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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: 207621
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Applicant's letter date: December 19, 2014 & October 5, 2015
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Product: TROXYCA ER (extended-release oxycodone
HCl and sequestered naltrexone HCl) capsule
Indication: (b) (4)
Applicant: Pfizer, Inc.
Review Division: Division of Anesthesia, Analgesia, and Addiction
Products
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1 Executive Summary

1.1 Introduction

Pfizer has submitted NDA 207621 for TROXYCA ER capsules (oxycodone hydrochloride, OC, extended-release with sequestered naltrexone hydrochloride, NTX) for the indication of management of pain severe enough to require daily, around-the-clock, long-term opioid treatment for which alternative treatment options are inadequate. The capsules are filled with individual pellets consisting of various layers designed to provide a controlled release profile for the OC component of the drug product and to adequately sequester the NTX when the product is used as labeled. The Applicant states that upon crushing, chewing or extraction of the product the NTX will be released and should abate the liking and euphoric effects of OC. The product will be available in strengths containing 10/1.2, 20/2.4, 30/3.6, 40/4.8, 60/7.2, and 80/9.6 mg/mg of OC/NTX and is intended for BID dosing. This NDA is a 505(b)(2) application and is relying on the Agency's findings of safety and the description of the pharmacology for OC and NTX in the labels of Roxicodone (NDA 21011) and Revia (NDA 18932), respectively.

1.2 Brief Discussion of Nonclinical Findings

Since NTX is not approved to be used intravenously, two toxicology studies were conducted to support the safety of a human abuse potential study with intravenous administration of OC and NTX. The studies were considered acceptable to support the safety of the clinical study. No other pharmacology or toxicology studies were required with either OC or NTX.

Genetic toxicology studies were conducted with two NTX drug substance and NTX-derived drug product degradants, (b) (4) that exceeded ICH Q3A(R2) and ICH Q3B(R2) thresholds for qualification. Both compounds tested negative in the Ames assay and the in vivo mouse micronucleus assay. In contrast, both impurities tested positive for clastogenic activity in the in vitro chromosome aberration assay. As per ICH S2(R1), an additional in vivo clastogenicity study will be required for both compounds to fully evaluate their clastogenic potential. Since these two compounds are both NTX drug substance impurities and NTX-derived drug product degradants and are in currently approved products, it is acceptable to conduct these studies as post-marketing requirements.

The TROXYCA ER formulation contains excipients that are intended to confer abuse-deterrent properties. When the ALO-02 product is consumed at the maximum theoretical daily dose (MTDD) of OC ((b) (4) g), several excipients exceeded levels used in products previously approved for chronic use. Written safety justification was provided by the Applicant. A literature-based justification of dibutyl sebacate (DBS) did not contain adequate information to support the safety of the levels in the product. It was communicated to the Applicant that as per the FDA excipient guidance, general

toxicology studies in two species, a full reproductive toxicology battery as well as carcinogenicity studies in two species (unless adequate justification for a waiver of the carcinogenicity studies is provided as per the FDA excipient guidance) will be required to qualify DBS as a novel excipient. The Applicant subsequently submitted two 26-week general toxicology studies which tested DBS up to the limit dose in rat and dog. No adverse effects were observed at any dose in either species and the NOAELs in rat and dog yielded exposure margins 35-fold and 117-fold, respectively, the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed. These studies adequately qualify DBS for chronic dosing from a general toxicology perspective. Additionally, three reproductive toxicology studies were submitted. Dibutyl sebacate can be considered qualified for male and female fertility (M & F rat: 35-fold) and embryofetal development (rat: 35-fold; rabbit: 21-fold) with acceptable safety margins. However, the fourth study in the reproductive toxicology battery, a pre- and post-natal study in rat, was not conducted. Given that DBS is currently in approved products, albeit at lower levels, and low toxicity was observed in the studies conducted, it is considered acceptable to conduct the pre- and post-natal study post-approval.

Carcinogenicity studies in two species will not be required with DBS based on the previous human experience, the lack of histopathological changes in modern chronic toxicology studies in two species, the negative genetic toxicology studies, and the summary review of the literature cited in the Applicant's written justification which included discussion of an older 2-year rat study. This approach is consistent with recent input received from the OND Associate Director of Pharmacology and Toxicology and the FDA guidance document titled: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, which notes that "The Centers recognize that existing human data for some excipients can substitute for certain nonclinical safety data, and an excipient with documented prior human exposure under circumstances relevant to the proposed use may not require evaluation in the full battery of toxicology studies outlined in this guidance."

With a post-marketing requirement for the pre- and post-natal study with DBS, the levels of excipients in this formulation when the product is used at the MTDD of OC can be considered acceptable.

1.3 Recommendations

1.3.1 Approvability

Nonclinical pharmacology/toxicology recommends approval of NDA 207621. The need to further characterize the clastogenic potential of the NTX drug substance impurities/ drug product degradants (b) (4) and the potential for toxicity of the excipient dibutyl sebacate on pre- and post-natal development are not considered approval issues. These studies may be conducted as post-marketing requirements.

1.3.2 Additional Non Clinical Recommendations

The Applicant has not provided adequate information to fully characterize the potential for developmental toxicity for dibutyl sebacate. A pre- and post-natal development study with dibutyl sebacate should be conducted as previous human experience is not considered adequate to characterize the endpoints that are evaluated in this study.

In addition, the impurities/degradants (b) (4) tested positive for clastogenic activity in the in vitro chromosome aberration assay and are considered to have clastogenic potential. As per ICH S2(R1), an additional in vivo test is required.

The following studies are recommended as post-marketing requirements:

1. Conduct a pre- and post-natal development study in the rat model to assess the potential impact of dibutyl sebacate on development.
2. Conduct an additional in vivo genetic toxicology assay to further characterize the clastogenic potential of (b) (4)
3. Conduct an additional in vivo genetic toxicology assay to further characterize the clastogenic potential of (b) (4)

1.3.3 Labeling

The table below contains the draft labeling submitted by the Applicant, the changes proposed by the reviewer and the rationale for the proposed changes. For the final version of the label, please refer to the Action Letter. Note: The recommended changes from the proposed labeling are in bold red (additions) or strikeout font.

<i>Applicant's proposed labeling</i>	<i>Reviewer's proposed changes</i>	<i>Rationale for changes</i>
USE IN SPECIFIC POPULATIONS (b) (4)	USE IN SPECIFIC POPULATIONS (b) (4) Pregnancy: May cause fetal harm (8.1).	Added by PMHT
Conversion from Tramadol to TROXYCA ER (b) (4)	Conversion from Tramadol to TROXYCA ER (b) (4) there has been no systematic assessment of conversion from tramadol to other opioids.	The word (b) (4) was replaced with a more accurate description of the pharmacology of tramadol.
0	8 USE IN SPECIFIC	The format has been

	<p>POPULATIONS</p> <p>8.1 Pregnancy Risk Summary Prolonged use of opioid analgesics during pregnancy may cause neonatal opioid withdrawal syndrome [see Warnings and Precautions (5.3)]. (b) (4)</p> <p>(b) (4)</p> <p>(b) (4)</p> <p>Animal reproduction studies with oral (b) (4) administration of oxycodone HCl in rats and rabbits during the period of organogenesis at doses equal to or 3-times, respectively, the human dose of 160 mg/day (b) (4) did not reveal evidence of teratogenicity or embryo-fetal toxicity. In several published (b) (4) studies, treatment of pregnant rats with oxycodone at clinically relevant doses and below resulted in neurobehavioral effects in offspring [See Data]. (b) (4)</p> <p>(b) (4)</p>	<p>changed to comply with the Pregnancy and Lactation Labeling Rule.</p> <p>The Pediatric and Maternal Health Team (PMHT) will add the appropriate language for the description of the human data.</p> <p>Exposure comparisons were based on body surface area comparisons at the human dose of 160 mg/day. The dose of 80 mg is the highest dosage form of this product which is labeled to be dosed twice per day.</p> <p>Human: 160 mg/day= 2.7 mg/kg (60 kg human)= 99 mg/m² Rat: 16 mg/kg= 96 mg/m² Rabbit: 25 mg/kg= 300 mg/m² Body surface area comparison at above doses: Rat/human=1x HED Rabbit/human= 3x HED</p> <p>To comply with PLLR, a description of the maternal toxicity should be added. (b) (4)</p> <p>(b) (4)</p> <p>This NDA</p>
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	<p style="text-align: center;">(b) (4)</p> <p>Clinical Considerations [Human data added by PMHT here].</p> <p>Data <u>Animal Data</u> In embryo-fetal development studies in rats and rabbits, pregnant animals received oral doses of oxycodone HCl administered during the period of organogenesis up to 16 mg/kg/day and up to 25 mg/kg/day, respectively;</p> <p>(b) (4) These studies revealed no evidence of teratogenicity or embryo-fetal toxicity due to oxycodone. The highest doses tested in rats and rabbits were equivalent to approximately 1-(b) (4) and (b) (4) 3- times an adult human dose of (b) (4) 160 mg/day, respectively, on a mg/m² basis. In published studies, offspring of pregnant rats administered oxycodone during gestation have been reported to exhibit neurobehavioral effects including altered stress responses, (b) (4) increased anxiety-like behavior (2 mg/kg/day IV from Gestation Day 8 to 21 and Postnatal Day 1, 3, and 5; 0.1-times an adult human dose of 160 mg/day on a mg/m² basis), and altered learning and memory (15 mg/kg/day orally from breeding through parturition; equivalent to an adult human dose of 160 mg/day on a mg/m² basis).</p>	<p>references Roxicodone via the 505(b)(2) pathway. The reviewer is not permitted to access the original reviews of a product referenced by the 505(b)(2) pathway and may only use what is in the label of the approved product.</p> <p>(b) (4)</p> <p>This label can be updated when the Roxicodone label is converted to PLLR (b) (4)</p> <p>(b) (4)</p> <p>Neurobehavioral findings from two published papers have been included (Davis, et al., 2010; Sithisarn, et al., 2013).</p>
<p>11 DESCRIPTION TROXYCA ER extended-release capsule contains pellets of oxycodone HCl with naltrexone</p>	<p>11 DESCRIPTION TROXYCA ER extended-release capsule contains pellets of oxycodone HCl with naltrexone</p>	<p>The Established Pharmacologic Class for oxycodone and naltrexone was added.</p>

<p>HCl at a ratio of 100:12 in each capsule strength for oral administration. The capsule strength describes the amount of oxycodone HCl/naltrexone HCl per capsule. Oxycodone HCl is an agonist and naltrexone HCl is an antagonist at the mu-opioid receptor.</p>	<p>HCl at a ratio of 100:12 in each capsule strength for oral administration. The capsule strength describes the amount of oxycodone HCl/naltrexone HCl per capsule. Oxycodone HCl is an opioid agonist and naltrexone HCl is an opioid antagonist at the mu-opioid receptor.</p>	
<p>12.1 Mechanism of Action Oxycodone Hydrochloride Oxycodone is a full opioid agonist and is relatively selective for the mu receptor, although it can bind to other opioid receptors at higher doses. The principal therapeutic action of oxycodone is analgesia. Like all (b) (4) opioid agonists, there is no ceiling effect for analgesia with oxycodone. Naltrexone Hydrochloride Naltrexone is (b) (4) opioid antagonist that reverses the subjective and analgesic effects of mu-opioid receptor agonists by competitively binding at mu-opioid receptors.</p>	<p>12.1 Mechanism of Action Oxycodone Hydrochloride Oxycodone is a full opioid agonist and is relatively selective for the mu receptor, although it can bind to other opioid receptors at higher doses. The principal therapeutic action of oxycodone is analgesia. Like all (b) (4) full opioid agonists, there is no ceiling effect for analgesia with oxycodone. Naltrexone Hydrochloride Naltrexone is an (b) (4) opioid antagonist that reverses the subjective and analgesic effects of mu-opioid receptor agonists by competitively binding at mu-opioid receptors.</p>	<p>The word (b) (4) was deleted and replaced with the correct term "full". (b) (4) was deleted (b) (4)</p>
<p>13 NONCLINICAL TOXICOLOGY</p> <p>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</p> <p>Carcinogenesis (b) (4)</p> <p>Mutagenesis Oxycodone HCl was genotoxic in an <i>in vitro</i> mouse lymphoma assay in the presence of metabolic activation. There was no evidence of genotoxic potential in an <i>in vitro</i></p>	<p>13 NONCLINICAL TOXICOLOGY</p> <p>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</p> <p>Carcinogenesis Long-term studies have not been performed in animals to evaluate the carcinogenic potential of TROXYCA ER or oxycodone.</p> <p>Mutagenesis Oxycodone HCl was genotoxic in an <i>in vitro</i> mouse lymphoma assay in the presence of metabolic activation. There was no evidence of genotoxic potential in an <i>in vitro</i></p>	<p>Current wording is from the Roxicodone label. No changes are recommended.</p>

<p>bacterial reverse mutation assay (<i>Salmonella typhimurium</i> and <i>Escherichia coli</i>) or in an assay for chromosomal aberrations (<i>in vivo</i> mouse bone marrow micronucleus assay).</p> <p>Impairment of Fertility Fertility studies have not been performed in animals to evaluate the potential impact on fertility of TROXYCA ER or oxycodone.</p>	<p>bacterial reverse mutation assay (<i>Salmonella typhimurium</i> and <i>Escherichia coli</i>) or in an assay for chromosomal aberrations (<i>in vivo</i> mouse bone marrow micronucleus assay).</p> <p>Impairment of Fertility Fertility studies have not been performed in animals to evaluate the potential impact on fertility of TROXYCA ER or oxycodone.</p>	
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2 Drug Information

2.1 Drug

TROXYCA ER (code name ALO-02) contains oxycodone HCl and naltrexone HCl. The naltrexone component is sequestered and is included in the formulation to deter abuse.

