CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208603Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208603
Supporting document/s:	SDN 1
Applicant's letter date:	12/14/2015
CDER stamp date:	12/14/2015
Product:	ARYMO ER (morphine sulfate extended release tablets)
Indication:	Management of pain severe enough to require daily, around-the-clock, long-term opioid treatment for which alternative options are inadequate
Applicant:	Egalet US, Inc.
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products
Reviewers:	Elizabeth A. Bolan, PhD and Grace S. Lee, PhD
Supervisor/Team Leader:	R. Daniel Mellon, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Christopher Hilfiger

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of 208603 are owned by Egalet US Inc. (Egalet) or are data for which Egalet has obtained a written right of reference.
Any information or data necessary for approval of 208603 that Egalet 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 Egalet.

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY 5

1.1 INTRODUCTION 5
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS 5
1.3 RECOMMENDATIONS 6

12 CLINICAL PHARMACOLOGY 12

2 DRUG INFORMATION 14

2.1 DRUG 14
2.2 RELEVANT INDS, NDAS, BLAS AND DMFS 14
2.3 DRUG FORMULATION 15
2.4 COMMENTS ON NOVEL EXCIPIENTS 16
2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN 17
2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN 18
2.7 REGULATORY BACKGROUND 18

3 STUDIES SUBMITTED 18

3.1 STUDIES REVIEWED 18
3.2 STUDIES NOT REVIEWED 18

4 PHARMACOLOGY 18

5 PHARMACOKINETICS/ADME/TOXICOKINETICS 19

6 GENERAL TOXICOLOGY 19

6.1 SINGLE-DOSE TOXICITY 19
6.2 REPEAT-DOSE TOXICITY 19

7 GENETIC TOXICOLOGY 23

7.1 *IN VITRO* REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES) 24
7.2 *IN VITRO* ASSAYS IN MAMMALIAN CELLS 28

8 CARCINOGENICITY 30

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY 30

10 SPECIAL TOXICOLOGY STUDIES 30

11 INTEGRATED SUMMARY AND SAFETY EVALUATION 30

12 APPENDIX/ATTACHMENTS 31

Table of Tables

Table 1. Composition of Arymo ER tablets 15
Table 2. Acceptability of levels of inactive ingredients in the 60 mg tablet at the MTDD of morphine 16
Table 3. Drug substance impurity specifications 17
Table 4. Drug product degradant specifications 18
Table 5. Compound, lot number and purity of test articles 19
Table 6. Summary of doses after initial titration phase 21
Table 7. Changes in body weight gain 22
Table 8. Ames assay positive controls 25
Table 9. (b) (4) Ames assay summary of results without S9 26
Table 10. (b) (4) Ames assay summary of results with S9 27
Table 11. Summary of in vitro chromosome aberration results with (b) (4) 29

Table of Figures

Figure 1. Structure of Morphine Sulfate.....14

1 Executive Summary

1.1 Introduction

Egalet submitted NDA 28603 for Arymo ER, an extended-release morphine sulfate (MS) product which contains excipients that are intended to confer abuse-deterrent properties. Arymo ER is formulated in strengths of 15, 30, and 60 mg MS per tablet and is intended for BID dosing. The indication for this product is management of pain severe enough to require daily, around-the-clock, long-term opioid treatment for which alternative options are inadequate. This NDA is a 505(b)(2) application and is relying, in part, on the Agency's findings of safety and efficacy of MS Contin (NDA 19516) to support their application.

No new pharmacology, general toxicology, genetic toxicology, reproductive and developmental toxicology, or carcinogenicity studies were conducted or required for this application. The Applicant has submitted studies to qualify (b) (4), a drug product degradant, which exceeds the ICH Q3B(R2) threshold for qualification. Justification has also been provided for several excipients, including polyethylene oxide (b) (4) (PEO; MW: 400,000), (b) (4) when the product is consumed at the maximum theoretical daily dose of morphine.

1.2 Brief Discussion of Nonclinical Findings

All impurities in the drug substance and degradants in the drug product are controlled at acceptable levels. The proposed drug product degradant specification for (b) (4), which exceeds the ICH the Q3B(R2) threshold for qualification, has been justified and is considered acceptable. For qualification, the Applicant submitted an Ames assay, an in vitro chromosome aberration (CA) assay, and a 13-week repeat-dose rat toxicology study with (b) (4) (b) (4) was negative for genotoxic potential in both the Ames and CA assays. The NOAEL in the 13-week rat study was the highest dose tested, but did not support the specification o (b) (4)% proposed by the Applicant. However, this application references MS Contin and the proposed specification is within the specification for (b) (4) in MS Contin. Therefore, the proposed specification for (b) (4) of (b) (4)% will be considered acceptable for this NDA.

Arymo ER contains excipients that are intended to confer abuse-deterrent properties. (b) (4). With the exception of the PEO, the levels of the excipients in this product are considered acceptable and do not require qualification. The levels of the PEO in this product, when used at the maximum theoretical daily dose (MTDD) of morphine, (b) (4) To support the safety of the levels of the PEO in this product, the Applicant is referencing MF (b) (4)). Master File (b) (4) has been found to be inadequate (b) (4) These (b) (4) entities could include (b) (4) and specifications for these impurities in the excipient master file may be required. However, because of the longstanding history of use of PEO in many

products which reference MF (b) (4) this deficiency will not be an approval issue for NDA 208603. The levels of PEO in Arymo ER when used at the MTDD of MS are considered acceptable from a pharmacology/toxicology perspective for this NDA. Pharmacology toxicology recommends that the Applicant conduct several studies as post-marketing requirements (PMR) to fully characterize the toxicity of the PEO. The recommended studies are outlined in Section 1.3.2.

The Applicant has submitted a literature review and proposed labeling changes for Section 8 (Pregnancy) in order to comply with the Pregnancy and Lactation Labeling Rule (PLLR). No new reproductive toxicology studies were submitted. In addition, a thorough review of the morphine literature was conducted by Dr. Grace Lee and is attached as an appendix to this review. The language for Section 8 of the label will be updated with this information.

1.3 Recommendations

1.3.1 Approvability

The recommendation from pharmacology/toxicology is that NDA 208603 be approved with four PMRs.

1.3.2 Additional Non Clinical Recommendations

Given the deficiencies noted in the review of DMF (b) (4) it is recommended that the Agency institute the following PMRs to Egalet. We encourage (b) (4) and Egalet to work together to address these deficiencies, given the daily dose of PEO that would be consumed in the Arymo ER product.

PMR 1. Analyze the PEO product employed in Arymo ER for low molecular weight impurities. Identify and quantitate the impurities. Submit a toxicological risk assessment for the exposure to the impurities taking into consideration the maximum theoretical daily dose of Arymo ER.

PMR 2. Conduct an embryo-fetal development study in the rat model to assess the potential impact of PEO on development. The study must be designed to adequately qualify the safety of the low molecular weight PEO components (impurities/degradants) in the PEO (b) (4) when the product is consumed up to the MTDD of Arymo ER.

PMR 3. Conduct an embryo-fetal development study in the rabbit model to assess the potential impact of PEO on development. The study must be designed to adequately qualify the safety of the low molecular weight PEO components (impurities/degradants) in the PEO (b) (4) when the product is consumed up to the MTDD of Arymo ER.

PMR 4. Conduct a pre- and post-natal development study in the rat model to assess the potential impact of PEO on development. The study must be designed to adequately qualify the safety of the low molecular weight PEO components

(impurities/degradants) [redacted] (b) (4) when the product is consumed up to the MTDD of Arymo ER.

1.3.3 Labeling

See Appendix 1 for the proposed labeling.

2 Drug Information

2.1 Drug

CAS Registry Number: 6211-15-0

Generic Name: Morphine sulfate

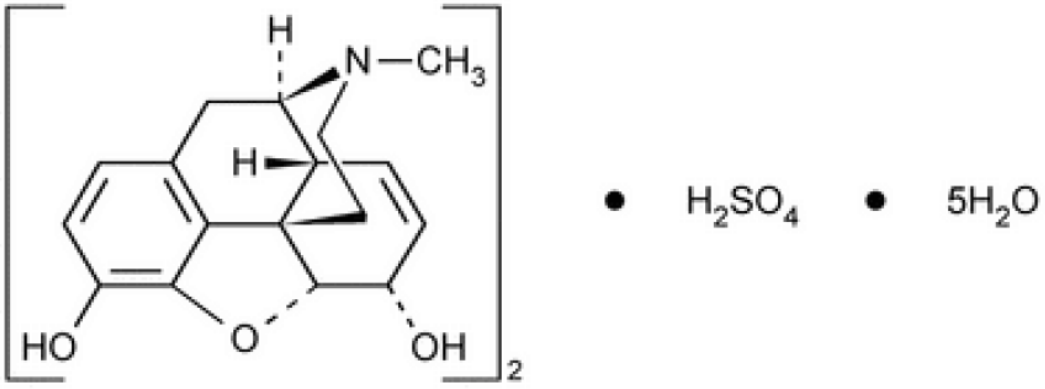
Code Names: EG-001

Chemical Name: 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol sulfate (2:1) (salt) pentahydrate

Molecular Formula/Molecular Weight: $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5\ H_2O$; 758.83 g/mol

Structure:

Figure 1. Structure of Morphine Sulfate



Pharmacologic Class: Opioid Agonist (EPC)

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA#	Drug Name	Div	Route	Marketing Status	AP Date	Company
19516	MS Contin	DAAAP	Oral	Approved	9/12/1989	Purdue

IND#	Drug	Status	Division	Indication	Sponsor
117317	EG-001	Active	DAAAP	Management of pain	Egalet

DMF#	Subject of DMF	Holder	Reviewer's Comment
		(b) (4)	Adequate
			Deficient (see discussion below)
			Adequate

2.3 Drug Formulation

The drug product is formulated as an extended-release oral tablet for once-a-day delivery in three strengths (15, 30, and 60 mg). The formulation consists of a matrix of morphine sulfate, polyethylene oxide 400,000 ((b) (4) and butylated hydroxytoluene (BHT). The formulation is intended to provide abuse-deterrent properties by resisting crushing/chewing and forming a viscous hydrogel on contact with liquid.

The composition of the drug product formulation is depicted in the table below.

Table 1. Composition of Arymo ER tablets

Component	15 mg Amount per Tablet mg (%)	30 mg Amount per Tablet mg (%)	60 mg Amount per Tablet mg (%)	Function	Reference to Quality Standards
Morphine Sulfate	15.00 (1.98)	30.00 (3.94)	60.00 (7.83)	Drug substance	USP
Polyethylene Oxide 400,000 (b) (4) (b) (4)			(b) (4)	Release controlling; Abuse-deterrent properties	USP/NF
Butylated Hydroxytoluene (b) (4)				(b) (4)	USP/NF
					Section 3.2.P.4.1 - (b) (4)
					Section 3.2.P.4.1 - (b) (4)
					Section 3.2.P.4.1 - (b) (4)
					USP
Total	759.21 (100.0)	761.69 (100.0)	766.53 (100.0)	-	-

[1] Refer to Section 3.2.P.2.1 (b) (4)
[2] (b) (4)

2.4 Comments on Novel Excipients

As with any single-entity opioid drug product approved for chronic use, there is no maximum daily dose listed in the labeling due to the development of tolerance. The development of tolerance necessitates increased doses with time in order to obtain the same desired effect. To establish the safety of the product for opioid-tolerant individuals, the Division has employed a "maximum theoretical daily dose" (MTDD) based on clinical use data. The clinical team has determined that the revised MTDD for extended-release morphine products is 1.5 g/day based on a recent review of data available to the Agency. The table below summarizes the MTDD of the excipients in this drug product and assumes that if these levels were to be reached, it would be via use of the highest dosage strength.

The quantitative composition of the 60 mg tablet and the amount of each inactive ingredient at the MTDD of MS is presented in the table below. The BHT can be found in approved products at higher amounts and requires no further qualification. The specific (b) (4) used in this product does not appear in the FDA Inactive Ingredients Database (IID). The Applicant has provided a reference to DMF (b) (4) from (b) (4) to support the safety of the coating. The quantitative composition of the (b) (4) has been reviewed and determined to be

acceptable from a toxicologic perspective. Therefore, the amount of [redacted] is considered acceptable in this formulation. The levels of polyethylene oxide MW 400,000 (PEO) when this product is used at the MTDD of MS is [redacted] g. [redacted]. Polyethylene oxide is discussed below.

Table 2. Acceptability of levels of inactive ingredients in the 60 mg tablet at the MTDD of morphine

Inactive Ingredient	***Total dosage via single 60 mg tablet, mg***	***Total Dose at MTDD (25 pills, mg)***	***Rationale***
Polyethylene oxide (MW 400,000)	[redacted]	[redacted]	Acceptable: see below and review of MF [redacted]
BHT			Acceptable: IID
[redacted]			Acceptable: See discussion above

IID: FDA Inactive Ingredients Database

Polyethylene Oxide

To support the safety of the levels of the PEO in this product, the Applicant is referencing MF [redacted] ([redacted] Master File [redacted] has been found to be inadequate [redacted] However, because of the longstanding history of use of PEO in many products which reference MF [redacted] this deficiency will not be an approval issue for NDA 208603. The levels of PEO in Arymo ER, when used at the MTDD of morphine, are considered acceptable from a pharmacology/toxicology perspective for approval, however, nonclinical post-marketing requirements will be issued in order to address the deficiency. Refer to the review of MF [redacted] for details.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Impurities.

The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substance for a maximum daily dose (MDD) of drug substance < 2 g/day is 0.15%. For this product, the clinical team determined that the MTDD of morphine is 1.5 g. The Applicant is obtaining the morphine drug substance for the marketed drug product from [redacted] (MF [redacted]). Morphinone contains a structural alert for mutagenicity but has been adequately qualified with studies in the DMF for genotoxic potential and can therefore be regulated as a typical DS impurity according to ICH Q3A(R2) thresholds for qualification. [redacted], therefor the specification of [redacted]%, which exceeds ICH Q3A thresholds for qualification, is considered acceptable. All drug substance impurity specifications are considered acceptable.

