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*APPLICATION NUMBER:*

**206940Orig1s000**

**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/BLA REVIEW AND EVALUATION**

Application number: 206940  
Supporting document/s: 025  
Applicant's letter date: February 20, 2015  
CDER stamp date: February 20, 2015  
Product: Eluxadoline ( (b)(4)® )  
Indication: For the treatment of pain and diarrhea associated with diarrhea-predominant irritable bowel syndrome (IBS-d)  
Applicant: Furiex Pharmaceuticals, Inc.  
Review Division: DGIEP  
Reviewer: Tamal Chakraborti, Ph.D.  
Supervisor: Sushanta Chakder, Ph.D.  
Division Director: Donna Griebel, MD  
Project Manager: Jennifer Sarchet RN, BSN

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## Introduction

In the previous review of NDA 206940 (SDN 025) dated February 23, 2015 [REV-NONCLINICAL-21 (Primary Review) Comm/Reference ID 3706020], other NDAs and an IND were referred, which is not necessary. This revised review replaces the above referenced review.

In this submission, the Applicant provided responses to the Agency's information requests (IRs) for the Chemistry, Manufacturing and Controls (CMC) issues related to residual solvents in the drug substance (DS).

The following table (from page 2 of Section 3.2.S.4.1 of the original NDA submission) shows the specifications of the DS.

**Table 3.2.S.4.1-1 Drug Substance Acceptance Criteria**

Parameters	Acceptance Criteria	Test Methods
Description	White to off-white powder	Visual examination
Identification by FTIR	Conforms to reference	Current USP <197K>
Identification by HPLC	Similar retention time for sample and reference solution peak	Assay by HPLC
Assay (b) (4)	(b) (4) % (w/w)	Assay by HPLC
Related Substances		Assay by HPLC
Impurity (b) (4)	NMT (b) (4) % w/w	
Any unspecified impurity	NMT %	
Total impurities	NMT (b) (4) %	
Residual Solvents		Residual Solvents by GC
(b) (4)	NMT (b) (4) ppm	
	NMT ppm	
	NMT ppm	
	NMT ppm	
	NMT ppm	
	NMT (b) (4) ppm	
	NMT ppm	
Water Content	NMT (b) (4) %	Current USP <921> Ia
Residue on Ignition/Sulphated Ash	NMT %	Current USP <281>
Heavy Metals	NMT ppm	Current USP <231>, Method II
Particle size	d <sub>50</sub> : (b) (4) μm	Laser Diffraction, Malvern
Microbiological Enumeration tests		
Total Aerobic Microbial Count (TAMC)	NMT (b) (4) CFU/g	Current USP <61>
Total Combined Yeasts & Molds Count (TYMC)	NMT (b) (4) CFU/g	
Test for Specified micro organisms		
<i>Escherichia coli</i>	Absence in (b) (4) g	Current USP <62>

(b) (4)

CFU = Colony forming units, FTIR = Fourier transform infrared spectroscopy, GC = Gas chromatography, HPLC = High pressure liquid chromatography, ICH = International Conference on Harmonization, NMT = Not more than, ppm = Parts per million, TAMC = Total aerobic microbial count, (b) (4) TYMC = Total combined yeasts and molds count, USP = United States Pharmacopeia

In the pharmacology review of NDA 206940 dated January 23, 2015 (page 14), it was stated “Residual solvent levels were well below the ICH Q3C limits and are acceptable”. The CMC team pointed out on February 11, 2015 that three residual solvents ((b) (4)) present in the DS are not listed in the ICH Q3C guidance.

In a teleconference with the Applicant on February 12, 2015, the FDA asked the Applicant to provide justifications for the proposed specification for (b) (4) at NMT (b) (4) ppm ((b) (4) %). Based on the available information in the literature, there were no safety concerns for the other two residual solvents. In this submission, the Applicant provided justifications for the proposed specification for (b) (4) at NMT (b) (4) ppm.

The following table (from page 15 of Section 3.2.S.3.2 of the original NDA submission) shows the list of the organic solvents and their ICH Q3C limits.

**Table 3.2.S.3.2-5 Organic Solvents Used in the Synthesis of Eluxadoline**

A large rectangular area of the document is completely redacted with a solid grey box. The redaction covers the entire content of Table 3.2.S.3.2-5. A small '(b) (4)' label is visible in the top right corner of the redacted area.

It is to be mentioned here that these three residual solvents were not present in nonclinical batches.

A rectangular area of text is redacted with a solid grey box. A small '(b) (4)' label is visible in the top right corner of the redacted area.