Oxycodone Hydrochloride

CAS Registry Number: 124-90-3

Generic Name: Oxycodone hydrochloride

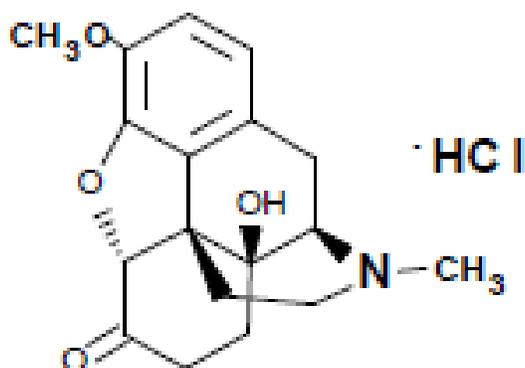
Code Name: NA

Chemical Name: (5 α)-14-hydroxy-17-methyl-3-(methoxy)-4,5-epoxymorphinan-6-one

Molecular Formula/Molecular Weight: C₁₈H₂₁NO₄•HCl; MW 351.83 g/mol

Structure:

Figure 1. Structure of Oxycodone Hydrochloride



Pharmacologic Class: Opioid agonist (Established Pharmacologic Class)

Naltrexone Hydrochloride

CAS Registry Number: 16676-29-2

Generic Name: Naltrexone hydrochloride

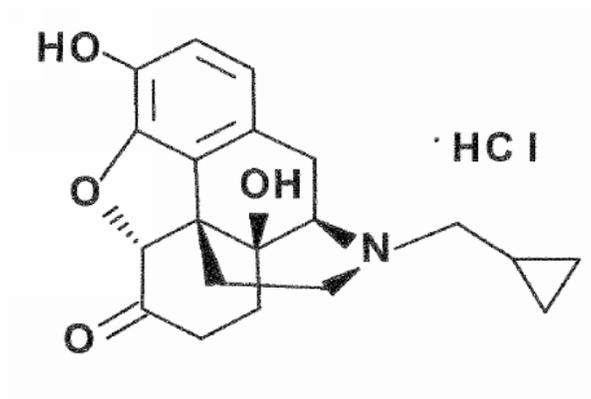
Code Name: NA

Chemical Name: morphinan-6-one, 17-(cyclopropylmethyl)-4,5- α -epoxy-3,14-dihydroxy-, hydrochloride

Molecular Formula/Molecular Weight: C₂₀H₂₃NO₄·HCl; MW= (b) (4) g/mol

Structure:

Figure 2. Structure of Naltrexone Hydrochloride



Pharmacologic Class: Opioid antagonist (Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND/NDA/MF	drug/compound	Sponsor	Division	status
IND 107037	ALO-02	Pfizer	DAAAP	Active
NDA 21011	Roxicodone	Mallinckrodt	DAAAP	Approved
NDA 18932	Revia	Teva	DAAAP	Approved
NDA 22321	Embeda	Pfizer	DAAAP	Approved
MF (b) (4)	Oxycodone HCl	(b) (4)	ONDQA	Acceptable
MF (b) (4)	Naltrexone HCl	(b) (4)	ONDQA	Acceptable

2.3 Drug Formulation

TROXYCA ER (ALO-02) is an extended release oxycodone hydrochloride (OC) capsule with properties put forth by the Applicant to confer abuse deterrence. The capsules are comprised of individual pellets containing layers of OC, naltrexone hydrochloride (NTX) and various excipients to provide a controlled-release profile for the OC and to sequester the NTX when the product is used as labeled. (b) (4)

The capsules contain (b) (4) ratio of OC to sequestered NTX and will be available in capsules containing 10/1.2, 20/2.4, 30/3.6, 40/4.8, 60/7.2, and 80/9.6 mg/mg of OC/NTX. If taken as prescribed, OC is released over a period of 12 hours. The Applicant states that upon crushing or chewing of the product the sequestered NTX will be released and should abate the liking and euphoric effects of OC (see CSS review by Dr. James Tolliver for details). (b) (4)

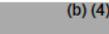
Table 1. Formulation of ALO-02 80 mg/9.6 mg Oxycodone/Naltrexone Capsule

Inactive Components:				
Talc	USP/NF			(b) (4)
Ammonio methacrylate copolymer (b) (4)	USP/NF			
Sugar spheres (b) (4)	USP/NF			
Ethylcellulose (b) (4)	USP/NF			
Hydroxypropyl cellulose (b) (4)	USP/NF			
Polyethylene glycol (b) (4)	USP/NF			
Dibutyl sebacate	USP/NF			
Diethyl phthalate	USP/NF			
Sodium lauryl sulfate	USP/NF			
Methacrylic acid copolymer (b) (4)	USP/NF			
Magnesium stearate	USP/NF			
Ascorbic acid	USP/NF			(b) (4)

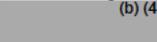
2.4 Comments on Novel Excipients

Table 2. Acceptability of Levels of Inactive Ingredients in the 80 mg/9.6 mg Oxycodone/Naltrexone Capsule at the MTDD of Oxycodone

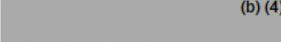
Inactive ingredient	Total dosage via single 80/9.6 mg/mg capsule, mg	Total dose at MTDD ((b) (4) capsules), mg	Acceptability, rationale
Talc		(b) (4)	Yes, IID
Ammonio methacrylate copolymer (b) (4)			Yes, IID, see below
Sugar spheres			Yes, GRAS
Ethyl cellulose			Yes, IID
Hydroxypropyl cellulose			Yes, IID
Polyethylene glycol (b) (4)			Yes, IID
Dibutyl sebacate			Yes, see below

Diethyl phthalate		(b) (4)	Yes, IID
Sodium lauryl sulfate		Yes, IID	
Methacrylic acid copolymer 		Yes, IID, see below	
		Yes, IID	
Ascorbic acid		Yes, IID	

IID= FDA Inactive Ingredients Database

 (b) (4) **(Ammonio methacrylate copolymer**  (b) (4) **) and**  (b) (4)
(Methacrylic acid copolymer  (b) (4) **)**



Therefore, the level  (b) (4) in the ALO-02 formulation,  (b) (4) does not present any unique toxicologic concerns and is considered acceptable.

Dibutyl Sebacate

Dibutyl sebacate (CAS # 109-43-3) is the dibutyl ester of sebacic acid. (b) (4)

Dibutyl sebacate is also used as a flavoring additive in food products, a plasticizer in food packaging and a lubricant in shaving lotions. Other commonly used names for dibutyl sebacate include: STAFLEX DBS, PX 404, Sebacic acid, dibutyl ester, Monoplex DBS, Kodaflex, and dibutyl decanedioate.

Figure 3. Structure of Dibutyl Sebacate



Although DBS is used as a flavoring agent and a plasticizer in food packaging, the levels for these applications are several orders of magnitude lower than the levels when this product is consumed at the MTDD of OC. According to the Joint FAO/WHO Expert Committee on Food Additives, the per capita intake for the United States is 0.08 mcg/kg.

Dibutyl sebacate is present in levels up to (b) (4) mg TDI in Embeda (NDA 22321) which is approved for chronic use. The TDI of DBS from the ALO-02 drug product if the MTDD of OC is consumed would be (b) (4) mg. (b) (4)

In support of the safety of levels of DBS, the Applicant originally submitted a literature-based written justification. A published report discusses the chronic toxicology of DBS (SMITH, 1953). One-year and two-year studies with DBS in rat with dietary administration yielded NOAELs that are 22-fold and 109-fold, respectively, above the amount of DBS in this product when calculated for the MTDD of OC based on body surface area comparisons. In the one-year study rats fed DBS at dietary concentrations of 0%, 0.01%, 0.05%, 0.25%, or 1.25% (equivalent to 0, 5, 25, 125, and 625 mg/kg) and 0%, 0.01%, 0.05%, 0.25%, 1.25%, or 6.25%, (equivalent to 0, 5, 25, 125, 625, and 3125 mg/kg) in the two-year study. Body weights and food intake were measured in both studies. No toxicokinetics were performed. Hematologic parameters (hemoglobin, total erythrocytes, total and differential leucocyte counts) were measured at 3, 6, and 9 months in the one-year study and 6, 12, 18, and 24 months in the two-year study. At study termination in both studies, necropsies were performed and histopathology was conducted on lung, heart, liver, spleen, adrenals, stomach, small intestine, thyroid, and brain. Multiple age-related changes were noted in both studies but no differences from control groups were observed. For both studies the NOAELs were the highest dose tested.

The Smith paper also described a multi-generation study in rats with limited endpoints using one concentration of DBS (SMITH, 1953). Male and female rats were fed diets containing 0% or 6.25% DBS for 10 weeks prior to mating. At weaning, the offspring were fed 0% or 6.25% DBS in the diet for 21 days. The number of females bearing young and number of rats per litter was measured and no treatment-related effects were noted. In the offspring, body weight and gross pathology were assessed. Body weights were reduced in both pre-weaning (M: -14%, F: -16%) and post-weaning (M: -24%, F: -34%) periods. No gross pathological changes were observed. Because of the decreases in body weights, no NOAEL can be calculated for this study. However, the one dose that was used was fairly high (6.25%; 3125 mg/kg) with an exposure of 109-fold higher than amount of DBS in this product when calculated for the MTDD of OC based on body surface area comparisons.

The studies in the Smith paper were conducted pre-GLP and assessed limited endpoints. The studies do not meet current standards. In the chronic toxicology studies, only nine tissues were examined microscopically and the tissues analyzed for gross pathology were not noted. Clinical chemistry, urinalysis, and complete hematologic parameters were not measured. Additionally, the animals were dosed by dietary administration and there is no confirmation of the amounts of DBS that were actually consumed by the animals. The reproductive toxicology assessment is also not up to current standards. Very limited fertility endpoints were measured and the study did not assess teratogenicity. With the exception of body weights, pre- and post-natal development endpoints were not measured. The study also tested only one dose and because fairly large reductions in body weight were observed post-weaning, no NOAEL could be established.

Although the studies in the Smith paper suggest low general toxicity for DBS, they are not up to current standards and this paper alone cannot be used to support the safety of DBS given the increase in total daily dose of this excipient. Dibutyl sebacate should be qualified as a novel excipient as per the FDA guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, available at, <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079250.pdf>.

Reviewer's note:

On July 2, 2015, the Applicant submitted the studies listed below to IND 107037. The studies were not submitted to NDA 207621. If the studies were necessary for approval of NDA 207621 and submitted to the NDA, they would have been considered a major amendment and the clock would have been extended. We queried the Applicant as to whether they wanted to submit the studies to the NDA in support of the safety of their drug product formulation. At the time of this information request, the review of the acceptability of the levels of the excipients in the formulation had not been completed. The Applicant stated that the literature-based risk assessments submitted with the NDA were considered acceptable to support the formulation and did not believe that the studies were necessary to support NDA 207621. Our information request (dated August 28, 2015) and the Applicant's response (dated September 2, 2015) are below.

Nonclinical studies submitted to IND 107037 on July 2, 2015:

- Study 8303450 (14GR232) - 26-Week Oral Gavage Chronic Toxicity Study with Dibutyl Sebacate in Rats
- Study 8303448 (14GR248) - 26-Week Oral Gavage Chronic Toxicity Study with Dibutyl Sebacate in Dogs
- Study 8304105 (14GR269) - Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with Dibutyl Sebacate in Rats
- Study 8304106 (14GR268) - Oral Gavage Embryo-fetal Development Study for Effects with Dibutyl Sebacate in Rabbits
- Study 8304104 (14GR267) - Oral Gavage Embryo-fetal Development Study for Effects with Dibutyl Sebacate in Rats
- Study 8303449 (14GR249) - 26-Week Oral Gavage Chronic Toxicity Study with Talc in Rats

Information request to applicant (August 28, 2015) and response from Applicant (September 2, 2015):

We note that you have submitted toxicology studies to IND 107037 (submission dated 02 July 2015) to support the safety of excipients in your drug product formulations, including Troxyca. Do you believe these studies are necessary to support your drug product formulation? If so, explain why these studies were not submitted to the NDA.

RESPONSE

Pfizer does not believe that the studies submitted to the IND 107,037 are necessary to support the NDA review of ALO-02. The excipients in the ALO-02 formulation are FDA Generally Recognized As Safety (GRAS) substances and/or are listed in the FDA Inactive Ingredient Guideline (IIG) Database. A literature based assessment of the safety of the excipients was performed and was included in the NDA in Module 4.3 (Derzi & Bell). The original assessments of excipient safety described in the NDA, including those for dibutyl sebacate and talc, were considered appropriate and adequate to support the Troxyca ER drug product formulation. The results from these additional studies with dibutyl sebacate and talc do not change their risk benefit profile and therefore were not submitted to the NDA.

Upon completion of our review of the original submission and determination that the referenced 1953 Smith study was not adequate to address the safety of the higher proposed dose of dibutyl sebacate, the Division contacted the Applicant to discuss the

deficiencies in the data and again inquired why they completed the new toxicology studies for this excipient. The Applicant maintains the studies were completed for another development program and are not necessary to support this application. Following further discussion of the nonclinical team's conclusion that the studies in the Smith paper were not adequate by current standards, the Applicant submitted the studies with DBS listed above to the NDA on October 5, 2015. This was considered a major amendment. The studies are reviewed in Appendix A. A summary of the data and a table with exposure margins are below.

Chronic toxicology of dibutyl sebacate

Two studies treating rats and dogs orally for 26 weeks with DBS were submitted by the Applicant. Both studies used doses of 0, 100, 300, and 1000 mg/kg DBS. In rats, slight non-dose dependent increases in thinning hair coat (males) and discoloration of the perineal area (females) were observed at the MD and HD. No associated pathology was noted and the findings were not considered adverse. No other treatment-related findings were noted in either males or females. In dogs, abnormal feces (M & F), increased salivation (F only), and swollen vulva (F) were observed in all treated groups in a non-dose-dependent manner. No associated pathology was noted in any case and the findings are not considered adverse. No other treatment-related findings were noted in the study in either males or females. The NOAEL for both rat and dog studies was the highest dose tested, 1000 mg/kg. At the NOAEL, the exposure margins are 35-fold in rats and 117-fold in dogs the amount of DBS in the ALO-02 drug product ((b) (4) mg DBS) if the MTDD of OC is consumed, based on a body surface area comparison. These studies demonstrate that DBS has relatively low toxicity via the oral route in the rat and dog. The high dose of 1000 mg/kg used in both studies is considered acceptable as it meets the criteria for the limit dose of 1000 mg/kg in general toxicology studies as per ICH M3(R2).