Table 3. Drug substance impurity specifications

Impurity	***Proposed Specification***	***Comments***
(b) (4)	NMT (b) (4) %	Acceptable
(b) (4)	NMT (b) (4) %	Contains a structural alert for mutagenicity but qualified as nongenotoxic in DMF and is therefore acceptable
(b) (4)	NMT (b) (4) %	Acceptable
(b) (4)	NMT (b) (4) %	(b) (4) Acceptable

Drug Product Degradants.
The qualification threshold according to the ICH Q3B(R2) guidance for impurities/degradants in the drug product for a MDD of a drug substance < 2 g is 0.2% or 3 mg, whichever is greater. For this product, DAAAP has determined that the MTDD of morphine is 1.5 g. The proposed specification of (b) (4) % for (b) (4) ((b) (4) exceeds the ICH Q3B(R2) qualification threshold. The Applicant has submitted studies to qualify (b) (4) The two genetic toxicology studies showed negative results and the 13-week repeat-dose toxicology study demonstrated that the toxicity of (b) (4) is similar to that of (b) (4) . The NOAEL for (b) (4) in the 13-week study, which was the highest dose tested, only supports a specification of (b) (4) % when calculated for a TDI of 1.5 g of morphine. However, the levels in this product are within the levels of the referenced product MS Contin . Therefore, the specification of (b) (4) % for (b) (4) will be considered acceptable for this product.

Table 4. Drug product degradant specifications

Degradant	***Proposed Specification***	***Comments***
(b) (4)	NMT (b) (4) %	Acceptable (see above)

2.6 Proposed Clinical Population and Dosing Regimen

This extended-release morphine product is planned to be marketed as 15, 30, and 60 mg tablets intended for BID dosing in adults. The indication is management of moderate-to-severe chronic pain when a continuous around-the-clock opioid analgesic is needed for an extended period of time. The formulation is intended to provide abuse-deterrent properties.

2.7 Regulatory Background

The Applicant is submitting NDA 208603 via the 505(b)(2) regulatory pathway and is relying on the Agency's previous findings of safety and efficacy for MS Contin (NDA 19516). The IND for development of this product (IND 117317) was originally opened by Egalet on August 22, 2013.

3 Studies Submitted

3.1 Studies Reviewed

The studies in the table below are located in the EDR in eCTD format.

Study Title	*Study #*
Bacterial Reverse Mutation Assay with (b) (4)	AE09LU.502ICH.BTL
In Vitro Mammalian Chromosome Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL)	AE09LU.341ICH.BTL
Morphine/(b) (4) Mixture: A 1 Week Dose Titration Followed by a 13 Week Toxicity Study in Sprague Dawley Rats by Oral Gavage Administration	HUD0440

3.2 Studies Not Reviewed

All studies submitted to the NDA were reviewed.

3.3 Previous Reviews Referenced

No previous reviews have been referenced.

4 Pharmacology

No new pharmacology studies were submitted by the Applicant. The Applicant is relying on the information in the label of the referenced product MS Contin.

5 Pharmacokinetics/ADME/Toxicokinetics

No new studies were submitted by the Applicant. The Applicant is relying on the information in the label of the referenced product MS Contin.

6 General Toxicology

No general toxicology data were submitted by the Applicant. A 13-week study in rat was submitted in support of qualification of the drug product degradant (b) (4)

6.1 Single-Dose Toxicity

No single-dose toxicology studies were conducted.

6.2 Repeat-Dose Toxicity

Study title: Morphine/(b) (4) Mixture: A 1 Week Dose Titration Followed by a 13 Week Toxicity Study in Sprague Dawley Rats by Oral Gavage Administration

Study no.:	HUD440
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 23, 2015
GLP compliance:	Yes
QA statement:	Yes

Table 5. Compound, lot number and purity of test articles

Compound	***Lot #***	***Purity***
Morphine sulfate (b) (4) %)	14EW161	99.5%
(b) (4) mixture (morphine sulfate (b) (4))	C11010	99.0%

Key Study Findings

- Body weight decreases were observed in most conditions, but no toxicologically relevant differences between the morphine only and the morphine with (b) (4) were noted.
- Clinical observations in this study were consistent with typical of opioid-mediated pharmacology hypoactivity, hyperactivity, hair loss, over-grooming, brown staining). No differences between the morphine only and the morphine with (b) (4) were noted.
- Several hematologic and clinical chemistry parameters differed from control but no differences were observed between the morphine only and morphine with (b) (4) groups.
- The NOAEL for morphine with (b) (4) in this study is the highest dose tested: 100 mg morphine with (b) (4).
- The (b) (4) used in this study will support a specification of (b) (4) % (see details of calculation below). The proposed specification of (b) (4) % is not supported by this study.
- The NOAEL in this study was the highest dose tested. The highest dose of 100 mg/kg of morphine (MS) used in this rat study had a concentration of (b) (4). Therefore, the rats at the 100 mg/kg dose of morphine received a total daily dose of (b) (4) mg/kg (b) (4) Using body surface area scaling for a human equivalent dose

(scaling factor of [redacted] or rat) [redacted]. The MTDD of morphine is 1.5 g. For a [redacted] Using body surface area scaling for a human equivalent dose (scaling factor [redacted] for human) [redacted]. The human equivalent dose at the NOAEL of the rat study of [redacted] mg/m^2 [redacted] is 0[redacted]% of the human equivalent dose at the MTDD of morphine of [redacted] mg/m^2. Therefore the study supports a specification of [redacted]% [redacted] at the MTDD of morphine.

Methods

Doses:	See table below
Frequency of dosing:	BID
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Reverse osmosis deionized water
Species/Strain:	Rat, Sprague-Dawley Crl:CD
Number/Sex/Group:	See table below
Satellite groups:	None
Unique study design:	Treated animals were habituated to the morphine dose by a dose escalation in the first week (titration phase) until the target dose was reached. After the titration phase, animals were dosed for 13 weeks.
Deviation from study protocol:	None

Table 6. Summary of doses after initial titration phase

Group	Treatment	Dose mg/kg/day (mg/kg b.i.d)	Number of animals	
			Males	Females
1	Control†	0	10	10
2	Low dose morphine	50 (25)	10	10
3	High dose morphine	100 (50)	10	10
4	Low dose morphine [redacted] mixture	50 (25)	10	10
5	High dose morphine [redacted] mixture	100 (50)	10	10

† Vehicle (deionised water) administered

Observations and Results

Mortality

A viability check was performed daily. One unscheduled death in the HD morphine group (Group 3) was observed. Following three episodes of convulsions on Study Days 91 and 92, the rat was euthanized. Macroscopic examination showed an incomplete deflation of all lung lobes and microscopic examination showed minimal focal inflammatory cell infiltrate in the liver.

Clinical Signs

Detailed clinical observations were conducted prior to initiation of treatment and weekly during treatment and recovery periods. Dose–dependent clinical signs typical of an opioid were observed in all treated groups. Increased activity (1-2 h post-dose), decreased activity (>6 h post dose), vocalization and irritable behavior were observed. Hair loss, over-grooming and brown fur staining (males only) were also noted throughout the study in treated groups. Similar clinical signs were noted across all treated groups.

Body Weights

Body weights were recorded prior to initiation of treatment and weekly during treatment. Lower body weight gains were seen in all male treated groups (Groups 2-5) and in Groups 3-5 in females. Slight body weight gain was observed in the female LD morphine only group. Although lower body weight gains were observed for the (b) (4) groups as compared to the morphine only groups at both doses, the magnitude of the changes were not toxicologically relevant. Concomitant decreases in food consumption were noted.

Table 7. Changes in body weight gain

Dose	***Body weight gain % control***	
	male	***female***
25 mg/kg MS	86	105
25 mg/kg MS + (b) (4)	76	93
50 mg/kg MS	80	93
50 mg/kg MS + (b) (4)	76	85

Food Consumption

Food consumption was recorded prior to study initiation and weekly during treatment. Consistent with the decreases in body weights, all groups except the female LD morphine only group showed slight decreases in food consumption as compared to control. In the female LD morphine only group, a slight increase was noted.

Ophthalmoscopy

Ophthalmoscopic examination was performed during Week 14. No treatment-related changes were noted.

Urinalysis

A slightly higher group mean of urinary pH was observed in all treated males with a similar trend in females. Group means of total urinary protein was lower in all treated males with a concomitant slight increase in total glucose. No changes in urinary protein or glucose were noted in females. No differences between the morphine and the morphine with (b) (4) groups were noted at either dose or for either sex.

Hematology

Standard hematology parameters were measured at termination of the treatment period. In males, significant but slight decreases in both HD groups were seen for red blood cells. Slight decreases were also seen for both LD groups but statistical significance was not reached. Increases in MCH, MCHC and MCV and decreases in RDW were observed for all treated males. In females, significant but slight increases were seen in hemoglobin in both (b) (4) groups but no changes were noted in hematocrit or red blood cells. Overall, the morphine only and morphine with (b) (4) groups showed a similar hematologic profile and no toxicologically relevant differences were observed.

Clinical Chemistry

Standard clinical chemistry parameters were measured at termination of the treatment period. Triglycerides were slightly lower in males at the HD with and without the (b) (4) However, no changes in cholesterol were observed in these groups. In females, triglycerides were lower in all treated groups and cholesterol values were slightly lower in all groups. In all parameters measured, no toxicologically relevant differences were seen between the morphine and morphine with (b) (4) groups.

Gross Pathology

Enlargement of the adrenals was seen in 2/10 males in the HD morphine and 2/10 males in the HD morphine with (b) (4) In females, enlargement of the adrenals was seen in 1/10 LD morphine and 1/10 HD morphine with (b) (4)

Organ Weights

Increased adrenal weights were observed in all treated males and females. The increases in weight were similar between morphine and morphine with (b) (4) for each dose and sex. No toxicologically relevant differences were seen between the morphine and morphine with (b) (4) groups for either sex.

Histopathology

Increased cortical hypertrophy of the adrenals was observed in all HD morphine groups with and without (b) (4) Males showed a higher incidence than females. Enlarged adrenals in males were also noted in both groups at the HD of morphine and all treated animals showed increases in adrenal weights. All other microscopic findings were similar to control or background incidence. No differences attributed to the (b) (4) were observed.

Toxicokinetics

No TK were conducted for this study.

Dosing Solution Analysis

The solutions utilized in the study were analyzed and found to be within acceptable concentration ranges.

7 Genetic Toxicology

No new genetic toxicology studies with morphine were submitted by the Applicant. The studies reviewed below have been submitted in support of the qualification of the degradant (b) (4) has been shown to be negative in the Ames assay and the in vitro chromosome aberration assay.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells

Study title: Bacterial Reverse Mutation Assay

Study no.:	AE09LU.502ICH.BTL (Egalet Study 067-EG-TOX-01)
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 8, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) NQS0802, 97.7%

Key Study Findings

- Under the conditions of this assay, (b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvr*A in either the presence or absence of S9.

Methods

Strains:	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537 and *Escherichia coli*: WP2 uvrA
Concentrations in definitive study:	50, 150, 500, 1500, and 5000 mcg +/- S9
Basis of concentration selection:	Preliminary toxicity study using 6.7 – 5000 mcg
Negative control:	DMF
Positive control:	See table below
Formulation/Vehicle:	DMF
Incubation & sampling time:	52 h at 37 degrees

Table 8. Ames assay positive controls

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. STBD3302V Exp. Date 31-Jul-2017 CAS No. 613-13-8 Purity 97.5%	1.0
TA100, TA1537			2.0
WP2 *uvr*A			15
TA98	None	2-nitrofluorene (Sigma Aldrich Chemical Co., Inc.) Lot No. S43858V Exp. Date 31-Mar-2016 CAS No. 607-57-8 Purity 99.4%	1.0
TA100, TA1535		sodium azide (Sigma Aldrich Chemical Co., Inc.) Lot No. MKBH5113V Exp. Date 30-Jun-2016 CAS No. 26628-22-8 Purity 99.6%	1.0
TA1537		9-aminoacridine (Sigma Aldrich Chemical Co., Inc.) Lot No. 09820CEV Exp. Date 31-Mar-2016 CAS No. 52417-22-8 Purity 99.4%	75
WP2 *uvr*A		methyl methanesulfonate (Sigma Aldrich Chemical Co., Inc.) Lot No. MKBR6050V Exp. Date 31-Oct-2017 CAS No. 66-27-3 Purity 100.0%	1,000

Study Validity

The study is valid. All strains were shown to contain the appropriate genetic markers. Suitable numbers of replicate plates and appropriate counting methods were utilized. The positive controls demonstrated clear increases in tester strain revertants while the vehicle control was within historical range for the tester strains for this vehicle.

Results

It is concluded that under conditions of the assays conducted, (b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvr*A in either the presence or absence of S9. The results are summarized in the tables below. No precipitate or reduction in bacterial lawn was observed at any concentration. For all strains, at least three concentrations of test article were able to be evaluated. All of the strains at all of the concentrations tested showed negative mutagenic responses in the presence and absence of exogenous metabolic activation with S9.

2 Page(s) have been Withheld in Full as B4 (CCI/TS) immediately following this page

7.2 *In Vitro* Assays in Mammalian Cells

Study title:
***In Vitro* Mammalian Chromosome Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL)**

Study no.:	AE09LU.341ICH.BTL (Egalet Study 067-EG-TOX-01)
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 10, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) NQS0802, 97.7%

Key Study Findings

- Under the conditions of this assay, (b) (4) is considered negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.

Methods

Cell line:	human peripheral blood lymphocytes
Concentrations in definitive study:	+/-S9: 50, 100, 200, 301 mcg/mL
Basis of concentration selection:	Preliminary toxicity study using 0.0301 to 301 mcg/mL (1 mM)
Negative control:	DMF
Positive control:	Methyl methanesulfonate (-S9) and Cyclophosphamide (+S9)
Formulation/Vehicle:	DMF
Incubation & sampling time:	-S9: 4 h and 20 h, +S9: 4 h

Study Validity

The study was deemed valid for the following reasons: the vehicle control cultures were within historical control ranges and the positive controls were significantly increased relative the vehicle controls.

Results

(b) (4) ((b) (4) did not induce structural or numerical aberrations in HPBLs when tested up to the limit dose of 1 mM (301 mcg/mL) in the absence or presence of S9. The data are summarized in the table below. The study is considered valid. Under the conditions of this assay, (b) (4) is considered negative for clastogenicity in both the presence and absence of metabolic activation in HPBLs.

1 Page(s) has been Withheld in Full as B4 (CCI/TS) immediately following this page

8 Carcinogenicity

No new studies were submitted by the Applicant. There are no carcinogenicity data for morphine in the published literature.

9 Reproductive and Developmental Toxicology

The Applicant has provided a literature review and has proposed language to update the Section 8 of the label in order to comply with PLLR. No new studies were submitted. In addition, a thorough review of the literature was conducted by Dr. Grace Lee and is attached as an appendix to this review. The language for Section 8 of the label will be updated with this information.

10 Special Toxicology Studies

No special toxicology studies were conducted.