The Applicant stated that a specification of NMT (b) (4) ppm was assigned as this was believed to be relatively conservative given the lack of perceived toxicological concern with this molecule. Nonclinical safety data for (b) (4) in the literature is limited. Per the Material Safety Data Sheet (MSDS, submitted by the Applicant), (b) (4) is not mutagenic in the Ames and mammalian cell culture assays. The oral LD<sub>50</sub> for (b) (4) in rats was reported to be (b) (4) mg/kg. (b) (4) is not listed as a carcinogen by the American Conference of Governmental and Industrial Hygienist (ACGIH), International Agency for Research on Cancer (IARC), and National Toxicology Program (NTP). The Applicant provided safety assessment for (b) (4) based on the European Chemicals Agency (ECHA) as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation, which includes additional compound specific nonclinical data for (b) (4). The following table (from page 2 of the cover letter of the submission dated February 20, 2015) shows the no-observed adverse effect level (NOAEL) of (b) (4).

**Table 1: No Observed Adverse Effect Levels for (b) (4) and (b) (4)**

Compound	NOAEL Repeat-Dose Rat (mg/kg)	Human Equivalent Dose (HED) (mg/kg)	Dose Multiples ** (b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)

\* ECHA REACH: Derived rat oral No Effect Level based on a repeat-dose inhalation study in rats (b) (4)  
 \*\*Dose multiples based on human dose of (b) (4) µg/kg) at the proposed specification limit of (b) (4) ppm. (b) (4) included for comparison.

Per the above ECHA document, the Derived No Effect Level (DNEL) was reported to be (b) (4) mg/kg/day based on the NOAEL of (b) (4) mg/kg/day in a repeated dose toxicity study by inhalation. However, the details of the study were not provided. The oral LD<sub>50</sub> for (b) (4) in rats ranged from (b) (4) mg/kg. A safe human dose of (b) (4) mg/day for (b) (4) was derived from the above LD<sub>50</sub> value using a 100-fold safety factor based on a 50 kg body weight. At NMT (b) (4) ppm ((b) (4) %) of (b) (4) in the eluxadoline DS, estimated exposure to (b) (4) from 200 mg/day (100 mg BID) dose of eluxadoline would be (b) (4) mg/day or (b) (4) µg/day, which is about (b) (4) times less than the above mentioned safe human dose ((b) (4) mg/day). Therefore, based on these, the proposed specification of (b) (4) at NMT (b) (4) ppm in the eluxadoline drug substance is acceptable.

(b) (4) The estimated safe dose of (b) (4) in humans was calculated to be (b) (4) mg/day per the ICH Q3A (PDE approach) using the NOAEL of (b) (4). At NMT (b) (4) ppm ((b) (4) %) of (b) (4) in the eluxadoline DS, estimated exposure to (b) (4) from 200 mg/day (100 mg BID) dose of eluxadoline would be (b) (4) µg/day ((b) (4) mg/day), which is about (b) (4) times less than the above mentioned safe human dose ((b) (4) mg/day). Therefore, the proposed specification for (b) (4) at NMT (b) (4) ppm in the eluxadoline DS is acceptable.

(b) (4) A PDE for (b) (4) mg/day was determined based on the negative results in a standard battery of genotoxicity tests and the absence of significant toxicity findings at a high dose of (b) (4) mg/kg/day in a 3-month oral toxicology study in rat (b) (4). At NMT (b) (4) ppm ((b) (4) %) of (b) (4) in the eluxadoline DS, estimated exposure to (b) (4) from 200 mg/day (100 mg BID) dose of eluxadoline would be (b) (4) mg/day, which is about (b) (4) times less than the PDE of (b) (4) mg/day. Therefore, the proposed specification of (b) (4) at NMT (b) (4) ppm in the eluxadoline DS is acceptable.

**Recommendations:** From a nonclinical standpoint, the specifications for (b) (4) at NMT (b) (4) ppm, (b) (4) at NMT (b) (4) ppm and (b) (4)

(b) (4) at NMT (b) (4) ppm in the eluxadoline drug substance are acceptable.

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/s/  
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TAMAL K CHAKRABORTI  
05/18/2015

SUSHANTA K CHAKDER  
05/18/2015

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
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## Introduction

Eluxadoline (JNJ-27018966/JNJ-27018966-AAA/ (b) (4)®) is a new molecular entity and acts as a mu-opioid receptor ( $\mu$ OR) agonist and delta-opioid receptor ( $\delta$ OR) antagonist. Under NDA 206940, the Applicant is seeking approval for eluxadoline for the treatment of diarrhea-predominant irritable bowel syndrome (IBS-d) in adult men and women. Eluxadoline has low oral bioavailability in all species tested, including humans. The recommended human dose is 100 mg twice daily (BID). Eluxadoline has not been marketed in any country.

In this submission, the Applicant provided responses to the Agency's information requests (IRs) for nonclinical and Chemistry, Manufacturing and Controls (CMC) issues.

The following table (from page 2 of Section 3.2.S.4.1 of the original NDA submission) shows the specifications of the drug substance (DS).