As per ICH M3(R2), a toxicology study in a nonrodent species of 9-month duration is typically required to support chronic use. As per the FDA guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, if toxicity and pharmacologic effect are absent in shorter duration studies, a 6-month study in nonrodent may be sufficient. Dibutyl sebacate is used in many approved products at lower levels. The rat 26-week toxicology study showed a NOAEL at the limit dose of 1000 mg/kg, demonstrating low toxicity. The toxicology study in dog with duration of 26 weeks is considered acceptable to qualify DBS for chronic use in lieu of a 9-month nonrodent study.

As a novel excipient, carcinogenicity studies in two species are required unless adequate justification for a waiver is provided as per the FDA guidance noted above. Dibutyl sebacate has been shown in the literature to be negative for genotoxic potential. Negative histopathology data in both the rat and dog at the limit dose of 1000 mg/kg were observed. Additionally, DBS has a long history of use as a flavoring additive in food products, a plasticizer in food packaging and as an excipient in many pharmaceutical products approved for chronic use, although at lower levels. The FDA excipient guidance document specifically states that Applicants may provide scientific

justification that carcinogenicity data are not necessary for an excipient. Specifically, the guidance states the following:

For example, based on negative genetic toxicology data (see ICH guidance S2B for recommended assays), limited systemic exposure, absence of accumulation based on nonclinical and clinical pharmacokinetic data, negative histopathology data from chronic toxicology studies performed at the maximum feasible dose (MFD) (absence of preneoplastic lesions and other toxicologic effects), and knowledge of other excipients in the same class, it may be reasonable to forego carcinogenicity testing.

The Applicant has not provided data to address all of these criteria. Specifically, there are no data to support the conclusion that DBS has limited systemic exposure, absence of accumulation in either nonclinical or clinical studies, and there was no discussion of knowledge of other excipients in the same class. However, carcinogenicity studies in two species will not be required with DBS based on the previous human experience, the lack of histopathological changes in modern chronic toxicology studies in two species, the negative genetic toxicology studies, and the summary review of the literature cited in the Applicant's written justification which included discussion of an older 2-year rat study. This approach is consistent with recent input received from the OND Associate Director of Pharmacology and Toxicology and the FDA guidance document titled: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, which notes that "The Centers recognize that existing human data for some excipients can substitute for certain nonclinical safety data, and an excipient with documented prior human exposure under circumstances relevant to the proposed use may not require evaluation in the full battery of toxicology studies outlined in this guidance."

Reproductive toxicology of dibutyl sebacate

The Applicant submitted three studies to characterize the developmental and reproductive toxicology of DBS. A fertility and early embryonic development study in rat and embryofetal development studies in rat and rabbit were submitted.

In the rat fertility study, both males and females were treated with DBS up to the limit dose of 1000 mg/kg. Dibutyl sebacate had no effect on body weight, food consumption or clinical signs in pregnant dams at any dose. No effects were noted on any of the fertility endpoints in either males or females. The NOAEL for maternal toxicity and effects on male and female fertility is 1000 mg/kg, the highest dose tested. The exposure margin at the NOAELs is 35-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed (^{(b) (4)} mg DBS) based on a body surface area comparison.

Pregnant rats were treated up to the limit dose of 1000 mg/kg in the embryofetal development study. No treatment-related effects were noted in any pregnant dams or on any of the Cesarean section parameters examined. The NOAEL for maternal toxicity and effects for developmental toxicity was 1000 mg/kg, the highest dose tested. The exposure margin at the NOAELs is 35-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed (^{(b) (4)} mg DBS) based on a body surface area comparison.

Pregnant rabbits were treated with 0, 100, 300, or 1000 mg/kg in the embryofetal development study. Reductions in body weight with concomitant reductions in food consumption, decreased feces production, abnormal stomach content and general debilitation of condition leading to moribund sacrifice were observed in high dose dams. Based on the adverse findings at the high dose, the NOAEL for maternal toxicity is the mid dose of 300 mg/kg. Two of the high dose dams aborted their litters which was likely secondary to maternal toxicity. No other embryofetal effects were noted at the high dose or at any other dose. Based on the aborted litters, the NOAEL for developmental toxicity is 300 mg/kg. The exposure margin at the NOAELs is 21-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed (^{(b) (4)} mg DBS) based on a body surface area comparison.

The Applicant did not conduct a pre- and post-natal development study with DBS. This study is required for full characterization of the developmental effects of DBS. The 1953 Smith paper described a multi-generation study in rats with very limited endpoints using one relatively high dose of DBS. Minor reductions in body weight pre-weaning and more substantial reductions post-weaning were seen in offspring of treated dams. No gross pathological changes were observed. Because of the decreases in body weights, no NOAEL can be calculated for this study. However, the dose that was used was fairly high (6.25%; 3125 mg/kg) with an exposure of 109-fold higher than amount of DBS in this product when calculated for the MTDD of OC based on body surface area comparisons. Because of the previous human experience, the high exposure margins seen in the studies conducted in the existing reproductive and developmental studies in rat and rabbit and the 6-month rat and dog general toxicology study, and because the MTDD of OC is not expected to be used in the pregnant population, the pre- and post-natal study may be conducted post-approval.

Table 3. Summary of Toxicology Studies with Dibutyl Sebacate

Species	Study	NOAEL (mg/kg) M&F	Safety Margin Based on BSA*
Rat	Fertility and early embryonic development	1000 (fertility & maternal toxicity)	35
	Embryofetal development	1000 (embryofetal & maternal toxicity)	35
	26-Week chronic toxicity	1000	35
Rabbit	Embryofetal development	300 (embryofetal toxicity)	21
		300 (maternal tox)	21
Dog	26-Week chronic toxicity	1000	117

*BSA in human: ^{(b) (4)} mg/kg (the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed)

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Impurities

Impurities in the oxycodone drug substance

The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substance for a MDD of drug substance < 2 g/day is 0.15%. For this extended-release OC product, the maximum theoretical daily dose (MTDD) is (b) (4) g. The Applicant is obtaining the OC drug substance from (b) (4) (DMF (b) (4)). The OC drug substance impurity specifications are outlined in the table below.



(b) (4) The threshold set for qualification of impurities according to ICH Q3A(R2) is 0.15%. The Applicant is proposing a specification of (b) (4)% for (b) (4) (b) (4) this specification can be considered acceptable from a pharmacology toxicology perspective.

All OC drug substance impurity specifications are considered acceptable from a pharmacology toxicology perspective.

Table 4. Drug Substance Impurity Specifications for Oxycodone

<i>Impurity</i>	<i>Specification limit</i>	<i>Acceptable?</i>
(b) (4)	NMT (b) (4)%	Yes
(b) (4)	NMT (b) (4)%	Yes
(b) (4)	NMT (b) (4)%	Yes
(b) (4)	NMT (b) (4)%	Yes
(b) (4)	NMT (b) (4)%	Yes
(b) (4)	NMT (b) (4)%	Yes

Impurities in the naltrexone drug substance

The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substance for a MDD of drug substance < 2 g/day is 0.15%. For this product, the MDD of NTX is (b) (4) mg (see below). The Applicant is obtaining the NTX drug substance from (b) (4) (DMF (b) (4)). The NTX drug substance impurity (b) (4) specifications are outlined in the table below.

Determination of the maximum daily dose of naltrexone

For this product, when the MTDD of OC (b) (4) g) is consumed, (b) (4) mg of NTX would be consumed. (b) (4)

The Applicant has set an in vitro dissolution specification for NTX in the drug product of NMT (b) (4). This specification was based on data from in vitro dissolution studies with ALO-02 (See CMC review by Dr. Benjamin Stevens for details). (b) (4)

It has also been shown that in the majority of patients no systemic NTX is detected and in the patients where NTX is detected, the levels are very low (mean: 4.05-1090 pg/mL, median: (b) (4) pg/mL; Studies B4531001 and B4531002; see Clinical Pharmacology review by Dr. Suresh Naraharsetti for details). Therefore, assuming (b) (4) release of the sequestered NTX, the maximum daily dose would be (b) (4) mg. The thresholds for qualification for impurities or degradants as per ICH Q3A(R2) and ICH Q3B(R2) will use this value.

(b) (4)
The impurity (b) (4) contains (b) (4) structural alert for mutagenicity (b) (4) has been demonstrated to be reactive with DNA resulting in genotoxicity and mutagenicity (b) (4)

As potentially genotoxic substances present a safety concern, the Agency maintains that such substances should be tested for their genotoxic potential or reduced to acceptable levels. Current Agency policy on acceptable levels for potentially genotoxic agents is NMT 1.5 mcg/day. At the current specification of (b) (4) % in the drug substance with a total daily intake of (b) (4) mg NTX, (b) (4) mcg would be consumed and the limit of NMT 1.5 mcg/day will be met. This specification is considered acceptable.

(b) (4)
(b) (4) both tested positive for clastogenicity in an in vitro chromosomal aberration assay. As detailed above, potentially genotoxic compounds require a specification to reflect a TDI of NMT 1.5 mcg/day. At the current specification of (b) (4) % in the drug substance with a total daily intake of (b) (4) mg NTX, (b) (4) mcg of each impurity would be consumed. In light of the new data, the drug substance specifications (b) (4) are not considered acceptable (b) (4) or the impurities must be adequately qualified. See detailed discussion below.

With the exception of (b) (4) all NTX drug substance impurity specifications are considered acceptable from a pharmacology toxicology perspective.

Table 5. Drug Substance Impurity Specifications for Naltrexone

Impurity	Specification limit	Acceptability
(b) (4)	NMT (b) (4) %	Yes
(b) (4)	NMT %	No
(b) (4)	NMT %	Yes
(b) (4)	NMT %	Yes
(b) (4)	NMT %	No
(b) (4)	NMT %	Yes
(b) (4)	NMT %	Yes
(b) (4)	NMT (b) (4) %	Yes

*Structural alert

Drug Product Impurities

The qualification threshold according to ICH Q3B(R2) in the drug product for a MDD of the drug substance administered >100 mg - 2 g per day (MDD of OC is (b) (4) g/day) is 0.2% or 3 mg TDI, whichever is lower. The MDD of NTX is <10 mg/day, therefore the qualification threshold according to ICH Q3B(R2) for degradants arising from the NTX component of the drug product is 1.0% or 50 mcg TDI, whichever is lower.

The Applicant has set the stability specifications for NTX-derived degradation products (b) (4) however, two impurities tested positive for clastogenicity in the in vitro chromosomal assay and will either require (b) (4) toxicologic qualification.

The Applicant has submitted studies for qualification of (b) (4) specification in the drug product for the impurities (b) (4). This product contains sequestered NTX. If the ICH Q3B(R2) specification is based on the total amount of NTX when the product is used at the MTDD of OC ((b) (4) g) the amount of NTX would be (b) (4) mg ((b) (4) pills of 80/9.6 mg/mg OC/NTX strength). Based on this amount, the ICH Q3B(R2) qualification threshold would be 0.2% or 3 mg TDI, whichever is lower (for a MDD >100 mg - 2 g). However, the Applicant has provided data (discussed above) demonstrating that little of the NTX is released in vivo and in vitro. Therefore, as stated above, the qualification threshold for NTX as per ICH Q3B(R2) for a MDD of <10 mg will be used (1% or 50 mcg TDI, whichever is lower).

The studies submitted by the Applicant were intended to provide qualification for a proposed (b) (4) specification for the two degradants which was (b) (4) specification (incorrectly based on a MDD of (b) (4) mg NTX). The Applicant conducted an Ames assay and an in vitro chromosomal aberration assay with each degradant and an in vivo micronucleus assay with the combination of the two. A 13-week repeat-dose toxicology study in rats was also conducted in order to qualify the proposed (b) (4) specification of the two impurities. Both impurities tested negative in the Ames and micronucleus assays. However, both degradants tested positive for clastogenicity in the in vitro chromosomal aberration assay. For a potentially genotoxic degradant, a specification reflecting a TDI of NMT 1.5 mcg/day would be necessary. At the MTDD of OC ((b) (4) g; (b) (4) capsules) with (b) (4) release of NTX ((b) (4) mg) a specification of

approximately (b) (4) would be needed. This specification is likely not feasible given the data submitted to date. Alternatively, data could be provided supporting a weight-of-evidence approach that the impurities are not clastogenic. Both impurities yielded a negative result in the in vivo micronucleus assay. As per ICH S2(R1), to make a weight-of-evidence argument in light of a positive finding in an in vitro mammalian assay (assuming a negative Ames Assay), a second in vivo assay with a negative result would be needed. Pharmacology Toxicology recommends these studies be conducted as post-marketing requirements.

(b) (4) both NTX drug substance impurities and ALO-02 drug product degradants. The drug product degradant specifications are (b) (4) for this product. (b) (4) These compounds are also likely to have been in many other NTX-containing products as impurities and degradants at specifications that meet ICH Q3A&B but not the Agency’s NMT 1.5 mcg/day threshold for potentially genotoxic compounds. Given the long history of clinical use of NTX and the lack of a safety signal, conducting the qualification studies as post-marketing requirements is considered acceptable from the pharmacology/toxicology perspective.

Table 6. ALO-02 Drug Product Degradant Specifications

Source	Degradant	Stability spec	Acceptability
<i>Oxycodone</i>	(b) (4)	NMT (b) (4) %	Yes
<i>Naltrexone</i>	(b) (4)	NM	No*
		NM	No*
		NMT %	Yes

*see qualification studies and discussion above

2.6 Proposed Clinical Population and Dosing Regimen

ALO-02 is intended to be used in adults for the management of pain severe enough to require daily, around-the-clock, long term opioid treatment for which alternative treatment options are inadequate. This product is intended to be dosed BID.