11 Integrated Summary and Safety Evaluation

This formulation of extended-release morphine sulfate uses a PEO-based formulation to confer abuse-deterrent properties. All excipients, with the exception of the PEO, can be found either in previously approved products or have acceptable daily intakes at higher levels and do not require further justification for the levels when the product is consumed at the MTDD of MS. To support the safety of (b) (4) PEO in this product, the Applicant is referencing MF (b) (4) Master File (b) (4) has been found to be inadequate (b) (4)

Because of the longstanding use of PEO in many products referencing MF (b) (4) this deficiency will not be an approval issue for NDA 208603 and the levels of PEO in this product are considered acceptable from a pharmacology/toxicology perspective. However, several PMRs will be issued in order to address the deficiency.

The Applicant is referencing MF (b) (4) ((b) (4) for the MS drug substance (DS). The specifications for all DS impurities are considered acceptable. One drug product degradant, (b) (4) ((b) (4) has been identified by the Applicant. The specification for (b) (4) exceeds the ICH Q3B (R2) qualification threshold. The Applicant submitted an Ames assay, an in vitro chromosome aberration (CA) assay, and a 13-week repeat-dose rat toxicology study for qualification of (b) (4) (b) (4) was negative for genotoxic potential in both the Ames and CA assays. The NOAEL in the 13-week rat study supports a specification of (b) (4) % for (b) (4) but does not support the specification of (b) (4) % proposed by the Applicant. However, this application references MS Contin and the specification is within that of the referenced product MS Contin. Therefore, the specification for (b) (4) of (b) (4) % will be considered acceptable for NDA 208603.

12 Appendix/Attachments

The following labeling recommendations for morphine regarding the developmental and reproductive toxicity potential are based upon a review of the literature completed by Grace S. Lee, Ph.D. The labeling recommendations for animal data are only addressed here. For the final version of the label, please refer to the Action Letter.

Recommended Labeling	Rationale/Comment
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** **[Human data to be provided by the clinical and maternal health review teams]** **Risk Summary Statement (nonclinical)** In published animal reproduction studies, morphine administered subcutaneously during the early gestational period produced neural tube defects (i.e., exencephaly and cranioschisis) at 5 and 16 times the human daily dose of 60 mg based on body surface area (HDD) in hamsters and mice, respectively, lower fetal body weight and increased incidence of abortion at 0.4 times the HDD in the rabbit, growth retardation at 6 times the HDD in the rat, and axial skeletal fusion and cryptorchidism at 16 times the HDD in the mouse. Administration of morphine sulfate to pregnant rats during organogenesis and through lactation resulted in cyanosis, hypothermia, decreased brain weights, pup mortality, decreased pup body weights, and adverse effects on reproductive tissues at 3-4 times the HDD; and long-term neurochemical changes in the brain of offspring which correlate with altered behavioral responses that persist through adulthood at exposures comparable to and less than the HDD [*see Animal Data*].	
Animal Data Formal reproductive and developmental toxicology studies for morphine have not been conducted. Exposure margins for the following published study reports are based on human daily dose of 60 mg morphine using a body surface area comparison (HDD). Neural tube defects (exencephaly and cranioschisis) were noted in following subcutaneous administration of morphine sulfate (35-322 mg/kg) on Gestation Day 8 to pregnant hamsters (4.7 times the HDD). A no adverse effect level was not defined in this study and the findings	EFD hamster data source: (Geber & Schramm, 1975)

Recommended Labeling	Rationale/Comment
cannot be clearly attributed to maternal toxicity.	
Neural tube defects (exencephaly), axial skeletal fusions, and cryptorchidism were reported following a single subcutaneous (SC) injection morphine sulfate to pregnant mice (100-500 mg/kg) on Gestation Day 8 or 9 at 200 mg/kg or greater (16 times the HDD) and fetal resorption at 400 mg/kg or higher (32 times the HDD). No adverse effects were noted following 100 mg/kg morphine in this model (8 times the HDD).	EFD mouse data sources: (Harpel & Gautieri, 1968; Iuliucci & Gautieri, 1971)
In one study, following continuous subcutaneous infusion of doses greater than or equal to 2.72 mg/kg to mice (0.2 times the HDD), exencephaly, hydronephrosis, intestinal hemorrhage, split supraoccipital, malformed sternebrae, and malformed xiphoid were noted. The effects were reduced with increasing daily dose; possibly due to rapid induction of tolerance under these infusion conditions. The clinical significance of this report is not clear.	EFD mouse data source: (Ciociola & Gautieri, 1983)
Decreased fetal weights ere observed in pregnant rats treated with 20 mg/kg/day morphine sulfate (3.2 times the HDD) from Gestation Day 7 to 9. There was no evidence of malformations despite maternal toxicity (10% mortality).	Data source: (Johannesson & Becker, 1972)
In a second rat study, decreased fetal weight and increased incidences of growth retardation (runt) were noted at 35 mg/kg/day (5.7 times the HDD) and there was a reduced number of fetuses at 70 mg/kg/day (11.4 times the HDD) when pregnant rats were treated with 10, 35, or 70 mg/kg/day morphine sulfate via continuous infusion from Gestation Day 5 to 20. There was no evidence of fetal malformations or maternal toxicity.	Data source: (Fujinaga & Mazze, 1988)
An increased incidence of abortion was noted in a study in which pregnant rabbits were treated with 2.5 (0.8 times the HDD) to 10 mg/kg/day morphine sulfate via subcutaneous injection from Gestation Day 6 to 10. In a second study, decreased fetal body weights were reported following treatment of pregnant rabbits with increasing doses of morphine (10-50 mg/kg) during the pre-mating period and 50 mg/kg/day (16 times the HDD) throughout the gestation period. No overt malformations were reported in either publication; although only limited endpoints were evaluated.	Data source: (Raye, Dubin, & Blechner, 1977; Roloff, Howatt, Kanto, & Borker, 1975)
In published studies, exposure to morphine during gestation and/or lactation periods is associated with alteration of maternal behaviors (e.g., decreased nursing and pup retrievals) in mice at 1 mg/kg or higher (0.08 times the HDD) and rats at 1.5 mg/kg or higher (0.2 times the HDD) and a host of	Abnormal maternal behavior data for mice are from: (Haney & Miczek, 1989) and for rats are from: (Cruz Ade, Maiorka, Canteras, Sukikara, & Felicio, 2010; Kinsley & Bridges, 1990; Miranda-Paiva et al., 2007; Moura, Canteras, Sukikara, & Felicio, 2010; Russell et al., 1989; Slamberova,

Recommended Labeling	Rationale/Comment
behavioral abnormalities in the offspring of rats, including altered responsiveness to opioids at 4 mg/kg/day (0.7 times the HDD) or greater.	Szilagyi, & Vathy, 2001; Sobor et al., 2010; Stafisso-Sandoz, Polley, Holt, Lambert, & Kinsley, 1998; Sukikara et al., 2011; Yim et al., 2006; Zagon & McLaughlin, 1977a) Data on altered responsiveness to morphine are from: (Bianchi et al., 1988; Chiang, Hung, Lee, Yan, & Ho, 2010; Chiou et al., 2003; Cicero, Nock, O'Connor, Adams, & Meyer, 1995; Davis & Lin, 1972; Eriksson & Ronnback, 1989; Gagin, Cohen, & Shavit, 1996; Johannesson & Becker, 1972; Koyuncuoğlu & Aricioğlu, 1993)
Exposure to morphine during gestation and/or lactation periods in rats has been reported to be associated with: decreased pup viability at 12.5 mg/kg/day or greater (2 times the HDD); decreased pup body weights at 15 mg/kg/day or greater (2.4 times the HDD); decreased litter size, decreased absolute brain and cerebellar weights, cyanosis, and hypothermia at 20 mg/kg/day (3.2 times the HDD); and alteration of behavioral responses (play, social-interaction) at 1 mg/kg/day or greater (0.2 times the HDD).	The data on decreased pup viability are from: (Davis & Lin, 1972; Eriksson & Ronnback, 1989; Fujinaga & Mazze, 1988; Hunter, Vangelisti, & Olsen, 1997; Siddiqui, Haq, Shaharyar, & Haider, 1995; Zagon & McLaughlin, 1977a, 1977b). The data on decreased pup body weights are from: (Davis & Lin, 1972; Eriksson & Ronnback, 1989; Fujinaga & Mazze, 1988; Hunter et al., 1997; Johannesson & Becker, 1972; Siddiqui, Haq, & Shah, 1997; Siddiqui et al., 1995; Zagon & McLaughlin, 1977a, 1977b). The data on decreased litter size are from: (Siddiqui et al., 1997; Siddiqui et al., 1995; Zagon & McLaughlin, 1977a, 1977b). The data on decreased absolute brain and cerebellar weights, cyanosis, and hypothermia are from: (Zagon & McLaughlin, 1977b). The data on alteration of behavioral responses are from: (Buisman-Pijlman, Gerrits, & Van Ree, 2009; H. H. Chen et al., 2015; Niesink, van Buren-van Duinkerken, & van Ree, 1999; Niesink, Vanderschuren, & van Ree, 1996)
Fetal and/or postnatal exposure to morphine in mice and rats has been shown to result in morphological changes in fetal and neonatal brain and neuronal cell loss, alteration of a number of neurotransmitter and neuromodulator systems, including opioid and non-opioid systems, and impairment in various learning and memory tests that appear to persist into adulthood. These studies were conducted with morphine treatment usually in the range of 4 to 20 mg/kg/day (0.7 to 3.2 times the HDD).	The data on morphological changes in fetal and neonatal brain and neuronal cell loss for mice and rats are from: (Droblenkov, Karelina, & Shabanov, 2010; Ghafari & Golalipour, 2014; Ghafari, Roshandel, & Golalipour, 2011; Golalipour, Ghafari, Kafshgiri, Moghadam, & Moharri, 2013; Harlan & Song, 1994; Kazemi, Saraei, Azarnia, Dehghani, & Bahadoran, 2011; Maharajan et al., 2000; Mei, Niu, Cao, Huang, & Zhou, 2009; Niu et al., 2009; Sadraie et al., 2008; Tenconi et al., 1989). The data on alteration of a number of neurotransmitter and neuromodulator systems in rats are from: (Basheer, Yang, & Tempel, 1992; Bhat, Chari, & Rao, 2006; Bhat, Chari, Rao, & Wirtshafter, 2006; Bianchi et al., 1988; Buisman-

Recommended Labeling	Rationale/Comment
	Pijlman et al., 2009; J. Chen, Ravis, & Walters, 1994; Chiou et al., 2003; De Vries et al., 1991; A. M. Di Giulio et al., 1988; A.M. Di Giulio, Tenconi, Mantegazza, & Gorio, 1989; Puppala, Matwyshyn, Bhalla, & Gulati, 2004; Sahraei et al., 2013; Tempel, Yiang, & Badheer, 1994; Vathy, Slamberova, Rimanoczy, Riley, & Bar, 2003; Yang et al., 2003; Yang et al., 2006) The data on impairment of behavioral responses in rats are from: (Ahmadalipour et al., 2015; Buisman-Pijlman et al., 2009; H. H. Chen et al., 2015; Chiou et al., 2003; Klausz et al., 2011; Nasiraei-Moghadam et al., 2013; Niu et al., 2009; Yang et al., 2003; Yang et al., 2006)
Additionally, delayed sexual maturation and decreased sexual behaviors in female offspring at 20 mg/kg/day (3.2 times the HDD), and decreased plasma and testicular levels of luteinizing hormone and testosterone, decreased testes weights, seminiferous tubule shrinkage, germinal cell aplasia, and decreased spermatogenesis in male offspring were also observed at 20 mg/kg/day (3.2 times the HDD).	Data on alteration of sexual maturation and behaviors in female offspring are from: (Siddiqui et al., 1997). Data on alteration of hormone and reproductive organs in male offspring are from: (Siddiqui et al., 1995).
Decreased litter size and viability were observed in the offspring of male rats that were intraperitoneally administered morphine sulfate for 1 day prior to mating at 25 mg/kg/day (4.1 times the HDD) and mated to untreated females.	Data source: (Cicero et al., 1995)
Decreased viability and body weight and/or movement deficits in both first and second generation offspring were reported when male mice were treated for 5 days with escalating doses of 120 to 240 mg/kg/day morphine sulfate (9.7 to 19.5 times the HDD) or when female mice treated with escalating doses of 60 to 240 mg/kg/day (4.9 to 19.5 times the HDD) followed by a 5-day treatment-free recovery period. Similar multigenerational findings were also seen in female rats pre-gestationally treated w th escalating doses of 10 to 22 mg/kg/day morphine (1.6 to 3.6 times the HDD).	Data source: (G. Friedler, 1978; G. Friedler & Cochin, 1972; G. Friedler & Wurster-Hill, 1974)
8.3 Females and Males of Reproductive Potential In published animal studies, morphine administration adversely effected fertility and reproductive endpoints in male rats and prolonged estrus cycle in female rats [*See 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility*].	Data source: (Cicero et al., 1991)(Cicero, Davis, LaRegina, Meyer, & Schlegel, 2002)(Ghowsi & Yousofvand, 2015)(James et al., 1980)(Yilmaz et al., 1999)(Siddiqui et al., 1995)(Siddiqui et al., 1997)
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**	
Carcinogenesis Long-term animal studies have not been completed	

Recommended Labeling	Rationale/Comment
to evaluate the carcinogenic potential of morphine.	
Mutagenesis No formal studies to assess the mutagenic potential of morphine have been conducted. In the published literature, morphine was found to be mutagenic in vitro increasing DNA fragmentation in human T-cells. Morphine was reported to be mutagenic in the in vivo mouse micronucleus assay and positive for the induction of chromosomal aberrations in mouse spermatids and murine lymphocytes. Mechanistic studies suggest that the in vivo clastogenic effects reported with morphine in mice may be related to increases in glucocorticoid levels produced by morphine in this species. In contrast to the above positive findings, in vitro studies in the literature have also shown that morphine did not induce chromosomal aberrations in human leukocytes or translocations or lethal mutations in Drosophila.	Data source: (Badr & Rabouh, 1983; Couch & Sawant, 1995; Das & Swain, 1982; Falek, Jordan, King, Arnold, & Skelton, 1972; Fuchs & Pruett, 1993; Knaap & Kramers, 1976; Sawant, Kozlowski, & Couch, 2001; Shafer, Xie, & Falek, 1994; Swain, Das, & Paul, 1980)
Impairment of Fertility No formal nonclinical studies to assess the potential of morphine to impair fertility have been conducted. Several nonclinical studies from the literature have demonstrated adverse effects on male fertility in the rat from exposure to morphine. One study in which male rats were administered morphine sulfate subcutaneously prior to mating (up to 30 mg/kg twice daily) and during mating (20 mg/kg twice daily) with untreated females, a number of adverse reproductive effects including reduction in total pregnancies and higher incidence of pseudopregnancies at 20 mg/kg/day (3.2 times the HDD) were reported.	Data source: (Cicero et al., 2002)
(b) (4)	
Female rats that were administered morphine sulfate intraperitoneally prior to mating exhibited prolonged estrous cycles at 10 mg/kg/day (1.6 times the HDD).	Data source: (Siddiqui et al., 1997; Siddiqui et al., 1995)
Exposure of adolescent male rats to morphine has been associated with delayed sexual maturation and following mating to untreated females, smaller litters, increased pup mortality, and/or changes in reproductive endocrine status in adult male offspring have been reported (estimated 5 times the plasma levels at the HDD).	Data source: (Cicero et al., 1991)

Morphine has a long history of use as pain medications, and there are numerous publications on the effects of morphine on reproductive and developmental systems in the literature. However, these studies have been conducted with mainly investigative research purposes, and none of these studies have been conducted according to the GLP standard. To update morphine Rx labeling, an extensive literature review has been conducted and major pivotal findings are described here. The studies that are more fitted into the three major types of studies to support drug development; namely, fertility, embryo-fetal developmental, and pre- and post-natal developmental toxicity studies, are described more extensively, and other studies that are considered more mechanistic studies are described collectively in this appendix.