**Table 3.2.S.4.1-1 Drug Substance Acceptance Criteria**

Parameters	Acceptance Criteria	Test Methods
Description	White to off-white powder	Visual examination
Identification by FTIR	Conforms to reference	Current USP <197K>
Identification by HPLC	Similar retention time for sample and reference solution peak	Assay by HPLC
Assay (% w/w calculated on an anhydrous basis)	(b) (4) % (w/w)	Assay by HPLC
Related Substances		Assay by HPLC
Impurity (b) (4)	NMT (b) (4) % w/w	
Any unspecified impurity	NMT %	
Total impurities	NMT (b) (4) %	
Residual Solvents		Residual Solvents by GC
(b) (4)	NMT (b) (4) ppm	
	NMT ppm	
	NMT ppm	
	NMT ppm	
	NMT ppm	
	NMT (b) (4) ppm	
	NMT ppm	
Water Content	NMT (b) (4) %	Current USP <921> Ia
Residue on Ignition/Sulphated Ash	NMT %	Current USP <281>
Heavy Metals	NMT ppm	Current USP <231>, Method II
Particle size	d <sub>50</sub> : (b) (4) μm	Laser Diffraction, Malvern
Microbiological Enumeration tests		
Total Aerobic Microbial Count (TAMC)	NMT (b) (4) CFU/g	Current USP <61>
Total Combined Yeasts & Molds Count (TYMC)	NMT (b) (4) CFU/g	
Test for Specified micro organisms		
<i>Escherichia coli</i>	Absence in (b) (4) g	Current USP <62>

(b) (4)

CFU = Colony forming units, FTIR = Fourier transform infrared spectroscopy, GC = Gas chromatography, HPLC = High pressure liquid chromatography, ICH = International Conference on Harmonization, NMT = Not more than, ppm = Parts per million, TAMC = Total aerobic microbial count, (b) (4) TYMC = Total combined yeasts and molds count, USP = United States Pharmacopeia

Please also refer to the page 14 of the pharmacology review of NDA 206940 dated January 23, 2015 (Residual Solvents), which incorrectly stated “Residual solvent levels were well below the ICH Q3C limits and are acceptable”. Upon re-examination of the levels of the residual solvents in the DS following the query from the CMC team on February 11, 2015, it was found out that three residual solvents ( (b) (4) ) in the drug substance are not listed in the ICH Q3C document.

The FDA/CDER/OPQ requested (dated February 11, 2015) for a teleconference to discuss several CMC issues including the issue of the residual solvent. Subsequently, a teleconference was held on February 12, 2015 between the FDA and the Applicant to discuss these issues. In that teleconference, the FDA asked the Applicant to provide justification in support of the specification especially for (b) (4) at NMT (b) (4) ppm ( (b) (4) %). The specifications for the other two solvents (e.g., (b) (4) at NMT (b) (4) ppm and (b) (4) ppm, respectively) were considered to be acceptable (as reviewed below). In this submission, the Applicant provided justification for the specification for (b) (4) at NMT (b) (4) ppm as requested by the FDA.

The following table (from page 15 of Section 3.2.S.3.2 of the original NDA submission) shows the list of the organic solvents and their ICH Q3C limits.

**Table 3.2.S.3.2-5 Organic Solvents Used in the Synthesis of Eluxadoline**



On page 4 of Section 3.2.S.4.5 of the original NDA submission under “Justification of Specification”, the Applicant stated “The proposed limits are justified based on batch data, the current organic solvents used in the drug substance synthesis and ICH Q3C or FDA recommendations for residual solvents. ICH Q3C does not list acceptance criteria specifically for (b) (4). The acceptance criteria for these three organic solvents also rely on safety information in the literature and international guidance documents to help establish the specification.” However, the examinations of the batch analysis data for eluxadoline drug substance used in nonclinical studies (listed in Module 2.6.7.4 of the original NDA submission) revealed these three residual solvents were not reported to be present in nonclinical batches and are not qualified in nonclinical toxicology studies. In addition, the Applicant did not provide adequate information (e.g., published literature, etc.) in support of the justification of these residual solvents at the specified level. The following review addresses the qualification these residual solvents in the eluxadoline drug substance.



(b) (4) The Applicant stated that a specification of NMT (b) (4) ppm was assigned as this was believed to be relatively conservative given the lack of perceived toxicological concern with this molecule. Nonclinical safety data for (b) (4) in the literature is limited. Per the Material Safety Data Sheet (MSDS, submitted by the Applicant), (b) (4) is not mutagenic in the Ames and mammalian cell culture assays. The oral LD<sub>50</sub> for (b) (4) in rats was reported to be (b) (4) mg/kg. (b) (4) is not listed as a carcinogen by the American Conference of Governmental and Industrial Hygienist (ACGIH), International Agency for Research on Cancer (IARC), and National Toxicology Program (NTP). The Applicant provided safety assessment for (b) (4) based on the European Chemicals Agency (ECHA) as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation, which includes additional compound specific nonclinical data for (b) (4). The following table (from page 2 of the cover letter of the submission dated February 20, 2015) shows the no-observed adverse effect level (NOAEL) of (b) (4).