2.7 Regulatory Background

The Applicant is submitting NDA 207621 via the 505(b)(2) regulatory pathway and is relying on the Agency’s previous findings of safety and efficacy for Roxicodone (NDA 21001) and Revia (18932). The IND for ALO-02 (IND 107037) was originally opened on January 26, 2010. (b) (4)

3 Studies Submitted

3.1 Studies Reviewed

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

Study Title	Study #
A Pharmacological Assessment of the Effect of Oxycodone and an Oxycodone/Naltrexone Combination on the Respiratory System of the Sprague Dawley Rat	694178
Single Dose Intravenous Tolerance and Irritation Study of PF-06412527 in New Zealand White Rabbits	12GR357
PF-06412527 (OxyNorm and Naltrexone): In Vitro Haemolysis and Plasma Compatibility Test in Human Peripheral blood	VPT1294
A 90-Day Toxicity Study of Naltrexone HCl, (b) (4) Administered by Oral Gavage to Rats with a 28-Day Recovery Period	ADZ00122
(b) (4) Bacterial Reverse Mutation Assay	AD11SS.503.BTL
(b) (4) Bacterial Reverse Mutation Assay	AD11ST.503.BTL
(b) (4) In Vitro Mammalian Chromosome Aberration Test	AD11SS.341.BTL
(b) (4) In Vitro Mammalian Chromosome Aberration Test	AD11ST.341.BTL
An <i>In-Vivo</i> Bone Marrow Micronucleus Assay in Sprague Dawley Rats (b) (4)	(b) (4) 655012
26-Week Oral Gavage Chronic Toxicity Study with Dibutyl Sebacate in Rats	8303450
26-Week Oral Gavage Chronic Toxicity Study with Dibutyl Sebacate in Dogs	8303448
Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with Dibutyl Sebacate in Rats	8304105
Oral Gavage Embryo-Fetal Development Study for Effects with Dibutyl Sebacate in Rats	8304104
Oral Gavage Embryo-Fetal Development Study for Effects with Dibutyl Sebacate in Rabbits	8304106

3.2 Studies Not Reviewed

All studies in NDA 207621 were reviewed.

3.3 Previous Reviews Referenced

No previous reviews have been referenced.

4 Pharmacology

4.1 Primary Pharmacology

Oxycodone is a semisynthetic opioid with highest affinity for the mu opioid receptor but it also shows weak affinity for the kappa opioid receptor. It is thought that OC exerts its primary pharmacodynamic effect of analgesia through activation of the mu opioid receptor. Naltrexone is a nonspecific opioid antagonist.

4.2 Secondary Pharmacology

The secondary pharmacologic effects of mu opioid agonists such as OC include dysphoria, euphoria, sedation, respiratory depression, decreased gastrointestinal motility, and physical dependence.

4.3 Safety Pharmacology

A single-dose intravenous tolerance and irritation study with the combination of OC and NTX in rabbit (Study # VFF1363) was conducted in support of a human abuse potential study with intravenous administration of OC and NTX (Study B4981002). Naltrexone has not been approved for the intravenous route of administration, therefore, supportive nonclinical studies were required. In the main phase of the study, six rabbits received single doses (6 mL) of saline or 3.33 mg/mL OC plus 0.4 mg/mL NTX intravenously over 2 minutes into the auricular vein. Rabbits were assessed three days later. No differences in local or systemic reactions between the groups were noted. Additionally, a formulation of 1 mg/mL OC and 0.12 mg/mL NTX was assessed for hemolytic potential and plasma compatibility in human peripheral blood (Study VPT1294). The formulation was not hemolytic in human blood at infusion rates up to 8 mL/min. No signs of precipitation, flocculation, or increased turbidity were observed in human plasma.

Taken together, these studies supported the intravenous administration of OC and NTX for Human Abuse Potential Study B4981002.

An additional safety pharmacology study was conducted. The goal of this nonclinical pharmacology study in rats was to determine whether a fixed ratio of NTX in combination with OC would antagonize the respiratory depressant effects of OC. Doses ranged from 50-400 mg/kg oral and 3-10 mg/kg IV of OC alone and 10-40 mg/kg oral and 10-400 mg/kg IV of OC plus 12% NTX in combination. This study evaluated the pharmacologic effects of OC and an OC/NTX combination on respiratory endpoints following a single oral gavage or intravenous dose in rats (Study 694178). The oral and IV doses of OC alone rapidly decreased respiratory rate and minute volume. Several deaths were seen at the higher doses with OC alone with both routes. Administration of a combination of OC and 12% NTX intravenously partially blocked the OC effects. When administered orally, the antagonist effect of the NTX was present but not as pronounced as IV administration. Complete inhibition of the respiratory depressant effects of OC was not observed with 12% NTX. However, the dose of OC when

administered in combination with NTX was ~7-fold higher than doses of OC alone (20 mg/kg vs 3 mg/kg) without causing a significant adverse effect on total minute volume.

5 Pharmacokinetics/ADME/Toxicokinetics

6 General Toxicology

No general toxicology studies were needed to support the safety of OC or NTX.

A 90-day repeat-dose study with two NTX impurities, (b) (4) was submitted in support of a specification which would exceed the ICH Q3B(R2) qualification threshold if the total daily dose of NTX were based on the full amount of NTX when the product is administered at the MTDD of OC. Since the NTX is sequestered, the maximum daily dose will be based on (b) (4) amount. See the discussion above for details.

6.2 Repeat-Dose Toxicity

Study title: A 90-Day Toxicity Study of Naltrexone HCl, (b) (4) Administered by Oral Gavage to Rats with a 28-Day Recovery Period

Study no.: ADZ00122
 Study report location: EDR 4.2.3.7.7
 Conducting laboratory and location: (b) (4)
 Date of study initiation: December 8, 2009
 GLP compliance: Yes
 QA statement: Yes

Table 7. Compound, Lot number and Purity of Test Articles

Compound	Lot #	Purity
Naltrexone HCl	0905000223	99.2%
(b) (4)	MMD-M09001-75	95.8%
(b) (4)	MDR-M09001-70A	100%

Figure 4. Structure

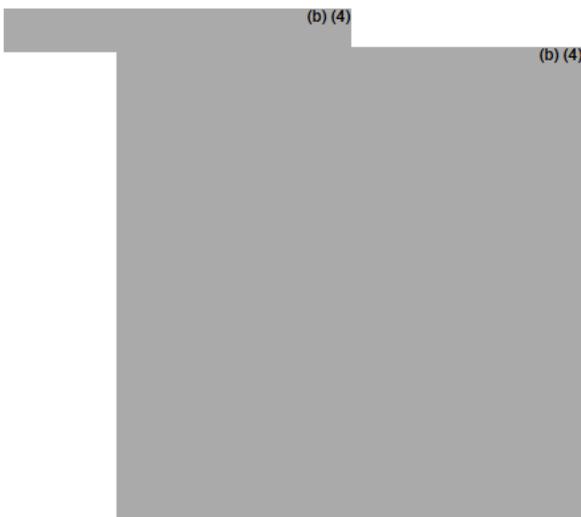


Figure 5. Structure



Key Study Findings

- No differences between any of the groups were observed.
- The NOAEL for the study is the highest dose tested (NTX: 3 mg/kg, (b) (4) 0.1 mg/kg and (b) (4) 0.1 mg/kg).
- For qualification (b) (4): A TDI of (b) (4) mg of NTX at the MTDD of OC ((b) (4) g) would be consumed. Assuming (b) (4) release of the sequestered NTX, the exposure would be (b) (4) mg/day. At the proposed specification (b) (4), (b) (4) mg of the impurity would be consumed. The NOAEL of 0.1 mg/kg (0.6 mg/m²) in rat provides a 56-fold safety margin over the 0.017 mg (0.0003 mg/kg; 0.01 mg/m²) human dose based on BSA.

Methods

Doses: See table below
 Frequency of dosing: Daily
 Route of administration: Oral gavage
 Dose volume: 5 mL/kg
 Formulation/Vehicle: Reverse osmosis deionized water
 Species/Strain: Rat, Sprague-Dawley Crl:CD(SD)
 Number/Sex/Group: See table below
 Satellite groups: TK groups: see table below
 Unique study design: None
 Deviation from study protocol: None that affected integrity of study

Table 8. Experimental Design

Group No.	No. of Animals				Dose Material	Dose Volume (mL/kg)	Dose Level (mg/kg/day)			Dose Concentration (mg/mL)		
	Toxicity (Recovery)		TK				(b) (4)	N	(b) (4)	N		
	M	F	M	F								
1	15 (5)	15 (5)	3	3	RODI water (pH 3.8)	5.0	0	0	0	0	0	0
2	15 (5)	15 (5)	18	18	Naltrexone HCl	5.0	0	0	3.0	0	0	0.60
3	15 (5)	15 (5)	18	18	(b) (4) + Naltrexone HCl	5.0	0.01	0.01	3.0	0.002	0.002	0.60
4	15 (5)	15 (5)	18	18	(b) (4) + Naltrexone HCl	5.0	0.03	0.03	3.0	0.006	0.006	0.60
5	15 (5)	15 (5)	18	18	(b) (4) + Naltrexone HCl	5.0	0.1	0.1	3.0	0.02	0.02	0.60
M = Males; F = Females; (b) (4) N = Naltrexone HCl; RODI – Reverse Osmosis Deionized water. Note: The dose concentrations were not corrected for purity.												

Observations and Results

Mortality

No test article-related mortality was seen in this study.

Clinical Signs

Detailed clinical observations were conducted prior to initiation of treatment and weekly during treatment and recovery periods. No treatment-related clinical signs were noted.

Body Weights

Body weights were recorded prior to initiation of treatment and weekly during treatment and recovery periods. No changes in body weights in males or females were seen

during the dosing period. Small non-dose dependent increases (females) and decreases (males and females) were seen near the end of the recovery period. These changes were minor and are not considered toxicologically relevant.

Food Consumption

Food consumption was recorded prior to study initiation, weekly during treatment and during the recovery periods. Some small decreases in food consumption were noted at the end of the treatment period in males. These changes were minor and are not considered toxicologically relevant. No differences between groups were noted in females.

Ophthalmoscopy

Ophthalmoscopic examination was performed prior to initiation of treatment and during the last week of the treatment period. No changes were noted in the ophthalmoscopic exams for any group.

Hematology, Clinical Chemistry, Gross Pathology, Organ Weights and Histopathology

Standard parameters were measured at termination of the treatment period for main study and recovery groups. A few changes in clinical chemistry and hematology parameters were noted but they were not dose-dependent and were within the historical control range. No toxicologically relevant changes in any parameters were observed.

Toxicokinetics

Although TK for all three compounds were conducted on Days 1 and 90, a large amount of variability was seen for the ^{(b) (4)} levels. This variability is most likely a result of the low levels tested and the measured values being close to the limit of detection of the assay. It can be concluded that exposure was demonstrated in this study, although exact TK values for the two impurities will not be calculated. Clinical levels of these impurities are not available and the exposure margins for the qualification of the proposed specification will be based on body surface area comparisons.

Dosing Solution Analysis

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

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay (b) (4)

Study no.: AD11SS.503.BTL
 Study report location: EDR 4.2.3.7.7
 Conducting laboratory and location: (b) (4)
 Date of study initiation: October 22, 2010
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4) MDR-M10013-06, 93.5%

Key Study Findings

- (b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 or *E. coli* strain WP2uvrA 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: Initial study used 1.5 – 5000 mcg
 Negative control: Water
 Positive control: See table below
 Formulation/Vehicle: Water
 Incubation & sampling time: 48-72 h at 37°C

Table 9. Ames Assay Positive Controls

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535 and TA1537	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03403ED Exp. Date 22-Jan-2012 CAS No. 613-13-8 Purity 99.8%	1.0
TA100			2.0
WP2 <i>uvrA</i>			15

TA98	None	2-nitrofluorene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03319JD Exp. Date 28-Feb-2011 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		sodium azide (Sigma Aldrich Chemical Co.) Lot No. 71980 Exp. Date 28-Dec-2010 CAS No. 26628-22-8 Purity 99.8%	1.0
TA1537		9-aminoacridine (Sigma Aldrich Chemical Co.) Lot No. 106F06682 Exp. Date 28-Oct-2011 CAS No. 90-45-9 Purity >97%	75
WP2 <i>uvrA</i>		methyl methanesulfonate (Sigma Aldrich Chemical Co., Inc.) Lot No. 76296KJ Exp. Date 02-Jun-2012 CAS No. 66-27-3 Purity 99.8%	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 *uvrA* in either the presence or absence of S9. The results of the confirmative assay are summarized in the tables below. No 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.

Table 10. (b) (4) Ames Confirmatory Assay Results –S9

Study Number: AD11SS.503.BTL
 Experiment: B3
 Exposure Method: Plate incorporation assay

Study Code: AD11SS
 Date Plated: 11/9/2010
 Evaluation Period: 11/15/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	(b) (4)	5000 µg	19	1	1.2	18 ^A , 20 ^A , 20 ^A
		1500 µg	25	4	1.6	27 ^A , 28 ^A , 20 ^A
		500 µg	19	7	1.2	24 ^A , 22 ^A , 11 ^A
		150 µg	19	5	1.2	18 ^A , 14 ^A , 24 ^A
		50 µg	14	6	0.9	20 ^A , 10 ^A , 11 ^A
		Water at pH 3.8 100 µL	16	6		14 ^A , 11 ^A , 23 ^A
TA100	(b) (4)	5000 µg	91	11	1.1	103 ^A , 85 ^A , 84 ^A
		1500 µg	86	4	1.0	91 ^A , 84 ^A , 83 ^A
		500 µg	83	17	1.0	73 ^A , 103 ^A , 73 ^A
		150 µg	93	11	1.1	89 ^A , 105 ^A , 85 ^A
		50 µg	89	10	1.1	82 ^A , 84 ^A , 101 ^A
		Water at pH 3.8 100 µL	84	4		88 ^A , 85 ^A , 80 ^A
TA1535	(b) (4)	5000 µg	9	1	0.7	8 ^A , 9 ^A , 9 ^A
		1500 µg	12	9	0.9	9 ^A , 22 ^A , 6 ^A
		500 µg	14	1	1.1	14 ^A , 15 ^A , 13 ^A
		150 µg	14	7	1.1	6 ^A , 17 ^A , 19 ^A
		50 µg	6	4	0.5	9 ^A , 1 ^A , 8 ^A
		Water at pH 3.8 100 µL	13	0		13 ^A , 13 ^A , 13 ^A
TA1537	(b) (4)	5000 µg	5	1	1.7	4 ^A , 6 ^A , 4 ^A
		1500 µg	5	1	1.7	6 ^A , 5 ^A , 5 ^A
		500 µg	7	2	2.3	8 ^A , 8 ^A , 4 ^A
		150 µg	3	2	1.0	4 ^A , 4 ^A , 0 ^A
		50 µg	5	1	1.7	5 ^A , 5 ^A , 6 ^A
		Water at pH 3.8 100 µL	3	2		4 ^A , 1 ^A , 5 ^A