Effects on Fertility. The effects of male fertility were examined in two mouse and six rat studies in which males were administered morphine, whereas the effects of female fertility were evaluated in one mouse study and five rat studies in which females were administered morphine. The male fertility studies showed that male-mediated adverse effects of morphine on development of offspring, and similar effects also have been seen in the female fertility studies.

Male Fertility

In the Friedler and Wurster-Hill (1974) study, male Charles River mice received twice daily subcutaneous (SC) injections of increasing doses of morphine sulfate (120-240 mg/kg) or saline for 5 days, followed by a 5-day drug-free recovery period, and then these males were mated with untreated females (G. Friedler & Wurster-Hill, 1974). The effects of pre-gestational exposure to morphine on the growth of two generations of progeny were investigated in this study. The F1 male and female offspring exhibited decreased birth weights, which persisted throughout the 12-week observation period. There was no difference in birth weights in F2 male and female offspring, but decreased body weight in the morphine group appeared by 1 week of age. There was no morphine-related increase in chromosome aberrations that were examined using cell cultures from newborn F1 offspring. The initial dose tested (120 mg/kg = 360 mg/m^2) is 9.7 times the human daily dose (HDD) of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

In the Badr and Rabouh (1983) study, a dominant lethal test with different time of mating (1-6 weeks) after treatment and a spermatocyte test (chromosomal aberrations in spermatogonia examined at 45-50 days after treatment) were carried out after daily injection of 10, 20, 40, or 60 mg/kg of morphine sulfate to male mice for 3 consecutive days (Badr & Rabouh, 1983). There were decreased number of live fetuses at Week 3, increased total embryonic losses especially at Weeks 1, 2, and 3, and increased mutation index at Week 3 at ≥10 mg/kg/day in the dominant lethal test, and increased abnormal cells at ≥10 mg/kg/day in the spermatocyte test. The dose level of 10

mg/kg/day (10 mg/kg = 30 mg/m^2) is only 0.8 times the HDD of 60 mg/ 60 kg person (37 mg/m^2) based on body surface area.

James et al., 1980 administered morphine sulfate to 42-day old male rats at a SC injection of 50 mg/kg/day for up to 9 weeks, and there were decreased serum concentrations of luteinizing hormone (LH) and testosterone, reduced weights of prostates and seminal vesicles, histology findings in pituitary (cellular atrophy, nuclear pyknosis, and condensation of granules), and quantitative reductions in spermatogenic cell populations at 50 mg/kg/day (James et al., 1980). No NOAEL was identified, but all findings were reversible at the end of the 13-week recovery period. The dose level of 50 mg/kg/day (50 mg/kg = 300 mg/m^2) is 8.1 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Cicero et al., (1991) implanted 25-27 days old male rats with a single 75 mg morphine or placebo pellet on Day 1, and two 75 mg morphine pellets on Days 4, 7, and 10 (Cicero et al., 1991). This treatment produced a delay in sexual maturation (decreased serum levels of testosterone and LH and reduced weights of the testes and seminal vesicles) in morphine-treated males. In addition, there were a slightly smaller mean litter size in the morphine group and the changes on endocrine in morphine-derived offspring (decreased serum testosterone levels, increased serum LH levels, modestly increased hypothalamic β-endorphin levels, slightly increased serum corticosterone levels, and higher adrenal weights in males; and increases in serum corticosterone levels and hypothalamic β-endorphin levels in females). Daily dose level of morphine were not reported in the paper; however, plasma levels of morphine were measured and free morphine blood levels were elevated initially up to 100 ng/mL for the first several days after pellet insertion. Based on data from 30 mg MS Contin reported in ClinicalPharmacologyonline.com, the 30 mg dose of MS Contin produces steady state levels of approximately 10 ng/mL. As MS Contin is the referenced drug product and has been reported to be bioequivalent and dose proportional, we can estimate that a 60 mg daily dose of Arymo should produce steady state plasma concentrations of about 20 ng/mL. Therefore, the exposures in this study are estimated to be about 5 times the plasma levels of a 60 mg dose of Arymo.

Cicero et al. (1995) treated adult male rats with a single intraperitoneal (IP) injection of 25 mg/kg morphine sulfate and at 24 h after treatment, treated males were mated with untreated females (Cicero et al., 1995). The authors report decreased litter size, increased pup mortality rates, decreased pup body weights, and also enhanced morphine-induced analgesic response (increased reaction time) in male offspring derived from morphine-exposed fathers. The dose level of 25 mg/kg (25 mg/kg = 150 mg/m^2) is 4.1 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Cicero et al. (2002) treated adult male rats with twice daily SC injections of increasing dose of 10-30 mg/kg/injection morphine sulfate to male rats for 14 days and then 20 mg/kg/injection for 2 days during the mating period (Cicero et al., 2002). The authors report decreased weights of prostates and seminal vesicles, decreased fertility index, and increased incidences of pseudopregnancy in the morphine group. The initial dose level of 20 mg/kg/day (20 mg/kg = 120 mg/m^2) is 3.2 times the HDD of 60 mg/ 60 kg person (37 mg/m^2) based on body surface area.

Ghowsi (2015) treated male rats (Groups 2 and 3) received twice daily SC injections of increasing doses of morphine sulfate from 10 mg/kg (20 mg/kg/day) to 68 mg/kg (136 mg/kg/day) for 30 consecutive days (Ghowsi & Yousofvand, 2015). The respective control groups (Groups 1 and 4) were also included in the study. Following the completion of the dosing period, males in Groups 3 and 4 were treated with methadone via drinking water for 14 days. The dose level of methadone was increased from 0.4 mg to 1 mg and then decreased to 0.4 mg. There were decreased serum levels of testosterone, LH, and follicle-stimulating hormone in the morphine group (Group 2), morphine + methadone group (Group 3) and the saline + methadone group (Group 4) and decreased weights of testes, prostates, and seminal vesicles in the Groups 2 and 3, relative to the control group [Group1]). The data appear that methadone did not ameliorate the morphine-induced effects on male reproductive parameters. No NOAEL for morphine was identified in the study because there were decreased serum levels of testosterone, LH, and FSH and decreased weights of testes, prostates, and seminal vesicles in rats in the morphine group. The initial dose level of 20 mg/kg/day (20 mg/kg = 120 mg/m^2) is 3.2 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Yilmaz et al. (1999) treated 30-32 days old male rats with twice daily SC injections of morphine HCl (5 mg/kg) or saline for 30 days, there was decreased mean body weight gain although no morphine-related changes in testicular weights were observed (Yilmaz et al., 1999). Also, there were no morphine-related changes in the seminiferous tubules and Leydig cells. Lower serum levels of testosterone and LH, but not FSH, were noted at 10 mg/kg/day. The dose level of 10 mg/kg/day (10 mg/kg = 60 mg/m^2) is 1.6 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Female fertility

Friedler (1978) investigated the effect of pre-gestational exposure to morphine on the growth of two generations of progeny (G. Friedler, 1978). Swiss-Webster inbred and Swiss outbred CD-1 mice received a SC injection of increasing doses of morphine sulfate or saline for 5 days, followed by a 5-day drug-free recovery period, and then these females were mated with untreated males. In Experiment 1, Swiss-Webster inbred mice received an initial dose of morphine (60 mg/kg), which was increased to a

daily maximum of 240 mg/kg on Day 5. In Experiment 2, Swiss outbred female mice received an initial dose of morphine (120 mg/kg), which was increased to a daily maximum of 420 mg/kg on Day 5. At 9 weeks of age, F1 male offspring Swiss outbred mice were mated to their respective F0 dams (Experiment 3). To investigate the pre-gestational influence on a second (F2) generation, F1 Swiss albino offspring were brother-sister mated (Experiment 4). There were no differences in pregnancy rate, litter size, or body weights of dams of the two treatment groups either during gestation or after parturition (Experiments 1-4). In all studies, there was a significant decrease in body weight of progeny in the morphine group. The growth retardation in males and females was evident at birth. In addition, pronounced movement deficits were observed in a few F2 offspring of the morphine group. The lowest dose level of 60 mg/kg/day (60 mg/kg = 180 mg/m^2) is 4.9 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

In the female fertility studies by Siddiqui et al. (1995, 1997)female rats received an IP injection of increasing doses of morphine sulfate (5-40 mg/kg/day) for 38 days prior to mating, and throughout gestation and lactation periods (Siddiqui et al., 1997; Siddiqui et al., 1995). Note that female rats received morphine at 20-40 mg/kg/day from the beginning of gestation until the end of the dosing period. Treated F0 females exhibited prolonged estrous cycles in which the assessment was performed from Day 25, where the dose level was 10 mg/kg/day (1.6-times of HDD), and decreased maternal body weight gain prior and during mating and gestation period. There were increased gestational lengths, decreased live litter size, increased stillbirths, decreased body weight and body weight gain of live pups, and morphine withdrawal signs in male and female pups in the morphine group at ≥20 mg/kg/day (3.2-times of HDD) during the gestation and lactation periods. The adult male offspring exhibited decreased testicular weight, decreased plasma levels of LH and testosterone and testicular testosterone concentrations, testicular histology findings (shrinkage of seminiferous tubules, Sertoli cell nuclei and tubular fluid in seminiferous tubules with signs of fibrosis and sclerosis, completely absent of germ cells, thin lamina propria, composed of only one to two layers, small Leydig cells containing small nuclei with largely condensed chromatin, dark cytoplasm, interstitial tissue containing fibrocytes, macrophages, and mast cells), increased hypothalamic noradrenaline on Postnatal Day (PND) 120 (at 17 weeks) at ≥20 mg/kg/day (3.2-times of HDD). Female offspring exhibited a delay in vaginal opening, decreased ovarian weights, decreases in plasma levels of LH and estradiol and ovarian concentrations of LH and progesterone, inhibition of adult lordosis behavior, and decreased hypothalamic norepinephrine level at ≥20 mg/kg/day (3.2-times of HDD). The lowest dose level of 10 mg/kg/day (10 mg/kg = 60 mg/m^2) is 1.6 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Female fertility and pre- and postnatal development studies by Zagon and McLaughlin (1977) report that female rats received twice daily IP injections of increasing doses 10-40 mg/kg to female rats for 5 days prior to mating and throughout the gestation and lactation periods (Zagon & McLaughlin, 1977a, 1977b). Females were mated with non-treated males, and dams were allowed to deliver. At weaning (PND 21), animals in the morphine group were divided into two groups (Groups 1 and 2). Offspring in Group 1 were not treated, whereas offspring in Group 2 received twice daily IP injections of 20 mg/kg morphine from PNDs 22 to 60. One morphine-treated F0 female found dead on GD 13 from unknown cause, and from Day 4 of treatment, F0 females in the morphine group exhibited opioid-related clinical signs. There was poor maternal behavior with offspring frequently scattered or buried. Findings of the F1 offspring included decreased number of pups, increased number of stillborn, decreased live offspring at 24 h and at weaning, and decreased pup body weight at birth and during pre- and post-weaning period. Within the first week after weaning, there were deaths of 4/10 offspring in Group 2 at 40 mg/kg/day. During the first week of the postnatal period, morphine-exposed pups were thin, cyanotic, and hypothermic. Throughout the 60-day postnatal period, offspring in Group 2 were docile, lethargic, and often unresponsive; these signs were less noticeable in offspring in Group 1. At sacrifice, decreased brain weights were observed in the morphine group. The lowest dose level of 20 mg/kg/day (20 mg/kg = 120 mg/m^2) is 3.2 times the HDD of 60 mg/ 60 kg person (37 mg/m^2) based on body surface area.

Friedler and Cochin (1972) treated Holtzman female rats with SC injections of increasing doses of morphine sulfate or saline for 5 1/2 days or 10 days, followed by a 5-day drug-free recovery period, and then these females were mated with untreated males (G. Friedler & Cochin, 1972). The dosing regimen for the 5 1/2 days dosing consisted of a single dose of 10 mg/kg on Day 1, twice daily doses of 15 mg/kg on Days 2-5, and a single dose of 22 mg/kg on Day 6, whereas the dosing regimen for the 10 days of dosing consisted of a starting dose of 10 mg/kg with 5 mg/kg increments to a maximum total daily dose of 60 mg/kg on the fifth day until the end of treatment. There were no morphine-related adverse effects at birth, but from 3 to 4 weeks of age, growth retardation in male and female offspring and decreased viability in female offspring were observed. The effects in offspring of dams with morphine exposure prior to mating were not eliminated by cross-fostering with untreated dams. There were no effects on similar control offspring cross-fostered to morphine-treated foster dams or nonfostered offspring of the saline-injected dams, revealing no detrimental effect of nursing by the morphine-treated dams on growth of offspring. The lowest dose level of 10 mg/kg/day (10 mg/kg = 150 mg/m^2) is 1.6 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area. Collectively, these data suggest that the adverse effects noted in the offspring are not due to lack of maternal care or to exposure of morphine via the breast milk in this model.

In addition to these fertility studies, Tang et al. (2015) showed that morphine exposure in pregnant female mice has been associated with reduced uterine receptivity and embryo implantation due to impaired luminal epithelial differentiation, decreased stromal cell proliferation, and poor angiogenesis at 50 mg/kg (Tang et al., 2015). The dose level of 50 mg/kg/day (50 mg/kg = 60 mg/m^2) is 4.1 times the HDD of 60 mg/ 60 kg person (37 mg/m^2) based on body surface area. These findings provide some mechanistic data that supports the previously reported findings of decreased implantations following morphine treatment. Although consistent with the other reported effects, this level of detail is not necessary for drug product labeling.

