-related effects on the local site. Vehicle-related findings were comparable in Injection Sites 1 (placebo) and 2 (CAM2038 q1w) between males and females and included minimal to mild cavitation, mild to moderate granulomatous inflammation, and minimal to mild pigment. Granulomatous inflammation included scattered macrophages with large cytoplasm (epithelioid) and occasional multinucleated giant cells which indicate a response to exogenous material. Overall, the granulomatous inflammation on Day 29 was interpreted as an attempt to clear the injected material described on Day 2.

Injection Site Microscopic Observations – Day 29 Recovery		
Group	1	1
Sex	Male	Female
Number Examined	2	2
Injection site 1 (placebo)		
cavitation		
-mild	1	2
inflammation, granulomatous	2	2
-mild	1	1
-moderate	1	1
pigment	2	2
-minimal	2	1
-mild	0	1
Injection site 2 (test article)		
cavitation	0	2
-minimal	0	1
-mild	0	1
inflammation, granulomatous		
-mild	2	2
pigment	2	2
-minimal	2	1
-mild	0	1
Group 1 – 32 mg/dose		

Day 113 (only Group 2)

There were no buprenorphine-related effects on the local site. Vehicle-related effects were not observed and were considered fully recovered. The observation of minimal extra medullary hematopoiesis and mild cyst in the pituitary gland of one animal were considered incidental because of the absence in the opposite sex and at other intervals.

Special Evaluation - None other than noted.

Toxicokinetics – none

Dosing Solution Analysis

The Sponsor provided documentation of the strength, purity, composition, stability, and other pertinent information for each batch or lot of the placebos and test articles used on study.

The placebos for CAM2038 q1w and CAM2038 q4w, and test articles, CAM2038 q1w buprenorphine subcutaneous injection (also referred to as CAM2038 q1w) and CAM2038 q4w buprenorphine subcutaneous injection (also referred to as CAM2038 q4w), were administered neat (undiluted), as received from the suppliers.

6.2 Repeat-Dose Toxicity

Study title: 9-Month Subcutaneous Toxicity Study of CAM2038 Drug Product in Dogs

Study no.: TO-15-531 (b) (4) # 2335-012)
 Study report location: EDR 4.2.3.2
 Conducting laboratory and location: (b) (4)
 Date of study initiation: July 29, 2015
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: See table below

Table 8. Summary of lot and purity

<i>Drug</i>	<i>Lot #</i>	<i>BUP purity</i>
CAM2038 q1w 32 mg	23506.002/23506.002A	101.6 %
CAM2038 q4w 128 mg	23521.001/23521.001A	100.9%
CAM2038 q4w 160 mg	23522.001/23522.001A	100.1%
CAM2038 q4w 192 mg	14PII0325-070	99%

Key Study Findings

Dogs were treated with CAM2038 q1w and q4w for 9 months with the following key study findings:

- The NOAEL in this study is 192 mg, the highest dose tested. The highest dose tested yielded exposure margins in dogs of 16-fold the human systemic exposure of 32 mg CAM2038 q1w based on AUC_{0-168h} comparisons.
- Very slight edema and minimal to moderate swelling/thickening of the skin (with subcutaneous tissue) was observed in all treated groups.
- Microscopic observations of foci of inflammation were observed in all treated groups, independent of BUP level. The localized inflammatory response ranged from acute to chronic inflammation depending on the time between dosing and microscopic evaluation.
- All CAM3028 groups containing BUP showed slowing of heart rate and dose-dependent lengthening of the QT/QTc interval on Day 1 post-dosing but not at the end of the study. This will be addressed clinically because it is a known pharmacologic effect of buprenorphine and is not specific to this formulation.

Methods

Doses: 32, 128, 160, 192 mg BUP
 Frequency of dosing: Saline, CAM2038 q1w vehicle and 32 mg: q1w;
 CAM2038 q4w vehicle, 128 mg, 160 mg and 192 mg: q4w

Route of administration: SC
 Dose volume: Saline and CAM2038 q1w: 0.64 mL; CAM2038 q4w: 0.58 mL
 Formulation/Vehicle: Saline control, vehicle controls for each presentation outlined in the table below
 Species/Strain: Dog/beagle
 Number/Sex/Group: 4/sex/group
 Age: 12-13 months
 Weight: M: 9-12 lbs; F: 7-10 lbs
 Satellite groups: None
 Unique study design: This study used a saline control and a vehicle control for each presentation (See table below). Injections were rotated across eight sites on back of the dog.
 Deviation from study protocol: None that affected the integrity of the study.

Table 9. Study design for 9-month dog study

Table A. Study Design							
Group	Test Article				Dose Volume (mL/dose)	Terminal Necropsy	
	Identity	Dose Level (mg/dose)	Dose Frequency	Concentration (mg/mL)		M	F
1	Saline Control	0	Once every week	NA	0.64	4	4
2	CAM2038 q1w Placebo Control	0	Once every week	NA	0.64	4	4
3	CAM2038 q4w Placebo Control	0	Once every 4 weeks	NA	0.58	4	4
4	CAM2038 q1w Drug Product	32	Once every week	50	0.64	4	4
5	CAM2038 q4w Drug Product	128	Once every 4 weeks	355.6	0.36	4	4
6	CAM2038 q4w Drug Product	160	Once every 4 weeks	355.6	0.45	4	4
7	CAM2038 q4w Drug Product	192	Once every 4 weeks	355.6	0.54	4	4
NA: Not applicable M – Male F – Female							

Table 10. Composition of treatment groups

Table B. Composition (mg) per dose						
Identity	CAM2038 q1w Placebo Control ^a	CAM2038 q4w Placebo Control ^a	CAM2038 q1w Drug Product ^b	CAM2038 q4w Drug Product ^c	CAM2038 q4w Drug Product ^c	CAM2038 q4w Drug Product ^c
Dose Group	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Dose Volume	0.64 mL	0.58 mL	0.64 mL	0.36 mL	0.45 mL	0.54 mL
BPN	—	—	32	128	160	192
NMP						
EtOH						
SPC						
GDO						
<i>BPN = buprenorphine; NMP = N-methyl-2-pyrrolidone; EtOH= ethanol; SPC = soy phosphatidylcholine; GDO = glycerol dioleate</i> ^aThe density of CAM2038 q1w placebo control and CAM2038 q4w placebo control is 0.957 and 0.978 g/mL, respectively. ^bThe density of the CAM2038 q1w drug product is 0.957. ^cThe density of the CAM2038 q4w drug products is 1.051 g/mL						

Dose Site Rotation			
Study Day	Injection Site Used		
	Groups 1, 2, 4	Groups 3, 5, 7	Group 6
1	1	1	1
8	4	-	-
15	5	-	-
22	8	-	-
29	2	4	4
36	3	-	-
43	6	-	-
50	7	-	-
57	1	5	5
64	4	-	-
71	5	-	-
78	8	-	-
85	2	8	8
92	3	-	-
99	6	-	-
106	7	-	-
113	1	2	4
120	4	-	-
127	5	-	-
134	8	-	-
141	2	3 ^a	5
148	3	-	-
155	6	-	-
162	7	-	-
169	1	6	8
176	4	-	-
183	5	-	-
190	8	-	-
197	2	7	4
204	3	-	-
211	6	-	-
218	7	-	-
225	1	7	5
232	4	-	-
239	5	-	-
246	8	-	-
253	2	7	8
260	3	-	-
267	6	-	-

- Dose not administered

^aOn Day 141, one male animal in Group 3 (animal number 224) was administered the placebo into dose site 4, rather than into dose site 3.

Observations and Results

Mortality

All dogs survived until scheduled necropsy.

Clinical Signs

Detailed clinical examinations were conducted weekly during the study and twice on the day of dosing (2 and 6 h post-dose). Injection sites were scored for erythema/eschar and edema using the Draize scale at 4, 24, 48, and 72 h post dose. Reduced incidence of soft feces, decreased appetite and activity and thin appearance were dose-dependently increased in BUP groups. Aggressive behavior and vocalizations were also seen during dosing of BUP groups. These findings are attributed to the known pharmacologic effects of BUP. Increased incidences of injection site scabbing were seen in Group 4 (CAM2038 q1w 32 mg) and Group 7 (CAM2038 q4w 192 mg) but the observations were not dose dependent and were considered secondary to the dosing procedure by the Applicant.

Body Weights

Body weights were measured prior to randomization (Day -6), on Day -1 and weekly, with the exception of Month 2 of the study where they were recorded once (on Day 56). Males in the CAM2038 q4w 192 mg (4.9%) had lower body weight gains at the end of the study as compared to saline and the CAM2038 q4w vehicle (14.8%). The decrease is attributed to the pharmacologic effects of BUP and was not considered adverse. No differences in females in any group were observed.

Food Consumption

Food consumption was measured daily during the study. Overall, no significant effects on food consumption were observed for any group.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted pretest and prior to terminal necropsy. No ophthalmic abnormalities were observed in any group.

ECG

All dogs received ECG examinations pretest, on Day 1 (predose and 4-6 h postdose) and on the last day of dosing (predose and 4-6 h postdose). All CAM3028 groups containing BUP showed slowing of heart rate and dose-dependent lengthening of the QT/QTc interval. The table below shows the magnitude of the changes, which are all greater than 10%. Buprenorphine is a known hERG channel blocker and has effects on QT prolongation in humans (Wedam et al., 2007). The finding is considered treatment-related but is attributed to the known pharmacologic effects of BUP. No ECG abnormalities were detected on the last day of dosing.

Table 11. Summary of QTc changes on Day 1

Group	CAM2038 Drug Product Dose (mg/dose)	QTc Pretest (sec)	QTc Day 1 Predose (sec)	QTc Day 1 Postdose (sec)	% of Day 1 Predose QTc
1	0 (saline control)	0.228	0.227	0.232	2.20%
2	0 (CAM2038 q1w placebo control)	0.219	0.224	0.232	3.57%
3	0 (CAM2038 q4w placebo control)	0.226	0.223	0.233	4.48%
4	32	0.225	0.220	0.244	10.91%
5	128	0.230	0.227	0.257	13.22%
6	160	0.231	0.225	0.259	15.11%
7	192	0.225	0.225	0.262	16.44%

Hematology

Hematological evaluations were conducted pretest and prior to terminal necropsy. No treatment-related changes in hematological parameters were seen.

Clinical Chemistry

Clinical pathology evaluations were conducted pretest and prior to terminal necropsy. No treatment-related changes in clinical chemistry parameters were observed.

Urinalysis

Urinalysis was conducted pretest and prior to terminal necropsy. No treatment-related changes in urinalysis were seen.

Gross Pathology

Dermal irritation was assessed using the Draize scale. All groups, with the exception of the saline control, showed Grade 1 (“very slight”) edema, which was attributed to the injection depot matrix. No differences were observed between the buprenorphine groups and their corresponding vehicles. Grade 2 (minimal) edema was observed in a few instances but was not dose-related. Grade 3 (moderate) edema was observed in one male dog in the CAM 2038 32 mg group on one day of dosing (Day 8 at a single injection site). The significance of this finding is unclear. No eschar or erythema were observed in any group.

Macroscopic changes were limited to edema, a swelling/thickening of the skin (with subcutaneous tissue) which was observed in all groups with the exception of the saline group. No differences were observed between the buprenorphine groups and their corresponding vehicles. Severity ranged from minimal to moderate and was attributed to the depot matrix and localized inflammatory reaction.

For weekly subcutaneous doses of saline (Group 1), there was no observed increase in edema over the course of dosing. For CAM2038 q1w placebo control or CAM2038 q1w drug product at 32 mg/dose (Groups 2 and 4, respectively) injection site observations consisted primarily of Grade 1 (“very slight”) presence of the injection depot matrix (IDM). Overall, Grade 1 IDM accounted for 41.4% to 100% and 64.1% to 100% of all

injection site observations made between any two consecutive injections given at either Site 1 or Site 8 for Groups 2 and 4, respectively. Following the second dose at Sites 1 and 8, there was an increased incidence of Grade 1 IDM ($\geq 73\%$ of all injection site observations) compared with the incidence noted following the first dose at each site. This likely represents the presence of residual IDM from the previous injection. Additionally, there was a small increase in the frequency of Grade 1 IDM observed at Site 8 (distal to the head) compared with Site 1 (proximal to the head), which may reflect differences in the fat content of the subcutaneous space at these two locations.

Consistent with the data from animals dosed once weekly, injection site observations related to administration of CAM2038 q4w placebo control or all dose levels of CAM 2038 q4w drug products consisted primarily of Grade 1 ("very slight") IDM. Grade 1 IDM accounted for 10.6% to 17.5% of all observations at Site 1 (proximal to the head) and 15.2% to 37.9% of observations at Site 8 (distal to the head). The frequency of Grade 1 IDM had no relationship to dose or the presence of buprenorphine; however, the frequency of Grade 1 IDM was increased at Site 8 compared with Site 1, which may reflect differences in the fat content of the subcutaneous space at these two locations. The frequency of Grade 1 IDM at Site 7, which was dosed three times (Days 197, 225, and 253), ranged from 61.5% to 71.9% (Appendix E, Table E). For CAM2038 q4w drug product at 160 mg/dose (Group 6), the incidence of Grade 1 IDM also increased as dose sites moved from the head to the tail with incidence at Sites 4, 5, and 8 of 29.9%, 37.1%, and 41.1%, respectively, of total injection site observations.

Organ Weights

A statistically significant increase in relative brain to body weight ratio was observed in males in the CAM2038 q1w 32 mg group. No effects were seen on absolute brain weights or body weights or in females of the same dose group. No microscopic correlates were seen in the brain. The finding is not considered to be adverse. No other effects on organ weights were observed.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Minimal fibrosis and minimal to mild mononuclear cell infiltrates were observed in the saline group in response to the injection. In all treated groups, histopathologic changes were observed at the injection sites and in the regional draining lymph node (axillary lymph node). No differences were noted between the CAM2038 q1w and q4w vehicles and the buprenorphine-containing formulations.

Toxicokinetics

Blood samples for TK were collected on Study Days 1, 141 and 225. Toxicokinetic parameters of BUP and nor-BUP are detailed in the tables below. Exposure to BUP and nor-BUP was similar for male and female dogs at all doses. At steady state (Days 141 and 225), BUP and nor BUP had a T_{max} of 8 h for CAM2028 q1w but the T_{max} values for CAM2028 q4w were between 1-2 h. It is not clear why the T_{max} values differ for the two products. The mean C_{max} and AUC_{0-168} values for BUP and nor-BUP were generally dose proportional or slightly greater than dose proportional. Mean AUC_{0-168} values for BUP and nor-BUP were generally similar between CAM2038 q1w 32 mg and CAM2038 q4w 128 mg groups.

The NOAEL in this study is the highest dose tested, 192 mg. The highest dose tested yielded exposure margins in dogs of 16-fold the human systemic exposure of 32 mg CAM2038 q1w based on AUC_{0-168h} comparisons (human data from Study HS-15-549).

The findings observed at necropsy and the clinical observations of edema correlated with microscopic observations of foci of inflammation. The localized inflammatory inflammatory responses ranged from acute to chronic inflammation depending on the time between dosing and microscopic evaluation. Fibrosis occurred with the chronic inflammation. The findings were not dependent on the amount of BUP.

Table 12. Mean BUP and nor-BUP TK parameters following SC administration of CAM2038 q1w to dogs (males and females combined)

Group	Day	Analyte	C_{max} (ng/mL)	T_{max} (hr) [day] ^b	C_{trough} (ng/mL)	$AUC_{0-168hr}$ (hr*ng/mL) [day*ng/mL] ^b	AUC_{INF} (hr*ng/mL) [day*ng/mL] ^b	$T_{1/2}$ (hr) [day] ^b	M:P ^a
4	1	buprenorphine	16.4	24 [1]	4.62	1640 [68.33]	1880 [78.33]	76.8 [3.20]	NA
		nor-buprenorphine	0.693	72 [3]	0.146	59.2 [2.47]	35.4 [1.48]	146 [6.08]	0.0406
4	141	buprenorphine	19.7	8 [0.33]	7.08	1850 [77.08]	NA	190 [7.92]	NA
		nor-buprenorphine	0.503	8 [0.33]	0.296	58.1 [2.42]	NA	230 [9.58]	0.0351
4	225	buprenorphine	19.4	8 [0.33]	8.61	2080 [86.67]	NA	138 [5.75]	NA
		nor-buprenorphine	0.600	8 [0.33]	0.283	63.2 [2.63]	NA	77.5 [3.23]	0.0336

NA - Not applicable
Note: AUC_{INF} reported for Day 1 only
a: $M:P = (AUC_{0-168hr \text{ nor-buprenorphine}} \times 467.64) / (AUC_{0-168hr \text{ buprenorphine}} \times 413.55)$
b: The value in hours was converted to days by dividing by 24.

Table 13. Mean BUP and Nor-BUP TK Parameters following SC Administration of CAM2038 q4w to Dogs (Males and Females Combined)

Day	Group	Analyte	Dose (mg/dose)	C _{max} (ng/mL)	T _{max} (hr) ^a	C _{trough} (ng/mL)	T _{last} (hr) ^a	AUC _{0-672hr} (hr*ng/mL) ^a	AUC _{0-168hr} (hr*ng/mL) ^a	M:P ^a	AUC _(12h) (hr*ng/mL) ^a	T _{1/2} (hr) ^a
1	5	buprenorphine	128	21.9	24.5 ^b [1.02]	2.97	672 [28.0]	5220 [217.50]	1980 [82.50]	NA	6010 [250.42]	270 [11.25]
		nor-buprenorphine	128	1.06	1 [0.04]	0.0608	552 ^b [23.0]	156 [6.50]	55.7 [2.32]	0.0334	209 [8.71]	133 [5.54]
	6	buprenorphine	160	22.4	72 [3.00]	3.96	672 [28.0]	5920 [246.67]	2160 [90.0]	NA	6990 [291.25]	256 [10.67]
		nor-buprenorphine	160	1.40	25 ^b [1.04]	0.229	672 [28.0]	338 [14.08]	110 [4.58]	0.0599	300 [12.50]	220 [9.17]
	7	buprenorphine	192	23.5	1.5 ^b [0.06]	5.01	672 [28.0]	7050 [293.75]	2360 [98.33]	NA	9490 [395.42]	349 [14.54]
		nor-buprenorphine	192	1.93	1 [0.04]	0.152	672 [28.0]	253 [10.54]	98.9 [4.12]	0.0405	257 [10.71]	145 [6.04]
141	5	buprenorphine	128	38.3	1 [0.04]	6.87	672 [28.0]	6600 [275]	1860 [77.50]	NA	NA	451 [18.79]
		nor-buprenorphine	128	1.41	0.75 ^b [0.03]	0.0628	636 ^b [26.5]	197 [8.21]	59.7 [2.49]	0.0334	NA	174 [7.25]
	6	buprenorphine	160	23.1	2 [0.08]	8.52	672 [28.0]	6780 [282.50]	1750 [72.92]	NA	NA	272 [11.33]
		nor-buprenorphine	160	1.88	2 [0.08]	0.353	672 [28.0]	389 [16.21]	110 [4.58]	0.0605	NA	97.3 [4.05]
	7	buprenorphine	192	60.8	1 [0.04]	10.0	672 [28.0]	10300 [429.17]	3200 [133.33]	NA	NA	324 [13.50]
		nor-buprenorphine	192	3.71	0.75 ^b [0.03]	0.257	672 [28.0]	378 [15.75]	128 [5.33]	0.0420	NA	203 [8.46]
225	5	buprenorphine	128	21.1	1 [0.04]	6.49	672 [28.0]	6780 [282.50]	1620 [67.50]	NA	NA	273 [11.38]
		nor-buprenorphine	128	1.29	1.00 [0.04]	0.0794	672 [28.0]	139 [5.79]	42.0 [1.75]	0.0231	NA	392 [16.33]
	6	buprenorphine	160	24.3	1 [0.04]	14.6	672 [28.0]	8530 [355.42]	2050 [85.42]	NA	NA	459 [19.13]
		nor-buprenorphine	160	2.01	1 [0.04]	0.449	672 [28.0]	345 [14.38]	96.4 [4.02]	0.0464	NA	136 [5.67]
	7	buprenorphine	192	33.0	1 [0.04]	12.3	672 [28.0]	11200 [466.67]	2720 [113.33]	NA	NA	370 [15.42]
		nor-buprenorphine	192	2.57	1 [0.04]	0.269	672 [28.0]	368 [15.33]	90.8 [3.78]	0.0359	NA	151 [6.29]

NA - Not applicable
Note: AUC_(12h) reported for Day 1 only
a: M:P = (AUC_{0-672hr} nor-buprenorphine X 467.64)/(AUC_{0-672hr} buprenorphine X 413.55)
b: Value is not a scheduled collection interval
c: The value in hours was converted to days by dividing by 24.

Dosing Solution Analysis

All dosing formulation samples had mean concentrations within the acceptance criteria with the exception of two animals in Group 2 (q1w vehicle) that had levels of BUP 1 to 2-fold greater than the LLOQ. No nor-buprenorphine or any other chromatographic peaks were detected in the samples suggesting that the contamination was not in vivo and most likely occurred ex vivo. This is not considered to affect the integrity of the study.

7 Genetic Toxicology

No genetic toxicology studies with BUP were conducted for NDA 210136. The referenced Subutex labeling provides the following information (updated 9/7/2017):

Mutagenicity

Buprenorphine was studied in a series of tests utilizing gene, chromosome, and DNA interactions in both prokaryotic and eukaryotic systems. Results were negative in yeast (*S. cerevisiae*) for recombinant, gene convertant, or forward mutations; negative in *Bacillus subtilis* "rec" assay, negative for clastogenicity in CHO cells, Chinese hamster bone marrow and spermatogonia cells, and negative in the mouse lymphoma L5178Y assay. Results were equivocal in the Ames test: negative in studies in two laboratories, but positive for frame shift mutation at a high dose (5 mg/plate) in a third study. Results were positive in the Green-Tweets (*E. coli*) survival test, positive in a DNA synthesis inhibition (DSI) test with testicular tissue from mice, for both in vivo and in vitro incorporation of [³H]thymidine, and positive in unscheduled DNA synthesis (UDS) test using testicular cells from mice.

The same information should be included in this labeling for this product.

8 Carcinogenicity

No new carcinogenicity studies with BUP were conducted for NDA 210136. The referenced Subutex labeling provides the following information (updated 9/7/2017):

Carcinogenicity

Carcinogenicity studies of buprenorphine were conducted in Sprague-Dawley rats and CD-1 mice. Buprenorphine was administered in the diet to rats at doses of 0.6, 5.5, and 56 mg/kg/day (estimated exposure was approximately 0.4, 3 and 35 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis) for 27 months. As in the buprenorphine/naloxone carcinogenicity study in rat, statistically significant dose-related increases in Leydig cell tumors occurred. In an 86-week study in CD-1 mice, buprenorphine was not carcinogenic at dietary doses up to 100 mg/kg/day (estimated exposure was approximately 30 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis).

The same information should be included in this labeling for this product with exposure margins adjusted accordingly.

To obtain exposure data in mice and rats treated like the above carcinogenicity studies in order to inform labeling, the Applicant conducted 28-day repeat-dose PK studies. The results of these studies are summarized below:

Study Title: A 28 Day Dietary Pharmacokinetic Study of Buprenorphine in Sprague-Dawley Rats

Study Number: 2335-001
 Study Completion Date: July 30, 2015

Study Design: Male and female Sprague-Dawley rats were treated via the diet with buprenorphine for 28-days in order to reach steady state.

Group Assignments			
Group Number	Target Dose Level (mg/kg/day)*	Number of Animals	
		Male	Female
1	0.6	9	0
2	5.5	9	0
3	56	9	0
4	0.6	0	9
5	5.5	0	9
6	56	0	9

*Actual dose levels, as determined on Days 1, 14 and 28 from mean food consumption values, are presented in the Pharmacokinetic Analysis Report presented in Appendix H.

Plasma samples were obtained at 1, 2, 4, 12, 13, 14, 16, and 24 hours post dose on Days 1 and 14 and at 1, 2, 4, 12, 13, 16, 24, 40, 56, and 72 hours post dose on Day 28.

PK results are depicted below:

Buprenorphine PK Summary Table								
Day	Target Dose (mg/kg)	Mean Actual Dose (mg/kg)	Mean Actual Dose Ratio	AUC _{0-24hr} (hr*ng/mL)	AUC _{0-24hr} Ratio	C _{max} (ng/mL)	C _{max} Ratio	T _{1/2} (hr)
1	0.6	0.528	1	16.4	1	1.52	1	NA
	5.5	2.75	5.2	90.4	5.5	6.83	4.5	NA
	56	23.6	44.7	700	42.7	56.3	37.0	NA
14	0.6	0.618	1	44.7	1	2.95	1	NA
	5.5	5.47	8.9	306	6.8	16.7	5.7	NA
	56	59.0	95.5	2200	49.2	127	43.1	NA
28	0.6	0.595	1	41.8	1	2.28	1	8.04
	5.5	5.22	8.8	208	5.0	10.4	4.6	10.0
	56	52.8	88.7	1680	40.2	87.3	38.3	17.4
NA – Not Applicable								

Norbuprenorphine TK Summary Table							
Day	Target Dose (mg/kg)	Mean Actual Dose (mg/kg)	AUC _{0-24hr} (hr*ng/mL)	AUC _{0-24h} /Dose (hr*kg*ng/mL/mg)	C _{max} (ng/mL)	C _{max} /Dose (kg*ng/mL/mg)	T _{1/2} (hr)
1	56	23.6	60.9	2.58	4.04	0.171	NA
14	56	59.0	22.3	0.379	1.54	0.0262	NA
28	56	52.8	13.8	0.262	0.893	0.0169	NA
NA – Not Applicable							

Study Title: A 28-Day Dietary Pharmacokinetic Study of Buprenorphine in CD1 Mice

Study Number: 2335-002
 Study Report Date: July 30, 2015

Study Design: Male and female CD1 mice were treated with 100 mg/kg buprenorphine via the diet for a total of 28-days to obtain exposure data in order to better inform the drug product labeling for this depot injection product. This dose was the NOEL for carcinogenicity in the referenced study.

Group Assignments				
Group Number	Target Dose Level (mg/kg/day)*	Number of Animals		
		Male	Female	
1	100	108	0	
2	100	0	108	

** Actual dose levels, as determined on Days 1, 14 and 28 from mean food consumption values for designated TK animals, are presented in the Pharmacokinetic Analysis Report presented in [Appendix H](#).

Plasma samples from 4 animals/group/time point were obtained via cardiac puncture such that data were obtained for 1, 2, 4, 12, 13, 14, 16, and 24 hours post-dosing on Days 1 and 14 and at 1, 2, 4, 12, 13, 14, 16, 24, 40, 56, and 72 hours post dosing on Day 28. The results are depicted below:

Buprenorphine TK Summary Table							
Day	Target Dose (mg/kg)	Mean Actual Dose (mg/kg)	AUC _{0-24hr} (hr*ng/mL)	AUC _{0-24hr} /Dose (hr*kg*ng/mL/mg)	C _{max} (ng/mL)	C _{max} /Dose (kg*ng/mL/mg)	T _{1/2} (hr)
1	100	68.6	817	11.9	47.5	0.693	11.1
14	100	80.1	1040	12.9	56.8	0.709	NA
28	100	84.0	1050	12.4	61.3	0.729	5.90

NA – Not Applicable

9 Reproductive and Developmental Toxicology

No new reproductive and developmental toxicology studies with BUP were conducted for NDA 210136. The referenced Subutex labeling provides the following information (updated 9/7/2017):

Section 13.1

Impairment of Fertility

Reproduction studies of buprenorphine in rats demonstrated no evidence of impaired fertility at daily oral doses up to 80 mg/kg/day (estimated exposure was approximately 50 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis) or up to 5 mg/kg/day IM or SC (estimated exposure was approximately 3 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis).

Section 8 Risk Summary (animal)

Reproductive and developmental studies in rats and rabbits identified adverse events at clinically relevant and higher doses. Embryofetal death was observed in both rats and rabbits administered buprenorphine during the period of organogenesis at doses approximately 6 and 0.3 times, respectively, the human sublingual dose of 16 mg/day of buprenorphine. Pre- and post-natal development studies in rats demonstrated increased neonatal deaths at 0.3 times and above and dystocia at approximately 3 times the human sublingual dose of 16 mg/day of buprenorphine. No clear teratogenic effects were seen when buprenorphine was administered during organogenesis with a range of doses equivalent to or greater than the human sublingual dose of 16 mg/day of buprenorphine. However, increases in skeletal abnormalities were noted in rats and rabbits administered buprenorphine daily during organogenesis at doses approximately 0.6 times and approximately equal to the human sublingual dose of 16 mg/day of buprenorphine, respectively. In a few studies, some events such as acephalus and omphalocele were also observed but these findings were not clearly treatment-related.

Section 8 (Animal Data)

Animal Data

The exposure margins listed below are based on body surface area comparisons (mg/m²) to the human sublingual dose of 16 mg buprenorphine via SUBUTEX.

No definitive drug-related teratogenic effects were observed in rats and rabbits at IM doses up to 30 mg/kg/day (estimated exposure approximately 20 times and 35 times, respectively, the human sublingual dose of 16 mg). Maternal toxicity resulting in mortality was noted in these studies in both rats and rabbits. Acephalus was observed in one rabbit fetus from the low-dose group and omphalocele was observed in two rabbit fetuses from the same litter in the mid-dose group; no findings were observed in fetuses from the high-dose group. Maternal toxicity was seen in the high-dose group but not at the lower doses where the findings were observed. Following oral administration of buprenorphine to rats, dose-related post-implantation losses, evidenced by increases in the numbers of early resorptions with consequent reductions in the numbers of fetuses, were observed at doses of 10 mg/kg/day or greater (estimated exposure approximately 6 times the human sublingual dose of 16 mg). In the rabbit, increased post-implantation losses occurred at an oral dose of 40 mg/kg/day. Following IM administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 mg/kg/day.

Buprenorphine was not teratogenic in rats or rabbits after IM or subcutaneous (SC) doses up to 5 mg/kg/day (estimated exposure was approximately 3 and 6 times, respectively, the human sublingual dose of 16 mg), after IV doses up to 0.8 mg/kg/day (estimated exposure was approximately 0.5 times and equal to, respectively, the human sublingual dose of 16 mg), or after oral doses up to 160 mg/kg/day in rats (estimated exposure was approximately 95 times the human sublingual dose of 16 mg) and 25 mg/kg/day in rabbits (estimated exposure was approximately 30 times the human sublingual dose of 16 mg). Significant increases in skeletal abnormalities (e.g., extra thoracic vertebra or thoraco-lumbar ribs) were noted in rats after SC administration of 1 mg/kg/day and up (estimated exposure was approximately 0.6 times the human sublingual dose of 16 mg), but were not observed at oral doses up to 160 mg/kg/day. Increases in skeletal abnormalities in rabbits after IM administration of 5 mg/kg/day (estimated exposure was approximately 6 times the human sublingual dose of 16 mg) in the absence of maternal toxicity or oral administration of 1 mg/kg/day or greater (estimated exposure was approximately equal to the human sublingual dose of 16 mg) were not statistically significant. In rabbits, buprenorphine produced statistically significant pre-implantation losses at oral doses of 1 mg/kg/day or greater and post-implantation losses that were statistically significant at IV doses of 0.2 mg/kg/day or greater (estimated exposure approximately 0.3 times the human sublingual dose of 16 mg). No maternal toxicity was noted at doses causing post-implantation loss in this study.

Dystocia was noted in pregnant rats treated intramuscularly with buprenorphine from Gestation Day 14 through Lactation Day 21 at 5 mg/kg/day (approximately 3 times the human sublingual dose of 16 mg). Fertility, and pre- and post-natal development studies with buprenorphine in rats indicated increases in neonatal mortality after oral doses of 0.8 mg/kg/day and up (approximately 0.5 times the human sublingual dose of 16 mg), after IM doses of 0.5 mg/kg/day and up (approximately 0.3 times the human sublingual dose of 16 mg), and after SC doses of 0.1 mg/kg/day and up (approximately 0.06 times the human sublingual dose of 16 mg). An apparent lack of milk production during these studies likely contributed to the decreased pup viability and lactation indices. Delays in the occurrence of righting reflex and startle response were noted in rat pups at an oral dose of 80 mg/kg/day (approximately 50 times the human sublingual dose of 16 mg).

The same information should be included in this labeling for this product with exposure margins adjusted accordingly.

To attempt to bridge the referenced studies and be able to compare these data with the exposures obtained following depot injection of CAM2038 products, the Applicant conducted the following studies. These studies permit some of the above information to

be expressed via exposure margins rather than based on body surface area; however complete PK bridging studies were not completed.

Study Title: A 12 to 14-Day Subcutaneous Pharmacokinetic Study of Buprenorphine in Gravid and Non-Gravid Sprague-Dawley Rats

Report Number: 2335-003
 Study Completion: July 29, 2015
 Study Design: Male and female Sprague-Dawley rats were treated subcutaneously with buprenorphine in 5% dextrose vehicle as follows:

Group Assignments							
Group Number	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Dose Concentration (mg/mL)	Number of Animals			
				Male	Female		
					Non-Gravid	Gravid	
1	0.1	1	0.1	0	0	15	
2	1	1	1.0	0	0	15	
3	5	2	2.5	0	0	15	
4	5	2	2.5	9	9	0	

Plasma samples were collected 0.5, 1, 2, 4, 12, and 24 hours post-dose on Days 1, 8 and 14. The results are depicted below.

Buprenorphine PK Summary Table (Gravid Females)							
Day	Dose (mg/kg)	Dose Ratio	AUC _{0-24hr} (ng*hr/mL)	AUC _{0-24hr} Ratio	C _{max} (ng/mL)	C _{max} Ratio	T _{1/2} (hr)
1	0.1	1	NA	NA	19.8	1	1.47
	1	10	384	1	94.8	4.8	4.93
	5	50	1140	3	229	11.6	9.50
8	0.1	1	77.5	1	24.1	1	2.55
	1	10	367	4.7	70.3	2.9	5.29
	5	50	1420	18.3	197	8.2	11.2
12	0.1	1	66.4	1	24.9	1	2.36
	1	10	351	5.3	63.5	2.6	6.80
	5	50	1290	19.4	171	6.9	30.6
NA – Not Applicable							

As noted in the table above, in gravid females, plasma levels (AUC and C_{max}) were less than dose proportional, and the half-life appeared to increase with increasing during of treatment. Plasma AUC also appeared to be lower in gravid females compared to nongravid male and females combined.

Buprenorphine PK Summary Table (Males and Non-Gravid Females)				
Day	Dose (mg/kg)	AUC _{0-24hr} (ng*hr/mL)	C _{max} (ng/mL)	T _{1/2} (hr)
1	5	1130	245	NA
8	5	1570	241	8.90
14	5	2330	230	39.4
NA – Not Applicable				

Study Title: A 12-Day Subcutaneous Pharmacokinetic Study of Buprenorphine in Gravid New Zealand White Rabbits

Study Number: 2335-004
 Study Completion: July 28, 2015
 Study Design: Pregnant female New Zealand White Rabbits were treated subcutaneously with buprenorphine (5 mg/kg/day) for 12 days. Plasma samples were obtained at 0.5, 1, 2, 4, 12, and 24 hours post injection on Days 1 and 8 and 0.5, 1, 2, 4, 12, 24, 40, 56, and 72 hours post injection on Day 12. Results are summarized below, reproduced from the study report.

Buprenorphine PK Summary Table				
Day	Dose (mg/kg)	AUC _{0-24hr} (ng*hr/mL)	C _{max} (ng/mL)	
1	5	1140	119	
8	5	1400	127	
12	5	1750	145	

10 Special Toxicology Studies

Off-Label Misuse: Intravenous Toxicity

As noted at several opioid drug product advisory committee meetings, there is an increased need to understand the potential adverse effects that could occur if an opioid drug product were to be abused via an unintended route of administration. During development, the Applicant conducted a non-GLP intravenous toxicology study testing one of the two formulations, CAM2038 Q1W. This study is reviewed below:

Study Title: Intravenous Administration of CAM2038 Q1W in Rats

Key Findings: Intravenous injection of CAM2038 Q1W into the tail vein of male Sprague-Dawley rats resulted in partial or complete occlusion of the injected vein with no evidence of blood in the section of the vein even at sacrifice on Day 7. The animals demonstrated effects consistent with systemic exposure to buprenorphine. No gross observations were made in visceral organs; however, lungs could not be examined due to the method of euthanasia.

Study Number: P-17-598
Regulatory Status: Non-GLP
Test Facility: Camurus AB, Lund Sweden
Study Report Date: October 3, 2017

Methods: GMP manufactured CAM2038 Q1W (entire contents of prefilled syringe = 8 mg CAM2038 in 0.16 mL per rat; ~ 27 mg/kg buprenorphine) was injected intravenously into the tail vein of 3 male MPF Sprague-Dawley rats obtained from (b) (4) under isoflurane anesthesia. Administration of the test item into the first animal was monitored for an hour to determine if severe or notable findings occurred prior to testing the material via IV administration into the second and third animal. Clinical observations were made over the first hour, hourly for the next six hours, and then at 11-12 hours and 24 hours post-injection. Observations were prolonged to 7 days post dose for Animals 2 and 3 (animals were observed daily during that period). Visual inspection of the injection site was completed during and after the administration. Body weights were recorded prior to dosing and daily thereafter. Animals were euthanized via CO₂ as noted above. At necropsy, injection sites on the tail were examined grossly and macroscopic evaluation of organs was completed in situ (color, shape, size). No microscopic examinations were made.