WP2 _{uvrA}	(b) (4)	5000 µg	23	1	0.8	22 ^A , 23 ^A , 23 ^A
		1500 µg	26	4	0.9	22 ^A , 29 ^A , 28 ^A
		500 µg	21	4	0.8	26 ^A , 18 ^A , 20 ^A
		150 µg	23	8	0.8	20 ^A , 18 ^A , 32 ^A
		50 µg	22	4	0.8	24 ^A , 17 ^A , 24 ^A
		Water at pH 3.8	100 µL	28	10	
TA98	2NF	1.0 µg	248	35	15.5	251 ^A , 281 ^A , 212 ^A
TA100	SA	1.0 µg	478	52	5.7	533 ^A , 430 ^A , 472 ^A
TA1535	SA	1.0 µg	301	9	23.2	296 ^A , 295 ^A , 311 ^A
TA1537	9AAD	75 µg	447	33	149.0	422 ^A , 485 ^A , 434 ^A
WP2 _{uvrA}	MMS	1000 µg	193	11	6.9	203 ^A , 195 ^A , 182 ^A

Key to Positive Controls

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

Key to Automatic & Manual Count Flags

^M: Manual count ^A: Automatic count

Table 11. (b) (4) Ames Confirmatory Assay Results +S9

Study Number: AD11SS.503.BTL Experiment: B3 Exposure Method: Plate incorporation assay		Study Code: AD11SS Date Plated: 11/9/2010 Evaluation Period: 11/15/2010				
Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	(b) (4)	5000 µg	27	3	0.8	24 ^A , 28 ^A , 29 ^A
		1500 µg	34	6	1.1	38 ^A , 37 ^A , 27 ^A
		500 µg	24	4	0.8	27 ^A , 24 ^A , 20 ^A
		150 µg	21	8	0.7	20 ^A , 29 ^A , 14 ^A
		50 µg	25	7	0.8	24 ^A , 19 ^A , 33 ^A
		Water at pH 3.8 100 µL	32	7		27 ^A , 28 ^A , 40 ^A
	TA100	(b) (4)	5000 µg	113	10	1.0
TA100	(b) (4)	1500 µg	100	4	0.9	97 ^A , 105 ^A , 99 ^A
		500 µg	108	3	1.0	110 ^A , 108 ^A , 105 ^A
		150 µg	113	12	1.0	122 ^A , 99 ^A , 117 ^A
		50 µg	120	16	1.1	102 ^A , 124 ^A , 134 ^A
		Water at pH 3.8 100 µL	109	2		108 ^A , 111 ^A , 107 ^A
	TA1535	(b) (4)	5000 µg	12	2	0.9
TA1535	(b) (4)	1500 µg	16	9	1.2	13 ^A , 10 ^A , 26 ^A
		500 µg	10	3	0.8	8 ^A , 13 ^A , 10 ^A
		150 µg	9	4	0.7	5 ^A , 8 ^A , 13 ^A
		50 µg	15	4	1.2	17 ^A , 11 ^A , 18 ^A
		Water at pH 3.8 100 µL	13	3		11 ^A , 17 ^A , 11 ^A
	TA1537	(b) (4)	5000 µg	7	4	0.9
TA1537	(b) (4)	1500 µg	5	1	0.6	5 ^A , 6 ^A , 5 ^A
		500 µg	10	2	1.3	8 ^A , 10 ^A , 11 ^A
		150 µg	13	5	1.6	8 ^A , 13 ^A , 17 ^A
		50 µg	8	1	1.0	8 ^A , 8 ^A , 9 ^A
		Water at pH 3.8 100 µL	8	2		9 ^A , 8 ^A , 6 ^A

WP2 _{uvrA}		(b) (4)	5000 µg	15	2	0.6	15 ^A , 17 ^A , 14 ^A
		1500 µg	17	3	0.6	18 ^A , 19 ^A , 14 ^A	
		500 µg	33	4	1.2	28 ^A , 34 ^A , 36 ^A	
		150 µg	24	1	0.9	23 ^A , 24 ^A , 24 ^A	
		50 µg	30	9	1.1	24 ^A , 40 ^A , 27 ^A	
		Water at pH 3.8	100 µL	27	8	34 ^A , 19 ^A , 29 ^A	
TA98	2AA	1.0 µg	263	7	8.2	256 ^A , 269 ^A , 263 ^A	
TA100	2AA	2.0 µg	858	29	7.9	824 ^A , 877 ^A , 872 ^A	
TA1535	2AA	1.0 µg	108	34	8.3	119 ^A , 70 ^A , 136 ^A	
TA1537	2AA	1.0 µg	33	10	4.1	22 ^A , 40 ^A , 37 ^A	
WP2 _{uvrA}	2AA	15 µg	195	24	7.2	198 ^A , 170 ^A , 218 ^A	

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

^M: Manual count ^A: Automatic count

Key to Plate Postfix Codes

CP Contaminated plate
N# Not counted

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

Study title: Bacterial Reverse Mutation Assay (b) (4)

Study no.: AD11ST.503.BTL
 Study report location: EDR 4.2.3.7.7
 Conducting laboratory and location: (b) (4)
 Date of study initiation: October 22, 2010
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4) MDR-M109007-70A, 95.6%

Key Study Findings

- (b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2_{uvrA} 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: Initial study used 1.5 – 5000 mcg

Negative control: Water

Positive control: See table below

Formulation/Vehicle: Water

Incubation & sampling time: 48-72 h at 37 degrees

Table 12. Ames Assay Positive Controls

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535 and TA1537	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03403ED Exp. Date 22-Jan-2012 CAS No. 613-13-8 Purity 99.8%	1.0
TA100			2.0
WP2 <i>uvrA</i>			15
TA98	None	2-nitrofluorene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03319JD Exp. Date 28-Feb-2011 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		sodium azide (Sigma Aldrich Chemical Co.) Lot No. 71980 Exp. Date 28-Dec-2010 CAS No. 26628-22-8 Purity 99.8%	1.0
TA1537		9-aminoacridine (Sigma Aldrich Chemical Co.) Lot No. 106F06682 Exp. Date 28-Oct-2011 CAS No. 90-45-9 Purity >97%	75
WP2 <i>uvrA</i>		methyl methanesulfonate (Sigma Aldrich Chemical Co., Inc.) Lot No. 76296KJ Exp. Date 02-Jun-2012 CAS No. 66-27-3 Purity 99.8%	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 WP2uvrA in either the presence or absence of S9. The results of the confirmative assay are summarized in the tables below. No 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.

Table 13. (b) (4) Ames Confirmatory Assay Results –S9

Study Number: AD11ST.503.BTL		Study Code: AD11ST				
Experiment: B3		Date Plated: 11/9/2010				
Exposure Method: Plate incorporation assay		Evaluation Period: 11/16/2010				
Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	(b) (4)	5000 µg	11	2	0.6	13 ^A , 9 ^A , 11 ^A
		1500 µg	20	8	1.1	17 ^A , 29 ^A , 15 ^A
		500 µg	13	8	0.7	9 ^A , 8 ^A , 23 ^A
		150 µg	18	3	1.0	20 ^A , 20 ^A , 14 ^A
		50 µg	18	5	1.0	22 ^A , 19 ^A , 13 ^A
		Water at pH 3.8 100 µL	18	2		17 ^A , 17 ^A , 20 ^A
TA100	(b) (4)	5000 µg	99	34	1.1	82 ^A , 139 ^A , 77 ^A
		1500 µg	70	12	0.8	82 ^A , 69 ^A , 59 ^A
		500 µg	76	18	0.8	91 ^A , 56 ^A , 80 ^A
		150 µg	84	7	0.9	92 ^A , 78 ^A , 83 ^A
		50 µg	78	3	0.8	79 ^A , 80 ^A , 75 ^A
		Water at pH 3.8 100 µL	93	3		91 ^A , 91 ^A , 97 ^A
TA1535	(b) (4)	5000 µg	11	2	1.2	9 ^A , 10 ^A , 13 ^A
		1500 µg	9	4	1.0	5 ^A , 13 ^A , 9 ^A
		500 µg	12	3	1.3	9 ^A , 15 ^A , 11 ^A
		150 µg	12	3	1.3	13 ^A , 15 ^A , 9 ^A
		50 µg	8	3	0.9	11 ^A , 6 ^A , 6 ^A
		Water at pH 3.8 100 µL	9	4		13 ^A , 10 ^A , 5 ^A
TA1537	(b) (4)	5000 µg	7	3	1.2	9 ^A , 8 ^A , 3 ^A
		1500 µg	4	2	0.7	6 ^A , 3 ^A , 3 ^A
		500 µg	3	2	0.5	3 ^A , 5 ^A , 1 ^A
		150 µg	4	2	0.7	4 ^A , 3 ^A , 6 ^A
		50 µg	8	6	1.3	5 ^A , 15 ^A , 4 ^A
		Water at pH 3.8 100 µL	6	3		6 ^A , 8 ^A , 3 ^A

WP2 _{uvrA}	(b) (4)	5000 µg	20	5	1.0	26 ^A , 17 ^A , 18 ^A
		1500 µg	16	2	0.8	15 ^A , 18 ^A , 15 ^A
		500 µg	21	6	1.1	23 ^A , 14 ^A , 26 ^A
		150 µg	27	2	1.4	29 ^A , 27 ^A , 26 ^A
		50 µg	25	8	1.3	33 ^A , 18 ^A , 24 ^A
Water at pH 3.8	100 µL	20	3		17 ^A , 20 ^A , 22 ^A	
TA98	2NF	1.0 µg	242	13	13.4	254 ^A , 228 ^A , 244 ^A
TA100	SA	1.0 µg	455	87	4.9	510 ^A , 355 ^A , 501 ^A
TA1535	SA	1.0 µg	285	80	31.7	237 ^A , 240 ^A , 377 ^A
TA1537	9AAD	75 µg	477	48	79.5	449 ^A , 533 ^A , 449 ^A
WP2 _{uvrA}	MMS	1000 µg	214	26	10.7	189 ^A , 240 ^A , 214 ^A

Key to Positive Controls

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

Key to Automatic & Manual Count Flags

^M: Manual count ^A: Automatic count

Table 14. (b) (4) Ames Confirmatory Assay Results +S9

Study Number: AD11ST.503.BTL			Study Code: AD11ST				
Experiment: B3			Date Plated: 11/9/2010				
Exposure Method: Plate incorporation assay			Evaluation Period: 11/16/2010				
Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes	
TA98	(b) (4)	5000 µg	28	6	1.6	28 ^A , 22 ^A , 33 ^A	
		2000 µg	28	12	1.6	42 ^A , 20 ^A , 23 ^A	
		1500 µg	24	9	1.4	15 ^A , 24 ^A , 33 ^A	
		500 µg	23	1	1.4	22 ^A , 24 ^A , 24 ^A	
		150 µg	24	7	1.4	23 ^A , 18 ^A , 31 ^A	
		50 µg	26	3	1.5	24 ^A , 29 ^A , 26 ^A	
		Water at pH 3.8	100 µL	17	3		15 ^A , 15 ^A , 20 ^A
		TA100	(b) (4)	5000 µg	119	6	1.2
		1500 µg	97	8	1.0	102 ^A , 88 ^A , 101 ^A	
		500 µg	102	12	1.0	112 ^A , 89 ^A , 106 ^A	
		150 µg	100	7	1.0	99 ^A , 107 ^A , 93 ^A	
		50 µg	93	12	0.9	82 ^A , 105 ^A , 93 ^A	
		Water at pH 3.8	100 µL	100	10		88 ^A , 107 ^A , 105 ^A
TA1535	(b) (4)	5000 µg	15	5	1.1	19 ^A , 10 ^A , 17 ^A	
		1500 µg	14	4	1.0	10 ^A , 17 ^A , 15 ^A	
		500 µg	13	1	0.9	13 ^A , 13 ^A , 14 ^A	
		150 µg	10	2	0.7	11 ^A , 8 ^A , 10 ^A	
		50 µg	11	3	0.8	9 ^A , 10 ^A , 15 ^A	
		Water at pH 3.8	100 µL	14	7		22 ^A , 11 ^A , 9 ^A
TA1537	(b) (4)	5000 µg	4	1	0.7	4 ^A , 4 ^A , 5 ^A	
		1500 µg	3	2	0.5	1 ^A , 5 ^A , 3 ^A	
		500 µg	10	5	1.7	6 ^A , 8 ^A , 15 ^A	
		150 µg	5	1	0.8	4 ^A , 6 ^A , 4 ^A	
		50 µg	7	1	1.2	8 ^A , 6 ^A , 6 ^A	
		Water at pH 3.8	100 µL	6	4		3 ^A , 10 ^A , 5 ^A
WP2 ^{uvrA}	(b) (4)	5000 µg	20	2	0.9	19 ^A , 23 ^A , 19 ^A	
		1500 µg	14	4	0.6	10 ^A , 17 ^A , 14 ^A	
		500 µg	25	5	1.1	19 ^A , 29 ^A , 27 ^A	
		150 µg	23	1	1.0	22 ^A , 23 ^A , 23 ^A	
		50 µg	29	4	1.3	28 ^A , 26 ^A , 33 ^A	
		Water at pH 3.8	100 µL	22	2		23 ^A , 23 ^A , 20 ^A
TA98	2AA	1.0 µg	236	32	13.9	268 ^A , 235 ^A , 205 ^A	
TA100	2AA	2.0 µg	709	63	7.1	774 ^A , 704 ^A , 648 ^A	
TA1535	2AA	1.0 µg	95	31	6.8	79 ^A , 131 ^A , 75 ^A	
TA1537	2AA	1.0 µg	45	9	7.5	55 ^A , 38 ^A , 41 ^A	
WP2 ^{uvrA}	2AA	15 µg	203	32	9.2	230 ^A , 210 ^A , 168 ^A	
Key to Positive Controls							
2AA	2-aminoanthracene						
Key to Automatic & Manual Count Flags							
M:	Manual count		A: Automatic count				

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Test (b) (4)

Study no.: AD11SS.341.BTL
Study report location: EDR 4.2.3.7.7
Conducting laboratory and location: (b) (4)
Date of study initiation: October 22, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) Lot MDR-
M10013-06, 93.5%

Key Findings

- It is concluded that (b) (4) is clastogenic in the in vitro chromosomal aberration assay in HPBLs in the presence of metabolic activation with a 4 h incubation and the absence of metabolic activation with a 20 h incubation.