Effects on Embryo-Fetal Development. Several literature reports indicate that morphine treatment during the early gestation period in mice and hamsters produced neural tube defects (i.e., exencephaly and cranioschisis), axial skeletal fusion, and cryptorchidism (Ciociola & Gautieri, 1983; Geber & Schramm, 1975; Harpel & Gautieri, 1968; Iuliucci & Gautieri, 1971), whereas morphine treatment during the organogenesis in rats and rabbits has not produced fetal abnormalities (Fujinaga & Mazze, 1988; Johannesson & Becker, 1972) (Raye et al., 1977; Roloff et al., 1975). Animal studies that were examined the fetal endpoints following morphine administration during the period of organogenesis were summarized below.

Harpel and Gautieri (1968) administered a single SC injection of 100, 200, 300, 400, or 500 mg/kg of morphine sulfate to pregnant CF-1 mice on Gestational Day (GD) 8 or 9 (Harpel & Gautieri, 1968). The GD 8 treatment increased incidences of exencephaly at ≥300 mg/kg [24 times the HDD] (5/54, 6/92, and 11/86 fetuses at 300, 400, and 500 mg/kg, respectively, vs. 1/387 in the untreated control group, 0/74 in the saline-treated control group, and 1/58 in the food deprivation group) and of axial skeletal fusions at ≥400 mg/kg [32 times the HDD] (4/46 and 5/45 fetuses at 400 and 500 mg/kg, respectively, vs. 0/134 in the untreated control group and 0/37 in the saline-treated control group, but also 8/32 in the food deprivation group). The GD 9 treatment increased fetal resorption at ≥400 mg/kg [32 times the HDD], a few incidence of exencephaly (2/53 fetuses at 100 mg/kg and 1/111 fetus at 400 mg/kg), and increased incidence of axial skeletal fusions ≥200 mg/kg [16 times the HDD] (10/41, 10/56, 18/58, and 9/32 fetuses, at 200, 300, 400, and 500 mg/kg, respectively). Regarding maternal toxicity, a table with food consumption was only presented in the paper, but based on this table, the maternal food consumption data could not be properly interpreted. Because of increased incidence of axial skeletal fusions after the GD 9 treatment at ≥200 mg/kg, the NOAEL of the study is 100 mg/kg. The NOAEL of 100 mg/kg/day (100 mg/kg = 300 mg/m^2) is 8.1 times the recommended human dose of 60 mg/60 kg person (37 mg/m^2) based on body surface area. These data suggest that the cause of the morphine-induced exencephaly cannot be attributed to decreased food consumption.

Iuliucci and Gautieri (1971) administered morphine sulfate or saline to pregnant CF-1 mice as a single SC injection of 200, 300, or 400 mg/kg on GD 8 or 9 (Iuliucci & Gautieri, 1971). The GD 8 treatment produced a few incidence of exencephaly (1/69, 3/63, and 0/63 fetuses at 200, 300, and 400 mg/kg, respectively) and increased incidence of a retardation of testicular descent (cryptorchidism) at ≥200 mg/kg (3/40, 3/32, and 3/32 fetuses at 200, 300, and 400 mg/kg, respectively). The GD 9 treatment produced exencephaly at 200 mg/kg (1/62 fetuses), cryptorchidism at 300 mg/kg (2/27 fetuses), and increased incidence of axial skeletal fusions at ≥200 mg/kg. Data from morphine-treated groups exhibited some adverse toxicity in fetuses in the morphine-treated groups; however, incidences of these findings were relatively low and/or not dose-dependent. However, according to the authors, these findings were consistent with findings from other published papers. Maternal toxicity included opioid-related clinical signs and decreased body weight gain in all dose levels of morphine and high incidences of deaths (43-52%) at 400 mg/kg. No NOAEL could be identified because the fetal findings were considered morphine-related in this study. The low dose level of 200 mg/kg/day (200 mg/kg = 600 mg/m^2) is 16.2 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Ciociola and Gautieri (1983) administered morphine sulfate to pregnant mice using subcutaneous infusion of 0.04%, 0.4%, or 4% (2.72 mg/kg, 27.2 mg/kg, or 272 mg/kg, respectively) via the miniature infusion pumps implanted on GD 7, 8, 9, or 10. The authors reported adverse effects on the fetuses including: exencephaly, hydronephrosis, intestinal hemorrhage, split supraoccipital, malformed sternebrae, and malformed xiphoid (Ciociola & Gautieri, 1983). However, these fetal findings were mainly observed in the low dose group (0.04%), and significantly increased incidence of exencephaly was observed in only 0.04% morphine implanted on GD 7 group, which overlaps the period of neural tube development in this species. The lack of a dose dependency to the findings makes the data difficult to interpret. The authors speculate that the lack of dose dependency may be due to tolerance at the higher doses tested in this study. Infusions of opioids have been reported to produce tolerance in mice at a greater rate than periodic bolus dosing (Madia, Dighe, Sirohi, Walker, & Yoburn, 2009). However, the Reviewer still questions the adequacy of the study and reliability of the findings in the study because the fetal findings in the paper occurred at a much lower daily dose level than in other mouse studies, and the paper does not provide the sufficient information for the study procedure and proper data interpretation. Thus, the Reviewer concludes that this paper alone is not adequate to include in the morphine drug label; however, since the findings are consistent with other publications (see above), the finding may be included in the Animal Data section of the label. The low dose level of 2.72 mg/kg (2.72 mg/kg = 8.16 mg/m2) is 0.2 times the HDD of 60 mg/60 kg person (37 mg/m2) based on body surface area.

Similar to mouse studies, in the Geber and Schramm (1975) SC injected morphine sulfate (35-322 mg/kg) on GD 8 to pregnant hamsters and report exencephaly in this species (Geber & Schramm, 1975). There were high incidences of neural tube defects (exencephaly and cranioschisis) in every dose groups of morphine, and thus, an NOAEL could not be identified. There was no maternal death with morphine treatment in the study although these pregnant hamsters exhibited typical opioid-related clinical signs. The lowest dose level of 35 mg/kg/day (35 mg/kg = 175 mg/m^2) is 4.7 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Johanness and Becker (1972) treated pregnant rats with SC injection of 20 mg/kg/day morphine sulfate on GDs 2, 3, 4, and 5 (Group 1), on GDs 7, 8, and 9 (Group 2); and on GDs 11, 12, and 13 (Group 3) (Note day of detection of vaginal plug = GD 1) (Johannesson & Becker, 1972). Six pregnant females died in the morphine groups, yet there was no difference in maternal body weight. There was decreased fetal weight from dams treated with morphine on GDs 7-9 at 20 mg/kg/day and no other findings. Because of decreased fetal body weight, no NOAEL could be identified. The dose level of 20 mg/kg/day (20 mg/kg = 120 mg/m^2) is 3.2 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Fujinaga and Mazze (1988) treated pregnant rats subcutaneously via implanted osmotic minipumps that delivered morphine sulfate (10, 35, and 70 mg/kg/day) at a constant rate for 15 days (Fujinaga & Mazze, 1988). No maternal toxicity was reported in the study; however, the study reported high incidences of decreased “pregnancy rates” at ≥35 mg/kg/day. This is an unusual description of the finding because the dose administration was initiated on GD 5 after conception occurred. Thus, morphine treatment should not affect “pregnancy rates” in this teratology study. Apparent nonpregnant uteri of rodents should be visualized for early implantation sites (e.g., immersion in ~10% ammonium sulfide) to ensure whether animals were pregnant or not (Yamada, Hara, Ohba, Inoue, & Ohno, 1985). However, it is not stated whether this procedure was conducted for nonpregnant rats in the study. If this verifying procedure was not applied in the study, it is possible that pre-implantation loss was incorrectly described as nonpregnancy. The paper states that because of the very low pregnancy rate at HD, no other reproductive and fetal parameters could be determined at 70 mg/kg/day. Lower fetal weight and higher incidences of runts were seen at 35 mg/kg/day. Although variations including increased incidences of slightly enlarged cerebral ventricle and generalized decreased ossification, no malformation was noted at 35 mg/kg/day (5.7 times the HDD). The NOAEL was 10 mg/kg/day in the study, and the NOAEL of 10 mg/kg/day (10 mg/kg = 60 mg/m^2) is 1.6 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

In the rabbit study by Roloff, et al. (1975) treated pregnant New Zealand rabbits via SC injection of 0, 2.5, 5, or 10 mg/kg of morphine sulfate in 6-hour intervals from GD 6 to

GD 14 (Roloff et al., 1975). The paper states that 70% of morphine-treated pregnant rabbits lost weight, whereas 84% of the control rabbits gained weight. There was a roughly dose-dependent increase in incidence of abortion (22% at 2.5 mg/kg, 46% at 5 mg/kg, and 46% at 10 mg/kg), and lower mean fetal body weight in the morphine groups (dose groups not stratified). Fetal visceral and skeletal examinations were not included in the study. Because of increased incidence of abortion and lower fetal weight at ≥25 mg/kg, no NOAEL was identified in the study. The dose level of 2.5 mg/kg/day (2.5 mg/kg = 30 mg/m^2) is 0.8 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Raye, et al. (1977) administered increasing doses of 10-50 or 10-100 mg/kg of morphine sulfate (SC) prior to mating and 50 or 100 mg/kg, respectively, throughout the gestation period to pregnant New Zealand rabbits. The authors report a decrease (but not dose-dependent) in daily food intake in the morphine groups (Raye et al., 1977). There were dose-dependent decreases in fetal crown-rump lengths, fetal body weights, and relative weights of placenta, liver, and kidneys at ≥50 mg/kg, compared to the *ad libitum* or pair-fed control groups. Fetal visceral and skeletal examinations were not included in the study. Because of decreases in fetal crown-rump lengths, fetal weights along with decreased relative weights of placenta, liver, and kidneys at ≥50 mg/kg, no NOAEL was identified in the study. The dose level of 50 mg/kg/day (50 mg/kg = 600 mg/m^2) is 16 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Effects of Pre- and Postnatal Development. Various morphine-related pre- and post-natal developmental (PPND) data have been reported by numerous published papers. Published literature has reported that exposure to morphine during the gestation and/or lactation periods is associated with alteration of maternal behaviors and a host of behavioral abnormalities in the offspring of animals. Morphine-related neonatal toxicity caused by prenatal and postnatal exposure in rats include the following: decreased litter size, pup viability, and pup body weights, and decreased absolute brain and cerebellar weights, cyanosis, hypothermia, and alteration of behavioral responses. Major findings are summarized below. However, some of PPND data are also described in the Fertility section above.

In the Johannesson and Becker study (1972) using SC injection of 20 mg/kg/day of morphine sulfate to pregnant rats from GD 17 to GD 20 (Note day of detection of vaginal plug = GD 1), there were decreases in pup body weights on PNDs 12-13 and 20-21 and analgesic response of morphine on PNDs 12-13 at 20 mg/kg/day (Johannesson & Becker, 1972). The dose level of 20 mg/kg/day (20 mg/kg = 120 mg/m^2) is 3.2 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

In the Fujinaga and Mazze study (1988) using SC implant of osmotic minipumps that delivered morphine sulfate (35 mg/kg/day) from GD 5 at a constant rate for 15 days, there were decreased in pup survival and decreased pup body weights at 35 mg/kg/day (Fujinaga & Mazze, 1988). Additionally, a cross-fostering experiment was conducted following the same dosing treatment as the PPND portion by Fujinaga and Mazze (1988) by replacing morphine-treated dams with replaced control dams that had delivered at approximately the same time immediately after the delivery. After cross-fostering, in the pups from morphine-treated dams with fostered by dams in the control group, there was decreased in live pups on PNDs 1 and 4, increased mortality rates on PND 1 and 4, and decreased body weight on PNDs 1-28. There were very slightly decreased body weights on PNDs 1-28 in the pups from control-treated dams with fostered by dams in the morphine group. These data suggest that the effects cannot be attributed soley to decreased maternal care. The dose level of 35 mg/kg/day (35 mg/kg = 120 mg/m^2) is 5.7 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

In the study by Koyuncuoğlu and Aricioğlu (1993), pregnant rats received thrice daily SC injection of 10 mg/kg during last 5 days of gestation and were allowed to deliver (Koyuncuoğlu & Aricioğlu, 1993). Subsequently, two pellets containing 75 mg morphine base (total 150 mg) were also subcutaneously implanted into all young male offspring from all groups. Three days after pellet implantation, all rats were given IP dose of 2 mg/kg naloxone and then observed. There were the higher occurrences of jumping, wet-dog shakes, teeth-chattering, diarrhea, defecation, and ptosis in the prenatally morphine-exposed group. The initial dose level of 30 mg/kg/day (30 mg/kg = 180 mg/m^2) is 4.9 times the recommended human dose of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

In the Davis and Lin (1972) study, pregnant Holtzman rats received SC injection of morphine sulfate from GD 4 to GD 17 (Davis & Lin, 1972). The dose level of morphine was increased by 5 mg/kg increment every two days from a single daily dose of 15 mg/kg to a final dose of 45 mg/kg. Maternal body weight gain was decreased in the morphine group. There was no difference in litter size at birth, but decreased birth weight and decreased pup viability during the first week of neonatal period were observed. Increased ambulation (line-crossed) and rearing for the open-field activity test and increased locomotor activity counts for the photocell actometer test were observed in the offspring in the morphine group on PNDs 30 and 70. There were no morphine-related effects on audiogenic seizure susceptibility. The authors stated that high pup mortality during the first week of the neonatal period is consistent with an opioid withdrawal syndrome seen in untreated human infants. The initial dose level of 15 mg/kg/day (15 mg/kg = 90 mg/m^2) is 2.4 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Maternal behaviors in rats and mice that were treated with morphine during gestation (especially late gestation) and/or lactation periods exhibited decreased maternal behaviors and increased non-maternal behaviors (Cruz Ade et al., 2010; Haney & Miczek, 1989; Kinsley & Bridges, 1990; Miranda-Paiva et al., 2007; Moura et al., 2010); (Russell et al., 1989; Slamberova et al., 2001; Sobor et al., 2010; Stafisso-Sandoz et al., 1998; Yim et al., 2006; Zagon & McLaughlin, 1977a). Examples of disruptive maternal behaviors are decreases in nursing, retrieving and grouping pups, and nest building; whereas non-maternal behaviors include eating, self-grooming, resting with eyes closed, rearing, sniffing, and manipulating nonnest shavings.