Results: There were no mortalities or clinical signs. Animals demonstrated expected pharmacological effects of buprenorphine (dazed facial expression, stereotyped repetitive behavior for approximately 11 hours). During injection in one animal a swelling was observed in the vein at the injection site. This impacted the ability to inject the entire volume in Rat 2. In Rat 3, a pale area at the injection site was noted which extended toward the base of the tail during injection.

At necropsy, there were no abnormalities noted in thymus, heart, bladder, liver, kidney, small intestine, colon, or stomach. Lungs could not be evaluated as the animals were sacrificed via CO₂ (expect irregular dark color from method of termination). In all three rats, formulation was found in the tail vein in the form of gel localized in the region of the injection site and extending toward the tail. There was no blood in this region of the tail vein. The test item appears to result in partial or complete occlusion of the vein.

Conclusions and Risk Assessment:

The above data suggest that if the drug product were to be administered intravenously, it will either gel rapidly and potentially block the injected vessel as it apparently did in the rat tail vein, or if the injected vein is larger and the product does not gel quickly, it

could result in a lung emboli or lodge in other small capillaries. The clinical significant of any blockade of blood vessels would depend upon the redundancy of vascular perfusion and degree of blockade, but it could result in tissue hypoxia and necrosis with impact on tissue functionality. Given the existing evidence of prolonged tissue residency, the vascular blockade of any tissue would be expected to take considerable time to clear.

Juvenile Animal Studies and Agreed PSP

The Applicant has requested a waiver of pediatric studies in all age groups citing that the studies would not likely be possible to conduct given the low incidence of patients possible to enroll. See the agreed PSP and clinical review for details. No juvenile animal studies were required given the fact that any pediatric patients who are eligible are already addicted to opioids prior to enrollment in the study.

11 Integrated Summary and Safety Evaluation

Braeburn Pharmaceuticals submitted a 505(b)(2) NDA in support of their subcutaneous (SC) depot buprenorphine drug product referred to during development as CAM2038 for the treatment of opioid use disorder. The NDA relies upon an FDA-previous finding of safety for Subutex (NDA 20732 - buprenorphine sublingual) and literature. There are actually two drug product formulations in this NDA, a once weekly (q1w) and a once monthly (q4w) formulation. The formulations (CAM2038 q1w and CAM2038 q4w) primarily differ (b) (4)

During development, the Division noted that as per any depot formulation development program, the fate of the injected material should be adequately characterized and any novel excipients should be adequately justified for safety as per the FDA guidance document on new excipients.

The results of the local tissue reaction assessment suggest that an expected foreign body reaction occurs which prolong localization of the gel matrix which lasts approximately a month or more following SC injection.

The most definitive life span study has been after administration of a single intramuscular (IM) dose of CAM2038 q1w and q4w with observations lasting up to 113 days (4 months). This study was conducted to evaluate effects after inadvertent IM dosing with CAM2038. Microscopically, the incidence and/or severity of injection site local toxicity observations on Day 2 (cavitation, myofiber degeneration/necrosis, and/or mixed leukocyte infiltration) were generally similar between q1w, q4w and their placebo test article injection sites. Compared with Day 2, microscopic changes were decreased on Day 29 (q1w) and absent on Day 113 (q4w), indicating progressing or full recovery over a period of approximately 1 month for q1w and 4 months for q4w. Similar local toxicity reversibility of inflammation was observed after termination of subcutaneous

dosing and is expected at an increased rate due to increased vascularity of subcutaneous sites.

The two highest (b) (4) doses of buprenorphine drug product q4w results in exposures to buprenorphine that exceed the referenced drug product Subutex. (b) (4)

The buprenorphine exposure following the 128 mg q4w dose exceeds the exposure at 24 mg via Subutex and therefore toxicology studies in two species were required. The Applicant provided a 9-month dog study only; however, the clinical team has concluded that adequate human experience exists and agreed to waive the 6-month rat study.

The definitive nonclinical study was a SC 9-month study of q1w and q4w in dogs with injections over rotating sites which provided adequate local and systemic safety justification for the proposed drug product. Collectively the local toxicity changes are consistent with a foreign body reaction of inflammatory cell infiltrates to a depot injection. Inflammation was considered reversible after dosing was terminated and was most noticeable in a single-dose intramuscular study in which q1w animals were observed for 29 days after the injection and q4w animals were observed for 113 days after injection. Both local inflammation and depot were either partially (Day 29) or completely (Day 113) in regression/reversed.

In addition to nonclinical test data, the extractables/leachables study does not support the human safety of potential leachables from the drug carrying system (e.g., syringe) as noted in Section 2.5 (Container Closure System).

Excipients - The drug product contains two new excipients. First, the once monthly injection q4w contains N-methyl-pyrrolidone (NMP) which during development was considered to be used in a new manner via this product (NMP was previously used in an FDA approved product for acute dental use and a product indicated for the treatment of advanced cancer). Both formulations contain glycerol dioleate (GDO), a diglyceride, which has also not previously been used in an FDA-approved drug product. Of note, the Agency recently approved a chronic depot injection product for a non-oncology indication that containing NMP at levels approximately (b) (4) times higher than this drug product with post-marketing requirements and labeling for potential adverse effects due to the NMP in that formulation. The review focuses on the safety of the CAM2038 formulations which are reviewed in Sections 4 through 10, and the two novel excipients NMP and GDO (which are reviewed in Appendices 1 and 2, respectively).

Approvability - From a nonclinical pharmacology toxicology perspective, there are inadequate data to support an approval recommendation at this time. Specifically, the Applicant has not provided an adequate extractable leachable evaluation of the container closure system. See Section 1.3 for specifics and recommendations.

12 Appendix/Attachments

Appendix 1: NMP Toxicological Risk Assessment

Review of Toxicity Data for NMP in Braeburn's CAM2038 q4w

CAM2038 q4w is a sterile, non-aqueous solution for subcutaneous injection indicated for the treatment of opioid use disorder. It contains 64 mg, 96 mg, 128 mg (b) (4) of buprenorphine base (BUP). This delivery system is designed to deliver buprenorphine over 4 weeks after subcutaneous injection. This injection depot platform contains soybean phosphatidylcholine (SPC), glycerol dioleate (GDO) and (b) (4) *N*-methyl-2-pyrrolidone (NMP) and is referred to by the Applicant as "FluidCrystal" technology. According to the Applicant, "once injected, CAM2038 spontaneously transforms from a low-viscous solution to a highly viscous crystalline gel that encapsulates the BUP and releases it at a steady rate as the depot degrades." In NDA 210136, Braeburn includes two products, CAM2038 q1w for once per week dosing and CAM2038 q4w for once per month dosing. Only the CAM2038 q4w contains NMP (b) (4). The quantitative composition of CAM2038 q4w is outlined in the table below.

Table 14. Composition of CAM2038 q4w Drug Product

Component	Standard	Function	Composition (mg) per dose		
			64 mg/ 0.18 mL	96 mg/ 0.27 mL	128 mg/ 0.36 mL
Buprenorphine Base (BUP)	Ph. Eur.	Active ingredient	64	96	128
<i>N</i> -Methyl-2-Pyrrolidone (NMP)	USP	(b) (4)	(b) (4)		
Soybean Phosphatidylcholine (SPC)	In-house				
Glycerol Dioleate (GDO)	In-house				
Total weight (mg) for all components per dose	Not Applicable	Not Applicable			

Regulatory History

As discussed during development, the use of NMP in this drug product formulation is technically novel. According to the Inactive Ingredients Database (IID, December 12, 2017), NMP is currently use as an excipient in two FDA-approved drug products, as noted below.

Table 15. FDA Inactive Ingredient Database Listing of N-methyl-2-pyrrolidone

Inactive Ingredient	Route	Dosage Form	Maximum Potency
Methypyrrolidone	Periodontal	Drug Delivery System	NA
Methylpyrrolidone	Subcutaneous	Injection	25.85%

The total daily dose via these drug products is not listed in the IID. According to the Applicant, the SC drug product is approved for an advanced cancer indication. As discussed with the Applicant during development, use in an advanced cancer indication is not adequate justification for the safety of this excipient for a chronic non-oncology indication.

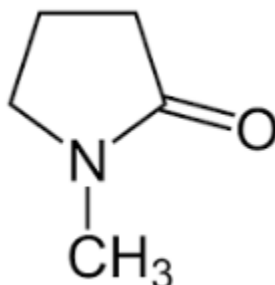
Each SC injection of the CAM2038 q4w (b) (4) BUP strength drug product contains (b) (4) NMP. CAM2038 q4w is intended to be administered once every 4 weeks with no limitation on the duration of treatment (i.e., chronic/lifetime). Although NMP can be found in previously approved products (one for acute periodontal use only, the second for an advanced cancer indication), the safety has not been characterized for this population (chronic use, not advanced cancer indication). Therefore, the use of NMP in CAM2038 q4w for a chronic non-oncology indication is novel.

The novelty of NMP for a chronic non-oncology indication was discussed with the Applicant over the course of development and the Agency noted that the Applicant would have to address the safety for this indication. Literature could be used to support the standard requirements for a novel excipient; however, the adequacy of the literature could only be determined during the NDA review. At the preNDA meeting in 2017, the Division noted that there was technically no chronic rodent toxicology study with NMP; however, we acknowledged that the carcinogenicity studies with NMP may be adequate to address this requirement for any novel chronic excipient.

Recently, the Agency approved a chronic drug product containing NMP for a noncancer indication with postmarketing requirements to provide new data on the reproductive and developmental effects of NMP and a mode of action assessment for mouse liver tumors. As such, although not listed in the IID to date, NMP is technically no longer a new excipient; however, outstanding safety concerns remain to be adequately addressed. These concerns also apply to this drug product.

N-methyl-2-pyrrolidone (NMP)

N-methyl-2-pyrrolidone (CAS No. 872-50-4) is a water-miscible, polar aprotic organic solvent used in the manufacture of paint removers, pesticides, herbicides, fungicides, pigments, cosmetics and pharmaceuticals. In rats, it has shown to be absorbed rapidly via inhalation, oral and dermal administration (World Health Organization, 2001). The structure is depicted below.

Figure 1. Structure of NMP (C₅H₉NO)

NMP is a commonly used solvent in pharmaceutical manufacturing. In fact, ICH Q3C(R6) specifically discusses NMP as a residual solvent in Part III of the document. The Expert Working Group (EWG) revisited the PDE for NMP in 2000 based on new data (chronic oral carcinogenicity study conducted by the NMP Producers Group (unpublished data) and a reproductive and developmental toxicology study published in 1994 (Hass et al., 1994). As noted in the ICH Q3C document, Hass et al. exposed pregnant Wistar rats to 150 ppm NMP for 6 hours a day from Gestation Day 7-20. The rats were allowed to deliver and offspring were evaluated. There was no evidence of maternal toxicity or physical abnormalities noted. However, there were reduced weight of the offspring, pre-weaning development was impaired, and the pups performed worst at higher cognitive function in solving a difficult task compared to control animals. A NOAEL was not established in this study. The EWG elected to keep NMP in Class 2 residual solvent and set an PDE of not more than (NMT) 5.3 mg/day, as follows:

Figure 2. PDE Calculation for NMP as per ICH Q3C(R6)

$$150 \text{ ppm} = \frac{150 \times 99.13}{24.45} = 608.16 \text{ mg/m}^3 = 0.608 \text{ mg/L}$$

$$\text{For continuous dosing} = \frac{0.608 \times 6}{24} = 0.152 \text{ mg/L}$$

$$\text{Daily dose} = \frac{0.152 \times 290}{0.33} = 133.58 \text{ mg/kg}$$

$$\text{PDE} = \frac{133.58 \times 50}{5 \times 10 \times 1 \times 5 \times 5} = \mathbf{5.3 \text{ mg/day}}$$

$$\text{Limit} = \frac{5.3 \times 1000}{10} = \mathbf{530 \text{ ppm}}$$

This PDE remains in the most current ICH Q3C document (Revision 6, Step 4, dated October 20, 2016).

ADME

NMP is readily absorbed across human skin, the gastrointestinal and respiratory tracts. It is rapidly distributed in the body to most organs including male sex organs. Wells and Digenis reported that after IV administration of 45 mg/kg of radiolabeled NMP to male Sprague Dawley rats, the radiolabel had a plasma half-life of 7-10 hours. Approximately 70% of the label was excreted in urine within 12 hours and 80% by 24 hours. At 6 hours, the highest radioactivity was present in the liver and intestines (Wells and Digenis, 1988).

Sitarek and Kilanowicz also studied the tissue distribution of radiolabeled IP NMP in female and male rats and noted that the highest levels at 4 hours were in the adrenals, kidneys, seminal vesicles, testes, muscles, liver, brain and lungs, as depicted in the tables below (Sitarek and Kilanowicz, 2006). NMP distributes widely to tissues and is cleared fairly rapidly.

Table 1. Radioactivity concentration in different male rat tissues after administration of ^{14}C -N-methyl-2-pyrrolidone in a single i.p. dose of 250 mg/kg b.w. (350 kBq/male)

Tissue	4 h	8 h	24 h	48 h	72 h
Liver	1.11 ± 0.11 ^a	0.89 ± 0.01	0.05 ± 0.01	0.04 ± 0.001	0.04 ± 0.001
Kidneys	1.40 ± 0.15	1.08 ± 0.02	0.11 ± 0.02	0.04 ± 0.001	0.04 ± 0.001
Spleen	0.69 ± 0.10	0.67 ± 0.10	0.05 ± 0.01	0.03 ± 0.002	0.03 ± 0.0007
Lungs	1.04 ± 0.13	0.86 ± 0.14	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.002
Brain	1.05 ± 0.11	0.91 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.0007
Adrenals	1.42 ± 0.10	1.03 ± 0.10	0.07 ± 0.01	0.06 ± 0.001	0.05 ± 0.0007
Sciatic nerve	0.96 ± 0.04	1.04 ± 0.03	0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.007
Muscles	1.32 ± 0.19	1.44 ± 0.01	0.03 ± 0.001	0.07 ± 0.004	0.02 ± 0.002
Fat	0.83 ± 0.30	0.67 ± 0.10	0.04 ± 0.01	0.06 ± 0.02	0.02 ± 0.001
Epididymis	0.89 ± 0.08	0.82 ± 0.02	0.05 ± 0.02	0.04 ± 0.005	0.03 ± 0.003
Testicles	1.28 ± 0.14	1.08 ± 0.009	0.05 ± 0.02	0.03 ± 0.005	0.03 ± 0.001
Seminal vesicles	1.33 ± 0.14	0.99 ± 0.08	0.09 ± 0.07	0.04 ± 0.003	0.02 ± 0.002
Whole blood	0.87 ± 0.04	0.70 ± 0.05	0.18 ± 0.003	0.17 ± 0.01	0.18 ± 0.01

i.p. – intraperitoneally; b.w. – body weight; a – mean radioactivity (kBq/g tissue) ± SD from 4 male rats at each time. To assess ^{14}C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12%, and muscles as 40% of whole b.w. [17].

Table 2. Concentration of radioactivity in different female rat tissues after administration of ¹⁴C-N-methyl-2-pyrrolidone in a single i.p. dose of 250 mg/kg b.w. (350 kBq/female)

Tissue	4 h	8 h	24 h	48 h	72 h
Liver	1.45 ± 0.16 ^a	0.88 ± 0.03	0.06 ± 0.01	0.04 ± 0.005	0.04 ± 0.001
Kidneys	1.70 ± 0.14	1.16 ± 0.22	0.09 ± 0.01	0.05 ± 0.002	0.06 ± 0.007
Spleen	0.87 ± 0.10	0.47 ± 0.05	0.05 ± 0.009	0.04 ± 0.002	0.03 ± 0.004
Lungs	1.70 ± 0.16	1.23 ± 0.06	0.06 ± 0.01	0.05 ± 0.001	0.05 ± 0.002
Brain	1.59 ± 0.19	1.14 ± 0.19	0.02 ± 0.002	0.02 ± 0.002	0.03 ± 0.0007
Adrenals	1.48 ± 0.18	0.98 ± 0.10	0.06 ± 0.005	0.06 ± 0.008	0.05 ± 0.003
Sciatic nerve	1.57 ± 0.16	1.52 ± 0.27	0.05 ± 0.007	0.05 ± 0.003	0.05 ± 0.0007
Muscles	2.02 ± 0.17	1.48 ± 0.12	0.04 ± 0.001	0.07 ± 0.004	0.04 ± 0.007
Fat	0.43 ± 0.08	0.82 ± 0.26	0.06 ± 0.006	0.06 ± 0.004	0.03 ± 0.006
Ovaries	1.66 ± 0.20	0.93 ± 0.16	0.05 ± 0.01	0.05 ± 0.008	0.04 ± 0.005
Uterus	1.19 ± 0.09	0.94 ± 0.03	0.04 ± 0.01	0.04 ± 0.005	0.04 ± 0.007

i.p. – intraperitoneally; b.w. – body weight; a – mean radioactivity (kBq/g tissue) ± SD from 4 female rats at each time. To assess ¹⁴C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12% and muscles as 40% of whole b. w. [17].

The Applicant conducted their own PK studies for NMP both in humans as well as rats and rabbits, which are reviewed below:

Study Title: Exploratory Evaluation of NMP (N-methyl-2-pyrrolidinone) Levels in Plasma from CAM2038 q4w Treated Healthy Volunteers from Study HS-13-487

Study Number: PK-15-551
Regulatory Status: Non-GLP

Methods: CAM2038 q4w was administered healthy subjects to obtain PK data for buprenorphine. Selected plasma samples were analyzed for NMP concentrations (96 mg q4w and 192 mg of q4w = ^(b)₍₄₎ mg and ^(b)₍₄₎ mg NMP, respectively). Samples from Study HS-13-487 analyzed are depicted below:

Treatment group	Treatment	No samples	Time points
D	CAM2038 q4w 96 mg	12/subject	All time points from pre-dose CAM2038 q4w to Day 5 (i.e. the Day 13 sample)
E	CAM2038 q4w 192 mg	12/subject	All time points from pre-dose CAM2038 q4w to Day 5 (i.e. the Day 13 sample)

Plasma samples from 1, 2, 4, 6, 10, 24 hours (Day 9) and 48 hours (Day 10) and also on Days 11, 12, and 13 were examined. The lower limit of quantitation was 30.0 ng/mL.

Results: Individual and mean PK values are presented in the Applicant's figure and table below:

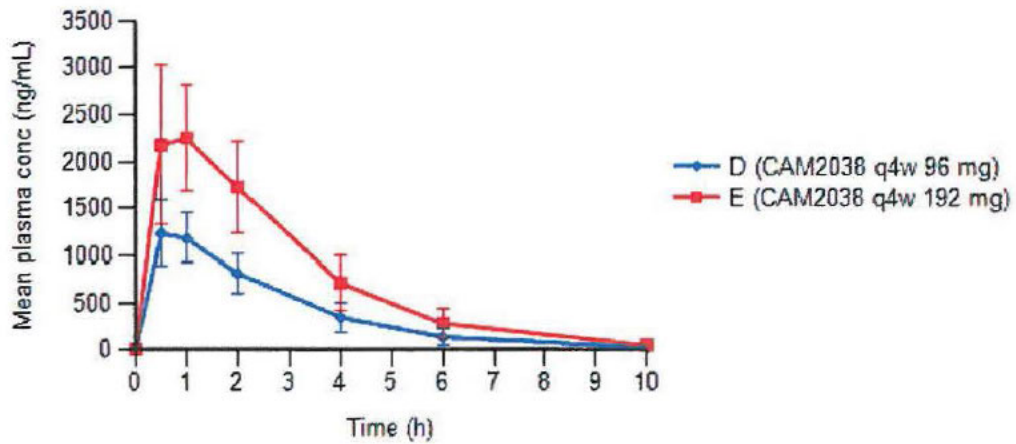


Figure 4. Group mean plasma concentrations of NMP; hours 0-10 (normal scale)

NMP levels were not detected within the LOQ at 24 hours in any individual. The mean AUC values can be used to compare exposures in humans to animals if similar PK data are available.

Table 2. PK parameters determined from NMP concentrations in plasma

Treatment group	Subject	tmax (h)	Cmax (ng/mL)	AUClast (ng/mL*h)	AUCinf (ng/mL*h)	T1/2(h)	
D (CAM2038 q4w 96 mg)	258	0.500	1080	4590	4590	1.21	
	260	1.03	1300	5310	5310	1.09	
	262	0.617	1210	4040	4040	0.932	
	264	0.483	962	4800	4800	1.71	
	267	0.500	1050	4530	4530	1.77	
	269	1.02	1620	8400	8400	1.91	
	273	0.517	1970	6920	6920	1.71	
	275	0.517	1750	6010	6010	1.67	
	277	0.500	1130	3800	3800	1.38	
	279	1.00	867	4970	4970	1.78	
	281	1.02	1120	5250	5250	1.26	
	283	1.03	1360	6180	6180	1.63	
	284	0.533	1580	4930	4930	1.22	
	287	0.500	1200	4880	4880	1.18	
	N	10	10	10	10	10	
	Mean	0.698	1300	5330	5330	1.46	
SD	0.251	320	1210	1210	0.311		
Min	0.483	867	3800	3800	0.932		
Median	0.525	1210	4950	4950	1.51		
Max	1.03	1970	8400	8400	1.91		
CV%	36.0	24.6	22.8	22.8	21.3		
E (CAM2038 q4w 192 mg)	259	1.00	1580	6720	6720	1.68	
	261	0.433	2150	7730	7730	1.2	
	263	1.02	1790	8390	8390	1.72	
	265	0.450	4350	14300	14300	2.06	
	266	1.00	1660	6910	6910	0.996	
	268	0.517	3280	8940	8940	1.42	
	270	0.500	1600	5110	5110	0.858	
	272	1.00	1420	7700	7700	1.96	
	274	0.500	2190	7750	7750	1.23	
	276	1.02	2340	9010	9010	1.47	
	278	0.983	2980	11400	11400	1.46	
	280	0.983	2600	11800	11800	1.4	
	282	1.00	3090	11700	11700	1.66	
	285	1.00	2680	11500	11500	1.47	
	286	0.483	2360	8550	8550	1.51	
	N	20	20	20	20	20	
Mean	0.792	2400	9170	9170	1.47		
SD	0.264	793	2470	2470	0.324		
Min	0.433	1420	5110	5110	0.858		
Median	0.983	2340	8550	8550	1.47		
Max	1.02	4350	14300	14300	2.06		
CV%	33.4	33.0	26.9	26.9	22.0		

Study Title: NMP (N-methyl-pyrrolidone). Toxicokinetic Study in Rats.

Study Number: TO-15-540
Regulatory Status: GLP, Audited
Study Report Date: June 17, 2016

Methods: NMP was injected subcutaneously to male and female Sprague-Dawley rats at doses of 100, 250, and 500 mg/kg. Plasma samples were obtained pre-dose, 20 minutes, 1, 2, 4, 8, 12, 24, 48, and 96 hours post dose in cohorts of animals to avoid excessive blood loss.

Results: Mean TK values are depicted below.

Table 4 Mean toxicokinetic parameters of N-methyl-2-pyrrolidone

Group	Dose (mg/kg)	Gender	T _{1/2} (hr)	T _{max} (hr)	C _{max} (ug/ml)	SE_C _{max} (ug/ml)	C _{max} /D (kg*ug/ml/mg)
1	100	Male	1.26	0.3	162	15	1.62
		Female	1.11	0.3	204	21	2.04
		Both	1.25	0.3	183	15	1.83
2	250	Male	1.13	1.0	321	11	1.28
		Female	2.14	1.0	411	8	1.64
		Both	2.28	1.0	366	21	1.46
3	500	Male	1.97	1.0	607	24	1.21
		Female	1.66	1.0	669	16	1.34
		Both	1.74	1.0	638	19	1.28

Group	Dose (mg/kg)	Gender	AUC _{0-t} (hr*ug/ml)	SE_AUC _{0-t} (hr*ug/ml)	AUC _{inf} (hr*ug/ml)	AUC _{inf} /D (hr*kg*ug/ml/mg)
1	100	Male	431	49	433	4.33
		Female	709	124	710	7.10
		Both	570	76	571	5.71
2	250	Male	1468	66	1468	5.87
		Female	2495	276	2496	9.99
		Both	1984	230	1986	7.94
3	500	Male	4002	258	4004	8.01
		Female	5674	572	5676	11.35
		Both	4838	403	4840	9.68

n=5/sex for each study Group. Blood sampling for toxicokinetics was performed using a spares sampling scheme with n=3/sex/time point for each Group.

The Applicant appropriately noted that in this study, female exposures were higher than males, but that for both sexes, NMP could be detected up to 8 to 12 hours after injection pending dose. T_{max} was 20 minutes following a dose of 100 mg/kg but 1 hour for doses of 250 and 500 mg/kg.

Study Title: NMP (N-methyl-2-pyrrolidone). Toxicokinetic Study in Rabbits

Study Number: TO-15-541
 Regulatory Status: GLP, Audited
 Study Date: June 17, 2015

Methods: NMP (50, 150, or 250 mg/kg) was administered via SC injection to White New Zealand Rabbits (five female) and plasma samples were obtained to characterize the PK profile of NMP in this species. Plasma samples were obtained pre-dose, 20

minutes, 1, 2, 4, 8, 12, 24, 48, and 96 hours. For several high dose animals, all time points were not obtained as the animals had to be euthanized early.

Results:

Table 2 Individual toxicokinetic parameters of N-methyl-2-pyrrolidone

Group	Animal	T _{1/2} (hr)	T _{max} (hr)	C _{max} (ug/ml)	C _{max} /D (kg*ug/ml/mg)
1	1	0.91	1.0	62.7	1.25
	2	0.90	1.0	41.9	0.84
	3	0.89	0.3	71.1	1.42
	4	0.87	1.0	66.4	1.33
	5	0.86	1.0	66.2	1.32
	N	5	5	5	5
	Mean	0.89	0.9	61.7	1.2
	SD	0.02	0.3	11.4	0.2
	CV%	2.3	34.6	18.6	18.6
2	6	1.05	1.0	166.0	1.11
	7	1.21	1.0	197.0	1.31
	8	1.21	1.0	87.7	0.58
	9	1.03	1.0	152.0	1.01
	10	1.01	1.0	219.0	1.46
	N	5	5	5	5
	Mean	1.10	1.0	164.3	1.1
	SD	0.10	0.0	50.2	0.3
	CV%	9.0	0.0	30.6	30.6
3	11		2.0	267.0	1.07
	12	2.22	1.0	320.0	1.28
	13		1.0	305.0	1.22
	14		1.0	310.0	1.24
	15	2.20	1.0	348.0	1.39
	N	2	5	5	5
	Mean	2.21	1.2	310.0	1.2
	SD	0.01	0.4	29.2	0.1
	CV%	0.6	37.3	9.4	9.4

Group	Animal	AUC ₀₋₂ (hr*ug/ml)	AUC ₀₋₄ (hr*ug/ml)	AUC _{0-t} (hr*ug/ml)	AUC _{inf} (hr*ug/ml)	AUC _{inf/D} (hr*kg*ug/ml/mg)
1	1	88	141	157	158	3.16
	2	72	134	157	158	3.16
	3	115	177	197	198	3.95
	4	113	183	207	207	4.15
	5	103	177	203	203	4.06
	N	5	5	5	5	5
	Mean	98	163	184	185	3.70
	SD	18	23	25	25	0.49
	CV%	18.5	14.2	13.4	13.4	13.4
2	6	280	523	667	668	4.45
	7	321	630	984	985	6.56
	8	145	284	343	343	2.29
	9	244	492	729	730	4.87
	10	348	647	890	891	5.94
	N	5	5	5	5	5
	Mean	268	515	723	723	4.82
	SD	79	145	247	247	1.65
	CV%	29.5	28.2	34.2	34.1	34.1
3	11	416				
	12	538	1110	1996	1997	7.99
	13	522				
	14	524				
	15	576	1113	1875	1877	7.51
	N	5	2	2	2	2
	Mean	515	1112	1935	1937	7.75
	SD	60	2	85	85	0.34
	CV%	11.6	0.2	4.4	4.4	4.4

Note that animal Nos. 11, 13 and 14 in Group 3 were terminated after 2 hours due to clinical signs.

As noted by the Applicant, NMP was detectable at 4 hours for the 50 mg/kg group and out to 8 and 12 hours for the 150 mg/kg and 250 mg/kg groups, respectively. T_{max} was about 1 hour.

There are limited data on SC route of exposure. As part of their safety assessment, the Applicant has concluded that the existing data on NMP suggest that comparable exposures occur via any route of administration (IV has slightly higher C_{max}) and therefore, extrapolation across studies testing different routes of administration is appropriate. The data submitted to support that conclusion are summarized in the Applicant's table below:

Table 1: Pharmacokinetic Parameters of Animals Treated with NMP by Oral, IV, SC or Inhalation and Humans Treated SC with CAM2038 q4w (192 mg)

Dose (mg/kg)	C _{max} (µg/mL/mg/kg) ^a	AUC _{0-t} (µg hr/mL/mg/kg) ^a	T _{max} (hr)	T _{1/2} (hr)	Reference
125 (oral) Rat	0.94	5.63	1.00	NR	Canerup et al., 2005
50 (oral) ^b Rat	0.67	3.00	2.25	2.25	DuPont, 1995b
100 (SC) Rat	1.83	5.70	0.30	1.19	CiToxLAB 2016a
50 (SC) Rabbit	1.23	4.18	0.90	0.89	CiToxLAB 2016b
50 (IV) ^b Rat	3.08	9.84	2.85	2.85	DuPont, 1995b
98 (inhalation) ^{b,c} Rat	0.91	5.10	1.55	1.55	DuPont, 1995b
2.8 (SC) Human ^{a,d}	0.86	3.30	0.80	1.47	Camurus, 2016

^aThe C_{max} and AUC values were adjusted based on mg/kg dose.

^bThe mean pharmacokinetic parameters for male and female animals was calculated

^cThe inhalation dose (410 mg/m³) was converted to an oral dose based on Equation 1 cited in the text (see Section 10).

^dThe dose of NMP in the 192 mg CAM2038q4w drug product is (b) (4) mg (total dose). This was converted to a mg/kg dose based on a 60 kg individual (b) (4) mg/kg).

Additional Information: NR – Not reported; IV - intravenous; SC – subcutaneous

According to the submission, the PK data were somehow adjusted for dose although the submission did not indicate how this was done. Regardless, review of the above summary table clearly indicates that the AUC in rat following IV administration of 50 mg/kg NMP is 3.3 times higher than the same dose administered via the oral route. C_{max} via IV is 4.6 times higher than oral in the rat. SC would likely be somewhere in between.

ADME Conclusions

Collectively, the above data do support the Applicants statement that, in contrast to buprenorphine, the NMP derived from CAM2038 q4w is cleared from the plasma relatively quickly. Based on their data, the NMP in the dosage form is eliminated from plasma within 24 hours.

For the purposes of this review, the worst-case MDD of NMP will be (b) (4) (b) (4) mg/day via the 128 mg q4w product, and 32 mg/day via the q1w product. A dose of (b) (4) mg/60 kg person = (b) (4) mg/m² on a body surface area basis. This daily dose is greater than any of the other two FDA approved drug products making this use novel in terms of dose, duration, and risk-benefit compared to the FDA-approved drug products listed in the IID (accessed 12/12/2017). Further, (b) (4) (b) (4) this dose is also (b) (4) times higher than the ICH Q3C(R6) PDE of 5.3 mg/day.

Finally, although the PK data presented suggest similar T_{max} and t_{1/2} values, the argument that all routes of administration will result in similar risk assessments in terms of toxicity is not convincing given the limited data provided.

TOXICOLOGICAL RISK ASSESSMENT

The Applicant has provided a review of the literature in support the safety of NMP. Although there are multiple toxicology studies in the literature with NMP, none of the studies use the subcutaneous route of administration. Bridging studies with appropriate TK have not been provided. Therefore, exposure margins will be based on body surface area comparisons.

General Toxicology

A 9-month general toxicology study in the dog testing CAM2038 q4w was conducted in support of this NDA. This study provides an adequate safety assessment of the NMP in the drug product formulation in the nonrodent species. The reader is referred to the body of this review for further details.

In addition, the Applicant summarized acute and repeat-dose general toxicology data from the published literature. This appendix will focus only on the published subchronic and toxicology studies submitted (Becci et al., 1983, Lee et al., 1987, Malek et al., 1997, Malley et al., 1999). The primary literature references and the NOAELs identified by the Applicant are depicted in the Applicant's table below:

Species	Route of Administration	Study Duration	Doses*	Reference
Mouse/ B6C3F1	Oral (diet)	28 Days	0, 500, <u>2500</u> , 7500, 10,000 ppm	Malek et al, 1997
Mouse/ B6C3F1	Oral (diet)	90 Days	0, <u>1000</u> , 2500, 7500 ppm	Malley et al., 1999
Rat/ CD	Oral (diet)	28 Days	0, 2000, <u>6000</u> †, 18,000‡, 30,000 ppm	Malek et al, 1997
Rat/ CD	Oral (diet)	90 Days	0, <u>3000</u> , 7500, 18000 ppm	Malley et al., 1999
Dog/ Beagle	Oral (diet)	90 Days	0, 25, 79, <u>250</u> mg/kg	Becci P, et al 1983
Rat/ CD	Inhalation	6 hr/day, 5 days/week for 4	0, 0.1, <u>0.5</u> and 10 mg/L	Lee et al, 1987

*The NOAEL is underlined; † NOAEL males; ‡ NOAEL females

Subchronic Toxicity

As noted in the table above, there are 90-day oral rat, mouse, and dog toxicology studies.

In the 90-day rat study, rats were administered 3000, 7500, or 18000 ppm NMP via the diet. Based on food consumption, male doses were 169, 433, and 1057 mg/kg and female doses were 217, 565, and 1344 mg/kg at the low-, mid-, and high-dose groups (LD, MD, and HD), respectively (Malley et al., 1999). The LD was considered the NOAEL for both sexes. There were no effects of NMP on mortality. The HD group demonstrated reduced body weights (~25% decrease compared to controls). The MD and HD male animals demonstrated increased foot splay values, low arousal, and

palpebral closure in the neurological assessments, consistent with a general sedative effect of the compound. HD animals demonstrated increased relative liver, kidney and lung weights and decreased relative brain and testes weights. Microscopically, the high-dose group female demonstrated hepatocellular hypertrophy which was considered an adaptive response. An increase in hemosiderin deposits in spleen was also noted in both HD males and females. There was no adverse histopathology in the CNS. The NOAEL was the low dose. These doses provide a safety margin of 7.2x and 9.3x for males and females for the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

In the 90-day mouse study, mice were fed diets containing 1000, 2500, or 7500 ppm NMP which resulted in mean daily doses of 277, 619, and 1931 mg/kg for both sexes based on food consumption data (Malley et al., 1999). There was no effect on mortality. There was a dose related increase in liver weights. Evidence of centrilobular hepatocellular hypertrophy was found in the HD animals, which was attributed to an adaptive response. The LD was considered the NOAEL/LOAEL based on liver weight increases. This dose provides a safety margin of 11.9x for the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

In the 90-day dog study, beagles were administered NMP via the diet resulting in daily doses of 25, 79, and 250 mg/kg (Becci et al., 1983). There was no impact on survival. HD animals demonstrated reduced body weight gain compared to controls (↓53% males, ↓37% females). Decreased cholesterol, serum albumin, and total protein levels in MD and HD male dogs was noted; however, there were no histological correlates to explain these findings. Platelet counts increased with dose at Week 8, but levels were subsequently decreasing by Week 12. Based on these unexplained changes, the HD was deemed the NOAEL which provides a 72x safety margin for NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

Chronic Toxicity

In a 2-year inhalation toxicology study in male rats, Lee et al report that 0.04 or 0.4 mg/L for 6 hours a day NMP, 5 days a week did not result in any clear treatment-related histopathology (Lee et al., 1987). This concentration in air, assuming 290 L/day (12 L/h) and 0.5 kg body weight, would result in 58 mg/kg dose (5x the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area).