**Table 1: No Observed Adverse Effect Levels for (b) (4)**

Compound	NOAEL Repeat-Dose Rat (mg/kg)	Human Equivalent Dose (HED) (mg/kg)	Dose Multiples ** (b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)

\* ECHA REACH: Derived rat oral No Effect Level based on a repeat-dose inhalation study in rats  
 \*\*Dose multiples based on human dose of (b) (4) µg/kg) at the proposed specification limit of (b) (4) ppm. (b) (4) included for comparison.

Per the above ECHA document, the Derived No Effect Level (DNEL) was reported to be (b) (4) mg/kg/day based on the NOAEL of (b) (4) mg/kg/day in a repeated dose toxicity study by inhalation. However, the above ECHA document did not provide the details of the study (e.g., species was not mentioned, test article was not specified, and there was no reference of the study).

(b) (4) ppm) in the DS was accepted under NDA 21506/21754 (Chemistry review of NDA 21506/21754 dated March 7, 2005 by Dr. Mark Seggel). Under IND (b) (4), the permitted daily exposure (PDE) for (b) (4) was calculated to be (b) (4) mg/day (pharmacology review of IND (b) (4)).

No PDE or concentration limit (ppm) has been established for (b) (4) (ICH Q3C). The PDE is derived from the no-observed effect level (NOEL), or the low-observed effect level (LOEL) in the most relevant animal study using various safety factors (ICH Q3C). The NOEL or LOEL data is not available for (b) (4). However, as mentioned above, the oral LD<sub>50</sub> for (b) (4) in rats ranged from (b) (4) mg/kg. A human dose of (b) (4) mg/day for (b) (4) (derived from the above LD<sub>50</sub> value using a 100-fold safety factor and based on a 50 kg body weight) was considered to be safe under NDA 202799. At NMT (b) (4) ppm ((b) (4) %) of (b) (4) in the eluxadoline DS, estimated exposure to (b) (4) from

200 mg/day (100 mg BID) dose of eluxadoline would be (b) (4) mg/day or (b) (4) µg/day, which is about (b) (4) times less than the above mentioned safe human dose ((b) (4) mg/day). Therefore, based on these, the specification of (b) (4) at NMT (b) (4) ppm in the eluxadoline drug substance does not appear to raise a safety concern and is acceptable.

(b) (4)  
The estimated safe dose of (b) (4) in humans was calculated to be (b) (4) mg/day per the ICH Q3A (PDE approach) under NDA 22453 (pharmacology review of NDA 22453 dated September 17, 2009 by Dr. William D. McGuinn) using the NOAEL of (b) (4)

At NMT (b) (4) ppm ((b) (4) %) of (b) (4) in the eluxadoline DS, estimated exposure to (b) (4) from 200 mg/day (100 mg BID) dose of eluxadoline would be (b) (4) µg/day ((b) (4) mg/day), which is about (b) (4) times less than the above mentioned safe human dose ((b) (4) mg/day). Therefore, the specification for (b) (4) at NMT (b) (4) ppm in the eluxadoline DS appears to be acceptable.

(b) (4)  
No adequate toxicological data are available for (b) (4). A PDE of (b) (4) mg/day for (b) (4) was used for the qualification of this chemical as a residual solvent in the drug substance under NDA 203188 (pharmacology review of NDA 203188 dated January 13, 2012 by Dr. Timothy W. Robison). A PDE for (b) (4) mg/day was determined (b) (4) based on the negative results in a standard battery of genotoxicity tests and the absence of significant toxicity findings at a high dose of (b) (4) mg/kg/day in a 3-month oral toxicology study in rats (details of the results were not provided).

At NMT (b) (4) ppm ((b) (4) %) of (b) (4) in the eluxadoline DS, estimated exposure to (b) (4) from 200 mg/day (100 mg BID) dose of eluxadoline would be (b) (4) mg/day, which is about (b) (4) times less than the above mentioned safe human dose ((b) (4) mg/day). Therefore, the specification of (b) (4) at NMT (b) (4) ppm in the eluxadoline DS appears to be acceptable.

## Recommendations:

The specifications for (b) (4) at NMT (b) (4) ppm, (b) (4) at NMT (b) (4) ppm and (b) (4) at NMT (b) (4) ppm in the eluxadoline drug substance appear to be acceptable.

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/s/  
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TAMAL K CHAKRABORTI  
02/23/2015

SUSHANTA K CHAKDER  
02/23/2015

Comments on NDA206940 eluxadoline

From A. Jacobs, AD

Date: 1/29/15

1. I concur that there are no approval issues and that the (b) (4)
2. I note the minimal systemic exposure for this product
3. I have conveyed other comments to the reviewer and supervisor and the comments have been addressed as appropriate.