Prenatal exposure to morphine has been shown to alter behavioral responses such as play and social interactions in rats. Buisman-Pijlman study showed that prenatal morphine treatment (at 10 mg/kg/day) increased play behivaior in rat pups on PND 21 (Buisman-Pijlman et al., 2009), whereas Chen et al. (2015) study showed prenatal morphine treatment (at increasing dose of 2 to 4 mg/kg/day) decreased the contact time in the social interaction test on PND 55 (H. H. Chen et al., 2015). The studies by Niesink et al. showed that prenatal exposure (at ≥1 mg/kg/day) increased social behaviors, related to play (pinning, boxing, and submissive postures), but not of other social behaviors, unrelated to play or non-social behaviors in rat offspring on PND 21 (Niesink et al., 1999; Niesink et al., 1996).

Rat offspring at juvenile and adolescent ages that were exposed to morphine pre- and post-natally or only prenatally exhibited impairment of various behavioral responses examined in the following tests: elevated plus-maze, Morris Water maze, Y-maze, objection recognition test, light-dark transition test, passive avoidance retention task, and forced swim test, and often the impaired effects were also observed in adulthood (Ahmadalipour et al., 2015; Buisman-Pijlman et al., 2009; H. H. Chen et al., 2015) (Chiou et al., 2003; Klausz et al., 2011; Nasiraei-Moghadam et al., 2013; Niu et al., 2009) (Yang et al., 2003; Yang et al., 2006). These studies were conducted with morphine treatment usually range of 4 to 20 mg/kg/day. The dose levels of 4 mg/kg/day (4 mg/kg = 24 mg/m^2) and 20 mg/kg/day (20 mg/kg = 120 mg/m^2) are 0.7-times and 3.2 times the HDD, respectively, of 60 mg/ 60 kg person (37 mg/m^2) based on body surface area. Some of these studies with behavioral test also have conducted for analyses of various protein levels or electrophysiological activities of brain tissues as described in the next following paragraphs.

Nasiraei-Moghadam, et al. (2013) study using oral administration of morphine sulfate at increasing dose of 0.01 mg/mL to 0.08 mg/mL via drinking water to pregnant rats from GD 1 to 13 (M1–13 group) or GD 1 to 21 (M1–21 group) (Nasiraei-Moghadam et al., 2013). Estimated daily dose level of morphine was from 0.73 to 5.84 mg/kg/day. Impaired passive avoidance retention task and increased Bax/Bcl-2 ratio and cleaved caspase-3 were observed in the hippocampus of adolescent and adult female offspring

in both M1–13 and M1–21 groups and adolescent male offspring in the M1–21 group. Additionally, there were decreased the expression of brain-derived neurotrophic factor (BDNF) precursor protein in adolescent female offspring in the M1–13 group and adolescent and adult female offspring in the M1–21 group and decreased ratio of phosphorylated CaMKII to CaMKII in adolescent male and female rats in both M1–13 and M1–21 groups. The initial dose level of 0.73 mg/kg/day (0.73 mg/kg = 4.38 mg/m^2) and the highest dose level of 5.84 mg/kg/day (5.84 mg/kg = 35.04 mg/m^2) are 0.12- and 0.95-times, respectively, the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

In the Niu (2009) study using twice daily SC injections of morphine sulfate (5 mg/kg for the first three injections and 10 mg/kg for all subsequent injections) to pregnant rats from GD 11 to GD 18, juvenile offspring exhibited poorer performance for the Y-maze task and loss of GABA-containing neurons in the dentate gyrus (Kinsley & Bridges) area (Niu et al., 2009). Additionally, there was decreased depotentiation (DP), but not long-term potentiation (LTP), of the EPSP slope in hippocampal DG area as well as decreases in both LTP and DP of the PS amplitude in the DG area. The dose level of 20 mg/kg/day (20 mg/kg = 120 mg/m^2) is 3.2 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Yang et al. administered twice daily SC injections of increasing dose of morphine HCl (2 mg/kg to 7 mg/kg) to female rats from 7 days prior to mating throughout the entire gestational period until 30 days postpartum. Offspring exhibited impaired performance of spatial learning (tested by Morris water maze task) on PNDs 28-31(Yang et al., 2003); (Yang et al., 2006). There was gross neuronal loss in all three representative brain regions (mid-brain, temporal cortex, and hippocampal CA1 subregion) from offspring in the morphine group on PND 14, decreased expression of PSD-95, nNOS, the phosphorylation of CREBSerine-133, and smaller long-term depression (LTD) generated by a low-frequency stimulation in hippocampal slices in the morphine group on PND 14. The dose level of 6 mg/kg/day (6 mg/kg = 36 mg/m^2, which was administered at the early gestation) is 0.97 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

Other studies in rats and mice also suggested that the impact of pre- and post-natal exposure to morphine can alter a number of neurotransmitter and neuromodulator systems, including noradrenaline (De Vries et al., 1991), met-enkephalin (Bianchi et al., 1988; A. M. Di Giulio et al., 1988; A.M. Di Giulio et al., 1989; Tempel et al., 1994; Tenconi et al., 1989), dopamine (J. Chen et al., 1994; Tenconi et al., 1989), and alteration of mu receptor binding or density (Bhat, Chari, & Rao, 2006; Vathy et al., 2003).

Morphological analyses of brain regions have conducted in rat and mouse offspring that were exposed to morphine pre- and post-natally or only prenatally, and morphine treatment have been shown to result in morphological changes in fetal and neonatal brain and neuronal cell loss (Buisman-Pijlman et al., 2009; Chiou et al., 2003; Ghafari & Golalipour, 2014; Ghafari et al., 2011; Harlan & Song, 1994; Kazemi et al., 2011; Maharajan et al., 2000; Mei et al., 2009; Niu et al., 2009; Yang et al., 2006).

Morphine-induced morphological changes in rat offspring included decreased thickness of the cortical plate along with suppressed cell proliferation in the cortical plate (Sadraie et al., 2008), and increases in the choroid plexus (CP) surface and ependymal cells in the lateral and third CP brain cavity and decreases in the lateral cavities, third ventricle surface, and central canal (ependymal canal) surfaces (Kazemi et al., 2011), and marked damage to neurons of the mesoaccumbocingulate system in adult offspring (Droblenkov et al., 2010).

The mouse studies showed that morphine treatment caused increases in the thickness of the external granular cell layer and molecular layer and decreases in the Purkinje cell layer, inner granular layer and total cerebellar cortex layer along with Purkinje cell loss (Ghafari et al., 2011), decreased thickness of the stratum pyramidal layer and increased thickness of the other hippocampal regions along with decreased number of pyramidal neurons in the hippocampal CA1, CA2, and CA3 subfields (Ghafari & Golalipour, 2014), decreased thickness of granular and polymorph layer in medial blade and lateral blade area of dentate gyrus in hippocampal coronal sections along with neuronal cells loss of dentate gyrus (Golalipour et al., 2013), and an increase in the number of PV-positive neurons and dendrites in layers II, III, and IV of the parietal cortex I (Maharajan et al., 2000).

Harlan et al. (1994) study showed decreased number of labeled cells, especially in the ventrolateral caudate-putamen of PND 0 rat pups that were prenatally exposed to morphine (20 mg/kg/day from GD 5 until term) and injected with BrdU on GD 14 (Harlan & Song, 1994). In brains of embryos injected with BrdU on GD 19, there were fewer labeled cells in the shell region of the nucleus accumbens in pups in the morphine group on PND 0, and in the caudate-putamen, the GD 19-labeled cells had migrated away from the ventricular and subventricular zones to occupy nearly the entire extent of the region. The labeled cells did not form clusters, as was seen for GD 14-labeled cells on PND 0. However, in one pup in the morphine group on PND 0, most of the GD 19-labeled cells were still adjacent to the ventricle, suggesting a severe deficit in cellular migration. Although the findings suggest an impact on neuronal migration during development, due to the minimal information presented in this manuscript, the finding is not recommended to be included in the labeling at this time.

In addition, the Nasiraei-Moghadam, et al. (2005) study administered morphine sulfate (0.01, 0.05, and 0.1 mg/mL [0.51, 2.5, and 5.1 mg/kg/day, respectively]) to pregnant rats from GD 0 until the sacrifice on GD 9.5 (Nasiraei-Moghadam et al., 2005). The morphine treatment led to decreased thickness of neuroectoderm, but the magnitude of the decrease was inversely related with increasing dose, namely, the embryos in the low dose group exhibited the most decreased thickness of neuroectoderm. Microscopic observations also revealed a neural groove, instead of a neural tube, in the morphine-exposed embryos on GD 9.5. The authors stated that these findings reflected a delay in neural tube development by morphine treatments in rats. Similarly, Niknam et al. study administered morphine sulfate (0.01, 0.05, and 0.1 mg/mL [0.51, 2.5, and 5.1 mg/kg/day, respectively]) to pregnant rats from GD 0 until the sacrifice on GD 12, 13, or 14, and morphine treatment caused decreases in the fronto-posterior length and the weight of the embryos (Niknam et al., 2013). Microscopic observations showed decreases in thickness of the white substance, grey substance, and ventricular layer and number of grey substance cells in the morphine group on GDs 12-14. There was an increase in the length of the ependimal duct in the morphine group, suggesting that the spinal cord is less complete on GD 12-14 by the morphine treatment. Although these studies somewhat suggest potential adverse effects of morphine on development of neural tube and spinal cord in rats, but available embryo-fetal developmental studies in rats have not reported any structural neurological defects even at the higher dose levels than the one used in these mechanistic studies.

Pre- and post-natally exposure to morphine has been shown to alter tolerance to morphine such as withdrawal and analgesic responses (Bianchi et al., 1988; Chiang et al., 2010; Chiou et al., 2003; Eriksson & Ronnback, 1989; Gagin et al., 1996; Johannesson & Becker, 1972; Koyuncuoğlu & Aricioğlu, 1993).

The Chiou (2003) study showed markedly decreased antinociceptive effect of supraspinal morphine (2 mg/kg, intracerebroventricular [ICV]) in offspring exposed to morphine in utero and via milk (Chiou et al., 2003). In the Chiang (2010) study, when the offspring received twice daily morphine injections for 4 days, the prenatally morphine-exposed rats more quickly developed a tolerance to morphine (decreased antinociceptive activity in the tail-flick tests) at 8-12 weeks age (Chiang et al., 2010). Similarly, the Johannesson and Becker (1972) study showed decreased analgesic responses (decreased reaction time on the hot plate) in 12-13 days old pups that were preternally exposed to morphine (Johannesson & Becker, 1972). On the other hand, an increased sensitivity to the analgesic effect of morphine (tested with the hot plate method) was seen in 80-90 days old male offspring that were prenatally treated with morphine (Eriksson & Ronnback, 1989). The Gagin 1996 study also showed higher analgesia in response to a morphine challenge in both tail-flick latency and hot-plate

latency tests and a greater preference for saccharin solution in rats prenatally exposed to morphine, compared to control groups (Gagin et al., 1996).

In addition to delayed sexual maturation and decreased sexual behaviors in female offspring described above, increased feminine behaviors were noted in adult male offspring in rats and hamsters that were pre- and/or post-natally exposed to morphine (Gagin, Cohen, & Shavit, 1997; Johnston, Payne, & Gilmore, 1992, 1994). In the Gagin (1997) study, adult male rat offspring that were prenatally exposed to morphine HCl exhibited normal rates of male copulatory behavior, but a significantly higher lordosis quotient, suggesting that prenatal exposure to morphine induced long-lasting feminizing effects (Gagin et al., 1997). The initial dose level of 3 mg/kg/day (3 mg/kg = 18 mg/m^2) and the highest dose level of 48 mg/kg (48 mg/kg = 288 mg/m^2) in the study are 0.49- and 7.8-times, respectively, the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area.

In the Johnston et al. (1994) study, adult female hamsters were once daily administered morphine (10 mg/kg, IP) for 4 days before mating and then throughout pregnancy (Johnston et al., 1994). Morphine was withdrawn during lactation by reducing the dose by 10% per day. When given estrogen and progesterone, 90% of male offspring that were pre- and postnatally exposed to morphine showed feminine behaviors, in comparison to 27% of males in the untreated group. The dose level of 10 mg/kg/day (10 mg/kg = 50 mg/m^2, which was administered at the early gestation) is 1.4 times the HDD of 60 mg/60 kg person (37 mg/m^2) based on body surface area. The Johnston et al. (1992) study using Duromorph (a long-acting form of morphine) also demonstrated increased feminine behavior in the male hamster offspring (Johnston et al., 1992).

Table 1 Safety margin (SM) for each finding from the embryo-fetal developmental toxicity studies. Safety margin is calculated with the lowest tested dose level that caused the finding.

	Mouse		Rat		Rabbit		Hamster	
	Dose Level (mg/kg/day)	**SM**	**Dose Levels (mg/kg/day)**	**SM**	**Dose Level (mg/kg/day)**	**SM**	**Dose Level (mg/kg/day)**	**SM**
Fetal Resorption	≥400	32x	-		-		-	
Exencephaly	≥200 ≥2.72	16x 0.2x	-		-		≥35	4.7x
Cranioschisis	-		-		-		≥35	4.7x
Axial skeletal fusion	≥200	16x	-		-		-	
Cryptorchism	≥200	16x					-	
Growth retardation (runt)	-		35	5.7x	-		-	
Decreased fetal body weight	-		≥20	3.2x	≥2.5	0.4x	-	
Increased incidence of abortion	-		-		≥2.5	0.4x	-	

SM for mg/m^2 basis with a human dose of 60 mg morphine for a body weight of 60 kg

Table 2 Safety margin (SM) for each finding from fertility and/or PPND studies Safety margin is calculated with the lowest tested dose level that caused the finding.