Malley et al. treated rats with oral 1600, 5000, or 15000 NMP via the diet for 2-years resulting in estimated daily intakes of 66.4, 207, and 678 mg/kg in males and 87.8, 283, and 939 mg/kg in females (Malley et al., 2001). NMP reduced body weights in HD males and females by 25 and 35% relative to controls which corresponded to lower food consumption and food efficiency. HD males demonstrated reduced survival which was associated with an increased incidence of chronic progressive nephropathy/uremia. Decreased survival in MD males was associated with an increase in pituitary and skin tumors which were not dose dependent and therefore, not likely treatment-related. As noted in the table below, NMP increased the incidence of chronic progressive

nephropathy, increased pigment containing macrophages in the spleen, produced hepatocellular fat accumulation, and generated testicular, bone and kidney damage in males. In females, the only findings were increased pigmented macrophages in the spleen and lymphoid depletion. The NOAEL was 5000 ppm (207 and 283 mg/kg in males and females, respectively) which suggests a safety margin of 18x and 24x for males and females, respectively, for the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

Figure 3. Non-neoplastic Lesions in 2-year Oral Rat Toxicology Study (Malley et al., 2001)

Lesion Concentration (ppm)	0	1600	5000	15 000
Male rats				
Severe chronic progressive nephropathy	7/62	10/62	12/62	25/62 [#]
Moderate accumulation of pigment containing macrophage, spleen	18/62	14/62	16/62	28/62 [#]
Hepatic centrilobular fatty change	4/62	8/62	4/62	19/62 [#]
Thrombus in renal vein of kidney	0/62	4/62	4/62	11/62 [#]
Polyarteritis, cecum	0/62	1/31	0/49	5/62*
Lymphoid depletion, mesenteric lymph node	10/62	10/30	9/48	28/60*
Hypertrophy/cystic degeneration, adrenal cortex	18/62	10/34	22/52	28/62*
Polyarteritis, testes	10/62	16/62	11/62	31/62 [#]
Bilateral degeneration/atrophy, seminiferous tubules	15/62	17/62	15/62	35/62 [#]
Bilateral oligospermia/germ cell debris in epididymides	11/62	14/62	12/62	35/62 [#]
Fibrous osteodystrophy, femur/knee	3/62	5/32	5/49	13/62*
Fibrous osteodystrophy, sternum	2/60	5/32	5/49	11/62*
Polyarteritis, mesenteric lymph node	0/60	0/30	0/48	5/62*
Female rats				
Moderate accumulation of pigment containing macrophage, spleen	38/62	35/62	36/62	51/62 [#]
Lymphoid depletion, mesenteric lymph node	15/58	18/47	11/41	29/60*

[#]Statistically significant by Cochran Armitage test for trend, $p < 0.05$.

*Statistically significant by Fisher's exact test, $p < 0.05$.

Malley et al. also treated mice for 18 months with oral NMP via the diet (600, 1200, or 7200 ppm) resulting in daily doses of 89, 173, or 1089 mg/kg in males and 115, 221, or 1399 mg/kg in females based on food consumption (Malley et al., 2001). There were no clear effects of NMP on body weight or food consumption; however, the NMP appeared to stick to the food hopper, so the sporadic changes in BW are difficult to interpret. There was no increased mortality in males or females. NMP significantly increased liver weights, likely the result of the increased incidence of liver tumors and hepatocellular hypertrophy in this study (see carcinogenicity section below). In males, absolute kidney weights were decreased in all dose groups although there were no histopathological correlates to explain this finding and the effects were not clearly dose dependent. Absolute testes weight was increased in the HD males; relative weights were increased in all treatment groups without histopathological correlates. The same can be said of the increased relative adrenal weight in HD males and lower ovarian weights in MD and HD females. In females, absolute brain weight increases in all treatment group without histopathological changes noted.

Table 16. Organ Weights 18-Month Oral Mouse Study (Malley et al., 2001)

Dosage (ppm)	0	600	1200	7200
Male mouse				
Absolute liver weight (mg)	1437.3 (407.8)	1308.7 (150.2)	1455.8 (388.3)	1776.9** (467.9)
Relative liver weight (% of body weight)	3.8 (1.0)	3.6 (0.3)	4.0* (1.5)	5.0** (1.1)
Absolute kidney weight (mg)	684.4 (70.1)	633.4** (54.5)	633.6** (68.2)	635.8** (58.3)
Relative kidney weight (% of body weight)	1.8 (0.2)	1.7 ** (0.2)	1.7** (0.2)	1.8 (0.2)
Absolute testes weight (mg)	228.8 (15.4)	235.0 (16.5)	233.8 (14.8)	238.2** (18.4)
Relative testes weight (% of body weight)	0.62 (0.06)	0.65* (0.07)	0.65* (0.08)	0.67** (0.08)
Absolute adrenal weight (mg)	5.4 (1.2)	5.5 (1.4)	5.2 (1.4)	5.6 (0.9)
Relative adrenal weight (% of body weight)	0.014 (0.003)	0.015 (0.004)	0.014 (0.004)	0.016* (0.003)
Female mouse				
Absolute liver weight (mg)	1273.6 (181.2)	1241.3 (159.9)	1293.8 (189.4)	1423.1 (691.8)
Relative liver weight (% of body weight)	3.9 (0.6)	4.0 (0.4)	4.0 (0.6)	4.6** (1.9)
Absolute brain weight (mg)	498.2 (24.5)	515.8** (24.2)	518.2** (26.6)	508.8* (24.5)
Relative brain weight (% of body weight)	1.6 (0.3)	1.7 (0.3)	1.6 (0.3)	1.7 (0.2)
Absolute ovarian weight (mg)	36.7 (44.4)	64.5 (263.8)	22.9* (28.2)	26.6* (25.9)
Relative ovarian weight (% of body weight)	0.111 (0.103)	0.203 (0.824)	0.074 (0.108)	0.085 (0.078)

*Statistically significant difference by one-way analysis of variance and Dunnett's tests, $p < 0.05$.

**Statistically significant difference by one-way analysis of variance and Dunnett's tests, $p < 0.01$.

Table 17. Summary of Test Substance-related Non-neoplastic Lesions in 18-month Oral Mouse Study (Malley et al., 2001)

Lesion	Concentrations (ppm)			
	0	600	1200	7200
Male mice				
Hepatocellular clear cell foci	0/50	1/50	1/50	6/50*
Hepatocellular basophilic foci	4/50	2/50	3/50	5/50
Hepatocellular eosinophilic foci	1/50	2/50	2/50	19/50*
Hepatocellular centrilobular hypertrophy	0/50	0/50	3/50	43/50*
Cellular alterations in liver	5/50	5/50	6/50	25/50*
Female mice				
Hepatocellular clear cell foci	0/50	0/50	0/50	1/50
Hepatocellular basophilic foci	2/50	2/50	1/50	10/50*
Hepatocellular eosinophilic foci	1/50	0/50	0/50	6/50*
Hepatocellular centrilobular hypertrophy	0/50	1/50	0/50	0/50
Cellular alterations in liver	3/50	2/50	1/50	17/50*

*Statistically significant by Cochran Armitage test for trend, $p < 0.05$.

As per the authors, the NOAEL was reported to be LD in males and the MD in females, the former apparently driven primarily by the liver effects in males at 1200 ppm. These NOAELs suggest exposure margins of 3.8x and 9.5x for the NMP dose of (b) (4) mg via the 128 mg q4w dosage form based on body surface area.

As discussed with the Applicant, this study could serve as the rodent chronic toxicology study requirements for NMP. Upon review, a dedicated rodent chronic toxicology study is not necessary.

REPRODUCTION AND DEVELOPMENTAL TOXICITY

The Applicant submitted a literature review to address the reproductive and developmental toxicology requirements for NMP in their formulation. In their review, the Applicant did not specifically delineate why they felt any given study addressed all standard endpoints of modern reproductive and developmental toxicology studies. The multigenerational studies below do contain some fertility and early embryonic study endpoints.

Male and Female Fertility and Early Embryonic Development

As per ICH S5(R2), fertility and early embryonic development studies are intended to test for toxic effects of a compound resulting from treatment prior to mating (males and females) through mating and implantation. For females, the studies should characterize effects on the estrus cycle, tubal transport, implantation, and development of preimplantation states of the embryo as well as mating behavior and fertility assessments. The acceptable dosing regimen for females is to dose them at least 2 weeks prior to mating through implantation in females (for rats implantation is generally by Gestation Day 6-7 of pregnancy). For males, the studies should evaluate the impact of a drug on functional effects (e.g., libido, epididymal sperm maturation) that may not be detected via histopathological examination of the male reproductive tissues. Although spermatogenesis in the rat lasts 63 days, acceptable dosing regimens require that the rats be tested for at least 28 days prior to mating.

Study Title: Multi-Generation Rat Reproduction Study with N-Methylpyrrolidone (Exxon, 1991; Project Number: 236535)

Exxon Biomedical Sciences (1991) conducted a study in which male and female rats were administered NMP via the diet with target dosing intended to administer 50, 160, and 500 mg/kg/day. The study contains some fertility and early embryonic development endpoints and is therefore discussed here in that context. Both male and females were dosed daily for 10 weeks prior to mating and throughout the mating period. Females were also dosed during gestation, lactation, and through weaning to Post-Partum Day (PPD) 21. Second generation pups (F1) were dosed directly starting on PND 21 for an additional 10 weeks and through gestation, lactation, and to weaning of the F2 pups. Paternal toxicity was noted in the high-dose (HD) animals as demonstrated by decreased body weights and food consumption. The HD group also demonstrated reduced fertility indices in both males and females and reduced survival of the F1 offspring in the HD group. Key summary tables from the report are reproduced below:

DOSE (MG/KG)	Male		Female		Gestational Index	Mean Days Gestation	Mean Litter Size	Mean		Percent	
	Mating Index	Fertility Index	Fertility Index	Fecundity Index				Live	Dead	Live	Dead
0	93.1	82.8	93.3	89.3	96.0	22.7	12.6	12.5	0.2	98.7	1.3
50	100.0	83.3	100.0	83.3	100.0	22.4	14.5	14.4	0.2	98.9	1.1
160	96.6	75.9	96.7	79.3	100.0	22.4	13.6	13.0	0.5	96.2	3.8
500	86.7	80.0	86.7	92.3	95.8	22.5	12.9	11.2	1.7	86.0	13.3

INDICES:

Male Mating Index (%) =	$\frac{\text{Number of males for which mating confirmed}}{\text{Number of males used for mating}} \times 100$
Male Fertility Index (%) =	$\frac{\text{Number of males impregnating females}}{\text{Number of males used for mating}} \times 100$
Female Fertility Index (%) =	$\frac{\text{Number of females for which mating confirmed}}{\text{Number of females paired}} \times 100$
Female Fecundity Index (%) =	$\frac{\text{Number of females pregnant}}{\text{Number of females sperm positive}} \times 100$
Gestational Index (%) =	$\frac{\text{Number of females with live litters}}{\text{Number of females pregnant}} \times 100$

Although not exactly dose dependent, the above data suggest that the doses of 160 and 500 mg/kg are associated with a slight reduction in male fertility index and the 500 mg/kg HD reduced fertility index in females. The Authors of the study suggest that this change is not likely biologically significant and considered them non-adverse. However, male rodents are very fertile compared to humans and evidence of changes in rodent fertility index does suggest some concern. There were no reported histopathological correlates in male testes in the study report. Toxicokinetic data or palatability data were not reported. As this study was conducted by dietary administration, the actual exposures in the MD and HD animals are estimates only and it is not known if there are palatability issues which impacted the actual dose consumed. This confounds the ability to make dose response conclusions with confidence.

	0	1	2	3	4	5	6	7	8	9	10	11
SURVIVORS												
0 MG/KG/DAY	312	311	311	311	310	221	221	221	221	221	221	221
50 MG/KG/DAY	359	355	353	353	352	240	240	240	240	240	240	240
160 MG/KG/DAY	300	299	298	298	298	209	209	209	209	209	209	209
500 MG/KG/DAY	267	209	200	196	192	155	153	152	151	151	151	150
GENERAL OBSERVATION												
NO OBSERVABLE ABNORMALITIES												
0 MG/KG/DAY	303	308	310	309	309	219	219	217	215	218	218	219
50 MG/KG/DAY	352	354	352	352	351	239	239	239	239	239	239	239
160 MG/KG/DAY	295	291	289	289	296	208	208	208	207	207	207	207
500 MG/KG/DAY	261	204	195	187	183	148	145	145	145	145	144	146
FOUND DEAD												
0 MG/KG/DAY	3	0	0	0	0	0	0	0	0	0	0	0
50 MG/KG/DAY	6	1	2	0	1	0	0	0	0	0	0	0
160 MG/KG/DAY	11	1	0	0	0	0	0	0	0	0	0	0
500 MG/KG/DAY	45	19	1	4	0	1	1	1	0	0	1	0
WITHOUT MILK												
0 MG/KG/DAY	8	0	0	1	1	0	0	0	2	0	0	0
50 MG/KG/DAY	0	1	4	1	2	0	0	0	0	0	0	0
160 MG/KG/DAY	2	7	2	7	8	0	3	0	0	0	0	0
500 MG/KG/DAY	22	3	0	3	4	2	3	1	2	0	0	0
PARTIALLY CANNIBALIZED (INCLUDED IN FOUND DEAD TOTALS)												
0 MG/KG/DAY	1	0	0	0	0	0	0	0	0	0	0	0
50 MG/KG/DAY	0	0	0	0	0	0	0	0	0	0	0	0
160 MG/KG/DAY	1	0	0	0	0	0	1	0	0	0	0	0
500 MG/KG/DAY	2	11	1	6	0	0	0	0	0	0	0	0
MISSING/PRESUMED CANNIBALIZED												
0 MG/KG/DAY	2	0	0	0	1	0	0	0	0	0	0	0
50 MG/KG/DAY	0	1	0	0	0	0	0	0	0	0	0	0
160 MG/KG/DAY	1	0	0	0	0	0	0	0	0	0	0	0
500 MG/KG/DAY	3	25	7	1	3	1	1	0	1	0	0	0

DOSE (MG/KG)	Live Birth Index %	Day 4 Survival Index %	Day 7 Survival Index %	Day 14 Survival Index %	Day 21 Survival Index %	Lactation Index %
0	98.7	99.4	100.0	100.0	100.0	100.0
50	98.9	98.1	100.0	100.0	100.0	100.0
160	96.2	99.3	100.0	100.0	95.2	95.2
500	86.7	71.9	96.8	97.4	98.6	93.0

INDICES:

Live Birth Index =	$\frac{\text{Number of live pups at birth}}{\text{Number of pups born}} \times 100$	Day 14 survival Index =	$\frac{\text{Number of live pups at day 14}}{\text{Number of live pups at day 7}} \times 100$
Day 4 survival Index =	$\frac{\text{Number of live pups at day 4 (precul}}{\text{Number of live pups at birth}} \times 100$	Day 21 survival Index =	$\frac{\text{Number of live pups at day 21}}{\text{Number of live pups at day 14}} \times 100$
Day 7 survival Index =	$\frac{\text{Number of live pups at day 7}}{\text{Number of live pups at day 4 (postcul}} \times 100$	Lactation Index =	$\frac{\text{Number of live pups at day 21}}{\text{Number of live pups at day 4 (postcul}} \times 100$

The NOAEL for parental and reproductive and developmental effects was reported by the study to be the MD of 160 mg/kg (960 mg/m²) which is 13.8x the maximum daily dose of NMP of (b) (4) mg via the 128 mg q4w drug product. However, the data above suggests a potential adverse effect of NMP on the MD pup survival as well even if not statistically significant. The LD of 50 mg/kg provides a 4.3x exposure margin. The study appears to be consistent with typical multigenerational study protocols from the time period and the study was conducted after GLPs were instituted. Although there is

a GLP statement, the statement is not signed, and the report is clearly marked as “Draft Not Subjected to QA Audit.” There were no assessments of sperm counts and sperm morphology. There were no specific evaluation of bone malformations using specialized staining. Further, it is worth noting that the dietary route of administration would result in lower C_{max} values than the SC route. As such, this is not a complete fertility and early embryonic development study by current standards or an ideal embryo-fetal development (EFD) study based on current models (See Exxon, 1992 for EFD data).

Male Fertility

Solomon et al. evaluated male fertility as part of a two-generation study in rat. Males were exposed to whole body inhalation of 10, 51, or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to end of mating period (100 days total) (Solomon et al., 1995). No effects on male fertility were observed but limited endpoints were assessed. Male mating and fertility indices were assessed and both were based only on reproductive performance (i.e., number of rats copulating or litters sired). Testes were weighed and reproductive organs were examined for gross lesions. No evaluation of spermatogenesis or histopathology of reproductive organs was performed. The NOEL for male reproductive performance is the highest dose tested, 68 mg/kg, which is 5.9 times the maximum daily dose of NMP of (b) (4) mg via the 128 mg q4w drug product.

Sitarek and Stetkiewicz administered NMP to male rats orally at doses of 100, 300 and 1000 mg/kg for 10 weeks prior to mating (Sitarek and Stetkiewicz, 2008). Males were mated with untreated females then necropsied and epididymis and testis were fixed for histopathological evaluation. The individual stages of spermatogenesis were assessed. Viability and development of offspring were observed up to 28 days. The doses of 100 and 300 mg/kg did not significantly alter fertility or spermatogenesis. However, significantly lower viability of the offspring in the first four days of life was seen at 300 mg/kg and above. Infertility and extensive damage to the seminiferous epithelium and the seminal tubule of the testis were observed at 1000 mg/kg (86x the MDD). The NOEL for male fertility is 300 mg/kg (26-fold the human dose based on BSA) although the NOEL for paternal effects of prenatal development of the progeny is 100 mg/kg which is 8.6 times the HDD based on BSA. This study appears to contain all the primary endpoints of a male fertility study. Exposure data are not available and oral dosing is expected to result in lower bioavailability compared to SC administration as such, the safety margin of 8.6 likely is an overestimate. The apparent male-mediated adverse impact on subsequent offspring are not endpoints tested in standard fertility studies nor are they addressed via pre- and postnatal development (PPD) studies because PPD study designs do not include paternal dosing. In terms of labeling, Section 13.1 and 8.1 could state:

Section 13.1

Adverse effects on testes and male fertility were noted in a published study in which rats were treated for 10 weeks with daily oral doses of NMP, an excipient in BRANDNAME ONCE MONTHLY at 86 times the MDD and resulted in male-mediated adverse effects on offspring (decreased pup weight and survival) at daily doses 26 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. No adverse effects were noted at oral doses 8.6 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY.

Section 8.1

Male-mediated adverse effects on offspring (decreased pup weight and survival) were noted in a published study in which male rats were treated orally for 10 weeks with NMP, an excipient in BRANDNAME ONCE MONTHLY at daily doses 26 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. No adverse effects were noted at oral doses 8.6 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY.

The proposed above statements do not include information from the Exxon 1991 study as the results of that study are difficult to interpret given the route of administration and uncertainty of the actual doses administered.

Female Fertility and Early Embryonic Development

Solomon et al. evaluated female fertility as part of their two-generation study in rat (Solomon et al., 1995). Females were exposed to whole body inhalation of 10, 51 or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to weaning with interruption between GD 20 to Day 4 post-partum (134 days total). Treated males were mated with treated females. Female mating and fertility indices were assessed and both were based only on reproductive performance (i.e., number of rats copulating or bearing litters). The uterus was weighed and reproductive organs were examined for gross lesions. Live and dead fetuses, resorptions, corpora lutea, and fetal weights were evaluated. The female fertility parameters that were evaluated appeared to be adequate in this study and no NMP-related effects were observed. The NOEL for female fertility is the highest doses tested, 68 mg/kg, which is 5.9-fold the human dose of (b) (4) mg NMP via the 128 mg q4w drug product based on BSA.

Sitarek et al. treated female rats with oral NMP 150, 450, or 1000 mg/kg for 5 days/week two weeks before mating, during mating, and through gestation and lactation (Sitarek et al., 2012). The fertility index was significantly lower at 450 and 1000 mg/kg (38.7 and 86 times the MDD). No standard early embryonic endpoints were examined (only fertility index = percentage of pregnant females in mating female group). However, examination of the HD females that did not get pregnant revealed evidence of early resorptions indicating intrauterine mortality of the embryos. The NOEL for fertility in this study is 150 mg/kg and is 12.9-fold the human dose of (b) (4) mg NMP based on BSA. The authors note that the 150 mg/kg dose “slightly toxic” (body weight changes) to the dams. It should be noted that although there was no effect on female fertility

parameters at 150 mg/kg, pup survival was decreased at this dose. Both the index of viability (% of pups born alive that survived to 4 days) and index of lactation (% of pups alive at 4 days that survived to 21 days) were significantly lower than control for all treated groups, as noted in the table below from the publication.

Table 18. Effect of NMP on Reproductive Performance in Female Rats and their Progeny (Sitarek et al., 2012)

	Effect of <i>N</i> -Methyl-2-Pyrrolidone on the Reproductive Performance of Female Rats and Viability of Their Progeny			
	Daily dose of NMP (mg/kg)			
	0	150	450	1000
Number of				
Mating females with males	24	26	28	22
Pregnant females	22	24	20	15
Died females*	0	0	0	2
Number of				
Live pups per litter	11.5 ± 3.5 ^a	10.4 ± 2.6	10.5 ± 3.4	0.33 ± 0.82 ^b
Dead pups per litter	0.18 ± 0.85	0	0.13 ± 0.34	0.80 ± 1.1 ^b
Sex ratio (F : M)	132 : 125	112:137	105:107	5:3
Indices				
Fertility	91.7	92.3	71.4 ^b	68.2 ^b
Viability	94.0	86.4 ^b	71.6 ^b	0
Lactation	96.1	78.2 ^b	43.4 ^b	0
Body weight gain of mothers from 0 to 20 GD (percentage of control)	100	87.7	75.6	40.8

*Two nonpregnant females died in the 30th and in the 32nd day of experiment, respectively.

^aMean ± SD.

^bSignificantly different ($p < 0.05$) from control value.

F, female; M, male; GD, gestation day.

Index of fertility—percentage of pregnant females in mating females group.

Index of viability—percentage of pups born alive that survived to 4 days.

Index of lactation—percentage of pups alive at 4 days that survived to 21 days.

The NOEL for female fertility is 150 mg/kg which is 12.9-fold the human dose of (b) (4) mg based on BSA. No NOAEL for pup effects can be reported (<12.9x the MDD). Given the limited endpoints, this study is technically not an adequate full assessment of early embryonic development. Given the lack of exposure data, the lack of adequate early embryonic development endpoints in these studies, and the likelihood that oral bioavailability is not 100%, a definitive SC EFD study should be completed.

In terms of labeling, for an impact on offspring, labeling (Section 8.1) could state:

Section 8.1

Decreased pup survival was noted following oral treatment of pregnant rats prior to and during gestation with NMP, an excipient in BRANDNAME ONCE MONTHLY at doses 12.9 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY. A NOAEL was not demonstrated.

Taken together, the literature reports provide an adequate assessment of the effects on male fertility for NMP and limited data on female fertility and early embryonic development. TK data are lacking. However, adverse effects in the offspring of both

treated males and females are observed at doses of NMP that have no effect on fertility index. The key results of these studies are summarized below.

Table 19. Summary Table of Effects of NMP on Male and Female Fertility and Early Embryonic Development

<i>Effect Reported</i>	<i>Species, Route</i>	<i>Treatment Period</i>	<i>Dose</i>	<i>Estimated Exposure Margin Based on BSA</i>	<i>Citation</i>	<i>Comment on Adequacy</i>
Reduced Fertility Index, Reduced pup survival	Rat, Dietary both males and females treated	Treated 10 weeks prior to mating and through gestation	500 mg/kg Reduced pup survival 160 mg/kg	43x 13.8x NOAEL 4.3x	(Exxon, 1991)	No sperm morphology to counts in males Draft unaudited report
Female Fertility						
Fertility decreased at 450 and 1000 mg/kg	Rat, oral	F: 5 days/wk 2 wks prior to mating, during mating, gestation and lactation	NOEL F fert: 150 mg/kg (12.9x)	38.7 X	(Sitarek et al., 2012)	No early embryonic development endpoints, only a fertility index determined.
~5% decrease in fetal weight in F1 offspring when dams along treated with HD of 68 mg/kg	Rat, inhalation	6 h/day, 7 d/wk M: 100 days total (from age 34 days to end of mating period) F: 134 days total (from age 34 d days to	68 mg/kg NOAEL = 30 mg/kg (2.6x)	5.9x	(Solomon et al., 1995)	No clear adverse effect on female fertility. Males and females dosed except at high dose only, so hard to interpret

		weaning with interruption between GD 20- Day 4 post-partum)				NOAEL for female fertility and early embryonic effects.
Male Fertility						
Male infertility and damage to seminiferous epithelium and tubules (at 1000 mg/kg)	Rat, oral	Treated from age 7 weeks 5 days/wk for 10 weeks and for 1 wk during mating, mated with untreated F	NOEL for M fertility: 300 mg/kg	26 X	(Sitarek and Stetkiewicz, 2008)	Endpoints are acceptable to assess male fertility
Survival rate of pups lower than controls at 300 mg/kg treated M untreated F			NOEL for effects on offspring of treated M: 100 mg/kg	8.6 X		Endpoint not examined in typical studies, but suggests male mediated adverse effects on offspring.

Embryofetal Development (EFD)

The Applicant's submitted a literature search as well as obtaining several unpublished studies to address the impact of NMP on embryofetal development. As per ICH S5(R2), embryo-fetal development studies are intended to detect adverse effects on the pregnant female and development of the embryo and fetus consequent to test article exposure of the female implantation to closure of the hard palate. The studies should characterize the toxicity profile of the test article compared to nonpregnant females as well as assess embryofetal death, altered growth and structural changes (including soft tissue and skeletal examination). The acceptable dosing regimen is to dose pregnant females from implantation to the closure of the hard palate (GD 6 to 18 for mice and rats; GD 6-20 for rabbits) and then sacrifice females one day prior to parturition (GD 18 mice; GD 20-21 rats; GD 29 rabbits). Fetuses should be examined for soft tissue and skeletal changes. At terminal examination, the following endpoints are recorded: macroscopic examination of all adults (histological evaluation), corpora lutea number,

number of live and dead implantations, individual fetal body weight, fetal abnormalities, and gross evaluation of placenta.

Embryofetal Development in Rat

Reports from the literature in rat are available for dermal, oral, and inhalation routes of administration. It has been shown that NMP causes embryo- and fetotoxicity as well as malformations. NMP also causes maternal toxicity. Based on body surface area comparisons, the data in the literature are inconsistent with regard to the dose at which various effects are observed. These inconsistencies are most likely due to differential exposure via the routes of administration tested and the lack of toxicokinetic data needed to more accurately compare exposure.

Becci et al. examined the teratogenic potential of NMP in rats following dermal administration of 75, 237, or 750 mg/kg NMP from GD 6-15 (6.5, 20.4, or 64.56x the MDD) (Becci et al., 1982). Maternal toxicity was noted at 750 mg/kg as decreased body weight gain. Reduced number of viable fetuses, increased resorptions, and skeletal abnormalities (missing sternabra extra or fused ribs, incomplete ossification of vertebra, incomplete closing of skull, fused atlas) were noted at 750 mg/kg group. There was no evidence of teratogenicity or maternal toxicity noted in the 75 or 237 mg/kg groups. The NOAEL for EFD provides a safety margin of 20.4x based on BSA.

Lee et al. treated pregnant rats via inhalation with 0.1 or 0.36 mg/L (15 or 52 mg/kg) for 6 hours per day from GD 6-15 (Lee et al., 1987). Evidence of maternal toxicity was limited to sporadic lethargy and irregular respiration. Evaluation of the fetuses at GD 15 revealed no evidence of teratogenicity or other adverse fetal effects. The HD was the NOAEL for EFD and predicts an exposure margin of 4.5x based on BSA.

Study Title: Developmental Toxicity Study in Rat with N-Methylpyrrolidone (Exxon, 1992; Project Number: 236534)

Exxon Biomedical Sciences (1992) conducted an embryofetal development study in which pregnant female rats were administered NMP via oral gavage at doses of 40, 125, and 400 mg/kg/day from Gestation Day 6 to 15. On Gestation Day 21, animals were euthanized and evaluated via standard EFD study endpoints including visceral and bone malformations of the fetuses for potential teratogenicity. There was no clear evidence of maternal toxicity at any dose although the HD group did have reduced uterine weights which indirectly impacted overall body weight gain in that group. Fetal body weights were reduced in HD animals by approximately 10-11% compared to controls. MD body weights were reduced by 3%. There was also an increase in the number of "stunted fetuses" in the HD group compared to controls and reduced ossification in the hindlimb proximal phalanges in the HD group as well. The NOAEL for maternal and fetal effects was reported to be the MD of 125 mg/kg (750 mg/m²) which is 10.8 times maximum daily dose of NMP of (b) (4) mg via the 128 mg q4w drug product. A

conservative evaluation given the slight decrease in fetal body weights in the MD would declare the LD the NOAEL which would result in a safety margin of 3.4x.

Parameter	0 mg/kg	40 mg/kg	125 mg/kg	400 mg/kg
Uterine weights		-3.9%	-1.7%	-10.9%*
BW Change GD 6-9	14 g	13 g	14.4 g	7.9 g*
BW Change GD 9-12	18.4 g	20.5 g	18.8 g	15.2 g*
Mean Fetal Body Weights: Males	5.46	5.35 (-2%)	5.32 (-2.6%)	4.90* (10%)
Mean Fetal Body Weights: Females	5.19	5.13 (-1%)	5.01* (-3.5%)	4.63* (-11%)
Stunted Fetuses	1/340 1/21 litters	1/393 1/25 litters	2/395 2/24 litters	12/397* 6/25 litters*
Sternebrae: Asymmetric	7	6	11	11
Sternebrae Unossified	0	1	3	4
Ossification Sites, Distal Phalanges	4.1	4.0	4.0	3.7*

* statistically significant

The study appears to be consistent with current protocols and was conducted after GLPs were instituted; however, although there is a GLP statement, the statement is not signed, the report is not QA'ed and is clearly marked as "Draft Not Subjected to QA Audit."

Hass et al. treated rats 0 or 165 ppm NMP via whole body inhalation on GD 4-20 (Hass et al., 1995). Increased pre-implantation loss, delayed ossification of the skeleton and decreased fetal body weight was observed in the absence of maternal toxicity. No NOAEL can be established in this study. The dose of 165 ppm (97 mg/kg) is approximately 8.4 times the human dose of (b) (4) mg NMP based on BSA. As this study did dose slightly earlier than a standard EFD study, implantation sites were assessed which could be used to inform embryonic development endpoints.

Solomon et al. evaluated embryofetal development as part of a two-generation study in rat (Solomon et al., 1995). Females were exposed to whole body inhalation of 10, 51 or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to weaning with interruption between GD 20 to Day 4 post-partum (134 days total). Males were exposed to whole body inhalation of 10, 51, or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to end of mating period (100 days total). Treated males were mated with treated females.

Gestation and viability indices were similar between control and exposed groups. No malformations or variations were attributed to exposure of NMP. The highest dose provides an exposure margin of 5.9x for the proposed human dose. The only finding was reduction of body weight of F1 offspring when both parents were exposed to 116 ppm NMP. Therefore, the NOEL for embryofetal development is the mid dose, 51 ppm (30 mg/kg), which is 2.9x the human dose of (b) (4) mg based on BSA.

Saillenfait et al. orally administered 125, 250, 500, or 750 mg/kg NMP to pregnant rats from GD 6-20 (Saillenfait et al., 2002). Maternal toxicity (decreased body weight gain and food consumption) was evident at 500 and 750 mg/kg. NMP increased resorptions at these same doses. Fetal malformations (imperforate anus, absence of tail, anasarca, malformations of the great vessels and cervical arches) were noted at 500 and 750 as well and one fetus in the 250 mg/kg group showed similar evidence of teratogenicity. Decreased fetal weights were evident at 250 mg/kg and above. The maternal NOAEL was 250 mg/kg (21.5x the MDD); however the developmental NOAEL was 125 mg/kg (10.8x the human dose of (b) (4) mg based on BSA. Given the presumed lack of 100% oral bioavailability, these margins may be lower following SC administration.

Saillenfait et al. exposed pregnant rats to NMP via whole body inhalation at 0, 30, 60 and 120 ppm (18, 35, or 71 mg/kg) 6 h/day from GD 6-20 (Saillenfait et al., 2003). Maternal toxicity was evident as transient body weight gain decreases at 60 and 120 ppm. Reduced fetal body weights were observed at 120 ppm. No effects were noted on the number of corpora lutea, implantation sites, dead or live fetuses. No NMP-related visceral or skeletal variations or malformations were observed. The NOEL for EFD effects is 60 ppm (35 mg/kg) is 3-fold the human dose of (b) (4) mg based on BSA. Transient maternal toxicity was observed at this dose as a 17% decrease in body weight GD 6-13.

Table 20. NOAEL and Adverse Effects in Published Rat EFD Studies

Study	Route	NOAEL for EFD	Exposure Margin (BSA)	Dose for AEs	Adverse Fetal Effects	Exposure Margin	Comment on Adequacy
(Becci et al., 1982)	Dermal	237 mg/kg	20.4x	750 mg/kg	Fetal lethality Increased resorptions Skeletal malformations	64.6x	No exposure data No maternal toxicity
(Lee et al., 1987)	Inhalation	52 mg/kg	4.5x		None	4.5x	No maternal toxicity No exposure data
Exxon, 1992	Oral	40 mg/kg	3.4x	125 mg/kg 400 mg/kg	Reduced ossification and Decreased Fetal body weight	10.8x	Unaudited draft report No exposure data No maternal toxicity
(Solomon et al.,	Inhalation	30 mg/kg	2.6		None	2.6x	In adequate dosing to

1995)							provide coverage for clinical
(Hass et al., 1995)	Inhalation	<97 mg/kg	<8.4x	97 mg/kg	Preimplantation loss Delayed ossification Decreased fetal body weight	8.4x	No exposure data No NOAEL
(Saillenfait et al., 2002)	Oral	125 mg/kg	10.8x	250 mg/kg	Increased resorptions Fetal malformations	21.5x	No maternal toxicity. No exposure data SM could be lower with SC
(Saillenfait et al., 2003)	Inhalation	35 mg/kg	3x		None	3x	Maternal toxicity noted at this dose, unclear significance

Collectively, the potential embryofetal effects in rat with NMP has been tested in rats via oral, dermal, and inhalation routes of administration. No studies with the SC route have been completed and exposure data are not available. The existing data suggest adverse effects on fetal development at exposures that 8.4 to 64.5 times the MDD of NMP via BRANDNAME ONCE MONTHLY. As these adverse effects appear to result in exposure margins that are smaller than the exposure margins for buprenorphine-related adverse effects as per the referenced data, the findings should be included in the product labeling. Recommended language for the label follows:

Preimplantation losses, delayed ossification and reduced fetal body weights were reported in published studies following treatment of pregnant rats during organogenesis with NMP, an excipient in BRANDNAME ONCE MONTHLY, via inhalation at 8.4 times the dose of NMP delivered by BRANDNAME ONCE MONTHLY. Reduced ossification and decreased fetal body weights at 10.8 times the MDD of NMP and fetal malformations and resorptions have also been reported following oral administration of 21.5 times the MDD of NMP delivered by BRANDNAME ONCE MONTHLY at the MDD based on a body surface area comparison.

It is not clear how the inhalation and oral routes of administration compare the proposed SC route of administration. As oral delivery is not likely 100% bioavailable and malformations may have a smaller safety margin that predicted by these data, a definitive SC EFD study in the rat should be completed. Given the indication and potential public health benefit of this drug product, the study can be completed as a Postmarketing Requirement (PMR) with the above labeling should this product be approved in this cycle.

Embryofetal Development in Rabbit

The Applicant submitted a 1991 study report by the International Research and Development Corporation¹ of a rabbit study which tested 0, 55, 175, and 540 mg/kg oral NMP daily from GD 6-18. Maternal toxicity (statistically significant decreases in body weights, reduced food consumption) was observed at 175 and 540 mg/kg. Post-implantation loss, increased incidence of cardiovascular malformations and skull malformations were observed at 540 mg/kg (93x the MDD). The NOEL for developmental toxicity in this study is 175 mg/kg which is 30-fold the human dose of (b) (4) mg based on BSA. Maternal toxicity was present at this dose. The report was a signed audited draft and did not have a signed statement of GLP compliance. The study appeared to evaluate the appropriate embryofetal development endpoints and the line listings of the data were provided. However, it is not a final study report and it is not clear how the oral route compares to the SC route. As such, the exposures margins provided are only estimates for the proposed drug product. Therefore, the study is not considered to adequately evaluate the toxicologic potential of NMP via this product in the rabbit. An embryofetal development study in the rabbit is recommended as a PMR.

In terms of the impact on labeling, suggesting language is as follows:

Post-implantation loss and increased cardiovascular and skull malformations were demonstrated in pregnant rabbits administered oral NMP, an excipient in BRANDNAME ONCE MONTHLY, at doses 93 times the human MDD of NMP via BRANDNAME ONCE MONTHLY in the absence of maternal toxicity. No adverse effects were reported at an oral dose 30 times to the MDD via BRANDNAME ONCE MONTHLY based on a body surface area comparison.

Embryofetal Development in the Mouse

Although not specifically identified or discussed by the Applicant, Becci et al cite a report from Schmidt in 1976² that apparently tested IP NMP in the mouse model (Becci et al., 1982). According to Becci:

Schmitt (1976) found that N-methylpyrrolidone caused dose-dependent embryotoxic and teratogenic effects in AJ JENA and C57BL mice when given in single or repeated i.p. doses on various days of gestation. The most pronounced embryotoxic effect of N-methylpyrrolidone was noted after a single i.p. administration of 165 mg/kg was given on the 7th day postconception. Twenty-three percent of all implanted fetuses died. The same dose level of N-methylpyrrolidone given on the 9th day caused the highest rate of fetal malformations, 18.6%.