Methods

Cell line: Human peripheral blood lymphocytes
Concentrations in definitive study: -S9 4h: 493, 985, 1970, and 3940 mcg/L; -S9 20h: 125, 250, 500, 1000, 1500, 2000, 2500, 3000, and 3500 mcg/mL; +S9 4h: 493, 985, 1970, and 3940 mcg/L
Basis of concentration selection: A preliminary toxicity test using concentrations 0.394-3940 mcg/mL (100 nM-10 mM) was conducted.
Negative control: Sterile water for injection
Positive control: -S9: Mitomycin C; +S9: Cyclophosphamide
Formulation/Vehicle: Sterile water for injection
Incubation & sampling time: 4 h +/- S9, 20 h -S9

Study Validity

The study appears to be valid for the following reasons: 1) The appropriate positive controls were employed according to FDA/CFR Redbook guidelines and produced expected results. 2) The appropriate number of cells was evaluated and two replicates of each test concentrations were tested which is in accordance with the current practice. 3) Metaphase cells with 46 centromeres were examined under oil immersion. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations. The counting method was in compliance with the currently accepted procedure and therefore considered valid. 4) According to the protocol a test article was considered to induce a positive response when the percentage of cells with

aberrations (minus gaps) was increased in a concentration-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group. A reproducibly statistically significant increase at the high concentration only or one other concentration only with no concentration-response was considered positive. The criteria for the evaluation of the positive results were considered valid. 5) The conditions of the assays were appropriate given the use of the limit dose for 4 h incubations and toxicity measured in the 21 h incubation (FDA/CFSAN Redbook guidelines). The dose selection based upon mitotic index was acceptable.

Results

Significant increases in structural aberrations were observed (b) (4) at the highest dose tested under S9-activated conditions. Additionally, a significant increase in structural aberrations was observed at the two highest concentrations for the 20 h time point in the absence of S9. No structural aberrations were observed at the shorter incubation in the absence of metabolic activation. The data are presented and summarized in the tables below. It is concluded that under conditions of the assay conducted, (b) (4) produces structural aberrations in the in vitro chromosomal aberration assay in HPBLs in the presence of metabolic activation with a 4h incubation and absence of metabolic activation with a 20 h incubation.

Table 15. Summary of Results (b) (4)

Treatment Time (hours)	Recovery Time (hours)	Harvest Time (hours)	S9	Mitotic Index Reduction ¹ at highest dose scored (µg/mL)	LED ² for Structural Aberrations (µg/mL)	LED ² for Numerical Aberrations (µg/mL)
4	16	20	-	47% at 3940	None	None
20	0	20	-	50% at 1500	1000	None
4	16	20	+	32% at 3940	3940	None

¹ Relative to the solvent control at the highest dose evaluated for chromosome aberrations

² LED = lowest effective dose level

Table 16. Summary of Cytogenetic Analysis Results (b) (4)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Water at pH 3.8	-S9	4	10.4	200	200	0.005	±0.071	0.0	0.5
(b) (4)									
985	-S9	4	9.6	200	200	0.000	±0.000	0.0	0.0
1970	-S9	4	8.7	200	200	0.000	±0.000	0.0	0.0
3940	-S9	4	5.5	200	200	0.005	±0.071	0.0	0.5
MMC, 0.6	-S9	4	5.4	200	100	0.220	±0.484	0.0	19.0**
Water at pH 3.8	+S9	4	10.9	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
985	+S9	4	10.0	200	200	0.005	±0.071	0.0	0.5
1970	+S9	4	8.7	200	200	0.005	±0.071	0.0	0.5
3940	+S9	4	7.4	200	200	0.040	±0.221	1.0	3.5**
CP, 10	+S9	4	4.2	200	100	0.190	±0.394	0.0	19.0**
Water at pH 3.8	-S9	20	10.7	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
250	-S9	20	10.3	200	200	0.000	±0.000	0.0	0.0
500	-S9	20	9.9	200	200	0.000	±0.000	0.0	0.0
1000	-S9	20	7.0	200	200	0.065	±0.318	0.0	5.0**
1500	-S9	20	5.3	200	100	0.410	±1.198	0.0	21.0**
MMC, 0.3	-S9	20	7.3	200	100	0.220	±0.440	0.0	21.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p<0.05; **, p<0.01; using the Fisher's Exact test.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Test (b) (4)

Study no.: AD11ST.341.BTL
Study report location: EDR 4.2.3.7.7
Conducting laboratory and location: (b) (4)
Date of study initiation: October 22, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) MDR-M109007-70A, 95.6%

Key Findings

- It is concluded that (b) (4) is clastogenic in the in vitro chromosomal aberration assay in HPBLs in the absence of metabolic activation and equivocal in the presence of metabolic activation.

Methods

Cell line: Human peripheral blood lymphocytes
Concentrations in definitive study: -S9 4h: 250, 500, and 1700 mcg/L; -S9 20h: 125, 250, and 1250 mcg/mL; +S9 4h: 250, 500, and 1600 mcg/L
Basis of concentration selection: A preliminary toxicity test using concentrations 0.392-3920 mcg/mL (100 nM-10 mM) was conducted.
Negative control: Sterile water for injection
Positive control: -S9: Mitomycin C; +S9: Cyclophosphamide
Formulation/Vehicle: Sterile water for injection
Incubation & sampling time: 4 h +/- S9, 20 h -S9

Study Validity

The study appears to be valid for the following reasons: 1) The appropriate positive controls were employed according to FDA/CFSAN Redbook guidelines and produced expected results. 2) The appropriate number of cells was evaluated and two replicates of each test concentrations were tested which is in accordance with the current practice. 3) Metaphase cells with 46 centromeres were examined under oil immersion. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations. The counting method was in compliance with the currently accepted procedure and therefore considered valid. 4) According to the protocol a test article was considered to induce a positive response when the percentage of cells with aberrations (minus gaps) was increased in a concentration-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group. A reproducibly statistically significant increase at the high concentration only or one

other concentration only with no concentration-response was considered positive. The criteria for the evaluation of the positive results were considered valid. 5) The conditions of the assays were appropriate given the use of the limit dose for 4 h incubations and toxicity measured in the 21 h incubation (FDA/CFSAN Redbook guidelines). The dose selection based upon mitotic index was acceptable.

Results

Substantial cytotoxicity was observed at the highest doses tested in the preliminary toxicity test. Significant increases in structural aberrations were observed (b) (4) at the highest dose tested under activated conditions. At this concentration, however, a reduction in the mitotic index of 61% was observed. Lower concentrations did not show increases in structural aberrations.

Dose-dependent significant increases in structural aberrations were observed at all three concentrations in the absence of S9 with 4h incubation. Significant increases in structural aberrations were observed at the highest concentration tested; all three in the absence of S9 with 20 h incubation. It is concluded that under conditions of the assay conducted, (b) (4) produces structural aberrations in the in vitro chromosomal aberration assay in HPBLs in the presence and absence of metabolic activation.

Table 17. Summary of Results (b) (4)

Treatment Time (hours)	Recovery Time (hours)	Harvest Time (hours)	S9	Mitotic Index Reduction ¹ at highest dose scored (µg/mL)	LED ² for Structural Aberrations (µg/mL)	LED ² for Numerical Aberrations (µg/mL)
4	16	20	-	52% at 1700	250	None
4	16	20	+	61% at 1600	1600	None

¹ Relative to the solvent control at the highest dose evaluated for chromosome aberrations

² LED = lowest effective dose level

Table 18. Summary of Cytogenetic Analysis Results (b) (4)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)		Structural (%)
				Numerical	Structural					
Water at pH 3.8	-S9	20	11.4	200	200	0.005	±0.071	0.0		0.5
(b) (4)										
125	-S9	20	9.3	200	200	0.005	±0.071	0.0		0.5
250	-S9	20	9.7	200	200	0.010	±0.100	0.0		1.0
1250	-S9	20	4.8	200	200	0.130	±0.352	0.0		12.5**
MMC, 0.3	-S9	20	5.1	200	100	0.240	±0.515	0.0		20.0**
Water at pH 3.8	-S9	4	13.0	200	200	0.000	±0.000	0.0		0.0
(b) (4)										
250	-S9	4	11.5	200	200	0.065	±0.247	0.0		6.5**
500	-S9	4	11.0	200	100	0.140	±0.377	1.0		13.0**
1700	-S9	4	6.3	200	100	0.290	±0.782	0.0		19.0**
MMC, 0.6	-S9	4	5.4	200	100	0.370	±0.677	0.0		27.0**
Water at pH 3.8	+S9	4	8.2	200	200	0.005	±0.071	0.0		0.5
(b) (4)										
250	+S9	4	8.8	200	200	0.000	±0.000	0.0		0.0
500	+S9	4	8.0	200	200	0.010	±0.100	0.0		1.0
1600	+S9	4	3.2	200	100	0.300	±0.969	1.0		14.0**
CP, 10	+S9	4	2.5	200	100	0.410	±0.842	0.0		28.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p<0.05; **, p<0.01; using the Fisher's Exact test.

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: An In Vivo Bone Marrow Micronucleus Assay in Sprague Dawley Rats (b) (4)

Study no: (b) (4) 655012 (11GR130)
 Study report location: EDR 4.2.3.7.7
 Conducting laboratory and location: (b) (4)
 Date of study initiation: May 2, 2011
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4) Lot MMD-

M09001-75, 89.5%; (b) (4)
 Lot MDR-M09001-70A, 95.6%

Key Study Findings

- (b) (4) were found to be negative in the in vivo micronucleus assay using rat bone marrow.

Methods

Doses in definitive study: See table below
 Frequency of dosing: Daily for two days
 Route of administration: Oral gavage
 Dose volume: 10 mL/kg
 Formulation/Vehicle: Water
 Species/Strain: Rat, Sprague Dawley
 Number/Sex/Group: 6/sex/group
 Satellite groups: TK groups: Vehicle 3/sex/group and HD 6/sex/group
 Negative control: Water
 Positive control: Cyclophosphamide

Table 19. Study Design for the MN Assay (b) (4)

Group Number	Treatment	Dosage Level (mg/kg/day)	Test Article Concentration ^a (mg/mL)
1/1A	Vehicle Control (b) (4)	0	0
2	(b) (4)	1	0.1
3		2	0.2
4/4A		4	0.4
5		1	0.1
6		2	0.2
7/7A	Positive Control	4	0.4
8		60	6

^a = The dosing formulations were (b) (4)

Study Validity

The micronucleus assay was deemed valid for the following reasons: 1) previous pharmacokinetic assessments demonstrated systemic exposure, 2) dosing appeared to be adequate based upon the results of the dose-ranging study, 3) preparation and administration of the test substance was acceptable, 4) the species and number of animals/sex/group were acceptable, 5) tissue sampling and analysis was acceptable, 6)

positive controls exhibited appropriate responses, and 7) the proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value.

Results

No treatment-related clinical signs were observed in the study. Mice treated (b) (4) showed numbers of PCEs that were similar to vehicle groups indicating the lack of bone marrow toxicity. Group mean frequencies of MN PCE in treated groups were similar to controls and within historical control ranges for the vehicle. Micronucleus data are summarized in the table below.

Table 20. Summary of Results for Bone Marrow Micronucleus Assay (b) (4) in Females

TREATMENT	ANIMAL No.	MN PCEs/ 2000 PCEs	% MN PCEs	PCEs	NCEs	PCE:TE Ratio
Purified water at pH 3.8 (Vehicle)	12331	1	0.05	503	497	0.50
	12356	1	0.05	634	366	0.63
	12379	2	0.10	502	498	0.50
	12381	1	0.05	652	348	0.65
	12383	1	0.05	439	561	0.44
Mean ± SD			0.06 ± 0.02			0.55 ± 0.09
(1 mg/kg/day)	(b) (4) 12335	0	0.00	583	417	0.58
	(b) (4) 12342	1	0.05	472	528	0.47
	(b) (4) 12350	1	0.05	547	453	0.55
	(b) (4) 12353	0	0.00	471	529	0.47
	(b) (4) 12363	1	0.05	351	649	0.35
Mean ± SD			0.03 ± 0.03			0.48 ± 0.09
(2 mg/kg/day)	(b) (4) 12330	2	0.10	539	461	0.54
	(b) (4) 12346	3	0.15	476	524	0.48
	(b) (4) 12347	0	0.00	547	453	0.55
	(b) (4) 12351	0	0.00	577	423	0.58
	(b) (4) 12352	0	0.00	455	545	0.46
Mean ± SD			0.05 ± 0.07			0.52 ± 0.05
(4 mg/kg/day)	(b) (4) 12326	2	0.10	612	388	0.61
	(b) (4) 12329	1	0.05	564	436	0.56
	(b) (4) 12338	2	0.10	440	560	0.44
	(b) (4) 12354	2	0.10	583	417	0.58
	(b) (4) 12372	2	0.10	548	452	0.55
Mean ± SD			0.09 ± 0.02			0.55 ± 0.07

(b) (4)	12328	1	0.05	646	354	0.65	
	12336	1	0.05	486	514	0.49	
	12343	4	0.20	459	541	0.46	
	(1 mg/kg/day)	12348	1	0.05	601	399	0.60
	12377	3	0.15	572	428	0.57	
Mean ± SD			0.10 ± 0.07	0.55 ± 0.08			
(b) (4)	12344	1	0.05	618	382	0.62	
	12349	0	0.00	590	410	0.59	
	12357	4	0.20	537	463	0.54	
	(2 mg/kg/day)	12358	1	0.05	615	385	0.62
	12359	2	0.10	479	521	0.48	
Mean ± SD			0.08 ± 0.08	0.57 ± 0.06			
(b) (4)	12323	2	0.10	596	404	0.60	
	12337	2	0.10	581	419	0.58	
	12355	0	0.00	568	432	0.57	
	(4 mg/kg/day)	12370	0	0.00	496	504	0.50
	12382	4	0.20	736	264	0.74	
Mean ± SD			0.08 ± 0.08	0.60 ± 0.09			
Cyclophosphamide (60 mg/kg)	12324	21	1.05	379	621	0.38	
	12339	19	0.95	371	629	0.37	
	12340	17	0.85	348	652	0.35	
	12360	9	0.45	525	475	0.53	
	12362	14	0.70	307	693	0.31	
Mean ± SD			0.80 ± 0.23*	0.39 ± 0.08*			