	Mouse			Rat		
	Dose Level (mg/kg/day)	**SM**	**Reference**	**Dose Level (mg/kg/day)**	**SM**	**Reference**
Decreased serum levels of testosterone and LH in males	-			10	1.6	(Ghowsi & Yousofvand, 2015; James et al., 1980; Yilmaz et al., 1999)
Decreased serum levels of FSH in males	-			20	3.2	(Ghowsi & Yousofvand, 2015)
Decreased weights of prostates, and seminal vesicles in males	-			20	3.2	(Cicero et al., 2002; Ghowsi & Yousofvand, 2015; James et al., 1980)
Decreased spermatogenic cell population	-			50	8.1x	(James et al., 1980)
Delayed sexual maturation in adolescent males	-			100 ng/mL	~5x[a]	(Cicero et al., 1991)
Decreased pregnancies and increased pseudo-pregnancies	-			20	3.2x	(Cicero et al., 2002)
Prolonged estrous cycle	-			10	1.6x	(Siddiqui et al., 1997; Siddiqui et al., 1995)
Reduced uterine receptivity and	50	4.1x	Tang, 2015	-	-	

	Mouse			Rat		
	Dose Level (mg/kg/day)	**SM**	**Reference**	**Dose Level (mg/kg/day)**	**SM**	**Reference**
embryo implantation						
Increased gestational lengths	-			20	3.2x	(Siddiqui et al., 1997; Siddiqui et al., 1995)
Alteration of maternal behaviors	1.0	0.08x	Haney et al., 1989	1.5	0.2x	(Cruz Ade et al., 2010; Kinsley & Bridges, 1990; Miranda-Paiva et al., 2007; Moura et al., 2010; Russell et al., 1989; Slamberova et al., 2001; Sobor et al., 2010; Stafisso-Sandoz et al., 1998; Sukikara et al., 2011; Yim et al., 2006; Zagon & McLaughlin, 1977a)
Decreased litter size	-			20	3.2x	(Siddiqui et al., 1997; Siddiqui et al., 1995; Zagon & McLaughlin, 1977a, 1977b)
Increased pup mortality	-			12.5	2x	(Davis & Lin, 1972; Eriksson & Ronnback, 1989; Fujinaga & Mazze, 1988; Hunter et al., 1997; Siddiqui et al., 1995; Zagon & McLaughlin, 1977a, 1977b)
Decreased pup body weights	-			15	2.4x	(Davis & Lin, 1972; Eriksson & Ronnback, 1989; Fujinaga & Mazze, 1988; Hunter et al., 1997; Johannesson & Becker, 1972; Siddiqui et al., 1997; Siddiqui et al., 1995; Zagon & McLaughlin, 1977a, 1977b)

	Mouse			Rat		
	Dose Level (mg/kg/day)	**SM**	**Reference**	**Dose Level (mg/kg/day)**	**SM**	**Reference**
Absolute brain and cerebellar weights	-			20	3.2x	(Zagon & McLaughlin, 1977b)
Cyanosis	-			20	3.2x	(Zagon & McLaughlin, 1977b)
Hypothermia	-			20	3.2x	(Zagon & McLaughlin, 1977b)
Alteration of behavioral response (play, social-interaction)	-			1	0.2x	(Buisman-Pijlman et al., 2009; H. H. Chen et al., 2015; Niesink et al., 1999; Niesink et al., 1996)
Impairment of behavioral responses	-			4	0.7x	(Ahmadalipour et al., 2015; Buisman-Pijlman et al., 2009; H. H. Chen et al., 2015; Chiou et al., 2003; Klausz et al., 2011; Nasiraei-Moghadam et al., 2013; Niu et al., 2009; Yang et al., 2003; Yang et al., 2006)
Morphological changes in fetal and neonatal brain, including neuronal cell loss	10	0.8x	(Ghafari & Golalipour, 2014; Ghafari et al., 2011; Golalipour et al., 2013)	5	0.8x	(Droblenkov et al., 2010; Harlan & Song, 1994; Kazemi et al., 2011; Maharajan et al., 2000; Mei et al., 2009; Niu et al., 2009; Sadraie et al., 2008; Tenconi et al., 1989)
Alteration of neurotransmitter and neuromodulator systems	-			2	0.3x	(Basheer et al., 1992; Bhat, Chari, Rao, et al., 2006; Bianchi et al., 1988; Buisman-Pijlman et al., 2009; J. Chen et al., 1994; Chiou et al., 2003; De Vries et al., 1991; A. M. Di Giulio et al., 1988; A.M. Di Giulio et al.,

	Mouse			Rat		
	Dose Level (mg/kg/day)	**SM**	**Reference**	**Dose Level (mg/kg/day)**	**SM**	**Reference**
						1989; Puppala et al., 2004; Sahraei et al., 2013; Tempel et al., 1994; Vathy et al., 2003; Yang et al., 2003; Yang et al., 2006)
Analgesic response to morphine (tail flick test, hot plate)	-			4	0.7x	(Bianchi et al., 1988; Chiang et al., 2010; Chiou et al., 2003; Cicero et al., 1995; Davis & Lin, 1972; Eriksson & Ronnback, 1989; Gagin et al., 1996; Johannesson & Becker, 1972; Koyuncuoğlu & Aricioğlu, 1993)
Decreased plasma and testicular levels of LH and testosterone in male offspring	-			20	3.2x	(Siddiqui et al., 1995)
Testicular histology findings in male offspring (e.g., decreased spermatogenesis)	-			20	3.2x	(Siddiqui et al., 1995)
Delay in vaginal opening in female offspring	-			20	3.2x	(Siddiqui et al., 1997)
Decreases in ovarian weights and	-			20	3.2x	(Siddiqui et al., 1997)

	Mouse			Rat		
	Dose Level (mg/kg/day)	**SM**	**Reference**	**Dose Level (mg/kg/day)**	**SM**	**Reference**
plasma levels of LH and estradiol and ovarian levels of LH and progesterone and inhibition of adult lordosis in female offspring						
Decreased serum testosterone levels and increased serum LH levels in adult male offspring	-			100 ng/mL of C_{max}	~5x[a]	(Cicero et al., 1991)

SM for mg/m^2 basis with a human dose of 60 mg morphine for a body weight of 60 kg

[a]Rationale for this SM can be found in the above paragraph describing the Cicero et al. (1991) study

Reference List

Ahmadalipour, A., Sadeghzadeh, J., Vafaei, A. A., Bandegi, A. R., Mohammadkhani, R., & Rashidy-Pour, A. (2015). Effects of environmental enrichment on behavioral deficits and alterations in hippocampal BDNF induced by prenatal exposure to morphine in juvenile rats. *Neuroscience, 305*, 372-383. doi: 10.1016/j.neuroscience.2015.08.015

Badr, F. M., & Rabouh, S. A. (1983). Effects of morphine sulphate on the germ cells of male mice. *Teratog Carcinog Mutagen, 3*(1), 19-26.

Basheer, R., Yang, J., & Tempel, A. (1992). Chronic prenatal morphine treatment decreases G alpha s mRNA levels in neonatal frontal cortex. *Brain Res Dev Brain Res, 70*(1), 145-148.

Bhat, R., Chari, G., & Rao, R. (2006). Effects of prenatal cocaine, morphine, or both on postnatal opioid (mu) receptor development. *Life Sci, 78*(13), 1478-1482. doi: 10.1016/j.lfs.2005.07.023

Bhat, R., Chari, G., Rao, R., & Wirtshafter, D. (2006). Prenatal cocaine and morphine alter brain cyclin-dependent kinase 5 (Cdk5) activity in rat pups. *Neurotoxicol Teratol, 28*(5), 625-628. doi: 10.1016/j.ntt.2006.06.006

Bianchi, M., Marini, A., Sacerdote, P., Cocco, E., Brini, A., & Panerai, A. E. (1988). Effect of chronic morphine on plasma and brain beta endorphin and methionine enkephalin in pregnant rats and in their fetuses or newborn. *Neuroendocrinology, 47*(2), 89-94.

Buisman-Pijlman, F. T., Gerrits, M. A., & Van Ree, J. M. (2009). Increased opioid release in specific brain areas in animals exposed to prenatal morphine and emotional stress later in life. *Neuroscience, 159*(1), 405-413. doi: 10.1016/j.neuroscience.2008.11.010

Chen, H. H., Chiang, Y. C., Yuan, Z. F., Kuo, C. C., Lai, M. D., Hung, T. W., . . . Chen, S. T. (2015). Buprenorphine, methadone, and morphine treatment during pregnancy: behavioral effects on the offspring in rats. *Neuropsychiatr Dis Treat, 11*, 609-618. doi: 10.2147/NDT.S70585

Chen, J., Ravis, W. R., & Walters, D. E. (1994). Perinatal exposure to morphine alters the density, but not the sensitivity, of dopamine receptors in the 10-day-old rat brain. *Research Communications in Alcohol and Substances of Abuse, 15*(3-4), 113-123.

Chiang, Y. C., Hung, T. W., Lee, C. W., Yan, J. Y., & Ho, I. K. (2010). Enhancement of tolerance development to morphine in rats prenatally exposed to morphine, methadone, and buprenorphine. *J Biomed Sci, 17*, 46. doi: 10.1186/1423-0127-17-46

Chiou, L. C., Yeh, G. C., Fan, S. H., How, C. H., Chuang, K. C., & Tao, P. L. (2003). Prenatal morphine exposure decreases analgesia but not K+ channel activation. *Neuroreport, 14*(2), 239-242. doi: 10.1097/01.wnr.0000054958.21656.a0

Cicero, T. J., Adams, M. L., Giordano, A., Miller, B. T., O'Connor, L., & Nock, B. (1991). Influence of morphine exposure during adolescence on the sexual maturation of male rats and the development of their offspring. *J Pharmacol Exp Ther, 256*(3), 1086-1093.

Cicero, T. J., Davis, L. A., LaRegina, M. C., Meyer, E. R., & Schlegel, M. S. (2002). Chronic opiate exposure in the male rat adversely affects fertility. *Pharmacol Biochem Behav, 72*(1-2), 157-163.

Cicero, T. J., Nock, B., O'Connor, L., Adams, M., & Meyer, E. R. (1995). Adverse effects of paternal opiate exposure on offspring development and sensitivity to morphine-induced analgesia. *J Pharmacol Exp Ther, 273*(1), 386-392.

Ciociola, A. A., & Gautieri, R. F. (1983). Evaluation of the teratogenicity of morphine sulfate administered via a miniature implantable pump. *J Pharm Sci, 72*(7), 742-745.

Couch, D. B., & Sawant, S. G. (1995). The clastogenicity of morphine sulfate in vivo. *Adv Exp Med Biol, 373*, 123-129.

Cruz Ade, M., Maiorka, P. C., Canteras, N. S., Sukikara, M. H., & Felicio, L. F. (2010). Morphine treatment during pregnancy modulates behavioral selection in lactating rats. *Physiol Behav, 101*(1), 40-44. doi: 10.1016/j.physbeh.2010.04.013

Das, R. K., & Swain, N. (1982). Mutagenic evaluation of morphine sulphate and pethidine hydrochloride in mice by the micronucleus test. *Indian J Med Res, 75*, 112-117.

Davis, W. M., & Lin, C. H. (1972). Prenatal morphine effects on survival and behavior of rat offspring. *Research Communications in Chemical Pathology adn Pharmacology, 3*(2), 205-214.

De Vries, T. J., Van Vliet, B. J., Hogenboom, F., Wardeh, G., Van der Laan, J. W., Mulder, A. H., & Schoffelmeer, A. N. (1991). Effect of chronic prenatal morphine treatment of mu-opioid receptor-regulated adenylate cyclase activity and neurotransmitter release in rat brain slices. *Eur J Pharmacol, 208*(2), 97-104.

Di Giulio, A. M., Restani, P., Galli, C. L., Tenconi, B., La Croix, R., & Gorio, A. (1988). Modified ontogenesis of enkephalin and substance P containing neurons after perinatal exposure to morphine. *Toxicology, 49*(1), 197-201.

Di Giulio, A. M., Tenconi, B., Mantegazza, P., & Gorio, A. (1989). The development of met-enkephalin innervation in the brain is altered by perinatal exposure to morphine. *Journal of Endocrinological Investigation, 12*(10 Suppl. 4), 5-8.

Dooley, R., Dooley, J., Antone, I., Guilfoyle, J., Gerber-Finn, L., Kakekagumick, K., . . . Kelly, L. (2015). Narcotic tapering in pregnancy using long-acting morphine: an 18-month prospective cohort study in northwestern Ontario. *Can Fam Physician, 61*(2), e88-95.

Droblenkov, A. V., Karelina, N. R., & Shabanov, P. D. (2010). Changes in neurons and gliocytes in the mesoaccumbocingulate system on perinatal exposure to morphine in rats. *Neurosci Behav Physiol, 40*(8), 848-851. doi: 10.1007/s11055-010-9334-0

Eriksson, P. S., & Ronnback, L. (1989). Effects of prenatal morphine treatment of rats on mortality, bodyweight and analgesic response in the offspring. *Drug Alcohol Depend, 24*(3), 187-194.

Falek, A., Jordan, R. B., King, B. J., Arnold, P. J., & Skelton, W. D. (1972). Human chromosomes and opiates. *Arch Gen Psychiatry, 27*(4), 511-515.

Friedler, G. (1978). Pregestational administration of morphine sulfate to female mice: longterm effects on the development of subsequent progeny. *J Pharmacol Exp Ther, 205*(1), 33-39.

Friedler, G., & Cochin, J. (1972). Growth retardation in offspring of female rats treated with morphine prior to conception. *Science, 175*, 654-656.

Friedler, G., & Wurster-Hill, D. (1974). Influence of morphine administration to male mice on their progeny. *Proceedings of National Research Council Committee on Problems of Drug Dependence*, 869-874.

Fuchs, B. A., & Pruett, S. B. (1993). Morphine induces apoptosis in murine thymocytes in vivo but not in vitro: involvement of both opiate and glucocorticoid receptors. *J Pharmacol Exp Ther, 266*(1), 417-423.

Fujinaga, M., & Mazze, R. I. (1988). Teratogenic and postnatal developmental studies of morphine in Sprague-Dawley rats. *Teratology, 38*(5), 401-410. doi: 10.1002/tera.1420380502

Gagin, R., Cohen, E., & Shavit, Y. (1996). Prenatal exposure to morphine alters analgesic responses and preference for sweet solutions in adult rats. *Pharmacol Biochem Behav, 55*(4), 629-634.

Gagin, R., Cohen, E., & Shavit, Y. (1997). Prenatal exposure to morphine feminizes male sexual behavior in the adult rat. *Pharmacol Biochem Behav, 58*(2), 345-348.

Garland, M., Abildskov, K. M., Kiu, T. W., Daniel, S. S., Weldy, P., & Stark, R. I. (2008). Placental transfer and fetal elimination of morphine-3-beta-glucuronide in the pregnant baboon. *Drug Metab Dispos, 36*(9), 1859-1868. doi: 10.1124/dmd.108.021352

Geber, W. F., & Schramm, L. C. (1975). Congenital malformations of the central nervous system produced by narcotic analgesics in the hamster. *Am J Obstet Gynecol, 123*(7), 705-713.

Ghafari, S., & Golalipour, M. J. (2014). Prenatal morphine exposure reduces pyramidal neurons in CA1, CA2 and CA3 subfields of mice hippocampus. *Iran J Basic Med Sci, 17*(3), 155-161.