¹ International Research and Development Corporation (IRDC). 1991. NMP (N-methyl-2-pyrrolidone): Developmental Toxicity Study in New Zealand White Rabbits.

² Schmidt, R. (1976). Tierexperimentelle Untersuchungen zur embryotoxischen und teratogenen Wirkung von N-Methyl-Pyrrolidon (NMP). Biol. Rundsch. 14:38-41.

A dose of 165 mg/kg in the mouse is approximately 7x the MDD in humans via BRANDNAME ONCE MONTHLY.

The Schmidt data were also summarized by the WHO as follows (World Health Organization, 2001):

NMP doses of 14–166 mg/kg body weight singly or repeatedly intraperitoneally administered to mice during various phases of pregnancy caused increased post-implantation loss and a reduced body weight of the fetuses. Morphological defects such as exencephaly, open eyelids, microphthalmia, cleft palate, oligodactyly, shortened or kinked tails, fusions and curvature of neck and chest vertebrae, and fusion of sternbrae and ribs were observed. The LOAEL for repeated doses was 74 mg/kg body weight administered on days 7–11 of gestation. No information on maternal toxicity is given in this study; thus, evaluation of the results is difficult (Schmidt, 1976).

The LOAEL dose of 74 mg/kg in the mouse is 3x the MDD in humans via BRANDNAME ONCE MONTHLY.

The Applicant submitted summaries of reproductive and developmental studies in mice conducted by BASF. These studies reportedly tested either 1/5th the LD₅₀ or 1/2 the LD₅₀ and employed the IP and PO route. The studies suggest significant adverse effects; however, the study summaries lacked adequate detail to inform risk and labeling for this product.

Study Title: Report on Testing of N-Methylpyrrolidone for Possible Mouse Teratogenicity (BASF 1970a; Study XIX/422)

NMP was administered to pregnant NMRI mice once daily on Gestation Day 11 to 15 at a dose that was reportedly one half the LD₅₀ via the IP and PO routes of administration. Doses were expressed as mm³/kg (= mL/kg). The high dose tested (1/2 LD₅₀ = 1525 mL/kg or 1570 mg/kg via the IP route and 2565 mL/kg or 2641 mg/kg via the PO route, were reported to cause an increase in cleft palate. At the low dose (1/5 the LD₅₀ = 610 mL/kg IP or 628 mg/kg and 1026 mL/kg PO or 1057 mg/kg), NMP was report to have no effect on resorptions, incidence of runts, or fetal weights. To covert mL/kg to mg/kg we used the density of NMP of 1.03 grams/mL. The NOAEL in this study for the IP injection was 45.5 times the human dose of (b) (4) mg NMP via this product. The conclusions of the study are limited in that the full period of organogenesis was not examined and given the sparsity of the details provided in these summary documents.

Study Title: Report on Testing of Pyrrolidone for any Teratogenic Effect in the Rat after Oral Administration (BASF 1970b; Study XIX/421)

NMP was administered NMP orally to pregnant Sprague-Dawley rat once daily on Gestation Day 6 to 15 at a dose 1700 mm³/kg (= mL/kg). This dose was presumably 1/5th the LD₅₀ in this species. The dose tested equals 1751 mg/kg. To convert mL/kg to mg/kg we used the density of NMP of 1.03 grams/mL. There was no maternal toxicity reported. There were no reported changes in percentages of resorptions compared to the control group. There were no effects on fetal weights or evidence of adverse effects on the fetus. The NOAEL in this study was 150 times the human dose of (b) (4) mg NMP via this product. The conclusions of the study are limited given the sparsity of the data presented in the summary document provided.

Peri- and Post-Natal Development (PPND)

As per ICH S5, PPND studies are intended to characterize the effects of a drug on the pregnant/lactating female and on development of the conceptus and the offspring after exposure of the female from implantation through weaning. Endpoints in the maternal animals should include clinical signs and mortalities, food intake and body weight changes, duration of pregnancy, and partition. Endpoints in the offspring include macroscopic examinations at necropsy, abnormalities, number of live/dead offspring, body weight at birth, pre- and post-weaning survival and growth, maturation and fertility of the offspring, physical development, sensory function and reflexes, and behavior. Specific functional testing methods is not outlined by ICH S5; however, studies that assess sensory function, motor activity, and learning and memory are recommended.

Hass et al. treated pregnant rats via whole body inhalation to 0 or 150 ppm (88 mg/kg = 7.6x the MDD of NMP via BRANDNAME ONCE MONTHLY) NMP for 6 h/day on GD 7-20 (Hass et al., 1994). The authors state that this dose level was chosen so as not to cause maternal toxicity or decrease viability of the offspring. Although the study did not include exposures during lactation, the offspring were allowed to be born and were evaluated via functional assessments, similar to a PPND study. Exposed offspring showed decreases in body weight and had delayed physical development. Performance in tasks including the reversal procedure (retesting with a new platform location) in the Morris Water Maze and operant delayed spatial alteration (Skinner boxes) was impaired in exposed offspring. No differences between NMP-exposed animals and control animals were seen in motor function (rotarod), activity level (open field) and learning tasks of low complexity. No NOAEL can be established for PPD parameters and the exposure at the dose employed (88 mg/kg) is approximately 7.6 times the dose of (b) (4) mg NMP in human based on BSA).

Sitarek et al. treated female rats with oral NMP 150, 450, or 1000 mg/kg for 5 days/week two weeks before mating, during mating, and through gestation and lactation (Sitarek et al., 2012). The authors considered the 150 mg/kg dose "slightly toxic" (body weight changes) to the dams. The incidence of stillbirths was increased at 1000 mg/kg. At 150 mg/kg there was no effect on fertility but pup survival was decreased. As previously noted in the fertility section of this appendix, both the index of viability (% of pups born alive that survived to 4 days) and index of lactation (% of pups alive at 4 days

that survived to 21 days) were significantly lower than control for all groups, as illustrated in the table below again reproduced from the publication.

Effect of *N*-Methyl-2-Pyrrolidone on the Reproductive Performance of Female Rats and Viability of Their Progeny

	Daily dose of NMP (mg/kg)			
	0	150	450	1000
Number of				
Mating females with males	24	26	28	22
Pregnant females	22	24	20	15
Died females*	0	0	0	2
Number of				
Live pups per litter	11.5 ± 3.5 ^a	10.4 ± 2.6	10.5 ± 3.4	0.33 ± 0.82 ^b
Dead pups per litter	0.18 ± 0.85	0	0.13 ± 0.34	0.80 ± 1.1 ^b
Sex ratio (F : M)	132 : 125	112:137	105:107	5:3
Indices				
Fertility	91.7	92.3	71.4 ^b	68.2 ^b
Viability	94.0	86.4 ^b	71.6 ^b	0
Lactation	96.1	78.2 ^b	43.4 ^b	0
Body weight gain of mothers from 0 to 20 GD (percentage of control)	100	87.7	75.6	40.8

*Two nonpregnant females died in the 30th and in the 32nd day of experiment, respectively.

^aMean ± SD.

^bSignificantly different ($p < 0.05$) from control value.

F, female; M, male; GD, gestation day.

Index of fertility—percentage of pregnant females in mating females group.

Index of viability—percentage of pups born alive that survived to 4 days.

Index of lactation—percentage of pups alive at 4 days that survived to 21 days.

In terms of typical PPND endpoints the offspring were only evaluated for general appearance, litter weight, mean pup weight, and mortality from birth to PND 21. As such, there are limited PPND endpoints in this report. No NOAEL was established for this study for these PPD endpoints. The low dose of 150 mg/kg is 12.9-fold the human dose of (b) (4) mg based on BSA.

In terms of labeling, based on these two published studies, the following can be included in Section 8.1:

Decreased pup survival was noted following oral treatment of pregnant rats prior to and during gestation and lactation with NMP, an excipient in BRANDNAME ONCE MONTHLY at doses 12.9 times the MDD. Developmental delays and impaired cognitive function were reported in pups born to pregnant rats treated with NMP via inhalation during gestation at doses 7.6 times the MDD of NMP via BRANDNAME ONCE MONTHLY based on a body surface area comparison.

The reports reviewed in the literature do not provide adequate exposure margins or complete methodology to accurately assess effects of NMP on PPD endpoints. Therefore a definitive PPND study the rat should be completed. Given the indication and potential public health benefit of this drug product, the study can be completed as a Postmarketing Requirement (PMR) with the above labeling if the application can be approved this cycle.

Overall Reproduction and Developmental Conclusions

The published literature on NMP clearly suggest the potential for adverse effects on development at doses that are typically about 7 times higher than the levels that would occur via this drug product. However, the studies are limited as they employ different routes of administration which likely have different bioavailabilities. Finally, as the exposure data do not exist for these other routes of administration and SC could result in increased exposures compared to oral studies, definitive studies for NMP should be completed via the SC route as post marketing requirements should this application be approved this cycle.

GENETIC TOXICOLOGY

The Applicant provided a literature review of published genetic toxicology studies, as summarized their table below.

Genotoxicity Assays with N-Methyl Pyrrolidone			
Test method	Test Organism/Species	Results	References
Salmonella microsome assay	TA97, TA98, TA100, TA2638 ^a , UTH8413, UTH8414	negative	Wells <i>et al.</i> 1988
	TA102, TA104 ^a	equivocal*	
	TA98, TA104 ^b	negative	
Ames Assay	TA1535, TA1537, TA 1538, TA98, TA100	Negative	(b) (4)
Aneuploidy induction	<i>Saccharomyces cerevisiae</i> Strain D61.M	positive	Mayer and Goin 1988
	<i>S. Cerevisiae</i> Strain D61.M	positive	
	<i>S. Cerevisiae</i> Strain D61.M	positive	Zimmermann <i>et al.</i> 1988
Bone marrow chromosome analysis	Chinese hamsters	negative	Engelhardt and Fleig 1993
CHO/HGPRT forward mutation assay	Chinese hamsters	negative	Hazelton Laboratories 1988a
Unscheduled DNA synthesis	Rat hepatocytes	negative	Hazelton Laboratories 1988b
Micronucleus test	NMRI mice	negative	Engelhardt and Fleig 1993

* The authors indicated that the increase was less than 2-fold over background without a clear linear dose-response relationship. No increases were observed in 6 other strains of *Salmonella typhimurium*.

a - Standard plate incorporation assay

b - Preincubation assay

NMP was tested in the Ames Assay using strains TA1535, TA1537, TA1538, TA98, TA100, TA102, and TA104 in the presence and absence of metabolic activation (Mortelmans *et al.*, 1986, Wells *et al.*, 1988). In strains TA102 and TA104, small increases in revertants above control were observed. However, the increases were less than two-fold above control values and therefore do not meet the criteria for a positive response per the OECD protocol. NMP was negative in the Ames Assay in all other strains tested.

NMP was found to be negative in the mouse micronucleus assay and Chinese hamster bone marrow assay (Engelhardt and Fleig, 1993).

In studies conducted by Hazelton Laboratories, NMP did not induce forward mutations *in vitro* in a CHO/HGPRT assay or in an unscheduled DNA synthesis (UDS) assay in rat primary hepatocyte cultures (HLA, 1988a; HLA, 1988b) at the limit dose of 5000 mcg/mL. In the CHO/HGPRT assay, NMP did not induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovaries (CHO) with and without metabolic activation. Of the six doses tested, only one with metabolic activation reached statistical significance for mutation but was within the background level for mutation. In the UDS assay, no significant increases in UDS were observed in hepatocytes from Fischer 344 rats. The UDS and CHO/GRPT studies were conducted according to OECD guidelines and by GLP. NMP was also negative in the Ames assay (b) (4)

NMP was found to induce aneuploidy in yeast (Mayer et al., 1988, Zimmermann et al., 1988). As discussed in Engelhardt and Fleig (1993), a cold shock treatment condition that destroys the spindles and has questionable relevance in intact mammals was used in these studies. Further work by Basler showed that various organic solvents that were known to be potent inducers of aneuploidy in yeast did not increase the frequency of micronucleated polychromatic erythrocytes in bone marrow cells of hamsters (Basler, 1986). Further, the weight of evidence suggests that the genotoxic potential for NMP is minimal.

CARCINOGENICITY

Two studies in rats and one in mice evaluating the carcinogenicity of NMP were identified in the literature.

Lee and colleagues exposed rats to 0, 0.04 or 0.4 mg/L NMP for 6 h/day 5 days/week via whole body inhalation for two years (Lee et al., 1987). No treatment-related tumors were identified in either males or females. The NOEL for carcinogenic effects of NMP is the high dose, 0.4 mg/L (58 mg/kg/day) yields and exposure margin of 5-fold the human dose of (b) (4) mg based on BSA.

Malley et al. assessed the carcinogenic effect of NMP in rats and mice (Malley et al., 2001). The administration of NMP was in the diet and food consumption was measured. Although not a GLP study, an adequate assessment of organs and tissues were conducted and it appears that methods are acceptable. In rats, NMP was administered in the diet at 0, 1600, 5000, or 15000 ppm for approximately two years. No carcinogenic effects of NMP were observed at any dose. The mean daily consumption at 15000 ppm, the NOEL for oncogenic effects was 678 mg/kg in males and 939 mg/kg in females. Therefore, the exposure margins at the NOEL for oncogenic effects is 58-fold and 81-fold in males and females, respectively, as compared to the human dose of (b) (4) mg based on BSA.

In mice, NMP was administered in the diet at 0, 600, 1200, or 7500 ppm for approximately 18 months. Increases in hepatocellular adenoma and carcinoma were observed in both males and females at the highest dose tested. In females, the hepatocellular carcinoma was within the historical control range. The mean daily consumption of NMP at 1200 ppm, the NOEL for oncogenic effects was 173 mg/kg in males and 221 mg/kg in females (Malley et al., 2001). Therefore, the exposure margins at the NOEL for oncogenic effects is 7.4-fold and 9.5-fold in males and females, respectively. The dose at which a statistically significant increase in hepatocellular adenomas and carcinomas were noted (7200 ppm) was estimated to result in daily exposures of 1089 mg/kg/day in male mice and 1399 mg/kg/day in female mice. Based on a body surface area basis, these doses were ~47 and 60 times the maximum human daily dose via the drug product.

Table 21. Summary of Neoplastic Lesions in Male and Female Mice

Lesion	Concentration (ppm)			
	0	600	1200	7200
Male mice				
Hepatocellular adenoma	5/50	2/50	4/50	12/50*
Hepatocellular carcinoma	4/50	1/50	3/50	13/50*
Pulmonary adenoma	1/50	2/50	3/50	5/50
Pulmonary adenocarcinoma	1/50	4/50	6/50	2/50
Total animals with benign tumors	15/50	5/50	8/50	18/50
Total animals with malignant tumors	9/50	7/50	11/50	14/50
Female mice				
Hepatocellular adenoma	2/50	2/50	1/50	7/50*
Hepatocellular carcinoma	0/50	0/50	0/50	3/50*
Total animals with benign tumors	10/50	6/50	5/50	12/50
Total animals with malignant tumors	12/50	5/50	9/50	10/50

*Statistically significant differences at $p < 0.05$ by the Cochran Armitage test for trend.

Table 22. Summary of NMP-related Non-neoplastic Lesions in Male and Female Mice

Lesion	Concentrations (ppm)			
	0	600	1200	7200
Male mice				
Hepatocellular clear cell foci	0/50	1/50	1/50	6/50*
Hepatocellular basophilic foci	4/50	2/50	3/50	5/50
Hepatocellular eosinophilic foci	1/50	2/50	2/50	19/50*
Hepatocellular centrilobular hypertrophy	0/50	0/50	3/50	43/50*
Cellular alterations in liver	5/50	5/50	6/50	25/50*
Female mice				
Hepatocellular clear cell foci	0/50	0/50	0/50	1/50
Hepatocellular basophilic foci	2/50	2/50	1/50	10/50*
Hepatocellular eosinophilic foci	1/50	0/50	0/50	6/50*
Hepatocellular centrilobular hypertrophy	0/50	1/50	0/50	0/50
Cellular alterations in liver	3/50	2/50	1/50	17/50*

*Statistically significant by Cochran Armitage test for trend, $p < 0.05$.

According to Malley et al., Van Esch and Kroes included an NMP solvent control arm in a toxicology studies in Swiss mice (Van Esch and Kroes, 1972). Mice presumably received 25 mg (~25 g body weight = 1000 mg/kg or 3000 mg/m²) NMP a total of 9 times over the 18-month assay (Day 14, Month 1.5, 3, 4.5, 6, 10, 14, and 17). No increased tumors were noted; however, a saline/water control arm was also not included in this study. One incidence of hepatocellular carcinoma was noted in the control group and one in one of the test arms. Although of limited utility given the study limitations, the dose tested was approximately 43 times the MDD and the dosing interval was punctuated, similar to that which will occur via the proposed dosing regimen.

The Applicant did not provide a mode of action (MOA) assessment to address the hepatic tumors in the mice. Although rodent liver tumors are frequently not believed to be relevant to humans, a MOA assessment is generally completed to put these findings into perspective (Holsapple et al., 2006). For example, a MOA assessment for phenobarbital-induced liver tumors in mice and rats has been undertaken and the data suggest that the increased cellular proliferation, decreased apoptosis, formation of altered hepatic foci, and ultimate liver tumors in rodents did not appear to translate to human risk (Elcombe et al., 2014). This conclusion was primarily driven by data that demonstrated that, unlike rodent hepatocytes, human hepatocytes did not proliferate in vitro in response to phenobarbital. That coupled with a lack of an epidemiological signal for increased tumors following extensive human exposure to phenobarbital support the conclusion that the risk of liver tumors in humans appear to be low for phenobarbital.

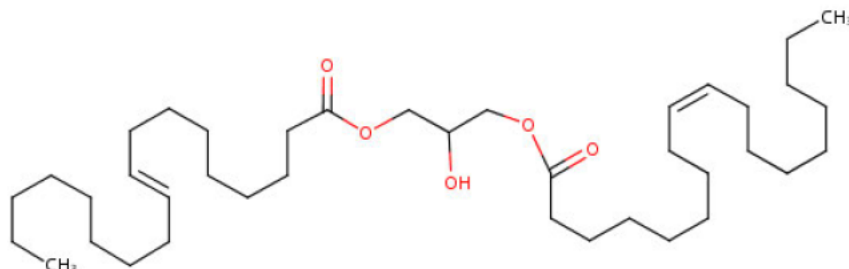
Like phenobarbital, repeat-dose toxicology studies with NMP demonstrate increased hepatocellular hypertrophy, suggesting that these liver tumors in mice may not correlate with increased human risk. However, as a similar MOA assessment has not been undertaken for NMP, we recommend that the product labeling include the following:

NMP, an excipient in BRANDNAME ONCE MONTHLY, produced an increase in hepatocellular adenomas and carcinomas in male and female mice at 47 and 60 times the maximum daily dose (MDD) of NMP via BRANDNAME ONCE MONTHLY. No tumors were noted at 7.4 and 9.5 times the MDD. The clinical significance of these findings is unclear. In 2-year inhalation and dietary studies in rats, NMP did not result in evidence of carcinogenicity.

Further, as this risk may not be relevant and could impact prescription practices, we recommend that a post-marketing requirement be instituted to conduct a MOA for NMP induced liver tumors, which could support removal of the above labeling statement.

Appendix 2: Toxicological Risk Assessment for Glycerol Dioleate (GDO) Glycerol Dioleate (GDO; CAS 25637-84-7):

Glycerol dioleate (aka Diolein, or glyceryl dioleate) is a diglyceride with two oleic acid molecules attached to glycerol in the 1,3 positions via an ester linkage.



GDO is included in both the CAM2038 q1w and q4w formulations. The highest proposed level is (b) (4) mg/32 mg dose via the weekly dosed product. The proposed concentration is (b) (4) mg/mL or (b) (4) % w/v. GDO is not listed in the FDA IID and is therefore considered a novel excipient that must be qualified for safety per the guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*. Diglycerides, like GDO, are endogenously formed via the hydrolysis of triglycerides. Diglycerides are metabolized by lipases to the free fatty acids and glycerol. The fatty acids are further metabolized via beta-oxidation or utilized as substrates for cellular metabolism.

PK studies in animals have demonstrated that after SC administration of CAM2038 vehicle that included (b) (4) % by weight GDO, levels of GDO were within the endogenous levels in rats. Metabolite levels were not examined.

The toxicological safety data submitted by the Applicant for GDO is comprised of the following studies:

Study Type and Duration	Route of Administration	Species	Test Article	GLP Compliance
Glycerol dioleate (GDO) (excipient)				
Safety Pharmacology				
CNS/FOB endpoints (within the 26-week study)	SC	Rat	GDO	GLP
Pharmacokinetic and Toxicology				
Single dose PK	SC	Rat	GDO	Non-GLP
Repeat dose TK (within the PPND study)	SC	Rat	GDO	Non-GLP (Bioanalysis only)
Single dose (acute) toxicity, including local tolerance 8 days 6 weeks	SC	Rat	GDO	GLP
Repeat dose toxicity, including local tolerance 28 days 26 weeks	SC	Rat	GDO	GLP
Genotoxicity in vitro bacterial reverse mutation in vitro mouse lymphoma in vivo micronucleus	In vitro In vitro In vivo	S. typhimurium Mouse lymphoma L5178Y Rat	GDO	GLP
Reproduction and Developmental Toxicity	PPND study for GDO. Reference to literature data for Diglyceride oil (DAG) for other reproduction and developmental endpoints			
Pre- and postnatal development, including TK	SC	Rat	GDO	GLP
Carcinogenicity	No studies – Reference to literature data for Diglyceride oil (DAG)			

BPN – buprenorphine; CNS – central nervous system; CVS – cardiovascular system; FC – FluidCrystal (placebo); CHO – Chinese hamster ovary; GDO – diacyl glycerol; GLP – Good Laboratory Practice; HEK293 cells – human embryonic kidney 293 cells; hERG - human ether-a-go-go related gene; IM – intramuscular; NBPN – norbuprenorphine; NTX – naltrexone; PK – pharmacokinetic; PPND – pre- and post-natal development; SC – subcutaneous; TK – toxicokinetic

As noted in the table above, the Applicant also references published studies with diacyl glycerol oils (DAG) as a surrogate molecule for GDO. According to the Applicant, DAG oils are largely GDO but also contain linoleic acids, and other fatty acids attached to glycerol. The composition of the DAG oils in the referenced articles summarized below are depicted in the Applicant's table below:

Figure 4. Comparison of Composition of GDO and DAG oil used in Studies Referenced by the Applicant

Study reference (see also Appendix I)	TO-10-405 / TO-12-446	TO-05-153 / TO-05-189 / TO-04-141 / TO-04-142 / TO-05-156 / TO-05-177	TO-04-146	Kasamatsu et al., 2005 (before heating / after heating)	Chengelis et al., 2006a / Chengelis et al., 2006b / Chengelis et al., 2006c	Morita et al., 2008b / Morita et al., 2008c	Soni et al., 2001	Morita et al., 2008a
Acylglycerol distribution (%)								
Monoacyl glycerol	(b) (4)			0.5 / 2.7	0.9-2.9	2.2	5 (indicated in text)	No detailed composition provided in the study.
Diacyl glycerol				85.5 / 80.3	82.9-83.9	86.1	75 (indicated in text)	
Triacyl glycerol				14.0 / 17.1	12.8-15.2	9.9	20 (indicated in text)	
Fatty acid composition (%)								
C16:0 (palmitic)				3.1 / 3.2	2.9-3.0	3.1	No detailed composition provided for the indicated study. The DAG oil prepared from high C18:1, C18:2 and C18:3 content raw materials.	No detailed composition provided for the indicated study. The DAG oil prepared from high C18:1, C18:2 and C18:3 content raw materials (soybean and rapeseed oil).
C18:0 (stearic)				1.3 / 1.3	1.1-1.2	1.1		
C18:1 (oleic)				41.1 / 42.3	35.6-37.6	39.5		
C18:2 (linoleic)				45.6 / 44.8	47.7-48.6	46.4		
C18:3 (linolenic)				8.7 / 8.1	6.3-6.5	6.2		
C20:0 (arachidic)				0.3 / 0.3	-	-		

SINGLE-DOSE PK

Study Title: A single dose study in rat evaluating circulating plasma glycerol dioleate levels following s.c. administration of FluidCrystal® Injection depot placebo formulation or glycerol dioleate. Study PK-15-539 (dated 12/21/2015)

In this non-GLP study, plasma levels of GDO were measured in rats following either a SC injection of GDO or of the proposed FluidCrystal (FC) delivery system containing GDO. Rats were treated with either 940 mg/kg of GDO or 432 mg/kg of FC placebo via SC injection. Plasma samples were collected at 6 hours, 2, 14, 42, 56, 63, and 72 days. A total of 10 rats per group were employed (three groups: Saline, GDO, or FC).

Maximum Plasma Concentrations of GDO (mcg/mL) for each dose group are below:

Treatment Group	Saline	GDO	FC
Highest Mean	3.5	3.8	3.4
Highest Maximum	5.0	6.9	4.7

The highest levels in the table above were based on any time points examined. The levels of GDO were slightly higher (38%) compared to saline; however, the levels via the FC vehicle dose were not different than controls.

The mean results are also plotted below, reproduced from the study report:

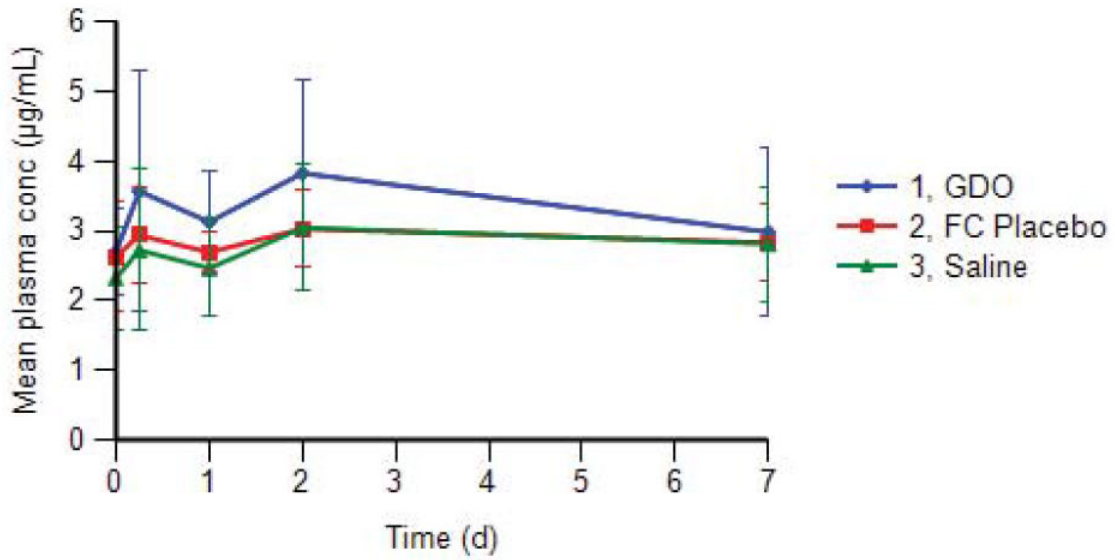


Figure 7. Group mean plasma concentrations of GDO; detailed on day 0-7 (linear scale)

Change from baseline are also depicted.

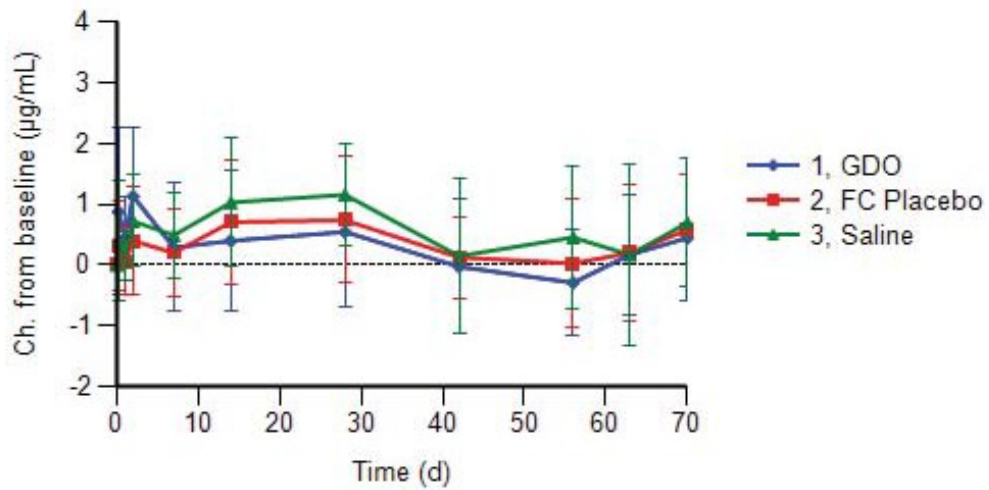


Figure 11. Group mean plasma concentrations of GDO, as absolute change from baseline; day 0-70 (linear scale)

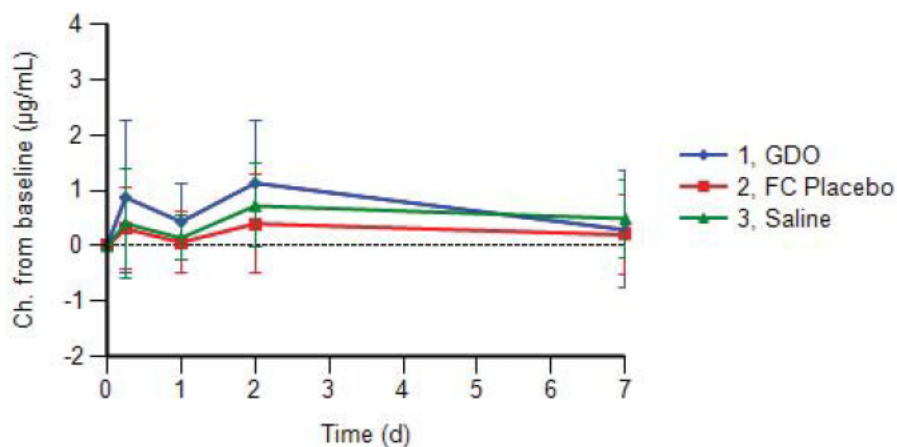


Figure 12. Group mean plasma concentrations of GDO, as absolute change from baseline; detailed on day 0-7 (linear scale)

In addition, the local tissue effect was evaluated via histopathology at Day 71. The report notes that for both animals dosed with GDO and FC, some remains of the test article were still observed at the injection site even 71 days after injection.

REPEAT-DOSE TOXICOLOGY

The Applicant conducted a 28-day and 26-week repeat-dose toxicology study for GDO. In addition, they have conducted a 9-month repeat-dose toxicology study with the drug product containing GDO (reviewed in the body of this review) to address the requirement for two species toxicity data for a novel excipient.

The review of the 26-week repeat-dose toxicology study with GDO and the drug product vehicle below was completed by Dr. Gary Bond.

Study title: A 26 Week Toxicity Study of Glycerol Dioleate and FluidCrystal® Vehicle by Subcutaneous Injection in Rats

Study no.:	TO-10-405 (Sponsor); 519932 (b) (4)
Study report location:	EDR (b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 12, 2011
GLP compliance:	Yes
QA statement:	Yes, signed

Drug, lot #, and % purity: Glycerol dioleate (GDO): sterile, Batch 3002413 (supplied packaged), purity NR (requested during the IND). The Applicant amended the report to include a CoA of the product, however, purity information was not provided.

2022IAS-I (CAM2038 q1w vehicle: SPC (b) (4)%, GDO (b) (4)%, EtOH 10%, (b) (4) (b) (4)%): sterile, Batch 3002396, purity NR (requested during IND). The Applicant amended the report to include a CoA of the product, however, purity information was not provided.

0.9% sodium chloride (negative control), Batch (10L09G61 and 11C09G61), purity NR

Key Study Findings

- The objective of this study was to determine the potential toxicity of glycerol dioleate (GDO) when given by subcutaneous injection (SC) once daily for 26 weeks to rats and to provide data to support the use of GDO in humans. The secondary objective was to determine the potential toxicity of a FluidCrystal® vehicle (FCV; Code Name 2022IAS-I), specifically the EtOH-based q1w FCV, when given by subcutaneous injections once weekly for 26 weeks to rats and to provide data to support its use in humans.

Group Number	Number of Animals		Test Item	Dose Level (mg/kg)	Dosage Volume (mL/kg)	Dosing Frequency
	Males	Females				
1	15	15	Control (0.9% sodium chloride w/v)	0	1.06	Daily
2	15	15	Glycerol dioleate	100	0.11	Daily
3	15	15	Glycerol dioleate	350	0.37	Daily
4	15	15	Glycerol dioleate	1000	1.06	Daily
5	15	15	2022IAS-I	1000	1.06	Weekly

- Dosing was rotated among 6 sites on the back resulting in ~30 injections per site. For the q1w FCV, both the incidence and severity of cystic granulomatous inflammation were lower at Site 3 (42 days after the last injection) compared with Site 2 (7 days after the last injection), suggestive of recovery.
- The subcutaneous administration to rats during 26 weeks of up to the limit dose

of 1000 mg/kg/day GDO was associated with no evidence of systemic effects, indicating the low order of systemic toxicity of GDO. In addition, subcutaneous administration of the dose 1000 mg/kg/week of the FluidCrystal® vehicle (FCV) formulation was not associated with any indication of systemic toxicity indicating low order of systemic toxicity for the FCV.

- Treatment-associated findings for GDO as well as q1w FCV directly attributed to the test items were localized and confined to the subcutaneous injection sites. There was no evidence of similar such reactions in any other tissue. The inflammation of the injection sites, for both GDO and the q1w FCV, had features of the not unexpected typical foreign body reaction. Injection site findings for GDO were primarily subcutaneous cystic granulomatous inflammation with sporadic mononuclear cell inflammation at all dose levels. The primary finding was generally dose-related and reached a severity usually no higher than marked (1 instance of severe in 1 high dose GDO female). Additional but less frequent GDO observations were mixed inflammation at 1000 mg/kg/day, fibrosis/fibroplasia, and granulation tissue that were confined to 350 or 1000 mg/kg/day. Additional, less frequent observations associated with q1w FCV were pigmented/foamy macrophages, mixed/mononuclear cell inflammation, fibrosis/fibroplasias, and granulation tissue.
- Also not unexpected and secondary to the inflammation at the injection sites was an inflammatory response were effects on hematology (increased white blood cells and fibrinogen).
- The NOAEL for GDO systemic toxicity is the highest dose tested of 1000 mg/kg/day (6000 mg/m²). This dose is (b) (4) times the single maximum dose of (b) (4) mg/kg/day via the q1w product. An acceptable LOAEL for GDO local toxicity is the low dose of (b) (4) mg/kg/day based on cystic granulomatous inflammation that appeared reversible with longer recovery periods.
- In summary, the adverse local effects noted in this study are anticipated findings following repeated subcutaneous injections of an insoluble material. There was no evidence of systemic toxicity.

Methods

Doses:

Group Number	Number of Animals		Test Item	Dose Level (mg/kg)	Dosage Volume (mL/kg)	Dosing Frequency
	Males	Females				
1	15	15	Control (0.9% sodium chloride w/v)	0	1.06	Daily
2	15	15	Glycerol dioleate	100	0.11	Daily
3	15	15	Glycerol dioleate	350	0.37	Daily
4	15	15	Glycerol dioleate	1000	1.06	Daily
5	15	15	2022IAS-I	1000	1.06	Weekly

Frequency of dosing: GDO – daily; 202IAS-I - q1w FluidCrystal vehicle (FCV)

Route of administration: Subcutaneous (SC)

Dose volume: See table

Formulation/Vehicle: FVC (ethanol-based q1w placebo)

Species/Strain: Rat/Han Wistar (CrL:WI Han)

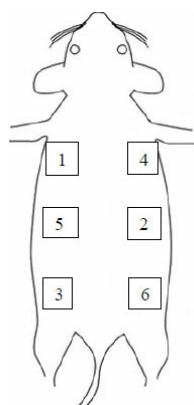
Number/Sex/Group: 15

Age: 6 weeks at shipping

Weight: 144-183 g (males) and 100-149 g (females)

Satellite groups: none

Unique study design: Dosing - The test and control items were administered by subcutaneous injection at volumes and frequencies indicated in the table. Six injection sites on the back of the rats (as defined in the diagram below) were used and the use of these sites were rotated in ascending numerical order throughout the study. For the controls and animals receiving GDO (Groups 1-4), each injection site typically received 30 injections (29-32 based on rotation of sites). For the animals receiving FCV (Group 5), Dose Sites 1 and 2 received 5 injections and the remaining dose sites received 4 injections.



Dose Site 1: Left shoulder
Dose Site 2: Right flank
Dose Site 3: Left rump
Dose Site 4: Right shoulder
Dose Site 5: Left flank
Dose Site 6: Right rump

Deviation from study protocol: Nothing considered significant to outcome of study. Calcium, phosphorus, sodium, potassium, chloride and creatinine were not measured in urine, which

was a deviation from the protocol (see results).

Observations and Results

Mortality

All animals were checked for viability early morning and again as late as possible on each day.