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/s/  
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ABIGAIL C JACOBS  
01/29/2015

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

**PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION**

Application number: 206940  
Supporting document/s: 000  
Applicant's letter date: June 26, 2014  
CDER stamp date: June 27, 2014  
Product: Eluxadoline ( (b)(4)® ) Tablets  
Indication: For the treatment of pain and diarrhea associated with diarrhea-predominant irritable bowel syndrome (IBS-d).  
Applicant: Furiex Pharmaceuticals, Inc.  
Review Division: Division of Gastroenterology and Inborn Errors Products (DGIEP)  
Reviewer: Tamal Chakraborti, Ph.D.  
Supervisor: Sushanta Chakder, Ph.D.  
Division Director: Donna Griebel, MD  
Project Manager: Jennifer Sarchet RN, BSN

**Disclaimer**

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 206940 are owned by Furiex Pharmaceuticals, Inc. or are data for which Furiex Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 206940 that Furiex Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 206940.

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# 1 Executive Summary

## 1.1 Introduction

Eluxadoline (JNJ-27018966/JNJ-27018966-AAA/ (b) (4)®) is a new molecular entity and acts as a mu-opioid receptor ( $\mu$ OR) agonist and delta-opioid receptor ( $\delta$ OR) antagonist. Under NDA 206940, the Applicant is seeking approval for eluxadoline for the treatment of diarrhea-predominant irritable bowel syndrome (IBS-d) in adult men and women. Eluxadoline has low oral bioavailability in all species tested, including humans. The recommended human dose is 100 mg twice daily (BID). Eluxadoline has not been marketed in any country.

## 1.2 Brief Discussion of Nonclinical Findings

Eluxadoline has been shown to be a  $\mu$ OR agonist and  $\delta$ OR antagonist, with moderate kappa OR ( $\kappa$ OR) agonist activity. In animal efficacy studies, eluxadoline has shown efficacy in normalizing GI transit and defecation in several animal models of altered GI function induced by stress, castor-oil or GI inflammation.

Eluxadoline did not cause significant inhibition of  $I_{Kr}$  in *in vitro* hERG assay up to 3  $\mu$ M concentration and had no significant effect on the rate and the force of contraction up to 10  $\mu$ M concentration in isolated guinea pig atrium. Eluxadoline did not show significant electrophysiological effects in isolated rabbit Purkinje fibers up to 10  $\mu$ M (> 1000 times the  $C_{max}$  of 3 ng/mL after 100 mg oral dose in humans). In anesthetized dogs, eluxadoline did not show any significant cardiovascular effect up to an IV cumulative dose of 1.443 mg/kg (124 times the  $C_{max}$  in humans at the 100 mg dose). In conscious telemetered monkeys, QT and QTc intervals were slightly prolonged (106% to 112%) at SC doses of 5, 15 and 30 mg/kg. In a respiratory safety pharmacology study in rats at IV doses of 5, 10 and 20 mg/kg, depressive changes in breathing, consistent with  $\mu$ OR agonists, were observed. No significant safety signals were identified in the CNS safety pharmacology studies in rats up to an oral dose of 300 mg/kg.

Chronic oral toxicology studies were conducted in rats (6-month) and monkeys (9-month) to support chronic use of eluxadoline. The no-observed-adverse-effect-levels (NOAELs) in rats and monkeys were 2000 and 200 mg/kg/day, respectively (about 11 and 14 times, respectively, the human AUC of 24 ng.h/mL after a single oral dose of 100 mg). In a 4-week oral toxicology study in juvenile rats, the NOAEL was 1500 mg/kg/day.

Eluxadoline was negative in the Ames test, chromosome aberration assay in human lymphocytes, the mouse lymphoma cell (L5178Y/TK<sup>+/+</sup>) forward mutation test and the *in vivo* rat bone marrow micronucleus test. Oral administration of eluxadoline for 104 weeks did not produce tumors in mice and rats at up to 14 and 36 times, respectively, the human AUC of 24 ng.h/mL after a single oral dose of 100 mg.

Eluxadoline at oral doses up to 1000 mg/kg/day (about 10 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg) was found to have no adverse effect on fertility and reproductive performance of male and female rats. Embryofetal development studies in rats and rabbits at oral/SC doses up to 1000/5 mg/kg/day (about 51 and 115 times, respectively, the human AUC after a single oral dose of 100 mg) did not cause any adverse effects on embryofetal development. A pre and postnatal development study in rats showed no evidence of any adverse effect on pre and postnatal development at oral doses of eluxadoline up to 1000 mg/kg/day (about 10 times the human AUC after a single oral dose of 100 mg).

### 1.3 Recommendations

#### 1.3.1 Approvability

From a nonclinical perspective, this application is recommended for approval for its proposed use as indicated in the label.

#### 1.3.2 Additional Non Clinical Recommendations

None

#### 1.3.3 Labeling

The proposed labeling of (b) (4)® appears to conform to the specific requirements on content and format of relevant nonclinical sections of the label for human prescription drugs under 21CFR 201.57. However, the following labeling changes are recommended.

### 8.1 Pregnancy

(b) (4)

#### *Animal Data*

Eluxadoline administered (b) (4) (about 51 and 115 times, respectively, the human AUC of 24 ng.h/mL after a single oral dose of 100 mg) and did not cause any adverse effects on embryofetal development. A pre and postnatal development study in rats showed no evidence of any adverse effect on pre and postnatal development at oral doses of eluxadoline up to 1000 mg/kg/day (about 10 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg).