MN = Micronucleated

NCE = Normochromatic Erythrocyte

TE = Total erythrocytes (PCE + NCE)

PCE = Polychromatic Erythrocyte

*Statistically different than vehicle control $p \leq 0.05$

* Except for cyclophosphamide treatment: single dose with 24 hour postdose bone marrow harvest

Table 21. Summary of Results for Bone Marrow Micronucleus Assay in Males

(b) (4)

TREATMENT	ANIMAL No.	MN PCEs/2000 PCEs	% MN PCEs	PCEs	NCEs	PCE:TE Ratio	
Purified water at pH 3.8 (Vehicle)	12270	17 ^a	0.43 ^a	584	416	0.58	
	12282	5	0.25	542	458	0.54	
	12288	0	0.00	432	568	0.43	
	12311	4	0.20	594	406	0.59	
	12317	4	0.20	501	499	0.50	
Mean ± SD			0.22 ± 0.15	0.53 ± 0.07			
(b) (4)	12264	10	0.50	615	385	0.62	
	12273	1	0.05	472	528	0.47	
	12274	11	0.55	571	429	0.57	
	(1 mg/kg/day)	12284	2	0.10	406	594	0.41
	12300	2	0.10	472	528	0.47	
Mean ± SD			0.26 ± 0.24	0.51 ± 0.08			
(b) (4)	12268	2	0.10	519	481	0.52	
	12275	11	0.55	651	349	0.65	
	12279	3	0.15	453	547	0.45	
	(2 mg/kg/day)	12280	4	0.20	520	480	0.52
	12308	3	0.15	475	525	0.48	
Mean ± SD			0.23 ± 0.18	0.52 ± 0.08			
(b) (4)	12257	20	1.00	667	333	0.67	
	12258	2	0.10	515	485	0.52	
	12261	3	0.15	543	457	0.54	
	(4 mg/kg/day)	12263	11	0.55	551	449	0.55
	12281	4	0.20	451	549	0.45	
Mean ± SD			0.40 ± 0.38	0.55 ± 0.08			

(b) (4) (1 mg/kg/day)	12265	6	0.30	495	505	0.50
	12266	3	0.15	639	361	0.64
	12271	4	0.20	474	526	0.47
	12283	4	0.20	489	511	0.49
	12315	1	0.05	393	607	0.39
Mean ± SD			0.18 ± 0.09		0.50 ± 0.09	
(b) (4) (2 mg/kg/day)	12260	11	0.55	557	443	0.56
	12285	1	0.05	561	439	0.56
	12294	0	0.00	193	807	0.19
	12297	2	0.10	597	403	0.60
	12305	7	0.35	648	352	0.65
Mean ± SD			0.21 ± 0.23		0.51 ± 0.18	
(b) (4) (4 mg/kg/day)	12259	18	0.90	650	350	0.65
	12262	12	0.60	570	430	0.57
	12269	2	0.10	479	521	0.48
	12292	3	0.15	473	527	0.47
	12303	3	0.15	566	434	0.57
Mean ± SD			0.38 ± 0.35		0.55 ± 0.07	
Cyclophosphamide (60 mg/kg)	12267	209	10.45	530	470	0.53
	12289	22	1.10	455	545	0.46
	12290	35	1.75	481	519	0.48
	12302	161	8.05	580	420	0.58
	12313	37	1.85	153	847	0.15
Mean ± SD			4.64 ± 4.30		0.44 ± 0.17	

MN = Micronucleated

NCE = Normochromatic Erythrocyte

TE = Total erythrocytes (PCE + NCE)

PCE = Polychromatic Erythrocyte

*Statistically different than vehicle control $p \leq 0.05$

* Except for cyclophosphamide treatment: single dose with 24 hour postdose bone marrow harvest

7.4 Other Genetic Toxicity Studies

None.

8 Carcinogenicity

No carcinogenicity studies have been conducted for OC.

9 Reproductive and Developmental Toxicology

The summary of reproductive and developmental toxicology studies conducted with OC is detailed in the product label of the referenced product and will be used for the product labeling in this product.

10 Special Toxicology Studies

No special toxicology studies were conducted.

11 Integrated Summary and Safety Evaluation

TROXYCA ER capsules contain extended-release OC and sequestered NTX. This NDA is a 505(b)(2) application and is relying on the Agency's findings of safety and the description of the pharmacology for OC and NTX in the labels of Roxicodone (NDA 21011) and Revia (NDA 18932), respectively.

Since NTX is not approved to be used intravenously, two toxicology studies were conducted to support the safety of a human simulated abuse potential study with intravenous administration of OC and NTX. The nonclinical studies were

considered acceptable to support the human study. No other pharmacology or toxicology studies were required with either OC or NTX.

No genetic toxicology studies were required for either OC or NTX. Genetic toxicology studies were conducted with two NTX drug substance impurities/NTX-derived drug product degradants, (b) (4). Both tested negative in the Ames assay and the in vivo mouse micronucleus assay. In contrast, both impurities tested positive for clastogenic activity in the in vitro chromosome aberration assay. The impurities (b) (4) are considered to have clastogenic potential and as per ICH S2(R1), an additional in vivo test is required. As these impurities are in currently marketed NTX products at similar levels to this product, these studies may be completed as post-marketing requirements. A 13-week repeat-dose rat toxicology study (b) (4) was also conducted. The NOAEL is the study provides a 56-fold safety margin over the amount that would be consumed at the MTDD of OC assuming (b) (4) release of NTX with (b) (4) specification.

The literature-based justification of dibutyl sebacate (DBS) did not contain adequate information to support the safety of the levels in the product. The Division required full qualification as a novel excipient for DBS. Two 26-week general toxicology studies which tested DBS up to the limit dose in rat and dog were conducted. No adverse effects were observed at any dose in either species and the NOAELs in rat and dog yielded exposure margins 35-fold and 117-fold, respectively, the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed. These studies adequately qualify DBS for chronic dosing from a general toxicology perspective.

Three reproductive toxicology studies were submitted. As in the chronic toxicology study in rat, which used the same doses as the fertility and embryofetal studies in rat, no toxicity was observed at any dose. In both rat fertility and embryofetal studies, the NOAELs for maternal toxicity and fertility/developmental effects were 1000 mg/kg, the highest dose tested with exposure margins of 35-fold. However, toxicities at the high dose of 1000 mg/kg in rabbits, including early sacrifice as a result of general debilitation of condition, were observed in the pregnant does. The rabbit embryofetal study used the same dosing as the rat and dog studies. Reductions in body weight and food consumption, decreased feces production, abnormal stomach contents and general debilitation of condition leading to moribund sacrifice were observed at the high dose only. No adverse effects were noted in the does at lower levels. Based on the adverse findings at the high dose, the NOAEL for maternal toxicity is the mid dose of 300 mg/kg. Two of the high dose does aborted their litters which was likely secondary to maternal toxicity. No other embryo-fetal effects were noted at the high dose or at any other dose. Based on the aborted litters, the NOAEL for developmental toxicity is 300 mg/kg. The exposure margin at the NOAELs is 21-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed ((b) (4) mg DBS) based on a body surface area comparison.

The Applicant did not conduct a pre- and post-natal development study with DBS. This study is required for full characterization of the developmental effects of DBS. Although the 1953 Smith paper describes a multi-generation study in rats, the study is not up to current standards and only used one relatively high dose of DBS. It cannot be used to characterize the developmental toxicity of DBS. Additionally, because of decreases in body weights of offspring, no NOAEL can be calculated for this study. However, the dose that was used was high (6.25%; 3125 mg/kg) with an exposure of 109-fold higher than amount of DBS in this product when calculated for the MTDD of OC based on body surface area comparisons. Because of the previous human experience, the high exposure margins seen in the studies conducted in the existing reproductive and developmental studies in rat and rabbit and the 6-month rat and dog general toxicology study, and because the MTDD of OC is not expected to be used in the pregnant population, the pre- and post-natal study may be conducted post-approval.

12 Appendix/Attachments

Appendix A

Study title: 26-Week Oral Gavage Chronic Toxicity Study with Dibutyl Sebacate in Rats

Study no.:	8303450
Study report location:	EDR 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 25, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dibutyl sebacate, Batch 0000134573, 98.57%

Key Study Findings

- Slight non-dose dependent increases in thinning hair coat (males) and discoloration of the perineal area (females) were observed at the MD and HD. No associated pathology was noted and the findings are not considered adverse.
- The NOAEL in this study is 1000 mg/kg, the highest dose tested. At the NOAEL, the exposure margin is 35-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed ((b) (4) mg DBS) based on a body surface area comparison.
- This study demonstrates that DBS has relatively low toxicity via the oral route in rat. The high dose of 1000 mg/kg used in this study is considered acceptable as

it meets the criteria for the limit dose of 1000 mg/kg in general toxicology studies as per ICH M3(R2).

Methods

Doses: 0, 100, 300, 1000 mg/kg/day
Frequency of dosing: Daily
Route of administration: Oral gavage
Dose volume: 10 mL/kg
Formulation/Vehicle: (b) (4) in water
Species/Strain: Rat, Crl:WI(Han)
Number/Sex/Group: 15/sex/group
Age: 6-7 weeks
Weight: M: 165 to 226 g; F: 120 to 169 g
Satellite groups: None
Unique study design: None
Deviation from study protocol: None that affected the integrity of the study.

Observations and Results

Mortality

No test unscheduled deaths were seen in this study.

Clinical Signs

Cageside observations were conducted once daily except on days when detailed observations were conducted. Detailed clinical observations were conducted prior to initiation of treatment and weekly during treatment. Non-dose dependent increases in discolored (yellow) haircoat around the perineal area were observed in females at the MD and HD. Additionally, small, non-dose dependent increases in thinning hair coat was observed in males at the MD and HD. Neither of these observations in either males or females was associated with any body weight changes or other pathology and is considered not adverse. No other treatment-related clinical signs were noted at any dose.

Body Weights

Body weights were recorded prior to initiation of treatment and weekly during treatment. No changes in body weights in males or females were observed.

Food Consumption

Food consumption was recorded weekly. No changes in food consumption in males or females were observed.

Ophthalmoscopy

Ophthalmoscopic examination was performed prior to initiation of treatment and during Weeks 13 and 26 for the control and high dose groups. No changes were noted in the ophthalmoscopic exams for any group.

Hematology, Clinical Chemistry, Urinalysis

Standard hematology, clinical chemistry, and urinalysis parameters were measured at Study Days 87 and 184. No toxicologically relevant changes in any parameters were observed.

Gross Pathology

No treatment-related gross pathological changes were noted in the study.

Organ Weights

No treatment-related changes in organ weights were noted in the study.

Histopathology

Adequate Battery: Yes. The standard battery of tissues was examined.

Peer Review: Yes.

Histological Findings

No histopathological findings were noted in the study.

Special Evaluation

No special evaluations were conducted.

Toxicokinetics

Toxicokinetics were not conducted.

Dosing Solution Analysis

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

Study title: 26-Week Oral Gavage Chronic Toxicity Study with Dibutyl Sebacate in Dogs

Study no.:	8303448
Study report location:	EDR 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 17, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dibutyl sebacate, Batch 0000134573, 98.57%

Key Study Findings

- Clinical signs of abnormal feces (M & F), increased salivation (F only), and swollen vulva (F) were observed in all treated groups in a non-dose-dependent manner. No associated pathology was noted in any case and the findings are not considered adverse.

- The NOAEL in this study is 1000 mg/kg, the highest dose tested. At the NOAEL, the exposure margin is 117-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed ((b) (4) mg DBS) based on a body surface area comparison.
- This study demonstrates that DBS has relatively low toxicity via the oral route in dog. The high dose of 1000 mg/kg used in this study is considered acceptable as it meets the criteria for the limit dose of 1000 mg/kg in general toxicology studies as per ICH M3(R2).

Methods

Doses:	0, 100, 300, 1000 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) in water
Species/Strain:	Dog, Beagle
Number/Sex/Group:	4/sex/group
Age:	13-14 months
Weight:	M: 6.1-11.1 kg; F: 6.1-10.2 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that affected the integrity of the study.

Observations and Results

Mortality

No unscheduled deaths were seen in this study.

Clinical Signs

Dogs were checked twice daily and cageside observations were conducted once daily except on days when detailed observations were conducted. Detailed clinical observations were conducted prior to initiation of treatment and weekly during treatment. Swollen vulva was observed in all treated female groups (V: 0/4, LD: 3/4, MD: 2/4, HD 4/4). The observations were transient and did not correlate with any other findings. The observation will be considered treatment-related but not adverse. In females, excessive salivation was observed in 1/4 MD and 3/4 HD dogs. No excessive salivation was noted in males. Abnormal feces were observed in all groups but a slightly higher incidence was noted in treated dogs (both males and females). The occurrence of abnormal feces was not dose-dependent or associated with decreases in food consumption or body weights and will not be considered adverse. One LD male fell from his cage prior to dosing on Day 85 and observations of "continuous tremors (entire body)" were noted after the fall. The dog was examined by a veterinarian and found to be normal and was later approved to be dosed. The tremors are considered to

be related to the fall and not the treatment because the dog was in the LD group and had not been dosed that day.

None of the clinical signs observed in either males or females was associated with any body weight changes or any other pathology and none were considered to be adverse.

Body Weights

Body weights were recorded prior to initiation of treatment and weekly during treatment. No changes in body weights in males or females were observed.

Food Consumption

Food consumption was recorded weekly. No changes in food consumption in males or females were observed.

Ophthalmoscopy

Ophthalmoscopic examination was performed prior to initiation of treatment and during Weeks 13 and 26. No changes were noted in the ophthalmoscopic exams for any group.

ECG

Electrocardiograms were recorded prior to the initiation of dosing and during Weeks 12 and 25 (pre-dose and 1 h post-dose). No ECG abnormalities were observed in the study.