There were six unscheduled deaths, although there were none later than Week 16. Four of these were sacrificed as moribund, due to treatment-related skin lesions associated with the injection sites (e.g., ulcerative dermatitis). The remaining two animals were found dead (one control animal related to the anesthesia necessary to obtain blood samples on Week 26 and one HD GDO animal related to nephroblastoma). The early sacrifices were related to dermal inflammatory reactions, unrelated to the SC foreign body response and considered relating to mechanical dosing and possible self-trauma/scratching. Starting at Week 20, any animal that was noted with such dermal lesions was single-housed until the lesion was considered sufficiently healed and the animal, where possible, was returned to its original cage without incident.

Unscheduled deaths are considered treatment-related and occurred only in HD GDO (1 animal) and FCV (3 animals) groups which were injected with the highest dose volume (1.06 mL/kg).

Clinical Signs

All animals received a detailed clinical examination weekly. All animals receiving daily injections were examined for reaction to treatment at frequent daily intervals. The onset, intensity and duration of any signs were recorded, starting immediately post dose and at least 1 h post dose. All animals receiving FCV were, up until Day 134 (Week 20), examined for reaction to treatment on the day of the weekly dosing and the following day only (i.e., 2 days per week). From Day 135 (Week 20), these animals were examined twice daily. From Week 20 onwards, any animal that was noted with a 'wet' lesion of the injection site was single housed until the lesion was considered sufficiently healed and the animal, where possible, could be returned to its original multi-animal cage. This was initiated, following the unscheduled deaths of 4 animals on the study, as a measure to improve the potential for recovery from the lesions and to lessen the likelihood of additional unscheduled deaths.

Observations were observed in cage (alertness), outside the cage in a standardized arena (e.g., alertness, diarrhea, tremors, respiration, aggression, other), during handling (e.g., body temperature, pupils, salivation, tail flick pain response, other).

No apparent systemic toxicity was observed in any group administered either test item (GDO or FCV).

Injection Site observations

Sites of control and GDO animals were examined for reaction to treatment at least once daily. Animals receiving FCV, up until Day 134 (Week 20), were examined at least once daily on the day of the weekly dosing and the following day only. From Day 135 (Week 20), these animals were examined for reaction to treatment at least twice daily.

Adverse signs of treatment were observed locally at the injection sites and included swellings, staining, and scabbing. Not unexpected staining of fur on the dorsal surface or neck was noted in any group throughout the dosing period. Sparse hair or baldness was observed in a dose volume-related manner with most high-volume animals (control and HD GDO) being effected and few LD GDO being effected (lowest dose volume). Greasy hair was also observed in GDO-treated groups. Greasy coats were also observed in HD GDO animals assumed related to the volume of GDO-based treatment.

Swellings (soft or firm) were noted at injection sites for MD and HD GDO groups and for the FCV group. Swellings were dose-dependent with respect to the number of animals affected, the number of dose sites with swellings, and size of the swellings increasing with dosage with essentially no swelling observed in LD GDO and control animals. Swellings were generally observed at earlier time points with increasing dosage. The swellings were consistent with the presence of the GDO at the injection sites, since GDO is viscous, insoluble oil that will slowly be cleared from the injection site. Swelling was also observed in some FCV animals. Lesions described as dry, wet, or scabbed were observed in some animals and were apparently related to scratching after dosing.

Body Weights

For controls and GDO animals, body weights were recorded once during the pre-dosing period and daily during the dosing period and before necropsy. For FCV animals, body weights were recorded weekly and at necropsy.

Body weight and body weight gains were not affected by treatment in any groups.

Food Consumption

Food consumption was measured once during the pre-dosing period and weekly thereafter.

Food consumption was not affected by treatment in any groups.

Ophthalmoscopy

An ophthalmic examination was performed on all animals pretrial and on all control and high dose (GDO and FVC) animals (GDO and FVC) during Weeks 13 and 26.

No ophthalmic effects were affected by treatment in any groups.

Modified Irwin Test

The examinations were conducted on 10 animals/sex/group for control and GDO dose groups (not FCV animals) 1 hour post dose on Day 1 and 1 day during Week 13 and Week 26. Evaluations included pain response, body temperature, and locomotor activity.

There were no behavioral or functional differences noted between groups for the Modified Irwin test.

ECG – not evaluated

Clinical Pathology Examinations

Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Haematology	Coagulation	Clinical Chemistry	Urinalysis
1-5	Week 13	X	X	X	X
1-5	Week 26	X	X	X	X

X = Sample collected

Hematology

Haematology Parameters

Red blood cell count Haemoglobin Haematocrit Mean cell volume Mean cell haemoglobin concentration Mean cell haemoglobin Reticulocytes Reticulocyte count (absolute) Red blood cell distribution width	Platelets Blood Smear ^a White blood cell count Neutrophils Lymphocytes Monocytes Eosinophils Basophils Large unstained cells

^a A blood smear was prepared from each hematology specimen and labelled, stained, stored and archived. The blood smears were not evaluated as it was considered that examination would not yield any further information.

Statistically significant (SS) increases in the WBC population were observed after treatment with GDO at 1000 mg/kg at Weeks 13 and 26. WBC counts in females and the mean number of specific WBC cell populations in male and female animals receiving 1000 mg/kg/day when compared with controls. These indices were also increased in the FCV groups but statistical analysis was not conducted compared to controls.

GDO treatment - At 13 weeks in HD GDO males, neutrophils and monocytes were statistically significantly (SS) increased 54 and 89%, respectively. Lesser but not SS increases were observed at 100 mg/kg (19% and 32%, respectively) and 350 mg/kg (22 and 33%, respectively). At 26 weeks in HD GDO males, neutrophils and monocytes were increased 122 and 78%, respectively. Lesser but not SS increases were observed

at 100 mg/kg (12 and 21%, respectively) and 350 mg/kg (30 and 36%, respectively). As will be discussed regarding these WBC/WBC cell population changes for GDO and FCV treated groups, such changes are consistent with the inflammation observed because of the SC dosing and resultant foreign body reaction.

Differences in White Blood Cells: Males

Group/Dosage (mg/kg/day)	Summary Information	Week 13		Week 26	
		Neut $\times 10^9/L$	Mono $\times 10^9/L$	Neut $\times 10^9/L$	Mono $\times 10^9/L$
1 (0)	Mean	0.78	0.09	0.76	0.14
	SD	0.24	0.03	0.19	0.05
	N	15	15	15	15
4 (1000)	Mean	1.20	0.17	1.69	0.25
	SD	0.35	0.07	1.06	0.12
	N	15	15	12	12
	P value	< 0.01	<0.001	<0.001	<0.001

At 13 weeks in HD GDO females, WBCs, neutrophils, lymphocytes, and monocytes were SS increased 51, 73, 44, and 140%, respectively. Lesser but not SS increases except for monocytes at 350 mg/kg, were observed at 100 mg/kg (8, 9, 1, and 25%, respectively) and 350 mg/kg (20, 17, 21, and 38%, respectively). At 26 weeks in HD GDO females, WBCs, neutrophils, lymphocytes, and monocytes were SS increased 57, 150, 33, and 160%, respectively. Lesser but not SS increases were observed at 100 mg/kg (8, 24, 4 and 43%, respectively) and 350 mg/kg (13, 38, 11 and 29%, respectively). As will be discussed regarding these WBC/WBC cell population changes for GDO and FCV treated groups, such changes are consistent with the inflammation observed because of the SC dosing and resultant foreign body reaction.

Differences in White Blood Cells: Females

Group/Dosage (mg/kg/day)	Summary Information	Week 13				Week 26			
		WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$	WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$
1 (0)	Mean	3.92	0.64	3.09	0.08	2.91	0.50	2.25	0.07
	SD	1.00	0.27	1.04	0.05	0.53	0.29	0.60	0.03
	N	14	14	14	14	14	14	14	14
4 (1000)	Mean	5.91	1.11	4.44	0.19	4.56	1.25	2.99	0.18
	SD	1.72	0.69	1.09	0.09	0.95	0.43	0.69	0.07
	N	13	13	13	13	13	13	13	13
	P value	<0.001	< 0.01	< 0.01	<0.001	<0.001	<0.001	< 0.01	<0.001

FVC treatment - For FCV males, there were increases in the WBC counts and number of specific WBC cell populations at 13 and 26 weeks compared to control. Most notable were a 206 and 161% increase in neutrophils at 13 and 26 weeks, respectively, and a 167 and 64% increase in neutrophils at 13 and 26 weeks, respectively (see table for all changes). These results are consistent with the observed inflammation at the injection site.

Differences in White Blood Cells: Males

Group/Dosage (mg/kg)	Summary Information	Week 13				Week 26			
		WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$	WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$
1 (0/day)	Mean	5.69	0.78	4.69	0.09	5.12	0.76	4.08	0.14
	SD	0.97	0.24	0.87	0.03	1.13	0.19	1.04	0.05
	N	15	15	15	15	15	15	15	15
5 (1000/week)	Mean	8.60	2.39	5.76	0.24	7.01	1.98	4.60	0.23
	SD	1.64	0.83	0.89	0.08	1.73	1.02	0.87	0.07
	N	15	15	15	15	14	14	14	14
	% difference from control	51	206	23	167	37	161	13	64

% difference from control added to table as no statistical analysis performed

For FCV females, there were increases in the WBC counts and number of specific WBC cell populations at 13 and 26 weeks compared to controls. Most notable were a 91 and 120% increase in neutrophils at 13 and 26 weeks, respectively, and a 75 and 129% increase in monocytes at 13 and 26 weeks (see table for all changes). These results are consistent with the observed inflammation at the injection site.

Differences in White Blood Cells: Females

Group/Dosage (mg/kg)	Summary Information	Week 13				Week 26			
		WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$	WBC $\times 10^9/L$	Neut $\times 10^9/L$	Lymph $\times 10^9/L$	Mono $\times 10^9/L$
1 (0/day)	Mean	3.92	0.64	3.09	0.08	2.91	0.50	2.25	0.07
	SD	1.00	0.27	1.04	0.05	0.53	0.29	0.60	0.03
	N	14	14	14	14	14	14	14	14
5 (1000/week)	Mean	5.73	1.22	4.23	0.14	4.28	1.10	2.91	0.16
	SD	1.51	0.43	1.22	0.07	0.97	0.44	0.76	0.06
	N	14	14	14	14	13	13	13	13
	% difference from control	46	91	37	75	47	120	29	129

% difference from control added to table as no statistical analysis performed

Coagulation

Coagulation Parameters

Activated partial thromboplastin time Fibrinogen	Prothrombin time

Statistically significant (SS) increases in fibrinogen were observed after treatment with GDO and FCV at 1000 mg/kg at Weeks 13 and 26.

At 13 and 26 weeks for GDO, increases in fibrinogen concentrations were 22 and 37% for males and 18 and 48% for females compared to controls. Other dose level values were lower. This occurrence can be related to the inflammation observed as a result of the SC dosing and resultant foreign body reaction.

Fibrinogen Concentration

Group/Dosage (mg/kg/day)	Summary Information	Week 13		Week 26	
		Males mg/dL	Females mg/dL	Males mg/dL	Females mg/dL
1 (0)	Mean	236	171	238	158
	SD	31	22	15	24
	N	15	14	14	13
4 (1000)	Mean	288	202	327	234
	SD	53	30	74	71
	N	15	14	13	14
	P value	<0.01	<0.001	<0.001	<0.001

At 13 and 26 weeks for FCV, increases in fibrinogen concentrations were 55 and 34% for males and 46 and 44% for females compared to controls. This occurrence can also be related to the inflammation observed because of the SC dosing and resultant foreign body reaction. Larger values for fibrinogen were noted than for GDO at the same dose.

Fibrinogen Concentration

Group/Dosage (mg/kg)	Summary Information	Week 13		Week 26	
		Males mg/dL	Females mg/dL	Males mg/dL	Females mg/dL
1 (0/day)	Mean	236	171	238	158
	SD	31	22	15	24
	N	15	14	14	13
5 (1000/week)	Mean	365	249	319	227
	SD	50	29	35	44
	N	13	13	14	12
	% difference from control	55	46	34	44

% difference from control added to table as no statistical analysis performed

Clinical Chemistry

Clinical Chemistry Parameters

Urea	Total protein
Glucose	Albumin
Aspartate aminotransferase	Globulin
Alanine aminotransferase	Albumin/globulin ratio
Alkaline phosphatase	Cholesterol
Creatine phosphokinase	Creatinine
Lactate dehydrogenase	Total bilirubin
Sodium	Calcium
Potassium	Inorganic phosphate
Chloride	Triglycerides

No treatment-related effects were observed for clinical chemistry when comparing GDO or FCV to control.

Urinalysis

Urinalysis Parameters

Microscopic evaluation of spun deposit Colour Turbidity Specific gravity Volume pH Protein	Glucose Bilirubin Ketones Leukocytes Blood Urobilinogen

No treatment-related effects were observed for urinalysis when comparing GDO or FCV to control. While calcium, phosphorus, sodium, potassium, chloride, and creatinine were not measured, which was a deviation from the protocol, this deviation was considered not to impact on the outcome of the study by the Sponsor. This reviewer agrees as values for these parameters were measured in the plasma with no treatment-related effects when comparing treated animals with controls. In addition, there were no treatment-related histological findings in the kidney which supports that these urinary indices were not affected by treatment.

Terminal Procedures and Evaluations

Terminal Procedures

Group No.	No. of Animals		Scheduled Euthanasia Day	Necropsy Procedures			Histology	Histopathology
	M	F		Necropsy	Tissue Collection	Organ Weights		
1	15	15	183	X	X	X	Full Tissue	Full Tissue ^a
2	15	15					Injection Sites	Injection Sites
3	15	15					Gross Lesions	Gross Lesions
4	15	15					Injection Sites	Injection Sites
5	15	15					Gross Lesions	Gross Lesions
Unscheduled deaths				X	X	-	Full Tissue	Full Tissue

X = Procedure to be conducted; - = Not applicable

^a See Tissue Collection and Preservation table for listing of tissues

Gross Pathology

After sacrifice, all animals were subjected to a complete necropsy examination. This included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal and pelvic cavities with their associated organs and tissues.

Externally visible injection site effects (e.g., swellings) were previously discussed in the clinical observations section. Most notable observations during gross necropsy compared to controls were injection site cysts. Nothing different in animals was observed for those sacrificed as moribund (1 HD GDO, 3 FCV animals due to skin

morbidity) or found dead (1 control, 1 HD – not GDO treatment-related) than reported in the following descriptions.

GDO group injection sites - Cysts, usually described as clear or pale, were the most notable feature at the sites dosed with GDO at all dose levels. The content of the cysts was likely GDO at the injection sites. This was expected since GDO is a slightly viscous oil, slowly cleared from the injection site. Cysts were most frequently observed in the animals that received 1000 mg GDO/kg/day, and a clear dose and volume related trend was apparent throughout the groups. Considering all 6 injection sites, the highest incidence at any given site in animals (not all sites affected) was 0/15 0/14 for controls, 2/15 and 1/15 (LD GDO), 10/15 and 8/15 (MD GDO), and 14/14 and 13/14 (HD GDO), in males and females, respectively. "Thickening" at sites was observed for 5/14 HD GDO males at one site with no more than 1 animal/site being affected from the other groups including control. This observation was of low occurrence (number of sites and animals) and its significance is unknown.

"Raw area" was noted only at one site and 1 male and 1 female at 1000 mg/kg/day. Scabs were observed at a very low incidence across all dose levels, most frequently at 1000 mg/kg/day where the highest incidence for 1 site was 6/14 males with no scabs in HD females. Scab formation/raw areas tended to correlate with ulcerative dermatitis at histopathology, and were considered secondary to trauma rather than an extension of the subcutaneous changes.

FCV group injection sites - Swelling and raised areas were recorded in animals that received 1000 mg FCV/kg/week at different sites with the highest incidence of 7/14 males and 6/13 females. The swellings likely represented test item at the injection sites. This was expected as the FCV is a formulation that when injected into the subcutaneous tissue self-assembles into a gel-like, firm, slowly degradable matrix. Almost one third of the sites had swellings and fewer had raised areas. There was generally no obvious trend for the distribution of the findings in relation to the various sites. Cysts were a less frequent observation with no more than 2/14 and 7/14 males and females being affected, respectively for a single site. Discoloration was treatment-related, but was not related clearly to the time since the administration of the last injection of FCV.

Scab formation was noted with the highest incidence for any site at 6/15 males and 2/13 females. Raw areas were noted infrequently with the highest incidence of 1/14 males and 1/13 females. These findings tended to correlate with ulcerative dermatitis at histopathology.

Other - Secondary to the injection site reactions, findings in the axillary/inguinal lymph nodes included enlargement of occasional nodes in 4 of 27 rats that received 1000 mg FCV/week, compared with none in controls. Lung discoloration was also noted in treated groups and not controls at a low incidence and not in a dose-responsive manner. Nothing else different was observed in early deaths (sacrificed as moribund or found dead).

Organ Weights

Organs were weighed at necropsy.

Organs Weighed at Necropsy

Brain	Liver
Epididymis ^a	Lung
Gland, adrenal	Ovary ^a
Gland, pituitary	Spleen
Gland, prostate	Testis ^a
Gland, thyroid	Thymus
Heart	Uterus
Kidney ^a	

^a – paired organs were not weighed together, but weights were combined for organ weight evaluation.

No treatment-related effects were observed for organ weights when comparing GDO or FCV to control.

Histopathology - Representative samples of the tissues listed the table below were collected from all animals, and preserved in 10% neutral buffered formalin, unless otherwise noted. Hematoxylin and eosin-stained issues from different groups were evaluated as listed in the terminal evaluation included previously above.

Adequate Battery – Yes.

Peer Review – Yes, by internal pathologist.

Tissue Collection and Preservation

Administration site	Liver
Animal identification	Lung
Artery, aorta	Lymph node, axillary
Bone marrow smear	Lymph node, inguinal
Bone marrow, femur	Lymph node, mandibular ^c
Bone marrow, sternum	Lymph node, mesenteric
Bone, femur with articulating surface	Muscle, skeletal
Bone, sternum	Nerve, optic ^a
Brain	Nerve, sciatic ^c
Cervix	Oesophagus
Epididymides	Ovaries
Eyes ^a	Oviducts ^c
Gland, adrenal	Pancreas
Gland, harderian ^a	Skin
Gland, lacrimal ^c	Small intestine, duodenum
Gland, mammary	Small intestine, ileum
Gland, parathyroid	Small intestine, jejunum
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary ^c	Stomach
Gland, seminal vesicle	Testes ^b
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Ureter ^c
Kidney	Urinary bladder
Large intestine, caecum	Uterus
Large intestine, colon	Vagina
Large intestine, rectum	

^a Preserved in Davidson's fixative

^b Preserved in Modified Davidson's fixative

^c Only one examined

Histological Findings - Treatment associated findings that were directly attributed to the test items were localized and confined to the subcutaneous injection sites. There was no evidence of reactions similar to these seen at the injection sites in any other tissue.

At the injection site, the region affected was the subcutis, with findings sometimes involving the panniculus muscle and occasionally the dermis. The microscopic features ranged from chronic active to chronic inflammation, a usual, expected foreign body reaction. Individual site information is provided in the tables after text.

Cystic granulomatous inflammation was the primary treatment-related finding for GDO and FCV with an incidence for number of animals and severity as listed in the following tables.

Incidence of Subcutaneous Cystic Granulomatous Inflammation at the 6 Injection Sites after SC dosing Daily with Glycerol Dioleate (GDO) or Weekly with FluidCrystal Vehicle (FCV) for 26 Weeks in Rats														
	# of animals/group		# of animals/group with inflammation at that site											
			Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Dose GDO (mg/kg)	M*	F*	M	F	M	F	M	F	M	F	M	F	M	F
0 (q1w placebo)	15	14	0	0	0	0	0	0	0	0	0	0	0	0
100	15	15	6	9	10	6	8	6	8	6	9	5	7	6
350	15	15	12	10	13	7	14	12	12	13	11	14	13	
1000	14	14	14	14	14	14	13	14	13	14	14	13	13	13
Dose FCV (mg/kg)														
1000	14	13	11	8	10	11	5	3	6	10	10	10	4	8

* - M (male), F (female)

Histopathological Severity* of Subcutaneous Cystic Granulomatous Inflammation at the 6 Injection Sites after SC dosing Daily with Glycerol Dioleate (GDO) or Weekly with FluidCrystal Vehicle (FCV) for 26 Weeks in Rats												
	Severity range (highest incidence severity)											
	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Dose GDO (mg/kg)	M	F	M	F	M	F	M	F	M	F	M	F
0 (q1w placebo)	0	0	0	0	0	0	0	0	0	0	0	0
100	1-2 (2)	1-2 (2)	1-3 (2)	1-2 (1)	1-2 (1-2)	1-2 (2)	1-2 (1)	1-2 (1-2)	1-2 (1)	1-2 (1)	1-2 (2)	1-2 (1)
350	1-3 (2)	1-3 (2)	1-4 (2)	1-2 (2)	1-3 (3)	1-3 (1)	1-3 (1-3)	1-3 (1)	1-3 (2)	1-3 (2)	2-4 (2)	1-3 (2)
1000	2-4 (2)	1-3 (3)	1-3 (3)	1-4 (3)	2-4 (3)	1-3 (3)	2-4 (3)	1-5 (4)	1-4 (3)	1-4 (2)	2-4 (3-4)	2-4 (2)
Dose FCV (mg/kg)												
1000	2-4 (3)	2-4 (4)	2-4 (3)	1-4 (3)	1-4 (4)	1-3 (1-3)	3-4 (3)	2-4 (3)	2-4 (3)	2-4 (2)	2-3 (2)	2-4 (2)

* - Grade 1-5 (minimal, mild, moderate, marked, severe)

** - M (male), F (female)

Based on the tables, the following text describes SC cystic granulomatous inflammation and other observed occurrences in GDO and FCV groups.

GDO injection sites – SC cystic granulomatous inflammation was the primary treatment-related finding. This was seen at all dose levels and correlated with the cysts observed at necropsy. There was a general dose-response for the cystic granulomatous response with respect to severity and incidence. Mononuclear cell inflammation was recorded at a relatively low incidence across the groups, with greater severity noted in animals that received 1000 mg/kg/day. Mixed inflammation was noted at 1000 mg/kg/day at both a low severity and incidence. Low incidences of fibrosis/fibroplasia and granulation tissue were confined to 350 or 1000 mg/kg/day, occasionally correlated with thickening.

Occasional ulcerative dermatitis was considered to be procedurally related, probably associated with mechanical trauma and scratching. This occurred at very low incidences at all dose levels of GDO, and correlated with necropsy findings including raw area and/or discoloration.

FCV injection sites - SC cystic granulomatous inflammation was the primary treatment-related finding. This correlated with the swelling/raised areas and less frequently cysts observed at necropsy in this particular group.

Pigmented macrophages (and less often foamy macrophages) were recorded at the majority of injection sites. Pigmented macrophages related to the necropsy observation of dark/discolored. Mixed/mononuclear cell inflammation, fibrosis/fibroplasia and granulation tissue were other features of the inflammatory reaction, sometimes correlating with raised area/swelling.

Reactions at the six injection sites in animals receiving 1000 mg/kg/week were similar overall with no consistent differences. However, both the incidence and severity of cystic granulomatous inflammation were lower at Site 3 (42 days after the last injection) compared with Site 2 (7 days after the last injection). Although this was suggestive of recovery, this tendency was not consistently demonstrated at all time points following the last injection.

Ulcerative dermatitis, epidermal hyperplasia, dermal fibrosis/inflammation and scab were noted at higher incidences compared with the incidence in control. This was possibly mechanically related (dosing) and due to self-trauma/scratching, rather than being directly attributed to treatment. This effect was not noted in animals that were individually housed, suggesting that the effect was due to external mechanical trauma from their littermates. Relatively few sites were affected overall, although the necropsy correlations, particularly scab, were more frequent.

Group	:	1	2	3	4	5
Test Item	:	Control (Saline)	-----	Glycerol dioleate	-----	2022IAS-I
Dosage (mg/kg)	:	0	100	350	1000	1000

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 1		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		15	8**	3***	0***	1	14	6***	5***	0***	4
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	1	3	0	0	0	2	3	1	0
mild		0	5*	6*	3	2	0	7**	4	3	1
moderate		0	0	3	6**	5	0	0	3	7**	3
marked		0	0	0	5*	4	0	0	0	3	4
Total Incidence		0	6*	12***	14***	11	0	9***	10***	14***	8
Inflammation, subcutaneous, mixed											
mild		0	0	0	1	1	0	0	0	1	2
moderate		0	0	0	0	1	0	0	0	2	2
Total Incidence		0	0	0	1	2	0	0	0	3	4
Inflammation, subcutaneous, mononuclear cell											
minimal		0	1	0	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	1	0	0	1	0	0	0	1	0
Fibrosis/fibroplasia, subcutaneous											
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	1	0	2	0	0	0	1	0
moderate		0	0	0	1	0	0	0	0	1	0
Total Incidence		0	0	1	1	3	0	0	0	2	0
Granulation tissue, subcutaneous											
mild		0	0	0	1	0	0	0	0	0	0
Total Incidence		0	0	0	1	0	0	0	0	0	0
Foamy macrophages, subcutaneous											
mild		0	0	0	0	1	0	0	0	1	2

moderate	0	0	0	0	0	0	0	0	0	3
Total Incidence	0	0	0	0	1	0	0	0	1	5
Pigmented macrophages, subcutaneous										
minimal	0	0	0	1	0	0	0	0	0	0
mild	0	0	0	0	2	0	0	0	0	0
Total Incidence	0	0	0	1	2	0	0	0	0	0
Haemorrhage, subcutaneous										
minimal	0	1	2	1	0	0	0	0	0	0
mild	0	0	0	1	1	0	0	0	1	0
Total Incidence	0	1	2	2	1	0	0	0	1	0
Dermatitis, ulcerative										
mild	0	0	0	0	1	0	0	0	0	1
moderate	0	0	0	0	1	0	0	0	0	2
Total Incidence	0	0	0	0	2	0	0	0	0	3
Epidermal hyperplasia										
mild	0	0	0	0	1	0	0	0	0	0
Total Incidence	0	0	0	0	1	0	0	0	0	0
Scab	0	0	0	0	4	0	0	0	0	0

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 2		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		15	3***	2***	0***	3	13	8*	8*	0***	2
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	2	3	0	0	4	1	2	1	
mild		0	7**	7**	5*	4	0	2	6*	4	
moderate		0	1	2	9***	5	0	0	5*	4	
marked		0	0	1	0	1	0	0	3	2	
Total Incidence		0	10***	13***	14***	10	0	6*	7**	14***	
Inflammation, mixed, subcutaneous											
minimal		0	0	0	0	0	0	0	1	0	
mild		0	0	0	0	0	0	0	1	0	
moderate		0	0	0	0	2	0	0	4	1	
Total Incidence		0	0	0	0	2	0	0	6*	1	
Inflammation, mononuclear cell, subcutaneous/muscle											
minimal		0	2	0	0	0	1	0	0	0	
mild		0	0	0	0	0	0	1	0	0	
moderate		0	0	0	0	0	0	0	2	0	
Total Incidence		0	2	0	0	0	1	1	2	0	
Fibrosis/fibropiasia, subcutaneous											
mild		0	0	1	1	2	0	0	1	0	
moderate		0	0	0	1	2	0	0	1	0	
Total Incidence		0	0	1	2	4	0	0	1	5	
Granulation tissue, subcutaneous											
mild		0	0	0	0	0	0	0	0	1	
Total Incidence		0	0	0	0	0	0	0	0	1	
Foamy macrophages, subcutaneous											
mild		0	0	0	0	0	0	0	0	1	
moderate		0	0	0	0	0	0	0	0	1	
Total Incidence		0	0	0	0	0	0	0	0	2	
Pigmented macrophages, subcutaneous											
minimal		0	0	0	0	0	0	0	1	0	
mild		0	0	0	0	0	0	0	0	5	
moderate		0	0	0	0	1	0	0	0	1	
Total Incidence		0	0	0	0	1	0	0	1	6	
Haemorrhage, subcutaneous											
minimal		0	0	0	0	2	0	0	1	1	
mild		0	0	0	1	3	0	0	0	1	
Total Incidence		0	0	0	1	5	0	0	1	2	
Fibrosis, dermal											
mild		0	0	0	0	1	0	0	0	0	
Total Incidence		0	0	0	0	1	0	0	0	0	

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 3		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		14	6**	1***	1***	4	14	9*	3***	0***	3
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	4	3	0	1	0	2	7**	2	1
mild		0	4	4	1	1	0	4	4	3	1
moderate		0	0	7**	9***	1	0	0	1	9***	1
marked		0	0	0	3	2	0	0	0	0	0
Total Incidence		0	8**	14***	13***	5	0	6*	12***	14***	3
Inflammation, mixed, subcutaneous											
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	1
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	3	0	0	0	0	1
Fibrosis/fibroplasia, subcutaneous											
mild		0	0	0	0	2	0	0	0	1	3
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	3	0	0	0	1	3
Foamy macrophages, subcutaneous											
mild		0	0	0	0	0	0	0	0	0	1
Total Incidence		0	0	0	0	0	0	0	0	0	1
Pigmented macrophages, subcutaneous											
minimal		0	0	0	0	0	0	0	0	0	1
mild		0	0	0	0	5	0	0	0	0	8
moderate		0	0	0	0	2	0	0	0	0	0
Total Incidence		0	0	0	0	7	0	0	0	0	9
Haemorrhage, subcutaneous											
minimal		0	1	0	0	0	0	0	0	1	0
mild		1	0	0	0	0	0	0	0	0	0
Total Incidence		1	1	0	0	0	0	0	0	1	0
Dermatitis, ulcerative											
mild		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0
Epidermal hyperplasia											
mild		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0
Fibrosis, dermal											
mild		0	0	0	0	2	0	0	0	0	1
Total Incidence		0	0	0	0	2	0	0	0	0	1

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 4		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		14	6**	2***	1***	6	13	8*	2***	0***	2
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	3	4	0	0	0	3	6*	1	0
mild		0	5*	2	1	0	0	3	2	0	4
moderate		0	0	4	7**	4	0	0	4	5*	5
marked		0	0	2	5*	2	0	0	0	7**	1
severe		0	0	0	0	0	0	0	0	1	0
Total Incidence		0	8**	12***	13***	6	0	6*	12***	14***	10
Inflammation, mixed, subcutaneous											
minimal		0	1	0	0	0	0	0	1	0	0
mild		0	0	0	0	1	0	0	0	0	0
moderate		0	0	0	1	0	0	0	0	4	0
Total Incidence		0	1	0	1	1	0	0	1	4	0
Inflammation, mononuclear cell, subcutaneous											
mild		0	0	0	2	1	0	0	0	0	2
Total Incidence		0	0	0	2	1	0	0	0	0	2
Fibrosis/fibroplasia, subcutaneous											
mild		0	0	0	1	0	0	0	1	1	0
moderate		0	0	0	1	2	0	0	0	1	0
Total Incidence		0	0	0	2	2	0	0	1	2	0
Granulation tissue, subcutaneous											
mild		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0
Foamy macrophages, subcutaneous											
mild		0	0	0	0	0	0	0	0	1	0
moderate		0	0	0	0	2	0	0	0	0	1
Total Incidence		0	0	0	0	2	0	0	0	1	1
Pigmented macrophages, subcutaneous											
minimal		0	0	0	0	0	0	0	0	0	2
mild		0	0	0	0	5	0	0	0	0	4
moderate		0	0	0	0	1	0	0	0	0	3
Total Incidence		0	0	0	0	6	0	0	0	0	9
Haemorrhage, subcutaneous											
minimal		0	1	1	0	0	0	0	0	0	0
mild		1	0	0	1	0	1	0	0	0	0
Total Incidence		1	1	1	1	0	1	0	0	0	0
Dermatitis, ulcerative											
minimal		0	0	0	0	0	0	0	1	0	0
mild		0	0	0	0	0	0	1	0	0	0
Total Incidence		0	0	0	0	0	0	1	1	0	0

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 5		(15)	(15)	(15)	(14)	(14)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		15	4***	2***	0***	1	14	10*	2***	1***	1
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	5*	1	1	0	0	3	3	1	0
mild		0	4	9***	5*	3	0	2	7**	7**	5
moderate		0	0	3	6**	4	0	0	1	4	3
marked		0	0	0	2	3	0	0	0	1	2
Total Incidence		0	9***	13***	14***	10	0	5*	11***	13***	10
Inflammation, mixed, subcutaneous											
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	1
moderate		0	0	0	0	1	0	0	0	0	1
Total Incidence		0	0	0	0	3	0	0	0	0	2
Inflammation, subcutaneous, mononuclear cell											
minimal		0	1	0	0	0	0	0	1	0	0
mild		0	0	1	0	1	0	0	0	0	0
Total Incidence		0	1	1	0	1	0	0	1	0	0
Fibrosis/fibroplasia, subcutaneous											
minimal		0	0	0	0	0	0	0	1	0	0
mild		0	0	1	0	2	0	0	0	0	1
moderate		0	0	0	2	4	0	0	0	0	0
Total Incidence		0	0	1	2	6	0	0	1	0	1
Granulation tissue, subcutaneous											
mild		0	0	0	1	0	0	0	0	0	0
moderate		0	0	0	0	0	0	0	0	0	1
Total Incidence		0	0	0	1	0	0	0	0	0	1
Foamy macrophages, subcutaneous											
mild		0	0	0	0	1	0	0	0	0	2
moderate		0	0	0	0	1	0	0	0	0	4
Total Incidence		0	0	0	0	2	0	0	0	0	6
Pigmented macrophages, subcutaneous/muscle											
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	7	0	0	0	0	4
moderate		0	0	0	0	1	0	0	0	0	1
Total Incidence		0	0	0	0	9	0	0	0	0	5
Haemorrhage, subcutaneous											
minimal		0	1	0	0	0	0	0	0	1	2
mild		0	0	0	1	1	0	0	0	1	0
Total Incidence		0	1	0	1	1	0	0	0	2	2
Dermatitis, ulcerative											
marked		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS									
		Males					Females				
		Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg	Grp 1 0 mg/kg	Grp 2 100 mg/kg	Grp 3 350 mg/kg	Grp 4 1000 mg/kg	Grp 5 1000 mg/kg
INJECTION SITE 6		(15)	(15)	(15)	(14)	(13)	(14)	(15)	(15)	(14)	(13)
No abnormality detected		15	8**	1***	0***	3	11	8	2***	1***	1
Inflammation, granulomatous, cystic, subcutaneous/muscle											
minimal		0	1	0	0	0	0	5*	2	0	0
mild		0	6*	11***	1	3	0	1	10***	5*	4
moderate		0	0	2	6**	1	0	0	1	4	3
marked		0	0	1	6**	0	0	0	0	4	1
Total Incidence		0	7**	14***	13***	4	0	6*	13***	13***	8
Inflammation, subcutaneous, mixed											
minimal		0	0	0	0	0	0	1	0	0	0
moderate		0	0	0	1	0	0	0	0	0	3
Total Incidence		0	0	0	1	0	0	1	0	0	3
Fibrosis/fibroplasia, subcutaneous											
mild		0	0	1	1	0	0	0	1	1	2
moderate		0	0	0	0	3	0	0	0	0	0
Total Incidence		0	0	1	1	3	0	0	1	1	2
Foamy macrophages, subcutaneous											
mild		0	0	0	1	1	0	0	0	0	2
moderate		0	0	0	0	0	0	0	0	0	1
Total Incidence		0	0	0	1	1	0	0	0	0	3
Pigmented macrophages, subcutaneous/muscle											
mild		0	0	0	0	3	0	0	0	0	7
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	4	0	0	0	0	7
Haemorrhage, subcutaneous											
minimal		0	0	1	0	1	1	1	1	0	0
mild		0	0	1	0	0	0	2	1	1	2
Total Incidence		0	0	2	0	1	1	3	2	1	2
Dermatitis, ulcerative											
mild		0	0	0	1	1	0	0	0	0	1
moderate		0	0	0	0	1	0	0	0	0	1
Total Incidence		0	0	0	1	2	0	0	0	0	2
Epidermal hyperplasia											
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	1	0	0	0	0	0
Fibrosis/inflammation, dermal											
mild		0	0	0	0	2	0	0	0	0	0
moderate		0	0	0	0	1	0	0	0	0	0
Total Incidence		0	0	0	0	3	0	0	0	0	0

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

Special Evaluation – None other than already listed.

Toxicokinetics – None conducted.

Dosing Solution Analysis - Test drug and CAM2038 q1w placebo (FCV, 202IAS-1) were used within expiration dates. The analysis was conducted under a separate protocol which was not found/referenced, which is not considered a critical issue considering the nature of the test materials.

GENETIC TOXICOLOGY

The Applicant complete a full standard battery of genetic toxicity studies with GDO (see table). All studies demonstrated a negative result under the conditions tested.