(b) (4)

(b) (4)

## 8.4 Pediatric Use

Eluxadoline was orally administered to juvenile rats at 500, 750, and 1500 mg/kg/day [about 16, 54 and 30 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg] for 4 weeks. There were no (b) (4)

Based on these results, the NOAEL for male and female juvenile rats was 1500 mg/kg/day (about 30 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg).

## 13 Nonclinical Toxicology

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

#### Carcinogenesis

Two-year oral carcinogenicity studies have been conducted with eluxadoline in CD-1 mice at doses up to 1500 mg/kg/day (about 14 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg) and in Sprague Dawley rats at oral doses up to 1500 mg/kg/day (about 36 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg). Oral administration of eluxadoline for 104 weeks did not produce tumors in mice and rats.

#### Mutagenesis

Eluxadoline was negative in the Ames test, the chromosome aberration test in human lymphocytes, the mouse lymphoma cell (L5178Y/TK<sup>+/-</sup>) forward mutation test and the in vivo rat bone marrow micronucleus test.

#### Impairment of Fertility

Eluxadoline at oral doses up to 1000 mg/kg/day (about 10 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg) was found to have no adverse effect on fertility and reproductive performance of male and female rats.

## 2 Drug Information

### 2.1 Drug

CAS Registry Number: 864821-90-9

Generic Name: Eluxadoline

Code Name: JNJ-27018966-AAA (b) (4)

JNJ-27018966

R497138

T3301

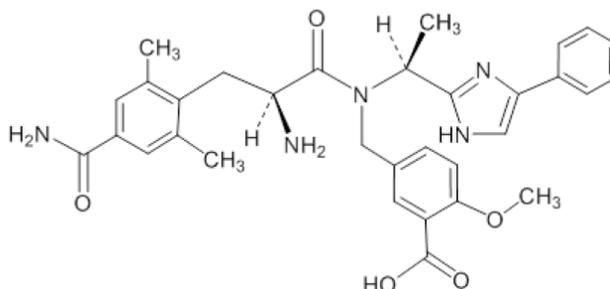
JNJ-27018966-AAC (b) (4)

Chemical Name: 5-[[[(2S)-2-amino-3-[4-(aminocarbonyl)-2,6-dimethylphenyl]-1-oxopropyl][(1S)-1-(4-phenyl-1H-imidazol-2-yl)ethyl]amino]methyl]-2-methoxybenzoic acid

Molecular Formula/Molecular Weight: C<sub>32</sub>H<sub>35</sub>N<sub>5</sub>O<sub>5</sub>/569.65 Daltons

Structure: The following (from page 1 of Section 3.2.S.1.2 of the submission) figure shows the chemical structure of eluxadoline.

Figure 3.2.S.1.2-1 Chemical Structure of Eluxadoline



Pharmacologic Class: Mu opioid receptor ( $\mu$ OR) agonist and delta opioid receptor ( $\delta$ OR) antagonist

### 2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 79,214 (JNJ-27018966, Furiex Pharmaceuticals, Inc.)

### 2.3 Drug Formulation

Eluxadoline drug product will be supplied as 75-mg and 100-mg film-coated tablets. The drug product is composed of eluxadoline and inactive ingredients listed in the table below (from page 1 of Section 3.2.P.1 of the submission).

**Table 3.2.P.1-1 Composition of Eluxadoline Tablets, 75-mg and 100-mg**

Ingredient	Function	75 mg Tablet Amount (mg/tab)	100 mg Tablet Amount (mg/tab)	% w/w
eluxadoline drug substance (Company Specification)	Active substance	75	100	(b) (4)
Silicified MCC (b) (4) NF				(b) (4)
Colloidal silica, NF, Ph Eur, JP				
Mannitol, USP, Ph Eur				
Crospovidone (b) (4) NF, Ph Eur				
Magnesium stearate, NF, Ph Eur, JP				
(b) (4)				
Opadry II (b) (4) (Company Specification)				
(b) (4)				
<b>Coated Tablet Weight</b>		<b>618 mg</b>	<b>824 mg</b>	-----

JP = Japanese Pharmacopeia; MCC = microcrystalline cellulose; NF = National Formulary; Ph Eur = European Pharmacopeia; USP = United States Pharmacopeia

(b) (4)

**2.4 Comments on Novel Excipients**

There are no novel excipients in the drug product (DP). The following table (from page 1 of Section 3.2.P.4 of the submission) shows a list of the compendial excipients of eluxadoline tablets and the respective compendial monograph. The compendial excipients met the acceptance criteria as described in their respective monograph.

**Table 3.2.P.4-1 Compendial Excipients**

Excipient	Compendial Reference
Silicified microcrystalline cellulose (b) (4)	NF
Colloidal silica	NF, Ph. Eur., JP
Mannitol	USP, Ph. Eur.
Crospovidone (b) (4)	NF, Ph. Eur.
Magnesium stearate	NF, Ph. Eur., JP
(b) (4)	USP, Ph. Eur.