Hematology, Clinical Chemistry, Urinalysis

Standard hematology and clinical chemistry parameters were measured on Study Days 89 and 183. Standard urinalysis parameters were measured on Study Day 183. No toxicologically relevant changes in any parameters were observed.

Gross Pathology

No treatment-related gross pathological changes were noted in the study.

Organ Weights

No treatment-related changes in organ weights were noted in the study.

Histopathology

Adequate Battery: Yes. The standard battery of tissues was examined.

Peer Review: Yes.

Histological Findings

No histopathological findings were noted in the study.

Special Evaluation

No special evaluations were conducted.

Toxicokinetics

Toxicokinetics were not conducted.

Dosing Solution Analysis

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

Study title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with Dibutyl Sebacate in Rats

Study no.:	8304105
Study report location:	EDR 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 21, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dibutyl sebacate (DBS); Batch 0000134573; 98.57%

Key Study Findings

- The NOAEL for maternal toxicity is 1000 mg/kg, the highest dose tested. No maternal toxicity was noted at any dose.
- The NOAEL for fertility and reproductive toxicity in both males and females is 1000 mg/kg, the highest dose tested, and yields an exposure margin of 35-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed ((b) (4) mg DBS) based on a body surface area comparison.
- No evidence of treatment-related maternal effects was observed in this study at any dose. The limit dose for a reproductive toxicology study as per ICH S5(R2) under most circumstances is 1 g/kg. Dibutyl sebacate has been shown to have relatively low toxicity in a 26-week rat toxicity study (Study 8303450). The high dose of 1000 mg/kg used in this study is considered acceptable.

Methods

Doses: 100, 300, 1000 mg/kg
Frequency of dosing: Daily
Dose volume: 10 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: (b) (4) in water
Species/Strain: Rat; Crl:CD(SD)
Number/Sex/Group: 20 sex/group
Satellite groups: None
Study design: Males were dosed for at least 28 days prior to mating, throughout the mating period, and through the day prior to termination. Females were dosed for at least 14 days prior to mating, throughout the mating period and through GD 7. Females were sacrificed on GD 14. Males were sacrificed after at least 10 weeks of treatment.

Deviation from study protocol: None that affected the integrity of the study.

The Sponsor states that the dose selection for this study was based on publically available data from a reproductive toxicology study which used 6.25% DBS in the diet (equivalent to 3125 mg/kg) (SMITH, 1953). They also note that the high dose of 1000 mg/kg for the study was chosen based the ICH recommended limit dose for an EFD study. The guideline ICH S5(R3) states that under most circumstances, 1 g/kg should be an adequate limit dose. A 26-week toxicity study in rat with DBS was conducted by the Sponsor and showed a NOAEL of 1000 mg/kg, demonstrating that DBS has relatively low toxicity. The high dose in this study provides a 35-fold exposure margin at the NOAEL for the intended use. The 1000 mg/kg dose is considered an adequate high dose.

Observations and Results

Mortality

No mortality was observed in this study.

Clinical Signs

Rats were briefly checked twice daily and detailed observations were made once daily during dosing. No test article-related clinical signs were noted.

Body Weight

Male body weights were recorded prior to randomization, on the first day of treatment, twice weekly during treatment and at termination. Female body weights were recorded prior to randomization, on the first day of treatment, twice weekly during the pre-mating and mating treatment phases and on GD 0, 3, 7, and 14. No test article-related effects on body weight were observed.

Food Consumption

Food consumption was recorded during the pre- and post-mating phases in both males and females. Food consumption was not measured during mating. No test article-related effects on food consumption were observed.

Toxicokinetics

Toxicokinetics were not conducted in this study or in the chronic toxicity study in rats.

Dosing Solution Analysis

The dosing solutions were acceptable. On Day 1, the low dose formulations did not meet specifications for concentration and homogeneity. Since the highest dose tested is the NOAEL, this was not considered to affect the integrity of the study.

Necropsy

No test article-related necropsy findings were noted at any dose.

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

Standard fertility parameters were assessed in males and females. No test article-related findings were observed for any parameter.

Study title: Oral Gavage Embryo-Fetal Development Study for Effects with Dibutyl Sebacate in Rats

Study no.:	8304104
Study report location:	EDR 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 19, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dibutyl sebacate; Batch 0000134573; 98.57%

Key Study Findings

- The NOAEL for maternal toxicity is 1000 mg/kg, the highest dose tested. No maternal toxicity was noted at any dose.
- The NOAEL embryofetal toxicity is 1000 mg/kg, the highest dose tested, yields an exposure margin of 35-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed ((b) (4) mg DBS) based on a body surface area comparison.

- No evidence of treatment-related maternal effects was observed in this study at any dose. The limit dose for a reproductive toxicology study as per ICH S5(R2) under most circumstances is 1 g/kg. Dibutyl sebacate has been shown to have relatively low toxicity in a 26-week rat toxicity study (Study 8303450). The high dose of 1000 mg/kg used in this study is considered acceptable.

Methods

Doses:	100, 300, 1000 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) in water
Species/Strain:	Rat; Sprague Dawley
Number/Sex/Group:	22 pregnant females/group
Satellite groups:	None
Study design:	Dosing on Gestation Days (GD) 6-17, sacrifice on GD 21
Deviation from study protocol:	None that affected the integrity of the study.

The Sponsor states that the dose selection for this study was based on publically available data from a reproductive toxicology study which used 6.25% DBS in the diet (equivalent to 3125 mg/kg) (SMITH, 1953). They also note that the high dose of 1000 mg/kg for the study was chosen based the ICH recommended limit dose for an EFD study. The guideline ICH S5(R3) states that under most circumstances, 1 g/kg should be an adequate limit dose. A 26-week toxicity study in rat with DBS was conducted by the Sponsor and showed a NOAEL of 1000 mg/kg, demonstrating that DBS has relatively low toxicity. The high dose in this study provides a 35-fold exposure margin at the NOAEL for the intended use. The 1000 mg/kg dose is considered an adequate high dose.

Observations and Results

Mortality

No mortality was observed in the study.

Clinical Signs

Rats were briefly checked twice daily and detailed observations were made once daily during dosing. No test article-related clinical signs were noted.

Body Weight

Body weights were recorded on GD 0, 4 and 6-21. No test article-related effects on body weight were observed.

Food Consumption

Food consumption was recorded on GD 0, 4, and 6-21. No test article-related effects on food consumption were observed.

Toxicokinetics

Toxicokinetics were not conducted in this study or in the chronic toxicity study in rats.

Dosing Solution Analysis

The dosing solutions were acceptable.

Necropsy

No other test article-related necropsy findings were noted at any dose.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

No test article-related effects were observed on any of the Cesarean section parameters.

Offspring (Malformations, Variations, etc.)

No test article-related effects were observed on any of the parameters measured in the offspring.

Study title: Oral Gavage Embryo-Fetal Development Study for Effects with Dibutyl Sebacate in Rabbits

Study no.:	8304106
Study report location:	EDR 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 19, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dibutyl sebacate; Batch 0000134573; 98.57%

Key Study Findings

- The NOAEL for maternal toxicity in rabbits is 300 mg/kg, based on reductions in body weight and food consumption, decreased feces production, abnormal stomach contents and moribund sacrifice at 1000 mg/kg.
- Litters were aborted in two 1000 mg/kg does (GD 19 and GD 21). The aborted litters are considered treatment-related but are most likely secondary to the maternally toxic effects of the test article.
- Other than the two aborted litters in the 1000 mg/kg group, no embryo-fetal effects were noted at any dose.

- The NOAEL for embryofetal toxicity in rabbits is 300 mg/kg, based on the aborted litters at 1000 mg/kg, yields an exposure margin of 21-fold the amount of DBS in the ALO-02 drug product if the MTDD of OC is consumed ((b) (4) mg) based on a body surface area comparison.

Methods

Doses:	100, 300, 1000 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) in water
Species/Strain:	Rabbit, Hra: (NZW)SPF
Number/Sex/Group:	22 pregnant females/group
Satellite groups:	None
Study design:	Dosing on Gestation Days (GD) 7-19, sacrifice on GD 29
Deviation from study protocol:	None that affected the integrity of the study.

Observations and Results

Mortality

One gavage error (confirmed by necropsy) resulting in unscheduled sacrifice occurred in each of the vehicle, MD and HD groups.

Two does in the HD group aborted and their litters (GD 19 and GD 21) and were therefore sacrificed. Three does in the HD group were sacrificed moribund with the reason for unscheduled termination given as general debilitation. All five HD unscheduled sacrifices showed similar toxicities (body weight loss, decreased food consumption, few or no feces) as observed in other HD dams that were sacrificed at the termination of the study. The five unscheduled deaths are attributed to treatment-related maternal toxicity.

Clinical Signs

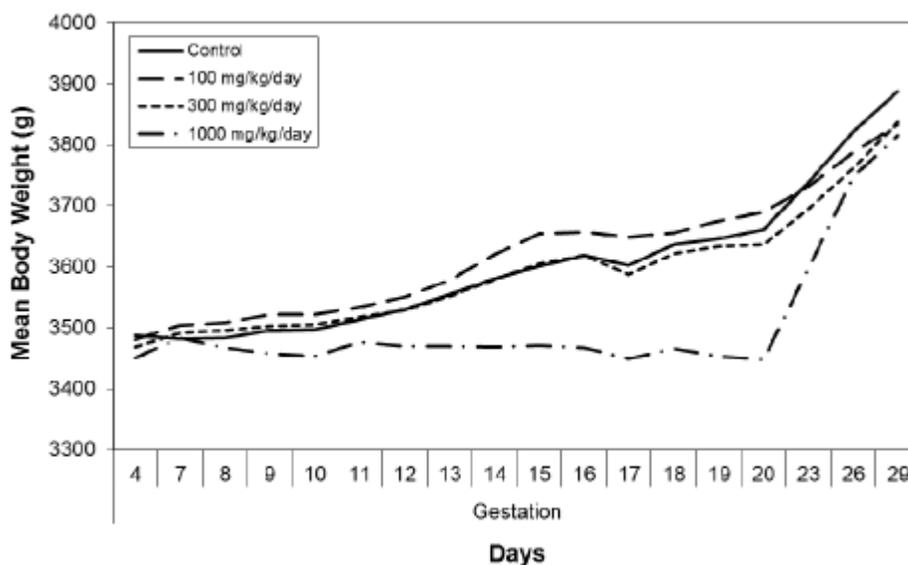
Does were briefly checked twice daily and detailed observations were made once daily during dosing. One vehicle and one MD rabbit had tonic convulsions after dosing and were euthanized. A perforated stomach (vehicle) and perforated lung (MD) were observed in necropsy confirming gavage errors in both animals. The convulsions were attributed to the gavage error. At the HD, decreased production of feces (few to none) was observed in most does. Two HD does showed evidence of aborted litters in the cages and were therefore sacrificed. Additionally, three HD does were sacrificed moribund because of general debilitation. All unscheduled sacrifices had decreased production of feces. Alopecia was observed in all groups including vehicle and not

considered test article-related. No test article-related findings were noted in the LD or MD groups.

Body Weight

Body weights were recorded on GD 0, 4, and 7-29. In the HD, decreases in mean BW were observed from GD 13-23. Decreases in body weight gain were seen GD 8-20 and reached statistical significance over the GD 10-11 and 12-13 intervals. Decreases were mainly seen during the dosing phase (GD 7-19) and recovered by the end of the study (GD 29). Slight increases in body weight were observed for the MD but the differences were attributed to normal variations in body weights and not considered test article-related. The data are presented in the figure below. No test article-related effects on body weight were observed in the LD or MD.

Figure 6. Mean Body Weights in Rabbits



Food Consumption

Food consumption was recorded on GD 0, 4, and 7-29. In the HD, decreases in food consumption paralleled decreases in body weights. No test article-related effects on food consumption were observed in the LD or MD.

Toxicokinetics

Toxicokinetics were not conducted in this study.

Dosing Solution Analysis

The dosing solutions were acceptable.

Necropsy

Abnormal stomach contents were noted in the five HD rabbits that were sacrificed prior to study termination. Observations included multicolored/green/white/brown stomach

contents that were semi-firm/firm/fibrous/solid/hairball. These findings were not observed in any of the scheduled necropsies. No other test article-related necropsy findings were noted at any dose.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Two HD does aborted their litters on GD 19 and 21. The loss of the litters is considered test article-related but secondary to the maternal toxicity caused by reduced food consumption and body weight loss. No other test article-related effects were observed on any of the Cesarean section parameters at any dose.

Offspring (Malformations, Variations, etc.)

No test article-related effects were observed on any of the parameters measured in the offspring. Two malformations were observed in the HD groups (lumbar vertebra-hemivertebra and thoracic centrum- fused) but were within historical control values. Several skeletal variations (bent hyoid, interparietal and sternebra incomplete ossification, sternebra bipartite ossification, unossified caudal vertebra, additional ossification site of cervical centrum) were increased in the HD but were within historical control values.

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/s/

ELIZABETH BOLAN
12/01/2015

RICHARD D MELLON
12/01/2015

I concur with Dr. Bolan's recommendation that from a pharmacology toxicology perspective, NDA 207621 may be approved. I also concur with the recommended post-marketing requirements and labeling recommendations.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 207621

NDA/BLA Number: 207621 Applicant: Pfizer

Stamp Date: December 19,
2014

**Drug Name: Extended-Release NDA/BLA Type: 505(b)(2)
Oxycodone HCl and
Naltrexone HCl**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA 207621**

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		A literature-based justification for the levels of excipients has been provided by the Applicant. The adequacy of these data will be determined upon review.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			Defer to CSS
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Phenanthrene-derivative opioid drug products may contain impurities (b) (4) which is a structural alert for mutagenicity. Therefore, the specification for these impurities in the drug substance must be reduced to reflect a maximal daily intake of NMT 1.5 mcg/day or adequate safety qualification must be provided. Upon preliminary review, (b) (4) appear to contain structural alerts and these impurities will require further evaluation to determine the appropriate specification. We recommend that you consult with your DMF holder to determine the levels of these impurities in the drug substance you are obtaining and if needed, to decrease the limit of these impurities.

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/s/

ELIZABETH BOLAN
02/06/2015

RICHARD D MELLON
02/06/2015