Study number/description	Doses	Results
TO-04-141: GLP <i>in vitro</i> reverse mutation in bacteria (Ames)	Glycerol dioleate (95.3 %): 0, 50, 160, 500, 1600, 5000 mcg/plate ± S9	Negative
TO-04-146: GLP <i>in vitro</i> reverse mutation in bacteria (Ames)	Glycerol dioleate (92.1 %): 0, 50, 160, 500, 1600, 5000 mcg/plate ± S9	Negative
TO-04-142: GLP <i>in vitro</i> gene mutation assay in mouse lymphoma cells (L5178Y TK ^{+/+})	Glycerol dioleate (95.3 %): 0, 156, 313, 625, 1250, 2500 and 5000 mcg/mL. – S9: 4 and 21 hours, +S9: 3 hours.	Negative
TO-12-446: GLP <i>in vivo</i> rat micronucleus assay	Glycerol dioleate (not reported*): 0, 500, 1000 and 2000 mg/kg SC in male and female rats (2 doses, 24 hours apart, n = 5/sex/group). Animals sacrificed 48 hours after first dose.	Negative

* - same batch as used in 26-week SC study in rats

NOTE: Genetic Toxicology studies were reviewed by Dr. Nikunj Patel of the Division of Anesthesia, Analgesia, and Addiction Products.

***In Vitro* Reverse Mutation Assays in Bacterial Cells (Ames)**

Study title: (b) (4) (Glycerol dioleate, (b) (4) %) - AMES TEST

Study no.: TO-04-141
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Not stated
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4) (Glycerol dioleate, (b) (4) %), Lot 2292/132, purity 95.3%

Key Study Findings

- All criteria were met for a valid assay.
- Glycerol dioleate concentrations of up to 5000 mcg/plate were negative for mutagenic potential in the bacterial reverse mutation assay under the conditions tested.

Methods

Strains: *Salmonella typhimurium* strains TA102, TA100, TA98, TA1537, and TA1535.
 Concentrations in definitive study: 50, 160, 500, 1600, and 5000 mcg/plate.
 Basis of concentration selection: A preliminary dose-range finding toxicity study conducted using the plate-incorporation method in bacterial strain TA98 with and without S9 showed no toxicity at glycerol dioleate doses of up to

5000 mcg/plate (no marked reductions in number of revertants or reduced growth of background lawn of non-revertants). Based on these results, 5000 mcg/plate was chosen as the highest dose in the definitive study.

Negative control:

DMSO (100 mcL/plate)

Positive control:

See table below

Formulation/Vehicle:

DMSO

Incubation & sampling time:

The plate-incorporation and pre-incubation methods were used for this assay. In each test, the plates were incubated at 37°C for approximately 72 hours, and the number of revertant colonies on each plate was counted.

Plate incorporation: A series of test tubes was prepared containing test item formulation (100 mcL), vehicle (100 mcL), or positive control solution (50 or 100 mcL), S-9 mix or 0.02 M phosphate buffer pH 7.4 (500 mcL), bacterial suspension (100 mcL, approximately 10⁹ bacteria/mL), and top agar (2 mL). After vortex mixing, the mixtures were spread on selective agar plates.

Pre-incubation: A series of test tubes was prepared containing test item formulation (100 mcL), vehicle (100 mcL), or positive control solution (25, 50, or 100 mcL), S-9 mix or 0.02 M phosphate buffer pH 7.4 (500 mcL), and bacterial suspension (100 mcL, approximately 10⁹ bacterial mL). Each test tube was incubated for 1 hour at 37°C with gentle shaking. Top agar (2 mL) was added, and after vortexing, the mixtures were spread on selective agar plates.

Positive Controls for the *in vitro* bacterial reverse mutation assays

Tester Strain(s)	S9	Positive Control	Concentration (mcg/plate)
TA102	-	Cumene hydroperoxide	Plate incorporation: 100 Pre-incubation: 25
TA100, TA1535	-	Sodium azide	1
TA98	-	2-nitrofluorene	1
TA1537	-	N-aminoacridine	80
TA100, TA98,	+	2-aminoanthracene	2

TA1537, TA1535			
TA102	+	2-aminoanthracene	4

Study Validity

The definitive studies utilized the plate incorporation and pre-incubation methods to examine the mutagenicity induction potential of glycerol dioleate on five strains of *S. typhimurium* (TA102, TA100, TA98, TA1537, and TA1535). The Sponsor examined an adequate number and type of strains required for a full bacterial reverse mutation battery. The concentrations of glycerol dioleate used were adequate to have at least three nontoxic doses for each strain tested with and without metabolic activation (no toxicity was observed at any of the doses tested). Each glycerol dioleate concentration, positive, and negative controls were tested in triplicate, in the presence or absence of S9 mix. Plates were examined to assess the quality of the background lawn and colonies. Revertant colonies were counted and compared to the negative controls for determination of statistically significant differences using ANOVA test, followed by Dunnett's test if statistically significant differences were shown with ANOVA. No precipitation of glycerol dioleate was observed at any concentration tested up to the 5000 mcg/plate maximal dose (limit dose per the ICH S2 (R1) Guidance). The assays were all considered valid based on the following criteria: the negative control counts fell within the normal ranges of laboratory historical controls, and all positive controls produced increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain. For all tester strains, the test article was considered to be positive for inducing mutations if the number of revertants showed a dose-dependent, reproducible, and statistically significant increase to at least 1.5-fold the concurrent negative controls. All criteria for a valid assay were met.

Results

No reductions in revertant colony counts occurred following exposure of any bacterial strain in the absence or presence of S9, indicating that glycerol dioleate was not toxic to any of the bacterial strains up to the maximum dose of 5000 mcg/plate. No precipitation of the test article was observed in the assay. All positive and negative controls were within acceptable ranges. In strain TA1537, glycerol dioleate caused a statistically significant increase in revertant colonies at 5000 mcg/plate with S9 in the plate incorporation test, however this increase was only 1.29-fold higher than the corresponding negative control value and therefore not considered to be positive for mutagenic activity. Glycerol dioleate did not increase mutant frequency by 1.5-fold over negative control values in any tester strain, with or without metabolic activation. Therefore, glycerol dioleate was not found to be mutagenic in this *in vitro* bacterial reverse mutagenicity assay.

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Study title: (b) (4) (Glycerol dioleate, (b) (4) %) – AMES TEST

Study no.: TO-04-146
Study report location: EDR

Conducting laboratory and location: (b) (4)

Date of study initiation: Not stated

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4) (Glycerol dioleate, (b) (4) %), Lot 2292/124-4, purity 92.1%

Key Study Findings

- All criteria were met for a valid assay.
- Glycerol dioleate concentrations of up to 5000 mcg/plate were negative for mutagenic potential in the bacterial reverse mutation assay under the conditions tested.

Methods

Strains: *Salmonella typhimurium* strains TA102, TA100, TA98, TA1537, and TA1535.

Concentrations in definitive study: 50, 160, 500, 1600, and 5000 mcg/plate.

Basis of concentration selection: A preliminary dose-range finding toxicity study conducted using the plate-incorporation method in bacterial strain TA98 with and without S9 showed no toxicity at glycerol dioleate doses of up to 5000 mcg/plate (no marked reductions in number of revertants or reduced growth of background lawn of non-revertants). Based on these results, 5000 mcg/plate was chosen as the highest dose in the definitive study.

Negative control: DMSO (100 mL/plate)

Positive control: See table below

Formulation/Vehicle: DMSO

Incubation & sampling time: The plate-incorporation and pre-incubation methods were used for this assay. In each test, the plates were incubated at 37°C for approximately 72 hours, and the number of revertant colonies on each plate were counted.

Plate incorporation: A series of test tubes was prepared containing test item formulation (100 mL), vehicle (100 mL), or positive control solution (50 or 100 mL), S-9 mix or 0.02 M phosphate buffer pH 7.4 (500 mL), bacterial suspension (100 mL, approximately 10⁹ bacteria/mL), and top

agar (2 mL). After vortex mixing, the mixtures were spread on selective agar plates.

Pre-incubation: A series of test tubes was prepared containing test item formulation (100 mcL), vehicle (100 mcL), or positive control solution (25, 50, or 100 mcL), S-9 mix or 0.02 M phosphate buffer pH 7.4 (500 mcL), and bacterial suspension (100 mcL, approximately 10^9 bacterial mL). Each test tube was incubated for 1 hour at 37°C with gentle shaking. Top agar (2 mL) was added, and after vortexing, the mixtures were spread on selective agar plates.

Positive Controls for the *in vitro* bacterial reverse mutation assays

Tester Strain(s)	S9	Positive Control	Concentration (mcg/plate)
TA102	-	Cumene hydroperoxide	Plate incorporation: 100 Pre-incubation: 25
TA100, TA1535	-	Sodium azide	1
TA98	-	2-nitrofluorene	1
TA1537	-	N-aminoacridine	80
TA100, TA98, TA1537, TA1535	+	2-aminoanthracene	2
TA102	+	2-aminoanthracene	4

Study Validity

The definitive studies utilized the plate incorporation and pre-incubation methods to examine the mutagenicity induction potential of glycerol dioleate on five strains of *S. typhimurium* (TA102, TA100, TA98, TA1537, and TA1535). The Sponsor examined an adequate number and type of strains required for a full bacterial reverse mutation battery. The concentrations of glycerol dioleate used were adequate to have at least three nontoxic doses for each strain tested with and without metabolic activation (no toxicity was observed at any of the doses tested). Each glycerol dioleate concentration, positive, and negative controls were tested in triplicate, in the presence or absence of S9 mix. Plates were examined to assess the quality of the background lawn and colonies. Revertant colonies were counted and compared to the negative controls for determination of statistically significant differences using the ANOVA test, followed by Dunnett's test if statistically significant differences were shown. No precipitation of glycerol dioleate was observed at any concentration tested up to the 5000 mcg/plate

maximal dose (limit dose per the ICH S2 (R1) Guidance). The assays were all considered valid based on the following criteria: the negative control counts fell within the normal ranges of laboratory historical controls, and all positive controls produced increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain. For all tester strains, the test article was considered positive for inducing mutations if the number of revertants showed a dose-dependent, reproducible, and statistically significant increase to at least 1.5-fold the concurrent negative controls. All criteria for a valid assay were met.

Results

No reductions in revertant colony counts occurred following exposure of any bacterial strain in the absence or presence of S9, indicating that glycerol dioleate was not toxic to any of the bacterial strains up to the maximum dose of 5000 mcg/plate. No precipitation of the test article was observed in the assay. All positive and negative controls were within acceptable ranges. Glycerol dioleate not increase mutant frequency by 1.5-fold of negative control values in any tester strain, with or without metabolic activation. Therefore, glycerol dioleate was not found to be mutagenic in this *in vitro* bacterial reverse mutagenicity assay.

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In Vitro Assay in Mammalian Cells

Study title: (b) (4) (Glycerol dioleate, (b) (4) %) - *IN VITRO*
MAMMALIAN CELL GENE MUTATION TEST PERFORMED WITH
MOUSE LYMPHOMA L5178Y CELLS

Study no.:	TO-04-142
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not stated
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) (Glycerol dioleate, (b) (4) %), Lot 2292/132, purity 95.3 %

Key Study Findings

- All criteria were met for a valid assay
- Glycerol dioleate concentrations of up to 5000 mcg/mL were negative for mutagenic potential

Methods

Cell line: Mouse lymphoma cell line L5178Y TK^{+/-}
Concentrations in definitive study: – S9: 4 and 21 hours, +S9; 3 hours: 156, 313, 625, 1250, 2500 and 5000 mcg/mL.

Basis of concentration selection: Exception: the 156 mcg/mL dose was omitted from the second duplicate with S9. A preliminary dose-range finding study was not indicated in the study report. The Sponsor indicated that the highest concentration of test-article used (5000 mcg/mL) is the maximum required by the OECD and ICH Guidelines and that the test article concentrations were separated by factors of 2.

Negative control: DMSO

Positive control: - S9; 4 and 21 hours: N-ethyl-N-nitrosourea (50 mcg/mL)
+ S9; 3 hours: 7,12 -dimethyl-1 ,2-benzanthracene (3.3 mcg/mL)

Formulation/Vehicle: DMSO

Incubation & sampling time: Duplicate cultures were used for each treatment. For cell cultures tested in the absence of S9 mix, cells (4.5×10^6) were suspended in an appropriate volume of normal medium and the vehicle (100 mL), test article solution (100 mL), or positive control solution (50 mL) were added. The cultures were incubated at 37°C with shaking for 4 or 24 hours in a final volume of 10 mL.

For cell cultures tested in the presence of S9 mix, cells (4.5×10^6) were suspended in an appropriate volume of normal medium and the vehicle (50 mL), test article solution (50 mL), or positive control solution (17 mL), and S9 mix (1 mL) were added. The cultures were incubated at 37°C with shaking for 3 hours in a final volume of 5 mL.

After the treatment periods, cultures were centrifuged and the supernatant replaced with fresh complete medium (15 mL per culture). Resuspended cultures were transferred to flasks and incubated for a period of 3 days (counted from the start of the experiment). Cell densities were adjusted daily to a density of 3×10^5 cells/mL. After the 3-day incubation,

aliquots of cultures were plated in medium containing trifluorothymidine for determination of mutant counts and cloning efficiency. Plates were incubated for 10 days and the mutant colonies were counted to determine mutant frequency. Large and small colonies were counted separately since large colonies have small changes to DNA (e.g., point mutations) whereas small colonies have larger defects such as chromosome aberrations. Large colonies were categorized as being diffuse and covering >50 % of the base of the culture well, whereas small colonies were more dense and covered < 25% of the well.

Study Validity

The study is valid based upon the following criteria: 1) the mutation frequencies of the negative and positive controls were within the range of the historical control data, 2) appropriate positive controls were used, which induced significant increases in mutation frequencies compared to the concurrent negative controls, 3) an appropriate rodent cell line was used (L5178Y), 4) the highest concentration of test article used in the study (5000 mcg/mL) is the maximum required by OECD and ICH guidelines, and 5) at least 3 analyzable concentrations were used.

According to the protocol, the test article would be considered to have shown mutagenic activity if the following criteria were met: statistically significant, dose-dependent and reproducible increases in mutant frequency in treated cultures compared to negative controls, at least 10% relative growth of the treated group at the end of the treatment period, and mean mutation frequency of at least 125 mutants/10⁶ cells higher in treated groups as compared to the negative control. The criteria for evaluation of mutagenic activity are considered valid.

Results

Glycerol dioleate was not toxic in the absence or presence of S9 (no reductions in relative total growth). Positive control cultures exhibited clear increases in mutation frequency. Mutation frequencies of negative and positive control cultures were within or close to the historical control range from the previous 10 studies performed by the testing laboratory and are considered acceptable. The test-article did not induce statistically significant increases in mutation frequency under the assay conditions.

In conclusion, glycerol dioleate was negative for mutagenic activity in this *in vitro* mammalian cell gene mutation assay with mouse L5178Y cells.

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In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Glycerol Dioleate: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow: 0 h and 24 h Subcutaneous Injection Dosing and 48 h Sampling

Study no: TO-12-446
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 9/11/12
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Glycerol dioleate, Lot 3002413, purity NR

Key Study Findings

- The criteria for a valid assay were met. Animals were adequately dosed according to a limit dose of 2000 mg/kg.
- Glycerol dioleate was negative for inducing the formation of micronuclei in the bone marrow erythrocytes of male and female rats after 2 subcutaneous doses of up to 2000 mg/kg, 24 hours apart.

Methods

Doses in definitive study: 0 and 24 hours: 0, 500, 1000, and 2000 mg/kg
 Frequency of dosing: Twice (one dose at 0 hours and one dose at 24 hours)
 Route of administration: Subcutaneous injection
 Dose volume: 0.53, 1.06, and 2.12 mL/kg for 500, 1000, and 2000 mg/kg doses, respectively and 2.12 and 10 mL/kg for negative and positive controls, respectively
 Formulation/Vehicle: Glycerol dioleate, sterile (GDO)
 Species/Strain: Wistar (Han) rats (CrI:WI(Han))
 Approximately 6 weeks of age at study start
 Males: 156.3-189.7 g, Females: 120.2-153.7 g
 Number/Sex/Group: Glycerol dioleate and negative control (saline) groups: 5/sex/group
 Satellite groups: Positive control group n = 3/sex
 Basis of dose selection: A preliminary dose-range finding study was conducted in which male and female rats (1/sex) were dosed subcutaneously with 2000 mg/kg glycerol dioleate in a dose volume of 2.12 mL/kg, at 0 and 24 hours. Rats were observed for clinical signs and mortality for up to 3 days after the second dose. No signs of toxicity were observed and there were no mortalities during

the 3-day observation period. Therefore, the Sponsor considered the maximum recommended dose of 2000 mg/kg acceptable for the micronucleus test based upon limit dose recommended in OECD guidelines.

Negative control: Saline, dosed at the same time point as the test article

Positive control: Cyclophosphamide 50 mg/kg, dosed at the same time points as the test article. N = 3/sex.

Study Validity

For the micronucleus test, Wistar rats (5/sex/group) were administered glycerol dioleate subcutaneously at doses of 0 (negative control, saline), 500, 1000, and 2000 mg/kg at 0 and 24 hours. A separate group of rats (3/sex) was dosed with the positive control (50 mg/kg cyclophosphamide) at the same time points. The doses of glycerol dioleate, positive, and negative controls were appropriate for the test to be considered valid. Bone marrow was collected from the femur of each rat 48 hours after the first dose and bone marrow smears were prepared for examination according to an established protocol. Bone marrow slides were examined by blinded operators. At least 2000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei and the frequency of micronucleated cells (MN-PCE). The PCE/NCE (normochromatic) ratio was determined by counting a minimum total of 1000 erythrocytes (PCE + NCE) per marrow preparation to assess potential systemic toxicity. The assay was considered valid based upon the following criteria: 1) administration of the test-article at the limit dose twice (24 hours apart) with sample collection occurring 24 hours after the second dose, and administration of negative and positive controls at the same time points, 2) the species (male and female Wistar rats) and the number of animals per group for the test-article (n = 5/sex), 3) a total of 2000 PCE per animal were examined for presence of micronuclei, 4) mean values of MN-PCE for negative controls were within the expected historical control range for the Wistar rats in the testing laboratory, and 5) the positive control (cyclophosphamide) exhibited an appropriate response in significantly increasing the number of MN-PCE. All criteria for a valid assay were met.

The test item was judged positive if an increase in the number of micronucleated polychromatic erythrocytes (MN-PCE) was obtained for one or more of the test item-treated dose groups (an increase greater than 10% over the expected historical control ranges for a group of animals). A negative result was indicated if no evident increases in the number MN-PCE relative to historical control frequencies.

Results

The results of the *in vivo* micronucleus test are summarized in the table below excerpted from the Sponsor's submission). The mean % MN-PCE in the negative control group was within the ranges observed in historical control animals of the same strain from the testing laboratory. The positive control induced strong and clear

increased in % MN-PCE which was accompanied by some bone marrow toxicity, as shown by a suppression of the PCE/NCE ratios as compared to the negative control group.

Animals treated with glycerol dioleate did not show any significant increases in the % MN-PCE and individual and mean values for % MN-PCE were close to or within the historical range for control animals. Some indication of bone marrow toxicity was observed in the high-dose male group, as noted by a suppression of the PCE/NCE ratio, although a large variation within the group precluded a definitive determination of toxicity.

In conclusion, glycerol dioleate administered subcutaneously in rats did not induce genotoxic activity in this *in vivo* rat bone marrow micronucleus test when tested up to a limit dose of 2000 mg/kg.

Summary of results from the *in vivo* rat micronucleus assay (table excerpted from Sponsor's submission)

Treatment	Dose (h)	Sex	No. of Rats Scored	Erythrocytes				PCE/NCE Mean ± S.D.
				Normochromatic Cells (NCE)	Polychromatic Cells (PCE)			
					No. of MN-NCE	PCE Analysed	No. of MN-PCE	
Saline (2.12 mL/kg/day)	0 + 24	M	5	2	10002	5	0.05	0.81 ± 0.12
		F	5	1	10005	4	0.04	0.84 ± 0.14
		M/F	10	3	20007	9	0.04	0.83 ± 0.12
Glycerol Dioleate (500 mg/kg/day)	0 + 24	M	5	0	10005	5	0.05	0.83 ± 0.08
		F	5	4	10002	2	0.02	0.86 ± 0.07
		M/F	10	4	20007	7	0.03	0.84 ± 0.07
Glycerol Dioleate (1000 mg/kg/day)	0 + 24	M	5	0	10006	1	0.01	0.80 ± 0.13
		F	5	1	10002	1	0.01	0.83 ± 0.11
		M/F	10	1	20008	2	0.01	0.81 ± 0.11
Glycerol Dioleate (2000 mg/kg/day)	0 + 24	M	5	4	10000	4	0.04	0.66 ± 0.16
		F	5	0	10002	3	0.03	0.81 ± 0.15
		M/F	10	4	20002	7	0.03	0.74 ± 0.16
Cyclophosphamide (50 mg/kg/day)	0 + 24	M	3	8	6005	98	1.63 Φ	0.66 ± 0.03
		F	3	16 α	6004	102	1.70 Φ	0.61 ± 0.03
		M/F	6	24	12009	200	1.67 Φ	0.63 ± 0.04

PCE = Polychromatic erythrocytes
MN-PCE = Micronucleated PCE

NCE = Normochromatic erythrocytes
MN-NCE = Micronucleated NCE
Φ = Positive response in PCE
α = Evident response in NCE

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CARCINOGENICITY

The Applicant did not conduct carcinogenicity studies for DGO. Rather, they referenced two published studies with DAG in mice and rats (Chengelis et al., 2006b, a). These publications were reviewed by R. Daniel Mellon, PhD.

Study Title: Chengelis CP, Kirkpatrick JB, Bruner RH, Freshwater L, Morita O, Tamaki Y, Suzuki H (2006a) A 24-month dietary carcinogenicity study of DAG (diacylglycerol) in rats. Food Chem Toxicol 44:98-121.

In the published GLP rat study intended primarily to compare the effects of dietary triglycerides (TG) to diacylglycerides (DAG) on carcinogenicity, rats were treated with diets supplemented with TG and DAG for 2 years as per the following table from the publication:

Figure 5. Treatment Groups; 2-year Rat Carcinogenicity Study DAG (Chengelis et al., 2006a)

Experimental design of a 24-month dietary carcinogenicity study of DAG in rats

Group	Treatment	Dietary fat/oil level			Number of animals	
		Standard (%) ^a	TG (%) ^b	DAG (%) ^b	Male	Female
<i>Dietary optimization groups</i>						
1	Control-1	4.5	–	–	50	50
2	Control-2	–	5.5	0	50	50
3	DAG-low	–	4.5	1	50	50
4	DAG-mid	–	2.75	2.75	50	50
5	DAG-high	–	0	5.5	50	50
<i>Ad libitum groups</i>						
6	Control-3	–	5.5	0	65	65
7	DAG-high	–	0	5.5	65	65

^a Diet was standard basal diet (PMI Certified Rodent Diet 5002) with 4.5% fat content.

^b Diet was WIL PMI Certified Rodent Diet 7247/1004740 with 5.5% fat content.

The composition of the oils tested are depicted below from the publication. As noted below, both the DAG and the TG groups contained comparable levels of oleic acid unsaturated fatty acids.

Figure 6. Fat Composition; 2-year Rat Carcinogenicity Study DAG (Chengelis et al., 2006a)

Composition of DAG and TG oils used on a dietary 24-month carcinogenicity study of DAG in rats

	DAG	TG
Monoglycerides (%)	0.90–2.90	0–1.08
Diglycerides (%)	82.85–83.94	0.57–3.16
Triglycerides (%)	12.77–15.18	95.32–98.35
Saturated fatty acids (%)		
Palmitic C16:0	2.89–3.04	5.23–5.62
Stearic C18:0	1.07–1.16	2.03–2.15
Unsaturated fatty acids (%)		
Oleic C18:1	35.62–37.60	32.77–34.11
Linoleic C18:2	47.71–48.62	47.72–49.12
Linolenic C18:3	6.31–6.49	6.98–8.26

Based on the composition of the DAG and TG oils used and food consumption, as reported in the publication, the dose of DAG in this study was determined, as depicted in the table below:

Figure 7. Mean DAG Consumption; 2-year Rat Carcinogenicity Study (Chengelis et al., 2006a)

Average test article consumption during a 24-month dietary carcinogenicity study of DAG in rats

Group	Regimen	Dietary fat/oil level		Average calculated DAG consumption (mg/kg/day)	
		TG (%)	DAG (%)	Males	Females
3	Restricted	4.5	1	356.2	477.5
4	Restricted	2.75	2.75	984.8	1326.2
5	Restricted	0	5.5	1982.4	2645.1
7	Ad libitum	0	5.5	1946.3	2507.2

Animals that were dosed with DAG/TG diets demonstrated lower survival, higher body weights and organ weights, changes in clinical chemistry, and increased incidence pituitary and mammary gland tumors compared to controls. All of these findings are consistent with a high fat diet. The authors conclude that DAG was no different than triglycerides with respect to tumor risk. The incidence of tumors in the study are depicted in the table below from the publication. The authors report that none of the neoplastic findings were statistically significant for common tumors. They employed statistical methods in accordance with published FDA guidelines and analyzed combined tumor types in accordance with McConnell (McConnell et al., 1986), consistent with current Agency practice on combining tumor incidence.

Figure 8. Tumor Incidence; 2-year Rat Carcinogenicity Study DAG (Chengelis et al., 2006a)

Incidence of primary neoplastic microscopic findings in a 24-month dietary carcinogenicity study of DAG in rats: all necropsies combined

	Group													
	0% DAG/ 0% TG ^a		0% DAG/ 5.5% TG ^b		1% DAG/ 4.5% TG ^b		2.75% DAG/ 2.75% TG ^b		5.5% DAG/0% TG ^b		5.5% TG ad lib ^b		5.5% DAG ad lib ^b	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
<i>Mammary gland</i>														
Number examined	36	47	26	48	18	47	29	47	26	47	24	60	31	63
Adenoma	0	0	0	1	0	1	0	3	0	1	0	4	0	2
Adenoma, multiple	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fibroadenoma	0	18	0	14	0	15	0	7	1	13	1	15	1	13
Fibroadenoma, multiple	0	4	0	3	0	4	0	5	0	4	0	4	0	2
Adenocarcinoma	1	6	0	10	0	9	0	12	1	14	3	13	0	8
Adenocarcinoma, multiple	0	4	0	6	0	6	0	8	0	8	0	4	0	6
Adenoacanthoma	0	0	0	1	0	2	0	2	0	0	0	0	0	0
Adenoacanthoma, multiple	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Overall mammary tumor burden	1	32	0	35	0	39	0	37	2	40	4	41	1	32
Percent affected (%)	3	68	0	73	0	83	0	79	8	85	17	68	3	51
<i>Pituitary gland</i>														
Number examined	48	49	50	50	42	48	41	48	50	50	64	64	64	65
Adenoma	27	37	37	37	26	40	32	39	33	37	49	53	45	56
Carcinoma	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Percent affected (%)	56	76	74	74	62	83	78	83	66	76	77	83	70	86

N = number of rats/sex/group; M = males; F = females.

^a Control group fed standard basal diet with 4.5% fat content on restricted feeding regimen.^b Diet was WIL Rodent Diet 7247/1004740 with 5.5% fat content, Groups 2–5 on restricted feeding regimen, Groups 6–7 ad libitum-fed.

With respect to the relevance of these findings to the safety of CAM2038, it is not clear how the exposure to GDO in humans compares to the estimated doses of GDO via DAG in the rat study above. However, if the q1w formulation were used chronically, and assuming the GDO were to be released ~equally from the injection site to the systemic circulation on any given day, the human would be exposed to $(b) (4)$ mg/m² GDO per day ($(b) (4)$ mg/week = $(b) (4)$ mg/60 kg/day x $(b) (4)$ = $(b) (4)$ mg/m²/day). The low dose of DAG in males ($(b) (4)$ mg/kg) is $(b) (4)$ mg/m² based on body surface area comparisons. Even if we adjust this dose based on 35.62% oleic acid fatty acid content in the diet, that would be $(b) (4)$ mg/m² oleic acid, which is still roughly $(b) (4)$ times the estimated mean daily dose of GDO in humans.

Study Title: Chengelis CP, Kirkpatrick JB, Bruner RH, Freshwater L, Morita O, Tamaki Y, Suzuki H (2006b) A 24-month dietary carcinogenicity study of DAG in mice. Food Chem Toxicol 44:122-137.

In a companion study to compare the carcinogenic potential of DAG to TG, mice were treated via the diet with up to 6% DAG oil for 2 years. Triglycerides (triacylglycerol oil) was used as the control article for fat intake. A separate control group was given the standard rodent diet (fat content of 4.5%).

Figure 9. Treatment Groups; 2-year Mouse Carcinogenicity Study DAG (Chengelis et al., 2006b)

Experimental design of a 24-month dietary carcinogenicity study of DAG in mice

Group	Treatment	Dietary fat/oil level			Number of animals	
		Standard (%)	TG (%)	DAG (%)	Males	Females
1	Control-1 ^a	4.5	0	0	50	50
2	Control-2 ^b	0	6.0	0	50	50
3	DAG-low ^b	0	4.5	1.5	50	50
4	DAG-mid ^b	0	3.0	3.0	50	50
5	DAG-high ^b	0	0	6.0	50	50

^a Diet was standard basal diet (PMI Certified Rodent Diet 5002) with 4.5% fat content.

^b Diet was WIL PMI Certified Rodent Diet 7247/1004740 with 6.0% fat content.

Figure 10. DAG Composition; 2-year Mouse Carcinogenicity Study DAG (Chengelis et al., 2006b)

Composition of DAG and TG oils used on a 24-month dietary carcinogenicity study of DAG in mice

	DAG	TG
Monoglycerides (%)	0.90–2.90	0–1.08
Diglycerides (%)	82.85–83.94	0.57–3.16
Triglycerides (%)	12.77–15.18	95.32–98.35
Saturated fatty acids (%)		
Palmitic C16:0	2.89–3.04	5.23–5.62
Stearic C18:0	1.07–1.16	2.03–2.15
Unsaturated fatty acids (%)		
Oleic C18:1	35.62–37.60	32.77–34.11
Linoleic C18:2	47.71–48.62	47.72–49.12
Linolenic C18:3	6.31–6.49	6.98–8.26

Figure 11. DAG Consumption; 2 -year Mouse Carcinogenicity Study (Chengelis et al., 2006b)

Average DAG consumption during a 24-month dietary carcinogenicity study of DAG in mice

Group	Dietary fat/oil level		Average calculated DAG consumption (mg/kg/day)	
	TG (%)	DAG (%)	Males	Females
3	4.5	1.5	1792	2509
4	3.0	3.0	3773	5286
5	0	6.0	7412	9796

The results of the study report that the effects of the DAG diet were not substantially different than the effects of the TG diet. Both higher fat diets decreased survival, increased body weights, lowered food consumption, and increased macroscopic and microscopic findings consistent with a high fat diet. As noted in the table below, there was an increased incidence of liver tumors in male mice; however, the authors attributed this to the increased lipid intake rather than specifically to DAG. It is not clear from the publication if these changes were statistically significant or not. The publication reports that the historical control incidence in the laboratory for male hepatocellular adenomas and carcinomas is 8% and 3%, respectively.

Figure 12. Tumor Incidence; 2-year Mouse Carcinogenicity Study DAG (Chengelis et al., 2006b)

Incidence of neoplasms in mice given diets containing 6.0% fat (DAG and/or TG) or the standard diet (4.5% fat) for 24 months

Finding	Group									
	0% DAG/0% TG ^a		0% DAG/6.0% TG ^b		1.5% DAG/4.5% TG ^b		3.0% DAG/3.0% TG ^b		6.0% DAG/0% TG ^b	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Liver										
Number examined	50	50	50	50	42	41	38	41	50	50
Hepatocellular adenoma	0	1	3	1	4	4	1	0	6	2
Hepatocellular carcinoma	2	1	8	0	11	2	6	0	4	0
Systemic										
Number examined	50	50	50	50	50	50	50	50	50	50
Histiocytic sarcoma	1	7	0	0	0	2	0	7	0	4

^a Diet was standard basal diet (PMI Certified Rodent Diet 5002) with 4.5% fat content.

^b Diet was WIL PMI Certified Rodent Diet 7247/1004740 with 6.0% fat content.

With respect to the relevance of these findings to the safety of CAM2038, it is not clear how the exposure to GDO in humans compares to the estimated doses of GDO via DAG in the mouse study above. However, if the q1w formulation were used chronically (worst-case exposure), and assuming the GDO were to be released ~equally from the injection site to the systemic circulation on any given day, the human would be exposed to (b) (4) mg/m² GDO per day ((b) (4) mg/week = (b) (4) mg/60 kg/day x (b) (4) = (b) (4)

mg/m²/day). The low dose of DAG in males ($(b) (4)$ mg/kg) is $(b) (4)$ mg/m² based on body surface area comparisons. Even if we adjust this dose based on 35.62% oleic acid fatty acid content in the diet, that would be $(b) (4)$ mg/m² oleic acid, which is still roughly $(b) (4)$ times the estimated mean daily dose of GDO in humans.

Conclusions of the Carcinogenicity Data. Although no carcinogenicity studies for GDO have been completed, it is a diglyceride and should be metabolically cleared via typical fat metabolic pathways. The data with DAG provided does suggest the potential for typical increase in tumors consistent with a high fat diet and do not suggest anything unexpected with DAG. Although a surrogate molecule for GDO, the two test articles would likely be cleared in a similar manner and the DAG oil tested does contain a high levels of oleic acid fatty acids making it a reasonable surrogate for GDO. Given the relatively low daily levels and rotating sites of administration compared the dietary levels the animals were exposed to, the risk of any tumor from the GDO appears minimal to none. A waiver for dedicated carcinogenicity studies, consistent with the parameters outlined in the FDA guidance to industry on novel excipients is warranted (i.e., negative genetic toxicology data, limited systemic exposure, absence of accumulation, negative histopathology data from chronic toxicology studies, and knowledge of other excipients in the same class).

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

The Applicant conducted one GLP pre- and post-natal developmental toxicity study for GDO. To address the remaining portions of the standard battery of reproductive and developmental toxicity studies for GDO, they are relying upon publications for the surrogate DAG oil (Morita et al., 2008a, Morita et al., 2008b).

Note: This published literature study is a combined Fertility and Early Embryonic Development study and Pre- and Post-Natal Development study

Study title: Morita O, Knapp JF, Tamaki Y, Nemec MD, Varsho BJ, Stump DG (2008a) Safety assessment of dietary diacylglycerol oil: a two-generation reproductive toxicity study in rats. Food Chem Toxicol 46:3059-3068.

Study report location:	N/A
Conducting laboratory and location:	Global R&D Safety Science, Kao Corporation, Japan WIL Research Laboratories, LLC, Ashland, OH, United States
Date of study initiation:	Unknown
GLP compliance:	Yes
QA statement:	Not reported
Drug, lot #, and % purity:	The article states: DAG oil was prepared by esterifying glycerol with free fatty acids from rapeseed oil and soybean oil using the method

reported by Huge-Jensen et al. (1988). The fatty acid composition of DAG oil is described in the table below. For dose calculation purposes, the test material was considered to be 100% pure, and 0.9260 g/mL was used as the specific gravity. The test material was stored at room temperature and protected from light.

Composition of DAG and TAG oil used on a two-generation reproductive toxicity study in rats

	DAG oil	TAG oil	Corn oil ^a
Monoglycerides (%)	2.16	0.17	–
Diglycerides (%)	86.06	6.13	2.8
Triglycerides (%)	9.91	91.09	95.8
Saturated fatty acids (%)			
Palmitic C16:0	3.05	5.60	9.2–16.5
Stearic C18:0	1.13	2.19	0–3.3
Unsaturated fatty acids (%)			
Oleic C18:1	39.53	35.19	20–42.2
Linoleic C18:2	46.36	47.50	39.4–65.6
Linolenic C18:3	6.21	7.07	0.5–1.5

^a D'alanzo et al. (1982) and Firestone (2006).

Key Study Findings

- The objective of this fertility/two-generation study was to evaluate potential adverse effects of DAG oil on reproductive processes. DAG oil was administered via gavage to rats (30/sex/group) for at least 70 days prior to mating, at dose levels of 0, 1.25, 2.5 or 5.0 mL/kg/day (0, 1160, 2320 and 4630 mg/kg/day). The rats were treated throughout the mating, gestation and lactation periods.
- Administration of DAG oil did not reveal any toxicologically significant effects on reproductive performance (mating, fertility and copulation/conception indices). DAG oil did not affect mean gestation lengths, the process of parturition, spermatogenic parameters, organ weights, histopathologic findings, mean numbers of pups born, implantation sites, and unaccounted sites. F2 pup viability, live litter sizes, body weights, mean age of attainment of balanopreputial separation and vaginal patency were similar to those in the control group. F1 pup viability may have been reduced from PND 0-1 but was within control range and unaffected at the low dose DAG group and in all F2 pups.
- Based on the results of this study, a dose level of 1.25 mL/kg/day (1160 mg/kg/day, HED of 186 mg/kg/day) was considered as the no-observed-adverse-effect level for fertility and early embryonic development and pre-

and post-natal development with the only possible effect as reduced F1 pup viability for PND0-1. At 40% oleic acid, the component of glycerol dioleate (GDO), the dose of GDO could be at most 464 mg/kg/day (2784 mg/m²). However, the actual levels are not known and the diacylglycerol composition could be heterogenous.