JP = Japanese Pharmacopeia; NF = National Formulary; Ph. Eur. = European Pharmacopeia; USP = United States Pharmacopeia

The film coat system for the 75-mg eluxadoline tablets is (b) (4) Opadry II (b) (4). This film coat is a mixture of six compendial ingredients: polyvinyl

alcohol partially hydrolyzed, USP/Ph Eur; titanium dioxide, USP/Ph Eur; macrogol/polyethylene glycol (PEG), NF/Ph Eur; talc, USP/Ph Eur; iron oxide yellow, NF/Ph Eur and iron oxide red, NF/Ph Eur. Opadry II (b) (4) met the USP acceptance criteria. The Applicant referred to (b) (4) Drug Master File DMF (b) (4) and also provided a copy of (b) (4) DMF letter of authorization in Section 1.4.2 of the submission.

The film coat system for the 100-mg eluxadoline tablets is (b) (4) Opadry II (b) (4). This film coat is a mixture of six compendial ingredients: polyvinyl alcohol partially hydrolyzed, USP/Ph Eur; titanium dioxide, USP/Ph Eur; macrogol/PEG, NF/Ph Eur; talc, USP/Ph Eur; iron oxide yellow, NF and iron oxide red, NF. Opadry II (b) (4) met the USP acceptance criteria. The Applicant referred to (b) (4) Drug Master File DMF (b) (4) and also provided a copy of (b) (4) DMF letter of authorization in Section 1.4.2 of the submission.

## 2.5 Comments on Impurities/Degradants of Concern

Impurities in eluxadoline drug substance (DS) included process-related impurities (known, potential and genotoxic), inorganic impurities, and residual solvents, which were discussed below. The following table (from page 2 of Section 3.2.S.4.1 of the submission) shows the specification of the DS.

**Table 3.2.S.4.1-1 Drug Substance Acceptance Criteria**

Parameters	Acceptance Criteria	Test Methods
Description	White to off-white powder	Visual examination
Identification by FTIR	Conforms to reference	Current USP <197K>
Identification by HPLC	Similar retention time for sample and reference solution peak	Assay by HPLC
Assay (% w/w) (b) (4)	(b) (4) % (w/w)	Assay by HPLC
Related Substances		Assay by HPLC
Impurity (b) (4)	NMT (b) (4) % w/w	
Any unspecified impurity	NMT %	
Total impurities	NMT (b) (4) %	
Residual Solvents		Residual Solvents by GC
(b) (4)	NMT (b) (4) ppm	
	NMT ppm	
	NMT ppm	
	NMT ppm	
	NMT ppm	
	NMT (b) (4) ppm	
	NMT ppm	
Water Content	NMT (b) (4) %	Current USP <921> Ia
Residue on Ignition/Sulphated Ash	NMT %	Current USP <281>
Heavy Metals	NMT ppm	Current USP <231>, Method II
Particle size	d <sub>50</sub> : (b) (4) μm	Laser Diffraction, Malvern
Microbiological Enumeration tests		
Total Aerobic Microbial Count (TAMC)	NMT (b) (4) CFU/g	Current USP <61>
Total Combined Yeasts & Molds Count (TYMC)	NMT (b) (4) CFU/g	
Test for Specified micro organisms		
<i>Escherichia coli</i>	Absence in (b) (4) g	Current USP <62>

CFU = Colony forming units, FTIR = Fourier transform infrared spectroscopy, GC = Gas chromatography, HPLC = High pressure liquid chromatography, ICH = International Conference on Harmonization, NMT = Not more than, ppm = Parts per million, TAMC = Total aerobic microbial count, (b) (4) TYMC = Total combined yeasts and molds count, USP = United States Pharmacopeia

### Organic Impurities:

Synthesis-Related Impurities and By-Products: In the original (b) (4) chemistry batches, up to 13 impurities were observed in the DS and 9 impurities were observed above the reporting threshold levels. These impurities were qualified in toxicology studies or were detected at a level at which qualification was not required. However, only one of these impurities, (b) (4)

was present at higher than ICH reportable levels in the latest batches. It is to be noted here that a number of these impurities were found (b) (4)

The following table (from page 3 of Section 3.2.S.3.2) shows the structure of the impurity (b) (4)

Related Substance	Structure
(b) (4)	

As mentioned above, of the potential impurities and degradation products in eluxadoline, only impurity (b) (4) has been observed at levels requiring ICH Q3A identification and qualification thresholds. Impurity (b) (4)

The current specification of (b) (4) in the DS is not more than (NMT) (b) (4) % w/w which will result in the maximum exposure of (b) (4) mg per day in humans at a dose of 100 mg BID . The following tables (from page 12 and 13 of Section 3.2.S.3.2) show nonclinical and clinical data, which justify that the proposed specification of NMT (b) (4) % is acceptable.