Ghafari, S., Roshandel, D., & Golalipour, M. J. (2011). Effect of intrauterine morphine sulfate exposure on cerebellar histomorphological changes in neonatal mice. *Folia Neuropathol, 49*(4), 328-334.

Ghowsi, M., & Yousofvand, N. (2015). Impact of morphine dependency and detoxification by methadone on male's rat reproductive system. *Iran J Reprod Med, 13*(5), 275-282.

Golalipour, M. J., Ghafari, S., Kafshgiri, S. K., Moghadam, M. H., & Moharri, A. R. (2013). Effect of maternal morphine sulfate exposure on neuronal plasticity of dentate gyrus in Balb/c mice offspring. *Pak J Biol Sci, 16*(6), 281-286.

Haney, M., & Miczek, K. A. (1989). Morphine effects on maternal aggression, pup care and analgesia in mice. *Psychopharmacology (Berl), 98*(1), 68-74.

Harlan, R. E., & Song, D. D. (1994). Prenatal morphine treatment and the development of the striatum. *Regulatory Peptides, 54*(1), 117-118.

Harpel, H. S., Jr., & Gautieri, R. F. (1968). Morphine-induced fetal malformations. I. Exencephaly and axial skeletal fusions. *J Pharm Sci, 57*(9), 1590-1597.

Hunter, M. A., Vangelisti, G. R., & Olsen, G. D. (1997). Chronic intermittent in utero exposure to morphine: effects on respiratory control in the neonatal guinea pig. *Biol Neonate, 72*(5), 293-304.

Iuliucci, J. D., & Gautieri, R. F. (1971). Morphine-induced fetal malformations II: influence of histamine and diphenhydramine. *Journal of Pharmaceutical Sciences, 60*(3), 420-425.

James, R. W., Heywood, R., & Crook, D. (1980). Effects of morphine sulphate on pituitary-testicular morphology of rats. *Toxicol Lett, 7*(1), 61-70.

Johannesson, T., & Becker, B. A. (1972). The effects of maternally-administered morphine on rat foetal development and resultant tolerance to the analgesic effect of morphine. *Acta Pharmacol Toxicol (Copenh), 31*(4), 305-313.

Johnston, H. M., Payne, A. P., & Gilmore, D. P. (1992). Perinatal exposure to morphine affects adult sexual behavior of the male golden hamster. *Pharmacol Biochem Behav, 42*(1), 41-44.

Johnston, H. M., Payne, A. P., & Gilmore, D. P. (1994). Effect of exposure to morphine throughout gestation on feminine and masculine adult sexual behaviour in golden hamsters. *J Reprod Fertil, 100*(1), 173-176.

Kazemi, M., Saraei, H., Azarnia, M., Dehghani, L., & Bahadoran, H. (2011). Effect of oral morphine consumption in female rats on development of brain cavities, central canal and choroid plexus of their embryos. *Cell Journal, 12*(4), 489-494.

Kinsley, C. H., & Bridges, R. S. (1990). Morphine treatment and reproductive condition alter olfactory preferences for pup and adult male odors in female rats. *Dev Psychobiol, 23*(4), 331-347. doi: 10.1002/dev.420230405

Klausz, B., Pinter, O., Sobor, M., Gyarmati, Z., Furst, Z., Timar, J., & Zelena, D. (2011). Changes in adaptability following perinatal morphine exposure in juvenile and adult rats. *Eur J Pharmacol, 654*(2), 166-172. doi: 10.1016/j.ejphar.2010.11.025

Knaap, A. G., & Kramers, P. G. (1976). Absence of mutagenic effects of morphine in Drosophila. *Mutat Res, 40*(2), 97-100.

Koyuncuoğlu, H., & Aricioğlu, F. (1993). Prenatal exposure to morphine or naloxone intensifies morphine dependence at maturity. *Pharmacology Biochemtstry and Behavior, 44*(4), 939-941.

Madia, P. A., Dighe, S. V., Sirohi, S., Walker, E. A., & Yoburn, B. C. (2009). Dosing protocol and analgesic efficacy determine opioid tolerance in the mouse. *Psychopharmacology (Berl), 207*(3), 413-422. doi: 10.1007/s00213-009-1673-6

Maharajan, P., Prencipe, R., Di Francesco, P., Paino, G., Ravagnan, G., & Maharajan, V. (2000). Maternal morphine alters parvalbumin immunoreactivity patterns in neonatal mouse brain. *Synapse, 35*(4), 265-271. doi: 10.1002/(SICI)1098-2396(20000315)35:4<265::AID-SYN4>3.0.CO;2-6

Mei, B., Niu, L., Cao, B., Huang, D., & Zhou, Y. (2009). Prenatal morphine exposure alters the layer II/III pyramidal neurons morphology in lateral secondary visual cortex of juvenile rats. *Synapse, 63*(12), 1154-1161. doi: 10.1002/syn.20694

Miranda-Paiva, C. M., Canteras, N. S., Sukikara, M. H., Nasello, A. G., Mackowiak, II, & Felicio, L. F. (2007). Periaqueductal gray cholecystokinin infusions block morphine-induced disruption of maternal behavior. *Peptides, 28*(3), 657-662. doi: 10.1016/j.peptides.2006.11.005

Moura, L. M., Canteras, N. S., Sukikara, M. H., & Felicio, L. F. (2010). Morphine infusions into the rostrolateral periaqueductal gray affect maternal behaviors. *Braz J Med Biol Res, 43*(9), 899-905.

Nasiraei-Moghadam, S., Sahraei, H., Bahadoran, H., Sadooghi, M., Salimi, S. H., Kaka, G. R., . . . Dashtnavard, H. (2005). Effects of maternal oral morphine consumption on neural tube development in Wistar rats. *Brain Res Dev Brain Res, 159*(1), 12-17. doi: 10.1016/j.devbrainres.2005.06.012

Nasiraei-Moghadam, S., Sherafat, M. A., Safari, M. S., Moradi, F., Ahmadiani, A., & Dargahi, L. (2013). Reversal of prenatal morphine exposure-induced memory deficit in male but not female rats. *J Mol Neurosci, 50*(1), 58-69. doi: 10.1007/s12031-012-9860-z

Niesink, R. J., van Buren-van Duinkerken, L., & van Ree, J. M. (1999). Social behavior of juvenile rats after in utero exposure to morphine: dose-time-effect relationship. *Neuropharmacology, 38*(8), 1207-1223.

Niesink, R. J., Vanderschuren, L. J., & van Ree, J. M. (1996). Social play in juvenile rats after in utero exposure to morphine. *Neurotoxicology, 17*(3-4), 905-912.

Niknam, N. A., Azarnia, M., Bahadoran, H., Kazemi, M., Tekieh, E., Ranjbaran, M., & Sahraei, H. (2013). Evaluating the effects of oral morphine on embryonic development of cerebellum in wistar rats. *Basic Clin Neurosci, 4*(2), 130-135.

Niu, L., Cao, B., Zhu, H., Mei, B., Wang, M., Yang, Y., & Zhou, Y. (2009). Impaired in vivo synaptic plasticity in dentate gyrus and spatial memory in juvenile rats induced by prenatal morphine exposure. *Hippocampus, 19*(7), 649-657. doi: 10.1002/hipo.20540

Puppala, B. L., Matwyshyn, G., Bhalla, S., & Gulati, A. (2004). Role of endothelin in neonatal morphine tolerance. *J Cardiovasc Pharmacol, 44 Suppl 1*, S383-385.

Raye, J. R., Dubin, J. W., & Blechner, J. N. (1977). Fetal growth retardation following maternal morphine administration: nutritional or drug effect? *Biol Neonate, 32*(3-4), 222-228.

Roloff, D. W., Howatt, W. F., Kanto, W. P., Jr., & Borker, R. C., Jr. (1975). Morphine administration to pregnant rabbits: effect on fetal growth and lung development. *Addict Dis, 2*(1-2), 369-379.

Russell, J. A., Gosden, R. G., Humphreys, E. M., Cutting, R., Fitzsimons, N., Johnston, V., . . . Stirland, J. A. (1989). Interruption of parturition in rats by morphine: a result of inhibition of oxytocin secretion. *J Endocrinol, 121*(3), 521-536.

Sadraie, S. H., Kaka, G. R., Sahraei, H., Dashtnavard, H., Bahadoran, H., Mofid, M., . . . Jafari, F. (2008). Effects of maternal oral administration of morphine sulfate on developing rat fetal cerebrum: a morphometrical evaluation. *Brain Res, 1245*, 36-40. doi: 10.1016/j.brainres.2008.09.052

Sahraei, H., Rostamkhani, F., Tekieh, E., Dehghani, L., Poorazizi, E., Meamar, R., & Kazemi, M. (2013). Identification of morphine accumulation in the rat embryo central nervous system: a c14-morphine administration study. *Int J Prev Med, 4*(Suppl 2), S222-228.

Sawant, S. G., Kozlowski, R. S., & Couch, D. B. (2001). The role of adrenal corticosteroids in induction of micronuclei by morphine. *Mutat Res, 498*(1-2), 129-133.

Shafer, D. A., Xie, Y., & Falek, A. (1994). Detection of opiate-enhanced increases in DNA damage, HPRT mutants, and the mutation frequency in human HUT-78 cells. *Environ Mol Mutagen, 23*(1), 37-44.

Siddiqui, A., Haq, S., & Shah, B. H. (1997). Perinatal exposure to morphine disrupts brain norepinephrine, ovarian cyclicity, and sexual receptivity in rats. *Pharmacol Biochem Behav, 58*(1), 243-248.

Siddiqui, A., Haq, S., Shaharyar, S., & Haider, S. G. (1995). Morphine induces reproductive changes in female rats and their male offspring. *Reprod Toxicol, 9*(2), 143-151.

Slamberova, R., Szilagyi, B., & Vathy, I. (2001). Repeated morphine administration during pregnancy attenuates maternal behavior. *Psychoneuroendocrinology, 26*(6), 565-576.

Sobor, M., Timar, J., Riba, P., Kiraly, K. P., Gyarmati, S., Al-Khrasani, M., & Furst, S. (2010). Does the effect of morphine challenge change on maternal behaviour of dams chronically treated with morphine during gestation and further on during lactation? *Pharmacol Biochem Behav, 95*(3), 367-374. doi: 10.1016/j.pbb.2010.02.015

Stafisso-Sandoz, G., Polley, D., Holt, E., Lambert, K. G., & Kinsley, C. H. (1998). Opiate disruption of maternal behavior: morphine reduces, and naloxone restores, c-fos activity in the medial preoptic area of lactating rats. *Brain Res Bull, 45*(3), 307-313.

Sukikara, M. H., Cruz, A. M., Felippe, E. C., Anselmo-Franci, J. A., Canteras, N. S., de Oliveira, C. A., & Felicio, L. F. (2011). Morphine-induced changes in opioid sensitivity in postpartum females: a unique progesterone response. *J Neuroendocrinol, 23*(11), 1134-1138. doi: 10.1111/j.1365-2826.2011.02182.x

Swain, N., Das, R. K., & Paul, M. (1980). Cytogenetic assay of potential mutagenicity in vivo of two narcotic analgesics. *Mutat Res, 78*(1), 97-100.

Tang, X., Chen, Y., Ran, H., Jiang, Y., He, B., Wang, B., . . . Wang, H. (2015). Systemic morphine treatment derails normal uterine receptivity, leading to embryo implantation failure in mice. *Biol Reprod, 92*(5), 118. doi: 10.1095/biolreprod.115.128686

Tempel, A., Yiang, J., & Badheer, R. (1994). Alterations in opioid peptides and the cAMP system in brains of morphine addicted newborns. *Regulatory Peptides, Suppl. 1*, S281-282.

Tenconi, B., La Croix, R., Donadoni, M. L., Di Giulio, A. M., Mantegazza, P., & Gorio, A. (1989). The developmental pattern of met-enkephalin innervation is altered in animals perinatally exposed to morphine. *Pharmacol Res, 21 Suppl 1*, 61-62.

Vathy, I., Slamberova, R., Rimanoczy, A., Riley, M. A., & Bar, N. (2003). Autoradiographic evidence that prenatal morphine exposure sex-dependently alters mu-opioid receptor densities in brain regions that are involved in the control of drug abuse and other motivated behaviors. *Prog Neuropsychopharmacol Biol Psychiatry, 27*(3), 381-393. doi: 10.1016/S0278-5846(02)00355-X

Yamada, T., Hara, M., Ohba, Y., Inoue, T., & Ohno, H. (1985). [Studies on implantation traces in rats. II. Staining of cleared uteri, formation and distribution of implantation traces]. *Jikken Dobutsu, 34*(3), 249-260.

Yang, S. N., Huang, L. T., Wang, C. L., Chen, W. F., Yang, C. H., Lin, S. Z., . . . Tao, P. L. (2003). Prenatal administration of morphine decreases CREBSerine-133 phosphorylation and synaptic plasticity range mediated by glutamatergic

transmission in the hippocampal CA1 area of cognitive-deficient rat offspring. *Hippocampus, 13*(8), 915-921. doi: 10.1002/hipo.10137

Yang, S. N., Liu, C. A., Chung, M. Y., Huang, H. C., Yeh, G. C., Wong, C. S., . . . Tao, P. L. (2006). Alterations of postsynaptic density proteins in the hippocampus of rat offspring from the morphine-addicted mother: Beneficial effect of dextromethorphan. *Hippocampus, 16*(6), 521-530. doi: 10.1002/hipo.20179

Yilmaz, B., Konar, V., Kutlu, S., Sandal, S., Canpolat, S., Gezen, M. R., & Kelestimur, H. (1999). Influence of chronic morphine exposure on serum LH, FSH, testosterone levels, and body and testicular weights in the developing male rat. *Arch Androl, 43*(3), 189-196.

Yim, A. J., Miranda-Paiva, C. M., Florio, J. C., Oliveira, C. A., Nasello, A. G., & Felicio, L. F. (2006). A comparative study of morphine treatment regimen prior to mating and during late pregnancy. *Brain Res Bull, 68*(5), 384-391. doi: 10.1016/j.brainresbull.2005.09.014

Zagon, I. S., & McLaughlin, P. J. (1977a). Effects of chronic morphine administration on pregnant rats and their offspring. *Pharmacology, 15*(4), 302-310.

Zagon, I. S., & McLaughlin, P. J. (1977b). Morphine and brain growth retardation in the rat. *Pharmacology, 15*(3), 276-282.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ELIZABETH BOLAN
10/04/2016

GRACE S LEE
10/04/2016

RICHARD D MELLON
10/04/2016

I concur with Drs. Bolan and Lee that NDA 208603 may be approved from a nonclinical pharmacology toxicology perspective and with the recommended post-marketing requirements and labeling.