- **NOTE:** A considerable amount of data was not included in this literature study, but what was reported supports the high dose NOAEL for DAG oil which likely contains some GDO for fertility and embryo-fetal development and for pre- and post-natal development.

Methods

Doses:	0, 1.25, 2.5, or 5.0 mL/kg/day (0, 1160, 2320 and 4630 mg/kg/day) with total volume made to 5 mL/kg/day with triacylglycerol (corn) oil.
Frequency of dosing:	- F0 animals were doses 70 days prior to pairing and continuing throughout the mating, gestation and lactation periods until euthanasia. - The offspring of the F0 generation (F1 litters) were potentially exposed in utero, through nursing during lactation (postnatal days [PND] 0–21) and orally by gavage following weaning (beginning on PND 22). - The F1 pups selected for breeding (30 rats/sex/group) were dosed for a minimum of 70 days prior to pairing and throughout mating, gestation and lactation as described for the F0 generation. - The offspring of the F1 generation (F2 litters) were potentially exposed in utero and through nursing during lactation (PND 0–21).
Dose volume:	5 mL/kg/day
Route of administration:	Oral gavage
Formulation/Vehicle:	Corn oil
Species/Strain:	Sprague-Dawley CrI:CD (SD)IGS BR rats
Number/Sex/Group:	F0 rats to each treatment group (n = 30 rats/sex/group)
Satellite groups:	None
Study design:	Combined Fertility and Early Embryonic Development study and Pre-and Post-Natal development study
Deviation from typical study review:	As this was a literature study, review of supporting data sets are not available for review. Summary tables were reviewed to confirm report assessments and conclusions.

Observations and Results

Mortality

One F0 male was found dead and one F0 male in the 50% DAG oil group (i.e., 2.5 mL/kg/day) was euthanized in extremis. Two, one, and one F1 males in the 0% DAG oil, 50% DAG oil and 100% TAG oil groups were found dead, respectively. One and two F1 females in the 0% and 25% DAG oil groups were euthanized in extremis. No relationship to treatment was reported evident for the rats found dead or euthanized, and no deaths were observed in either sex at both generations in the 100% DAG group (data not shown).

Clinical Signs

No treatment-related clinical signs were detected in males and females in either generation that survived to scheduled euthanasia (data not shown).

Body Weight (F0 and F1 females and males)

During the pre-mating treatment interval, F0 and F1 body weight was measured weekly. Following evidence of mating, female body weight was recorded on Gestation Days (GD) 0, 4, 7, 11, 14, and 20 and on Lactation Days (LD) 1, 4, 7, 14, and 21.

No DAG oil related effects on mean F0 or F1 weekly body weights, body weight gains and cumulative body weight gains were noted for either sex in the 25%, 50% and 100% DAG oil groups for the pre-mating period F0 (Fig. 1) and F1 (Fig. 3) generation females or F0 (Fig. 2) and F1 (Fig. 4) generation males.

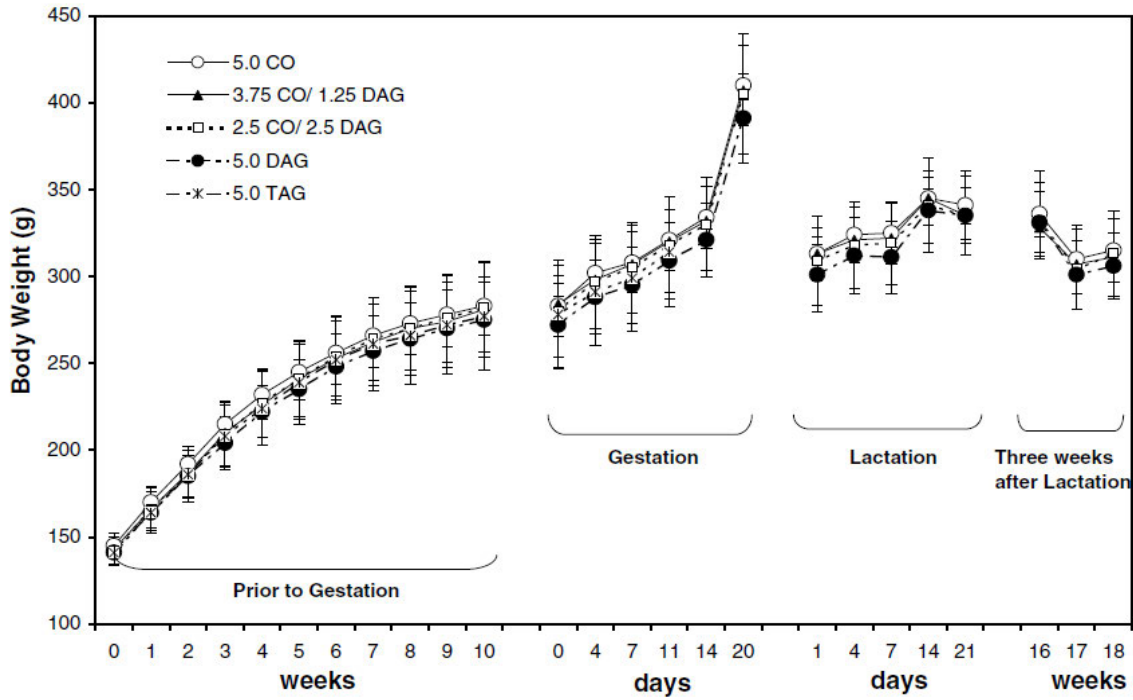


Fig. 1. Mean F₀ female body weights prior to gestation, during gestation and lactation, and three weeks after lactation.

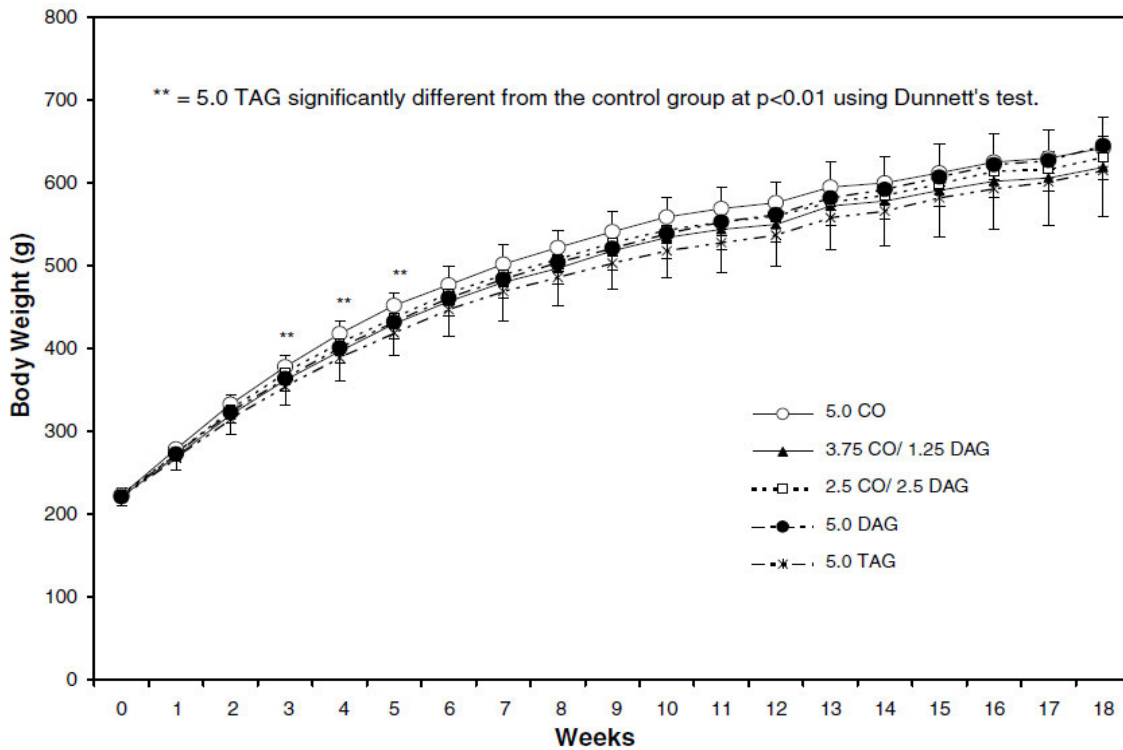


Fig. 2. Mean F₀ male body weights on a two-generation reproductive toxicity study of DAG oil in rats.

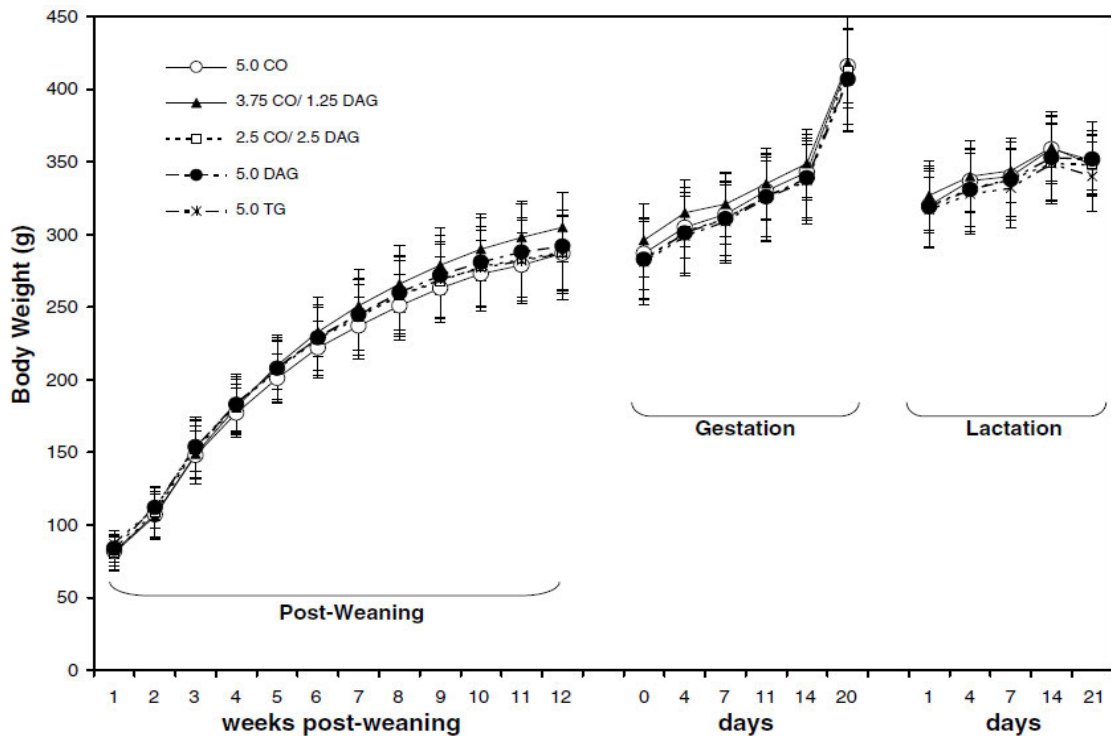


Fig. 3. Mean F₁ female body weights prior to mating, during gestation and lactation.

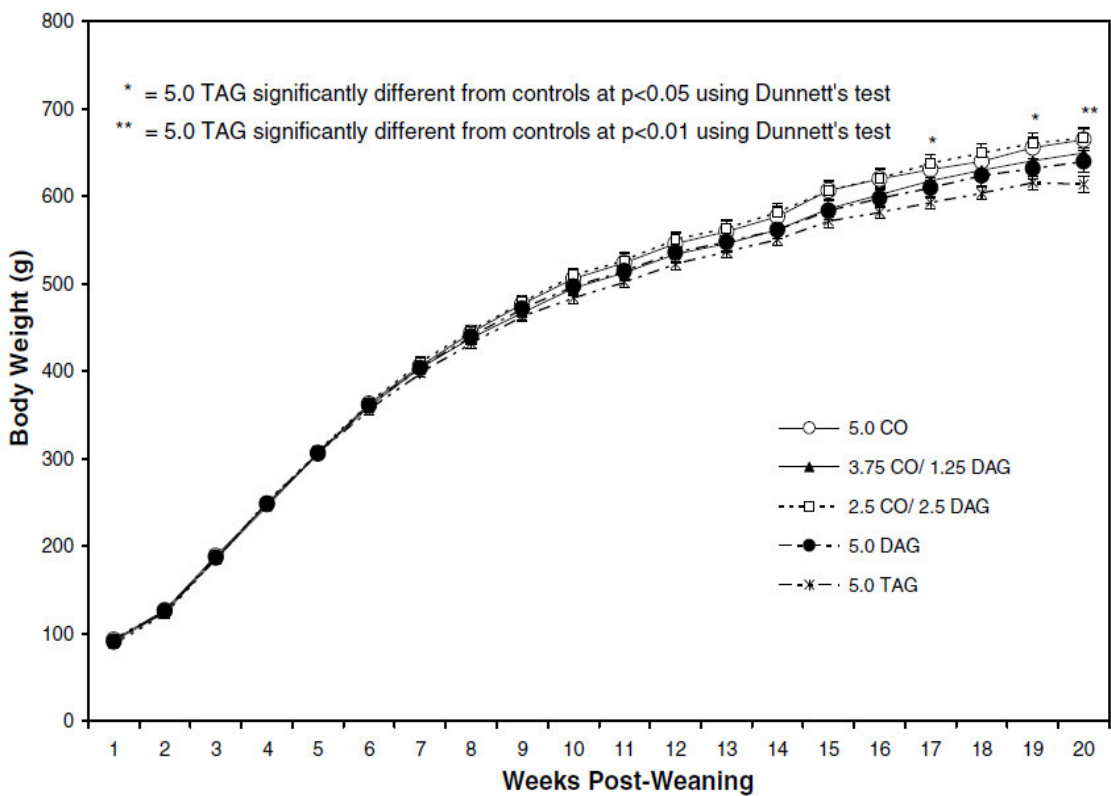


Fig. 4. Mean F₁ male body weights on a two-generation reproductive toxicity study of DAG oil in rats.

Food Consumption (F0 and F1 females and males)

During the pre-mating treatment interval, F0 and F1 food consumption was measured weekly. Following evidence of mating, female food consumption was recorded on Gestation Days (GD) 0, 4, 7, 11, 14, and 20 and on lactation days (LD) 1, 4, 7, 14, and 21.

Mean F0 and F1 weekly food consumption and food efficiency in the DAG oil-treated males and females were reported to be unaffected by test article administration (data not shown).

Toxicokinetics – none

Dosing Solution Analysis – not reported

Necropsy (Gross and Histopathology)

All surviving F0 and F1 parental animals were euthanized following weaning of the F1 and F2 litters, respectively. All surviving unselected F1 pups and all F2 pups were euthanized by carbon dioxide inhalation on PND 21. A complete necropsy was done on all parental animals (F0 and F1) dying spontaneously, euthanized in extremis or surviving until scheduled euthanasia. The necropsy included macroscopic examination of external surfaces, all orifices, the cranial cavity, external surfaces of the brain and spinal cord, and the thoracic, abdominal and pelvic cavities including viscera. The following tissues were preserved from all F0 and F1 animals and examined microscopically from all animals that were found dead or euthanized in extremis and from 10 rats/sex/group in the control (0% DAG oil group) and high dose group (100% DAG oil group) that survived to the scheduled euthanasia: adrenal glands, brain, cervix, coagulating glands, epididymis (right), kidneys, liver, ovaries, oviducts, pituitary gland, prostate, seminal vesicles, spleen, testis (right), thymus, uterus, vagina, vas deferens and all gross lesions. A quantitative histopathologic evaluation of five sections from the inner third of each ovary from 10 control and high-dose group F0 and F1 females was performed to enumerate the total number of primordial follicles and corpora lutea.

No DAG or TAG oil-related macroscopic or microscopic findings were observed for the parental animals in either generation.

Study-Specific Observations

Upon completion of parturition, litters were sexed, pups were examined for gross abnormalities, and the numbers of live and dead pups were recorded. All pups were identified using special markings on PND 0. Litters were culled to eight pups (four/sex, when possible) on PND 4. Pups were examined daily for survival. Each pup was weighed and received a detailed physical examination on PND 1, 4, 7, 14, and 21. Intact pups dying from PND 0 to 4 were necropsied using a fresh dissection technique.

A detailed gross necropsy was performed on all pups dying between PND 5 and weaning.

At weaning on PND 21, 30 pups/sex/group (minimum of one pup/sex/litter) were randomly selected to comprise the F1 generation. These pups were administered DAG oil/corn oil formulations or TAG oil beginning on PND 22. Each selected male pup was observed for balanopreputial separation beginning on PND 35 (separation of the prepuce from the glans penis). Each selected female pup was observed for vaginal patency beginning on PND 25. Examinations for both the males and females continued daily until a positive finding was recorded. The body weight of the weanling was recorded on the day of attainment of the developmental landmark.

All surviving F0 and F1 males were euthanized following the weaning of the F1 and F2 pups, respectively. Immediately upon euthanasia, the reproductive tract of each F0 and F1 male used for breeding was exposed by a ventral mid-line incision. The right epididymis was excised and weighed, and an incision was made in the distal right cauda epididymis. The right cauda epididymis was then placed in Dulbecco's phosphate buffered saline (maintained at approximately 37°C) with 10 mg/mL bovine serum albumin. After a 10-min incubation, sperm motility was determined. Where possible, at least 200 spermatozoa (motile and non-motile) from each male were analyzed. Sperm morphology was evaluated for all F0 and F1 males by light microscopy. Abnormal forms of sperm (double heads, double tails, microcephalic, or megacephalic, etc.) from a differential count of 200 spermatozoa per animal were recorded, if possible. The left testis and epididymis from all F0 and F1 males were weighed, homogenized and evaluated for sperm numbers.

F0 and F1 males

In the F0 males, several statistically significant differences were observed in mean absolute and/or relative left epididymis, right testis, and seminal vesicle/coagulating gland weights compared to the 0% DAG oil group males (Table 2 from publication below). These changes were not considered DAG or TAG oil-related because of the lack of dose relationship, lack of consistency between left and right organs and lack of correlating macroscopic and microscopic changes in these organs.

Table 2
Organ weights for F₀ males on a two-generation reproductive toxicity study of DAG oil in rats

Organ	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
<i>Absolute (g)</i>					
Brain	2.20 ± 0.075	2.15 ± 0.083	2.16 ± 0.097	2.14 ± 0.127	2.12 ± 0.099**
Pituitary gland	0.0154 ± 0.00217	0.0146 ± 0.00212	0.0149 ± 0.00189	0.0141 ± 0.00178	0.0147 ± 0.00258
Liver	23.76 ± 3.344	22.37 ± 2.532	23.20 ± 3.363	24.79 ± 4.950	23.17 ± 3.583
Kidneys	4.52 ± 0.422	4.33 ± 0.400	4.49 ± 0.391	4.48 ± 0.548	4.24 ± 0.412
Seminal vesicles/coagulating gland	2.42 ± 0.373	2.19 ± 0.310*	2.33 ± 0.378	2.17 ± 0.297**	2.26 ± 0.217
Prostate	1.00 ± 0.195	1.10 ± 0.206	1.07 ± 0.184	1.05 ± 0.158	1.06 ± 0.243
Left testis	1.8 ± 0.157	1.87 ± 0.164	1.85 ± 0.163	1.81 ± 0.139	1.83 ± 0.171
Right Testis	1.71 ± 0.320	1.86 ± 0.175	1.82 ± 0.136	1.74 ± 0.250	1.82 ± 0.154
Left epididymis	0.69 ± 0.064	0.72 ± 0.080	0.74 ± 0.055*	0.73 ± 0.067	0.73 ± 0.077*
Right epididymis	0.71 ± 0.074	0.72 ± 0.066	0.74 ± 0.069	0.71 ± 0.079	0.73 ± 0.064
<i>Relative to final body weight (g/100 g)</i>					
Final body weight (g)	642 ± 68.0	621 ± 61.4	632 ± 59.8	648 ± 103.9	617 ± 60.6
Brain	0.346 ± 0.0352	0.349 ± 0.0346	0.344 ± 0.0312	0.337 ± 0.0483	0.346 ± 0.0309
Pituitary gland	0.002 ± 0.0005	0.002 ± 0.0005	0.002 ± 0.0005	0.002 ± 0.0003	0.002 ± 0.0006
Liver	3.694 ± 0.2320	3.603 ± 0.2509	3.658 ± 0.2506	3.815 ± 0.3169	3.745 ± 0.3171
Kidneys	0.707 ± 0.0473	0.700 ± 0.0604	0.713 ± 0.0567	0.698 ± 0.0765	0.689 ± 0.0545
Seminal vesicles/coagulating gland	0.382 ± 0.0752	0.356 ± 0.0633	0.370 ± 0.0659	0.343 ± 0.0652	0.369 ± 0.0415
Prostate	0.157 ± 0.0315	0.178 ± 0.0386	0.171 ± 0.0322	0.166 ± 0.0376	0.173 ± 0.0413
Left testis	0.282 ± 0.0298	0.304 ± 0.0339	0.294 ± 0.0317	0.285 ± 0.0395	0.298 ± 0.0343
Right testis	0.269 ± 0.0553	0.301 ± 0.0341*	0.290 ± 0.0284	0.275 ± 0.0547	0.297 ± 0.0315*
Left epididymis	0.108 ± 0.0125	0.117 ± 0.0166	0.118 ± 0.0126*	0.115 ± 0.0201	0.120 ± 0.0131*
Right epididymis	0.112 ± 0.0142	0.117 ± 0.0129	0.117 ± 0.0103	0.111 ± 0.0202	0.118 ± 0.0108

* Significantly different from the control group at 0.05 using Dunnett's test.

** Significantly different from the control group at 0.01 using Dunnett's test.

Spermatogenic assessment parameters in the F₀ and F₁ males were unaffected by DAG or TAG oil exposure (Table 4).

Table 4
Spermatogenic evaluation of male rats on a two-generation reproductive toxicity study of DAG oil

Parameter	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
<i>F₀ males</i>					
No. rats examined	30	30	28	29	30
Sperm concentration					
Left testis (×10 ⁶ /g)	78.0 ± 10.62	80.6 ± 14.55	76.5 ± 13.22	82.4 ± 9.90	83.5 ± 12.70
Left epididymis (×10 ⁶ /g)	434.7 ± 88.15	450.7 ± 89.28	468.6 ± 94.50	453.5 ± 85.57	480.8 ± 109.46
Motility (%)	88.0 ± 7.3	90.0 ± 5.7	92.0 ± 2.8	89.0 ± 6.3	90.0 ± 4.0
Morphology (% normal)	99.2 ± 3.19	99.7 ± 0.52	99.8 ± 0.42	99.4 ± 1.51	99.5 ± 0.67
<i>F₁ males</i>					
No. rats examined	28	30	28	29	28
Sperm concentration					
Left testis (×10 ⁶ /g)	68.2 ± 13.50	73.5 ± 11.83	72.5 ± 11.67	74.8 ± 11.45	74.1 ± 12.47
Left epididymis (×10 ⁶ /g)	462.6 ± 111.61	483.5 ± 91.53	457.2 ± 123.26	458.6 ± 111.27	444.0 ± 77.46
Motility (%)	82.0 ± 16.9	84.0 ± 5.1	85.0 ± 4.8	85.0 ± 5.3	85.0 ± 5.0
Morphology (% normal)	99.9 ± 0.33	99.9 ± 0.26	99.9 ± 0.28	99.8 ± 0.34	99.4 ± 0.95

None significantly different from the control group.

Fertility Parameters

Reproductive performance parameters for the F₀ generation were unaffected by DAG and TAG oil administration at any dose level (Table 5). Parameters evaluated included mating and fertility indices, estrous cycle lengths, pre-coital intervals, gestation lengths, sex ratios, live litter size, postnatal survival and offspring weights. The only statistically significant differences were slightly lower F₀ mean litter proportions of litter viability during PND 0-1 in the 100% DAG oil group and the 100% TAG oil group (98.8% and 97.2% per litter, respectively) compared to the 0% DAG oil group. The differences were slight, were comparable to the historical control mean (99.0 ± 1.24%) and were higher

than the mean minimum value (94.3% per litter) in the historical control data. In addition, only 5 of 372 pups in the 100% DAG oil group and 10 of 370 pups in the 100% TAG oil group died during PND 0–1 and survival in these groups were not lower than the control group from birth to PND 4. On this basis, the low dose DAG group (1.25 mL/kg) was considered to be unaffected.

Table 5
Reproductive parameters and litter data for F₀ generation on a two-generation reproductive toxicity study of DAG oil in rats

Parameter	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
No. mating pairs	30	30	30	30	30
Precoital interval (days)	2.6 ± 2.18	2.6 ± 1.81	2.8 ± 2.00	2.7 ± 1.96	3.1 ± 2.07
Mating index (%)	100.0	96.7	96.7	100.0	93.3
Fertility index (%)	86.7	80.0	93.3	90.0	90.0
Gestation length (days)	22.0 ± 0.45	22.0 ± 0.47	21.9 ± 0.26	21.9 ± 0.27	22.1 ± 0.55
Total no. pups/litter	14.1 ± 2.84	14.3 ± 2.32	14.4 ± 1.87	13.9 ± 2.01	13.9 ± 1.34
No. live pups/litter	13.8 ± 2.99	14.3 ± 2.42	14.1 ± 1.79	13.8 ± 1.95	13.7 ± 1.49
Sex ratio (% per litter)	53.4 ± 15.48	49.5 ± 11.37	53.5 ± 9.61	49.3 ± 13.14	49.6 ± 14.17
Pup survival (% per litter)					
PND 0	98.0 ± 4.55	99.3 ± 2.38	98.3 ± 6.48	99.5 ± 1.68	98.6 ± 3.53
PND 0–1	100.0 ± 0.00	100.0 ± 0.00	99.3 ± 2.20	98.8 ± 2.63*	97.2 ± 5.66**
PND 1–4 (precull)	99.4 ± 2.11	100.0 ± 0.00	98.1 ± 4.42	99.1 ± 2.88	99.5 ± 1.84
PND 0–4 (precull)	97.4 ± 5.21	99.3 ± 2.38	95.7 ± 7.73	97.4 ± 4.57	95.4 ± 7.50
PND 4 (postcull)–21	98.3 ± 5.04	99.5 ± 2.55	97.8 ± 6.85	97.7 ± 7.78	97.7 ± 9.83

* Significantly different from the control group at 0.05 using Dunnett's test.

** Significantly different from the control group at 0.01 using Dunnett's test.

Reproductive performance parameters for the F₁ generation was unaffected by DAG and TAG oil administration at all dose levels (Table 6).

Table 6
Reproductive parameters and litter data for F₁ generation on a two-generation reproductive toxicity study of DAG oil in rats

Parameter	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
No. mating pairs	30	30	30	30	30
Precoital interval (days)	3.5 ± 2.01	3.5 ± 2.81	2.6 ± 1.05	3.5 ± 2.56	3.6 ± 2.53
Mating index (%)	100.0	93.3	96.7	93.3	96.7
Fertility index (%)	89.7	86.7	90.0	90.0	86.7
Gestation length (days)	22.1 ± 0.32	22.2 ± 0.49	22.2 ± 0.37	22.0 ± 0.44	22.0 ± 0.29
Total no. pups/litter	14.3 ± 2.83	13.3 ± 3.45	13.2 ± 3.04	12.5 ± 2.98	13.9 ± 2.01
No. live pups/litter	14.1 ± 2.89	13.0 ± 3.35	12.6 ± 2.94	12.3 ± 3.03	13.7 ± 1.95
Sex ratio (% per litter)	51.6 ± 17.46	46.4 ± 18.52	50.7 ± 13.63	51.2 ± 13.51	53.6 ± 15.47
Pup survival (% per litter)					
PND 0	98.7 ± 4.64	98.3 ± 5.35	96.0 ± 8.23	98.5 ± 4.03	99.0 ± 3.62
PND 0–1	98.9 ± 3.13	99.7 ± 1.31	98.4 ± 6.58	99.7 ± 1.60	95.1 ± 19.96
PND 1–4 (precull)	96.5 ± 14.16	99.3 ± 2.73	95.0 ± 19.47	96.0 ± 19.24	98.8 ± 3.78
PND 0–4 (precull)	94.8 ± 15.58	97.3 ± 5.81	90.8 ± 20.74	94.2 ± 19.31	93.4 ± 20.03
PND 4 (postcull)–21	93.1 ± 22.29	99.0 ± 3.46	99.5 ± 2.45	99.5 ± 2.45	98.4 ± 5.61

None significantly different from the control group.

F₀ Dams

Survival: No treatment-related changes (discussed previously)
 Clinical signs: No treatment-related changes (discussed previously)
 Body weight: No treatment-related changes (see Table 2)
 Food consumption: No treatment-related changes (see Table 3) were observed.
 Organ weights: In the 100% DAG oil group F₀ females, mean absolute and relative (to final

body weight) uterus weights were increased compared to the 0% DAG oil group (Table 3). This increase was attributed to the slightly increased number of females in this group that were in estrus or proestrus at the time of necropsy. Therefore, this change was not considered DAG oil-related.

weights for F₀ females on a two-generation reproductive toxicity study of DAG oil in rats

	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
Uterine weight (g)	2.00 ± 0.102	2.00 ± 0.097	2.02 ± 0.092	1.98 ± 0.091	2.01 ± 0.090
Uterine gland	0.0192 ± 0.00360	0.0179 ± 0.00297	0.0191 ± 0.00357	0.0187 ± 0.00349	0.0181 ± 0.00317
Uterine weight (g)	12.85 ± 1.416	12.49 ± 1.410	12.78 ± 1.519	12.22 ± 1.562	12.60 ± 1.701
Uterine gland	2.39 ± 0.167	2.42 ± 0.322	2.42 ± 0.217	2.40 ± 0.227	2.34 ± 0.214
Uterine weight (g)	0.1629 ± 0.03185	0.1590 ± 0.03256	0.1512 ± 0.02285	0.1535 ± 0.02847	0.1584 ± 0.01751
Uterine gland	0.68 ± 0.182	0.67 ± 0.165	0.78 ± 0.239	0.82 ± 0.270*	0.73 ± 0.246
Uterine weight (g)	317 ± 16.8	314 ± 21.2	316 ± 22.7	310 ± 18.8	314 ± 28.4
Uterine gland	0.634 ± 0.0345	0.641 ± 0.0562	0.641 ± 0.0529	0.643 ± 0.0444	0.644 ± 0.0630
Uterine weight (g)	0.006 ± 0.0010	0.006 ± 0.0010	0.006 ± 0.0012	0.006 ± 0.0011	0.006 ± 0.0010
Uterine gland	4.055 ± 0.3833	3.979 ± 0.3169	4.044 ± 0.3843	3.941 ± 0.3585	4.010 ± 0.3597
Uterine weight (g)	0.755 ± 0.0527	0.771 ± 0.0763	0.768 ± 0.0740	0.777 ± 0.0697	0.749 ± 0.0639
Uterine gland	0.051 ± 0.0095	0.051 ± 0.0098	0.048 ± 0.0081	0.050 ± 0.0094	0.051 ± 0.0078
Uterine weight (g)	0.214 ± 0.0589	0.214 ± 0.0575	0.247 ± 0.0752	0.265 ± 0.0852*	0.233 ± 0.0800

* significantly different from the control group at 0.05 using Dunnett's test.

Uterine content:

Conducted, with no report of adverse effects.

Necropsy

observation: Discussed previously.

Toxicokinetics: None.

F₁ Generation

Survival: Conducted, with no report of adverse effects (no data).

Clinical signs: Conducted, with no report of adverse effects (no data).

Body weight: No apparent effects on pup weight were observed based on Figure 5.

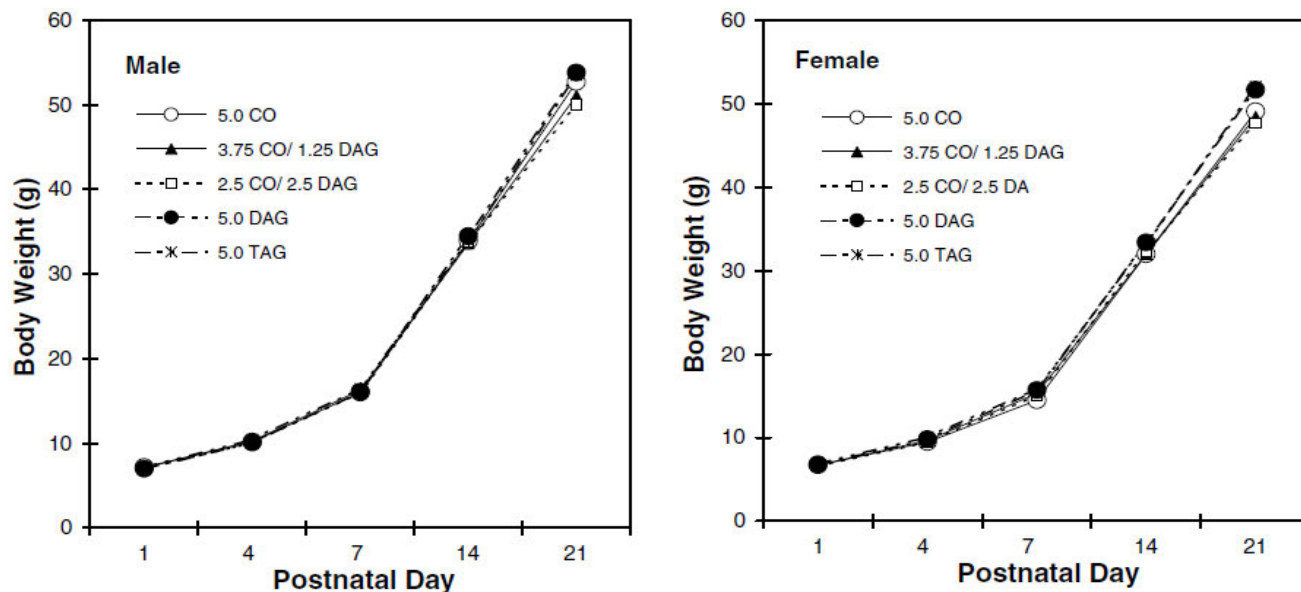


Fig. 5. Male and female F₁ pup body weights on a two-generation reproductive toxicity study of DAG oil in rats.

Food

consumption: Reported previously.

Organ weights: No DAG or TAG oil-related effects on organ weights (absolute and relative to final body weight) in animals of the F₁ generation were observed when the DAG and TAG oil-treated animals were compared to the 0% DAG oil group (data not shown).

Sexual

Development: In F₁ male weanlings, the mean age of attainment of balanopreputial separation and mean body weights on the day of attainment were unaffected by DAG and TAG oil administration (Table 7). In the F₁ female weanlings, the mean age of attainment of vaginal patency and mean body weights on the day of attainment were similar between the control group and the treated groups (Table 7).

Table 7

Developmental landmarks for F₁ male and female rats on a two-generation reproductive toxicity study of DAG oil

Balanoepreputial separation	Male				
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
Day of attainment	44.3 ± 2.70	44.1 ± 2.23	44.5 ± 3.05	43.8 ± 2.05	43.4 ± 2.22
Body weight (g)	228.2 ± 25.92	226.6 ± 19.02	229.1 ± 17.61	224.1 ± 13.87	227.0 ± 17.60
No. rats examined	29	30	30	30	30
Vaginal patency	Female				
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG	5.0 TAG
Day of attainment	34.4 ± 2.28	34.9 ± 2.33	35.4 ± 1.96	34.3 ± 1.80	35.0 ± 2.01
Body weight (g)	123.2 ± 15.20	126.1 ± 18.08	128.3 ± 14.70	124.9 ± 18.62	133.1 ± 16.35
No. rats examined	30	30	30	30	30

None significantly different from the control group.

Neurological
assessment:

Not reported (presumably not conducted).

Reproduction:

No treatment-related effect. See fertility parameters above.

F₂ Generation

Survival:

Conducted, with no report of adverse effects (no data).

Body weight:

Statistically significant increases in male and female F₂ pup body weight gains were noted in all DAG oil-treated groups during PND 1–4 compared to the 0% DAG oil group values (data not shown). However, no dose-related trends were apparent. During PND 4–7, mean male and female pup body weight gains in the 25% DAG oil group were statistically significantly increased compared to the 0% DAG oil group. Mean body weight gains in the DAG oil-treated groups were similar to those in the control group during PND 7–14 and 14–21. The increases in mean body weight gain in the DAG oil-treated groups resulted in higher (often statistically significant) mean F₂ male and female pup body weights compared to the 0% DAG oil group during the pre-weaning period. The greatest differences were reported to be observed on PND 4 (Fig. 6). Mean male pup weights in the 100% DAG oil group were 6.0–12.1%.

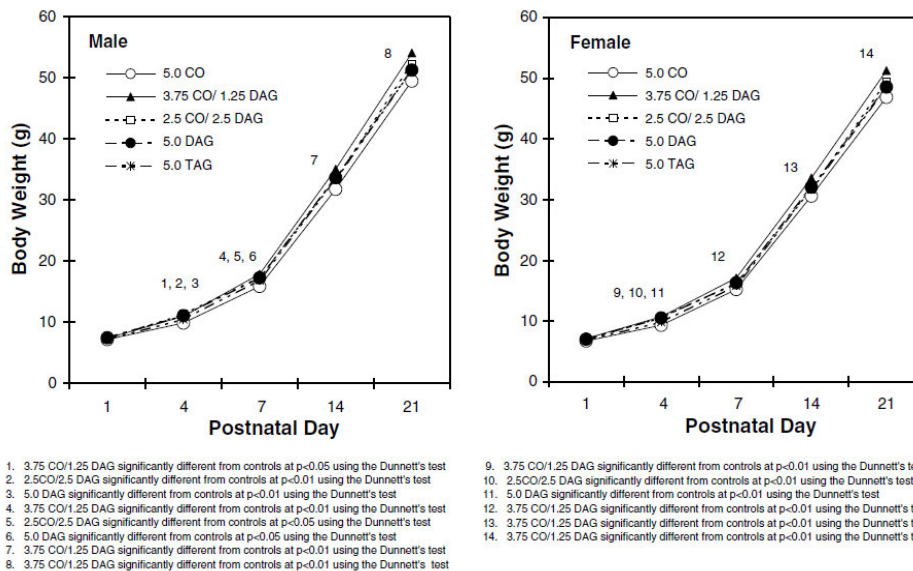


Fig. 6. Male and female F₂ pup body weights on a two-generation reproductive toxicity study of DAG oil in rats.

External evaluation: Conducted, with no report of adverse effects (no data).
 Male/Female ratio: No treatment-related effects (see fertility parameters section above)
 Other: No.

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9.2 Embryonic Fetal Development

NOTE: This study from the published literature was reviewed by Dr. Jay Chang.

Study title: Morita O, Knapp JF, Tamaki Y, Varsho BJ, Stump DG, Nemec MD (2008b) Effects of dietary diacylglycerol oil on embryo/fetal development in rats. Food Chem Toxicol 46:2510-2516.

Study no.: N/A
Study report location: N/A
Conducting laboratory and location: Global R&D Safety Science, Kao Corporation, Japan
 WIL Research Laboratories, LLC, Ashland, OH, United States
Date of study initiation: Unknown
GLP compliance: Yes
QA statement: Not reported
Drug, lot #, and % purity: The article states: DAG oil was prepared by esterifying glycerol with free fatty acids from rapeseed oil and soybean oil using the method reported by Huges-Jensen et al. (1988). The

fatty acid composition of DAG oil is described in the table below. For dose calculation purposes, the test material was considered to be 100% pure, and 0.9260 g/mL was used as the specific gravity. The test material was stored at room temperature and protected from light. DAG oil is reported to contain 40% oleic fatty acid. If the glycerol contains two oleic acid residues it would be glycerol dioleate (GDO).

It is not clear how much GDO is actually in this DAG product as the diacylglycerides may contain any combination of the above fatty acid moieties.

Composition of DAG and TAG oil used on a two-generation reproductive toxicity study in rats

	DAG oil	TAG oil	Corn oil ^a
Monoglycerides (%)	2.16	0.17	–
Diglycerides (%)	86.06	6.13	2.8
Triglycerides (%)	9.91	91.09	95.8
Saturated fatty acids (%)			
Palmitic C16:0	3.05	5.60	9.2–16.5
Stearic C18:0	1.13	2.19	0–3.3
Unsaturated fatty acids (%)			
Oleic C18:1	39.53	35.19	20–42.2
Linoleic C18:2	46.36	47.50	39.4–65.6
Linolenic C18:3	6.21	7.07	0.5–1.5

^a D'alanzo et al. (1982) and Firestone (2006).

Key Study Findings

Pregnant rats were treated with DAG from GD 7-16 with the following key findings:

- No mortality or treatment-related clinical or internal findings were noted in any of the groups. Compared to animals in control group, mean maternal body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights, and food consumption were not affected by DAG oil administration. Intrauterine growth and survival were not affected by DAG oil administration.

- No DAG oil-related fetal malformations or developmental variations were noted. A maternal maximum tolerated dose for DAG oil was not achieved in this study.
- Based on the results of this study, a dose level of 5.0 mL/kg (4630 mg/kg/day) was considered as the NOAEL for both maternal and developmental toxicity. This dose correlates to 27780 mg/m².

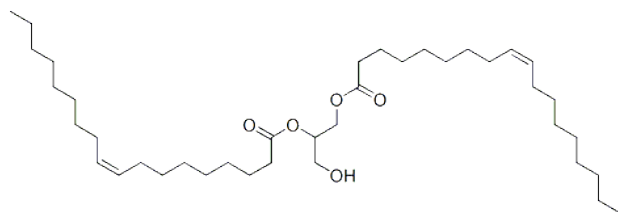
Methods

Doses:	0, 1.25, 2.5 or 5.0 mL/kg/day (0, 1160, 2320 and 4630 mg/kg/day) with total volume made to 5 ml/kg/day with triacylglycerol (corn) oil.
Frequency of dosing:	Once daily on Days 6-17 of gestation
Dose volume:	5 mL/kg/day
Route of administration:	Oral gavage
Formulation/Vehicle:	Triacylglycerol (corn) oil
Species/Strain:	sexually mature Sprague–Dawley Cri:CD®(SD)IGS BR rats
Number/Sex/Group:	25 mated females/group
Satellite groups:	none
Study design:	Standard embryo-fetal protocol
Deviation from typical study review:	As this was a literature study, review of supporting data sets did not occur. Summary tables were reviewed to confirm report assessments and conclusions.

Table 2.6.7.10-2 Comparison of compositions of GDO and the DAG oil used in the studies referenced as supportive for GDO safety

Study Reference	TO-10-405 / TO-12-446	TO-05-153 / TO-05-189 / TO-04-141 / TO-04-142 / TO-05-156 / TO-05-177	TO-04-146	Kasamatsu et al., 2005 (before heating / after heating)	Chengelis et al., 2006a / Chengelis et al., 2006b / Chengelis et al., 2006c	Morita et al., 2008b / Morita et al., 2008c	Soni et al., 2001	Morita et al., 2008a
Acylglycerol distribution (%)								
Monoacyl glycerol	0.2	0.5	4.2	0.5 / 2.7	0.9-2.9	2.2	5 (indicated in text)	No detailed composition provided in the study.
Diacyl glycerol	96.0	95.3	92.1	85.5 / 80.3	82.9-83.9	86.1	75 (indicated in text)	
Triacyl glycerol	4.0	4.0	3.5	14.0 / 17.1	12.8-15.2	9.9	20 (indicated in text)	
Fatty acid composition (%)								
C16:0 (palmitic)	0.3	0.3	0.3	3.1 / 3.2	2.9-3.0	3.1	No detailed composition provided for the indicated study. The DAG oil prepared from high C18:1, C18:2 and C18:3 content raw materials.	No detailed composition provided for the indicated study. The DAG oil prepared from high C18:1, C18:2 and C18:3 content raw materials (soybean and rapeseed oil).
C18:0 (stearic)	2.4	2.4	2.0	1.3 / 1.3	1.1-1.2	1.1		
C18:1 (oleic)	90.0	91.5	91.8	41.1 / 42.3	35.6-37.6	39.5		
C18:2 (linoleic)	5.5	3.5	3.6	45.6 / 44.8	47.7-48.6	46.4		
C18:3 (linolenic)	0.7	0.7	0.3	8.7 / 8.1	6.3-6.5	6.2		
C20:0 (arachidic)	0.0	1.1	0.1	0.3 / 0.3	-	-		

Glycerol dioleate (GDO; CAS No.25637-84-7): also known as 1,3-DIOLEIN



Observations and Results

Mortality

Animals were observed twice daily throughout the study for mortality and morbidity.

All females survived to the scheduled necropsy on Gestation Day 20.

Clinical Signs

Individual clinical observations were recorded daily prior to dose administration and approximately 1 h following dosing. The observations included, but were not limited to, evaluations for changes in appearance of skin and fur, eyes, mucous membranes, respiratory and circulatory system, autonomic and central nervous systems, somatomotor activity, and behavior.

At the daily examination or 1 h following dose administration, no treatment-related clinical findings were noted in the animals treated with 1.25, 2.5, and 5.0 mL/kg of DAG. On Gestation Days 16 and 17, dried red material around the mouth was noted 1 h after dose administration in three females treated with the highest dose of DAG. This finding was only noted once for each female and therefore was not attributed to DAG oil administration. Clinical findings observed in the DAG oil-treated groups occurred infrequently or in a manner that was not dose-related (data not shown).

Body Weight

Individual body weights were recorded on Gestation Days 0, 6–18 (daily), and 20. Gravid uterine weight was recorded and net body weight (the Day 20 body weight exclusive of the weight of the uterus and contents) and net body weight change (the Day 0–20 body weight change exclusive of the weight of the uterus and contents) were calculated and determined for each gravid female at the scheduled laparohysterectomy.

Mean maternal body weights, body weight gains, net body weights, and net body weight gains in the 3.75 CO/1.25 DAG, 2.5 CO/2.5 DAG and 5.0 DAG groups were unaffected by DAG oil administration (Fig. 1; Table 2).

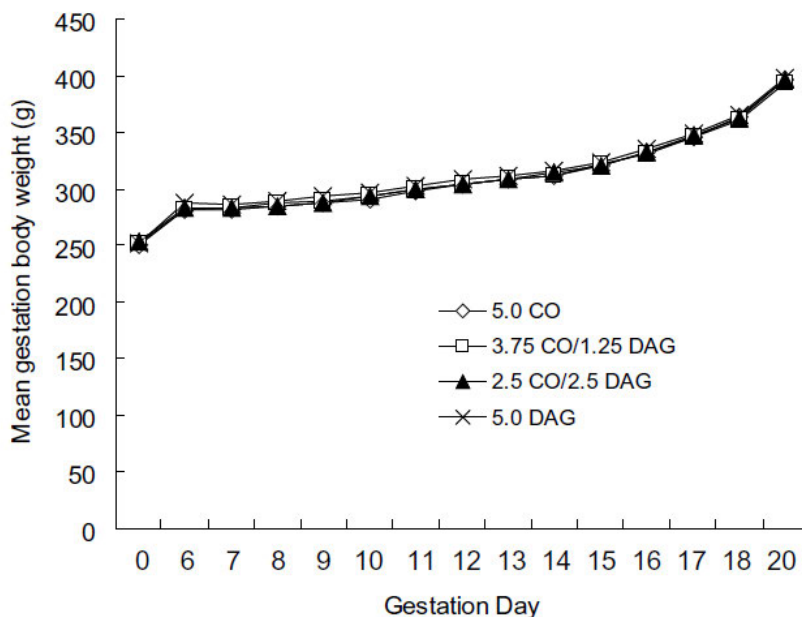


Fig. 1. Mean gestation body weights on an embryo/fetal developmental toxicity study of DAG oil in rats.

Table 2

Summary of maternal weight data (mean $\pm$ SD, g) on an embryo/fetal developmental toxicity study of DAG oil in rats

Parameter	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
Number of gravid females	24	24	24	25
Maternal absolute weight (GD 0)	251 $\pm$ 13.5	252 $\pm$ 13.0	253 $\pm$ 14.4	252 $\pm$ 13.3
Maternal absolute weight (GD 20)	397 $\pm$ 19.5	394 $\pm$ 20.2	396 $\pm$ 21.7	398 $\pm$ 22.3
Net body weight	314.3 $\pm$ 19.93	312.0 $\pm$ 16.96	312.5 $\pm$ 17.08	316.1 $\pm$ 16.02
Net body weight change	63.6 $\pm$ 11.42	60.0 $\pm$ 8.73	59.7 $\pm$ 9.39	63.9 $\pm$ 11.99
Gravid uterine weight	82.8 $\pm$ 13.69	82.1 $\pm$ 8.53	83.1 $\pm$ 12.83	81.4 $\pm$ 14.69

None significantly different from the control group.

Food Consumption

Food consumption, determined as g/animal/day and g/kg/day, in the 3.75 CO/1.25 DAG, 2.5 CO/2.5 DAG, and 5.0 DAG groups, was unaffected by DAG oil administration (Table 3).

Table 3

Summary of maternal food consumption (mean $\pm$ SD, g/animal/day) on an embryo/fetal developmental toxicity study of DAG oil in rats

Gestation day	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
GD 0–6	21 $\pm$ 1.7	21 $\pm$ 1.9	21 $\pm$ 2.1	22 $\pm$ 2.1
GD 6–9	19 $\pm$ 1.8	19 $\pm$ 1.6	19 $\pm$ 1.6	20 $\pm$ 2.1
GD 9–12	20 $\pm$ 1.9	20 $\pm$ 2.0	20 $\pm$ 1.8	21 $\pm$ 1.8
GD 12–18	22 $\pm$ 1.7	22 $\pm$ 1.8	22 $\pm$ 1.4	22 $\pm$ 1.3
GD 6–18	21 $\pm$ 1.5	21 $\pm$ 1.6	21 $\pm$ 1.2	21 $\pm$ 1.4
GD 18–20	26 $\pm$ 2.2	26 $\pm$ 1.5	26 $\pm$ 1.9	26 $\pm$ 1.4

None significantly different from the control group.

Toxicokinetics - none

Dosing Solution Analysis – not reported

Cesarean Section Data:

Euthanasia was performed by carbon dioxide inhalation on all females on Gestation Day 20. The thoracic and abdominopelvic cavities were opened and the contents were examined. The number of corpora lutea on each ovary was recorded. The uterus was weighed, and the number and location of all fetuses and early and late resorptions as well as the total number of implantation sites were recorded. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide for the detection of early implantation loss.

No DAG oil-related internal findings were noted in the maternal animals on Gestation Day 20 (data not shown). Intrauterine growth and survival were unaffected by DAG oil administration at all doses (Table 4). Parameters evaluated included mean numbers of corpora lutea, implantation sites and viable fetuses, mean fetal weights, and mean litter proportions of preimplantation loss, early resorptions, late resorptions, and fetal sex ratios.

Table 4

Summary of maternal and fetal data (mean $\pm$ SD) on an embryo/fetal developmental toxicity study of DAG oil in rats

Parameter	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
Number of corpora lutea	17.5 $\pm$ 2.69	17.3 $\pm$ 2.54	17.9 $\pm$ 2.47	17.2 $\pm$ 2.40
Number of implantation sites	16.1 $\pm$ 2.55	16.1 $\pm$ 1.60	16.4 $\pm$ 2.59	15.8 $\pm$ 1.72
Number of viable fetuses	15.4 $\pm$ 2.45	15.4 $\pm$ 1.61	15.4 $\pm$ 2.47	14.8 $\pm$ 2.90
Preimplantation loss (% per litter)	7.6 $\pm$ 13.61	6.0 $\pm$ 7.30	8.2 $\pm$ 10.76	7.4 $\pm$ 9.13
Early resorptions (% per litter)	4.0 $\pm$ 4.41	4.6 $\pm$ 5.15	5.9 $\pm$ 6.96	6.8 $\pm$ 12.55
Late resorptions (% per litter)	0.3 $\pm$ 1.45	0 $\pm$ 0.0	0 $\pm$ 0.0	0.4 $\pm$ 1.82
Fetal weight (g)	3.5 $\pm$ 0.37	3.5 $\pm$ 0.22	3.6 $\pm$ 0.20	3.5 $\pm$ 0.23
Males (% per litter)	48.9 $\pm$ 13.99	49.2 $\pm$ 15.32	50.3 $\pm$ 17.49	46.4 $\pm$ 13.18

None statistically different from the control group.

Offspring (Malformations, Variations, etc.):

Live fetuses were examined for external morphological abnormalities, weighed, sexed, and euthanized by intrathoracic injection of sodium pentobarbital. The fetuses were then dissected for visceral examination using a fresh dissection technique, which included examination of the heart and major blood vessels. Heads from approximately one-half of the fetuses in each litter were placed in Bouin's fixative for subsequent soft-tissue examination by a free-hand section technique. The heads from the remaining fetuses were examined by mid-coronal slice. All fetal carcasses were eviscerated and fixed in 100% ethyl alcohol, macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson. Findings of anatomical departures from normal were recorded as either malformations (those structural anomalies that alter general body conformity, disrupt or interfere with body function, or may be incompatible with life) or developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity, representing slight deviations from normal).

Results listed in Table 4, 5, and 6 indicate that there were no untoward effects from treatment with DAG oil containing GDO.

Table 5
Summary of fetal malformations on an embryo/fetal developmental toxicity study of DAG oil in rats

Parameter	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
Number of fetuses (litters) examined	369 (24)	369 (24)	370 (24)	371 (25)
External malformations				
Cleft palate	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % ($\pm$ SD)	0	0	0	0.3 $\pm$ 1.25
Vertebral agenesis	0 (0)	1 (1)	0 (0)	0 (0)
Mean litter % ($\pm$ SD)	0	0.2 $\pm$ 1.20	0	0
Visceral malformations				
Kidney and ureter absent	1 (1)	0 (0)	0 (0)	0 (0)
Mean litter % ($\pm$ SD)	0.2 $\pm$ 1.13	0	0	0
Skeletal malformations				
Vertebral anomaly	0 (0)	1 (1)	0 (0)	0 (0)
Mean litter % ($\pm$ SD)	0	0.2 $\pm$ 1.20	0	0
Bent limb bones	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % ($\pm$ SD)	0	0	0	0.3 $\pm$ 1.25
Total no. of malformations	1 (1)	2 (2)	0 (0)	1 (1)
% Per litter with malformations	0.2 $\pm$ 1.13	0.5 $\pm$ 1.66	0.0 $\pm$ 0.0	0.3 $\pm$ 1.25

% Per litter with malformations not statistically different from the control group.

Table 6

Summary of fetal developmental variations on an embryo/fetal developmental toxicity study of DAG oil in rats

Parameter	Dose concentration			
	5.0 CO	3.75 CO/1.25 DAG	2.5 CO/2.5 DAG	5.0 DAG
Number of fetuses (litters) examined	369 (24)	369 (24)	370 (24)	371 (25)
External variations	0 (0)	0 (0)	0 (0)	0 (0)
Visceral variations				
Major blood vessel variation	1 (1)	1 (1)	0 (0)	0 (0)
Mean litter % (±SD)	0.3 ± 1.46	0.2 ± 1.13	0	0
Renal papilla (e) not developed	0 (0)	1 (1)	0 (0)	2 (2)
Mean litter % (±SD)	0	0.3 ± 1.70	0	0.5 ± 1.86
Hemorrhagic ring around iris	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.2 ± 1.18
Skeletal variations				
Cervical centrum #1 ossified	101 (19)	117 (23)	84 (20)	112 (24)
Mean litter % (±SD)	26.4 ± 28.0	31.8 ± 22.6	22.1 ± 20.15	31.2 ± 21.55
14th Rudimentary rib	23 (10)	26 (13)	26 (12)	30 (13)
Mean litter % (±SD)	6.1 ± 8.59	7.2 ± 12.53	7.1 ± 9.66	8.0 ± 12.98
Pubis unossified	8 (2)	1 (1)	0 (0)	0 (0)
Mean litter % (±SD)	2.3 ± 10.22	0.3 ± 1.28	0	0
Sternebra(e) #5 and/or 6 unossified	15 (8)	7 (5)	9 (5)	10 (3)
Mean litter % (±SD)	3.8 ± 6.51	1.8 ± 3.98	2.3 ± 6.28	2.4 ± 7.8
Hyoid unossified	5 (2)	2 (2)	2 (1)	2 (1)
Mean litter % (±SD)	1.5 ± 5.93	0.5 ± 1.67	0.5 ± 2.40	0.5 ± 2.5
Bent rib	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.3 ± 1.25
25 Presacral vertebrae	1 (1)	0 (0)	0 (0)	0 (0)
Mean litter % (±SD)	0.3 ± 1.28	0	0	0
Reduced ossification of the 13th rib	1 (1)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0.2 ± 1.07	0	0	0.2 ± 1.18
7th Cervical rib	5 (4)	3 (3)	4 (4)	4 (4)
Mean litter % (±SD)	1.5 ± 3.7	0.8 ± 2.16	1.2 ± 2.76	1.0 ± 2.37
Sternebra(e) malaligned (slight or moderate)	0 (0)	1 (1)	0 (0)	2 (2)
Mean litter % (±SD)	0	0.3 ± 1.28	0	0.5 ± 1.73
Sternebra(e) #1, 2, 3 and/or 4 unossified	1 (1)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0.3 ± 1.28	0	0	0.3 ± 1.25
Reduced ossification of vertebral centra	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.3 ± 1.25
Ischium unossified	2 (1)	0 (0)	0 (0)	0 (0)
Mean litter % (±SD)	0.6 ± 2.92	0	0	0
Unco-ossified vertebral centra	0 (0)	0 (0)	0 (0)	1 (1)
Mean litter % (±SD)	0	0	0	0.2 ± 1.11
% Per litter with variations (mean ± SD)	37.3 ± 24.32	39.8 ± 20.82	29.7 ± 20.66	41.0 ± 21.31

% Per litter with variations not statistically different from the control group.

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Prenatal and Postnatal Development

As noted in the review above, the Morita et al. (2008a) combination Fertility and Early Embryonic Development study and Pre- and Post-Natal development study does not include any behavioral or cognitive testing of the F1 offspring and therefore is not considered an adequate pre- and post-natal development study by current standards.

To address this data gap, the Applicant conducted a dedicated pre- and post-natal developmental toxicology study with DGO. The study below was reviewed by R. Daniel Mellon, PhD.

Study title: Glycerol Dioleate (GDO): A Subcutaneous Pre- and Post-Natal Reproductive and Developmental Toxicity Study in Female Sprague-Dawley Rats

Study no.: TO-16-569
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: May 30, 2016
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: GDO, PP1/924, purity not specified, testing indicated (b) (4) % (b) (4) content, (b) (4) % (b) (4) content. Material from Camurus AB, Lund, Sweden

Key Study Findings

Female Sprague-Dawley Rats were treated daily from GD 6 through parturition and to post-prandial day 20 (PPD20) with 100, 350, or 1000 mg/kg GDO per day via subcutaneous injection with the following key findings:

1. Reduced pup viability and increased dead pups at birth were demonstrated in the HD females largely driven by effects in a single dam; however, a treatment-related effect cannot be ruled out because additional dams were impacted and the findings were outside of the historical control range for the laboratory.
2. There was a reduction in F1 reproductive performance (female fertility and conception rats) and an increase in the mean number of days to mating and reduced mating index in the LD and HD animals. These findings were not clearly dose-dependent and reportedly outside the normal control ranges for the laboratory. Due to the lack of dose dependency, the study director considered them to be "of questionable relationship to the test item".
3. Toxicokinetic data suggest that although there was an initial increase in systemic exposure to GDO on GD 6 in LD and MD animals compared to control levels, there were no differences via this route of administration at any other time point and the levels were basically within the levels of the saline treated control animals, suggesting minimal to no elevations over endogenous levels during the treatment period.
4. The study report concludes that the NOAEL for reproductive performance of the F0 group to be the MD of 350 mg/kg/day (2100 mg/m²). I (RDM) concur. This dose is (b) (4) times the human maximum daily dose of (b) (4) mg/60 kg x (b) (4) = (b) (4) mg/m², based on body surface area.

5. The study report concludes that the NOAEL for growth and development of the F1 offspring to be the MD as well. I (RDM) concur. This dose is $\frac{(b)(4)}{(b)(4)}$ times the human maximum daily dose of $\frac{(b)(4)}{(b)(4)} \text{ mg/60 kg} \times \frac{(b)(4)}{(b)(4)} = \frac{(b)(4)}{(b)(4)} \text{ mg/m}^2$, based on body surface area.
6. The study notes that “Findings on the reproductive performance of F1 females (fertility and conception rate indices) of questionable relationship to the test item were observed at dose levels $> \frac{(b)(4)}{(b)(4)} \text{ mg/kg/day}$.” I (RDM) concur that these are unusual findings but difficult to interpret given the lack of clear dose dependency.
7. Given the slow clearance of the GDO from the local injection site (as evident by the local tissue reactions) and the TK data, and the exposure margins noted, the adverse effects reported above are not likely to be clinically significant, particularly given the indication and the potential risks associated with continued illicit opioid use.

Methods

Doses: 100, 350, or 1000 mg/kg GDO per day
 Frequency of dosing: Daily
 Dose volume: 0.11 to 1.06 mL/kg
 Route of administration: SC
 Formulation/Vehicle: Controls received 0.9% saline injections
 Species/Strain: Sprague Dawley rats
 Number/Sex/Group: 25 Dams

Treatment Group ³	Targeted Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals		
			F ₀ Generation		
			M ²	Main	TK
				F	F
1. Control ¹	0	1.06	130	25	4
2. GDO	100	0.11		25	10
3. GDO	350	0.37		25	10
4. GDO	1000	1.06		25	10

¹Group 1 animals received the reference item alone (0.9 % Sodium Chloride for Injection USP (Saline)).

²Untreated sexually-mature males used to induce pregnancies only.

³Six injection sites on the back of the rats were used and were rotated throughout the study.

M: male; F: female; TK: toxicokinetic

Satellite groups: 10 per treatment group
 Study design: See above table. Males were not treated. Females were dosed from Gestation Day 6 to Post-partum Day 20

Deviation from study protocol: Based on test item density the doses of the material administered were slightly outside of the targeted doses (102, 344, and 986 mg/kg/day estimates). These differences do not compromise the study conclusions.

Observations and Results (Optional Table)

F₀ Dams

Survival: There was one mortality in the MD group on PPD16. Two unscheduled euthanasias were completed due to cannibalism of the pups by the dams, as noted below:

Group/Dose Level	Dam No.	Day of death	Fate	Reason for Euthanasia
2 – 100 mg/kg/day	2517	PPD1	Unscheduled euthanasia	Pups cannibalism
3 – 350 mg/kg/day	3512	PPD16	Found dead	-
4 – 1000 mg/kg/day	4514	PPD1	Unscheduled euthanasia	Pups cannibalism

Dam 3512 demonstrated evidence of deteriorating condition starting on PPD14 (decreased activity, decreased muscle tone, hunched back, dehydration, thinness, weakness, massive body weight loss from PPD 10 to 14. Lower body weights were noted in this dam's pups likely due to poor maternal health and behavior. Necropsy suggested cause of death related to lung edema and inflammation as well as inflammation in the kidney. Spleen and lymph nodes were enlarged, evidence of hemorrhage in the stomach lining, and decreased thymic weights were consistent with severe physiological stress. These findings were restricted to this animal and therefore not thought to be due to the GDO.

Clinical signs: Primarily restricted to injection site reactions. Described as slight to severe edema and erythema. The injection site demonstrated lump/firm swelling consistent with residual GDO at the site of injection. GDO did not appear to absorb systemically rapidly and was slowly cleared from the injection site.

One HD dam (4514) demonstrated signs of difficult parturition which correlated with poor maternal performance. She was terminated early due to cannibalism of the pups.

Modified Draize scoring for injection site reactions increased in a treatment dependent fashion in all treatment arms.

Body weight: A treatment-related trend in increased body weights was demonstrated in MD and HD animals starting from PPD7 to 21. However, body weight losses were noted in all groups from PPD17 to 21.

Food consumption: No treatment-related effects noted.

Uterine content: There was a higher number of pup deaths in the HD

group animals, largely driven by the seven dead pups from Animal 4514 discussed above. However, dead pups (2-5 per dam) were noted in four other dams as well.

Group/Dose Level	Post-implantation loss (%)	Live Birth Index (%)	Dead Pups
1 – 0 mg/kg/day	6.9	93	4
2 – 100 mg/kg/day	7.4	93	7
3 – 350 mg/kg/day	7.2	93	1
4 – 1000 mg/kg/day	12	88	19*

*Statistically significant when compared to Group 1: $p \leq 0.01$, Fisher

There was no reported effects on gestation indices, duration of parturition, corpora lutea counts, implantation sites, sex ratio, pre-implantation loss, or number of live or malformed pups noted.

There was an increased mean post-implantation loss percentage and decreased live birth index in the HD group as well, also largely driven by Dam 4514. If this dam were eliminated from the analysis, the Applicant notes that although the HD group was still numerically higher than the controls, the incidence rates were close to the historical control levels.

Necropsy observation: Macroscopic examination of the dams demonstrated local tissue injection site hemorrhage and presence of clear cysts/gelatinous material which is presumed to be the test item and related injection site damage.

There were two dams that did not litter. One demonstrated evidence of complete resorption (control animal), the other was not pregnant (MD).

Toxicokinetics: Levels (AUC) of GDO were higher in the LD and MD groups compared to the HD groups and controls on GD6, however, levels were comparable to controls on GD18 and PPD20. As the differences between these days all fluctuate within a factor of three and do appear to be clearly dose dependent or time dependent, these values were interpreted to be within the normal range.

Treatment group*	AUC _{0-24h} (µg·h/mL)		
	GD6	GD18	PPD20
1; Control (saline)	5.05	26.53	7.15
2; GDO 100 mg/kg/day	15.8	35.04	8.44
3; GDO 350 mg/kg/day	13.3	33.58	10.6
4; GDO 1000 mg/kg/day	9.63	25.24	7.59

* n=3/timepoint, refer to Text Table 9 in Section 7.16.

Dosing Solution Analysis: At the completion of the treatment period, a sample of the (b) (4) test item was returned to (b) (4) for analysis to confirm stability. The sample was within the prespecified ranges.

Other: None

F₁ Generation

Survival: Pup viability index was lower in the HD group on PND4.

Group/Dose Level	Day 4 Viability Index (%)	Day 7 Survival Index (%)	Day 14 Survival Index (%)	Day 21 Lactation Index (%)
1 – 0 mg/kg/day	97.9	100	100	100
2 – 100 mg/kg/day	93.6	100	100	99.5
3 – 350 mg/kg/day	95.1	100	100	100
4 – 1000 mg/kg/day	86.6	99.4	98.3	98.3

There were no effects on sex ratio, pup viability, survival or lactation indices between PPD7 and PPD21.

There were four post-weaning F₁ mortalities in the control animals, two in the LD group and one in the HD group. No clear treatment-related effects were demonstrated. Mortality/euthanasia was mostly due to euthanasia with deteriorating condition and evidence of hydrocephalus. Because the findings were more evidence in the control animals, the effects were not considered treatment-related.

Group/Dose Level (mg/kg/day)	F ₁ Animal No.	Day of death	Fate	Reason for Euthanasia
1 – 0	1017	PPD34	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
	1018	PPD26	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
	1020	PPD26	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
	1520	PPD40	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
2 – 100	2063	PPD66	Found dead	-
	2074	PPD23	Unscheduled euthanasia	Deteriorating Condition-Hydrocephalus
4 – 1000	4082	PPD61	Unscheduled euthanasia	Deteriorating Condition

Clinical signs: No clear GDO related findings were reported. Clinical signs noted were distributed across the treatment groups.

Body weight: No clear GDO related effects reported.

Food consumption: Not recorded

Physical development: The following developmental landmarks were recorded:

<i>Physical Assessment</i>	<i>Reflexological Development</i>	<i>Sensory Development</i>
Pinna unfolding ¹ (PPD1 to PPD4)	Righting reflex ¹ (PPD2 to PPD4)	Pupillary closure †* (PPD21)
Eye opening ¹ (PPD12 to PPD20)	Negative geotaxis ¹ (PPD8 to PPD20)	Auricular startle response ¹ (PPD12 to PPD 20)
Preputial Separation† (PPD35 to PPD46)		Visual placing response †* (PPD21)
Tooth eruption ¹ (PPD7 to PPD20)		
Vaginal Opening† (PPD26 to PPD37)		

¹ Reported as the mean day of positive development per litter, by sex, and for combined sexes when applicable.

† Post weaning; on selected pups only (2 pups/sex/litter) and assessed until all pups in a given litter had a positive response.

†*Post weaning; on selected pups only (2 pups/sex/litter).

The report states there were no changes in any of these parameters.

There were no changes in vaginal opening or preputial separation reported.

Neurological assessment: The functional observational battery was assessed as per the following:

Open-field evaluation*	Involuntary motor movements (such as convulsion and tremors) Respiration Rearing Urination † Defecation † Gait Arousal Mobility Abnormal or stereotyped behavior
Manipulative evaluation	Ease of removal from the cage Handling reactivity Palpebral closure Eye observations (eg., lacrimation, exophthalmus) Salivation Piloerection Approach response † Auditory test (Click response) † Tail pinch response † Touch response † Pupil response to light Extensor thrust reflex Cornea reflex
Physiological evaluation	Body temperature
Neuromuscular evaluation**	Grip strength (forelimbs and hindlimbs) Landing foot splay***
Motor activity**	Figure 8 maze E-Water maze

* Animals were placed in the open-field area and observed for a period of at least 2 minutes.

** The neuromuscular evaluation and motor activity were performed following the completion of each series of manipulative evaluation, except E-water maze.

***Exceeded 5 evaluations when considered necessary.

†Was observed following the 2 minute interval while still in the open field.

No treatment-related changes in any FOB parameters were reported. There were no clear effects on motor activity or the E-Water Maze (learning and memory). Although this is not a complex learning task, it will be considered adequate given the low systemic exposures and endogenous nature of the test item.

Reproduction: There were no changes reported in the F1 offspring estrus cyclicity. F1 parenteral performance is summarized in the table below:

Group/Dose Level	Mean Day to Mating	Male Mating Index (%)	Female Fertility Index (%)	Conception Rate (%)
1 – 0 mg/kg/day	4.8	66.7	79.2	90.5
2 – 100 mg/kg/day	8.4	47.8	50.0	60.0
3 – 350 mg/kg/day	4.8	66.7	58.3	73.7
4 – 1000 mg/kg/day	7.7	52.2	56.5	68.4

The reduced indices in all treatment groups do not appear to be dose-dependent. The results are also outside of the historical control range for these animals. The clinical significance of these findings is not clear.

At Cesarean section of the F1 females on GD15, there were no changes in corpora lutea, number of implantation sites, or pre-implantation losses; however, there was an apparent increase in post-implantation losses and reduced live embryos in the LD and HD groups compared to controls. As these were within the range of the historical control values and not clearly dose dependent, the effects are not likely treatment-related.

Group/Dose Level	Post-Implantation Loss (%)	Live Embryos Index (%)
1 – 0 mg/kg/day	5.6	94
2 – 100 mg/kg/day	16	84
3 – 350 mg/kg/day	5.8	94
4 – 1000 mg/kg/day	11	89

Other: Toxicokinetic data were obtained from the TK animals on GD6, GD18, and PPD20 at 1, 2, 6, and 24 h after the injection per the following schedule.

No. of rats / timepoint		Pre-dose	Toxicokinetic timepoints (post-dose)			
			1 h	2 h	6 h	24 h
Group 1	3	√		√		√
Groups 2-4	3 (Subset 1)	√			√	
	3 (Subset 2)		√			√
	3 (Subset 3)			√		

F₂ Generation – no evaluations of the F₂ offspring were made.

Survival:

Body weight:

External evaluation:

Male/Female ratio:

Other:

Conclusions of Reproductive and Developmental Effects of GDO

During the development of this drug product (see EOP2 meeting minutes), the Division noted the following:

While your plan to rely on published literature may be acceptable, we note that this strategy may be risky as these articles typically do not include any underlying data and the designs of the studies may not have been conducted in the spirit of Good Laboratory Practices. We also note that if no specific information can be found for GDO, toxicity information for a “related” compound such as diacylglycerol (DAG), which contains GDO, may be supportive only if the levels of GDO tested in those studies can be clearly discerned and adequate levels of GDO were generated under the conditions tested, or if a persuasive argument can be made to support that the “related” compound would be predicted to have a similar or greater toxicity profile than GDO. We remind you that your safety risk assessment for GDO must address all of the standard toxicological endpoints per the FDA guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079250.pdf>. This includes evaluations for reproductive toxicology that include assessing: (1) the potential to affect fertility or early embryonic development to implantation; (2) teratology in both a rodent species and a mammalian nonrodent species; and (3) effects on prenatal and postnatal development, including maternal function.

The existing safety assessment lacks any embryo-fetal development data on GDO in rabbits. Further, although fertility and early embryonic development data and embryofetal development data in rats have been published with DAG, there is no clear understanding how much GDO is present in the DAG oil administered to the animals. However, oleic acid is an omega-9 monounsaturated fatty acid. Unlike omega-6 fatty acids, omega-9 fatty acids can be produced endogenously. Omega-9 fatty acids, including oleic acid, can help reduce the risk of heart disease by raising the “good” cholesterol, high-density lipoprotein. They also lower the “bad” cholesterol, low-density lipoprotein. Oleic acid can be found in many dietary sources including canola oil, sunflower oil, almonds, beef, chicken and eggs. The World Health Organization recommends that the intake of oleic acid be 15-30% of daily energy intake per day (equal to 40-80 g for men and 25-50 g for women). One serving of almonds (1 ounce) has 9 grams of oleic acid. One tablespoon of canola oil contains 8.4 g oleic acid. The q1w product has (b) (4) mg of GDO ((b) (4) mg/day). Therefore, although embryofetal development studies have not been conducted with GDO in rabbit, the dietary intake of oleic acid, the metabolite of GDO, far exceeds levels in this product and a rabbit study may not be necessary.

As such, an embryo-fetal study with GDO in rats and rabbits and a fertility and early embryonic development study in rats are recommended unless adequately justified otherwise. Given the existing limited data, the apparent limited systemic exposure to GDO, the unknown exposures and effects of GDO metabolites, these studies may be completed as post-marketing requirements if there is a favorable risk:benefit assessment of the NDA.

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/s/

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