**Table 3.2.S.3.2-3 Toxicological Coverage of (b) (4) in Eluxadoline – Nonclinical Studies**

Batch Number	Impurity % (w/w)	Study Description	NOAEL (po) (mg/kg/day)	Coverage at NOAEL (mg/kg/day)	HED in 60 kg Human (mg/day)				
<b>Reproductive Toxicity</b>									
ZR497138PFA011 (tested May 2010)	(b) (4)	Segment 1 (rat) Study 1808-003	(b) (4)	(b) (4)	(b) (4)				
30205959		Segment 2 (rabbit) Study tox8376							
30205959		Segment 2 (rat) Study tox8398							
ZR497138PFA141		Segment 3 (rat) Study 1808-019							
<b>Chronic Toxicity</b>									
ZR497138PFA071		6 month rat Study 1808-007				(b) (4)	(b) (4)	(b) (4)	(b) (4)
ZR497138PFA011 (retested Jun 2011)		9 month primate Study 1808-004							
<b>Carcinogenicity</b>									
ZR497138PFA111		Rat Study 1808-008 Mouse Study 1808-009				(b) (4)	(b) (4)	(b) (4)	(b) (4)
ZR497138PFA141	Rat Study 1808-008 Mouse Study 1808-009								
ZR497138PFA151	Rat Study 1808-008 Mouse Study 1808-009								
ZR497138PFA171	Rat Study 1808-008 Mouse Study 1808-009								

<sup>a</sup> (b) (4) mg/kg was the NOEL for female general toxicity and for fertility and reproductive performance in males and females. The NOAEL for male general toxicity was (b) (4) mg/kg/day.

<sup>b</sup> NOAEL for rabbit fetuses; Maternal NOAEL (b) (4) mg/kg.

<sup>c</sup> NOAEL for rat fetuses; Maternal NOAEL (b) (4) mg/kg.

HED = human equivalent dose; NOEL = No observed effect level; NOAEL = No observed adverse effect level

**Table 3.2.S.3.2-4 Toxicological Coverage of (b) (4) in Eluxadoline – Human Studies**

Batch Number	Impurity % (w/w)	Study Description	NOAEL (po) (mg/kg/day)	Coverage at NOAEL (mg/kg/day)	HED in 60 kg Human (mg/day)
<b>Human Studies</b>					
31517808	(b) (4)	Phase 2; 200 mg BID highest dose administered for 12 weeks Study 27018966IBS2001	---	---	(b) (4)
31746876	(b) (4)	Phase 2; 200 mg BID highest dose administered for 12 weeks Study 27018966IBS2001	---	---	(b) (4)
ZR497138PFA041	(b) (4)	Phase 2; 200 mg BID highest dose administered for 12 weeks Study 27018966IBS2001	---	---	(b) (4)
ZR497138PFA161	(b) (4)	Phase 3 <sup>a</sup> : 100 mg BID highest dose	----	----	(b) (4)
ZR497138PFA181	(b) (4)	Phase 3 <sup>a</sup> : 100 mg BID highest dose	----	----	(b) (4)
A11KD0108	(b) (4)	Phase 3 <sup>a</sup> : 100 mg BID highest dose	----	----	(b) (4)
A11KD0109	(b) (4)	Phase 3 <sup>a</sup> : 100 mg BID highest dose	----	----	(b) (4)
A12BD0275	(b) (4)	Phase 3 <sup>a</sup> : 100 mg BID highest dose	----	---	(b) (4)

HED = human equivalent dose; NOAEL = no observed adverse effect level

<sup>a</sup> Used in both Phase 3 clinical trials (27018966IBS3001 and 27018966IBS3002).

In addition, per the FDA comments on CMC (Chemistry Manufacturing, and Controls) Pre-NDA meeting package, (b) (4) impurity was considered (FDA meeting minutes dated February 14, 2014) toxicologically qualified and that the limit of NMT (b) (4) % was found to be acceptable.

**Genotoxic Impurities:**

A genotoxic impurity assessment of process related impurities was performed using Derek analysis with the mutagenicity endpoint. No potential genotoxic impurities in the synthesis of eluxadoline were identified.

**Inorganic Impurities:**

(b) (4) is present in the DS at (b) (4) ppm ( (b) (4) %). The following table (from page 7 of Section 3.2.S.4.5 of the submission) shows the (b) (4) levels in representative batches of eluxadoline DS.

**Table 3.2.S.4.5-1 (b) (4) Levels in Representative Batches of Eluxadoline Drug Substance**

Batch	Batch Use	(b) (4) Level	Analytical Method
A11KD0108	Phase 3 clinical	(b) (4) ppm	AA
A11KD0109	Phase 3 clinical	(b) (4) ppm	AA
A11KD0110	Phase 3 clinical, pharmaceutical development	(b) (4) ppm	AA
A12BD0275	Phase 3 clinical	(b) (4) ppm	AA
ZR497138PFA141	Carcinogenicity and stability	(b) (4) ppm	AA
ZR497138PFA151	Carcinogenicity, pharmaceutical development and stability	(b) (4) ppm	AA
ZR497138PFA161	Phase 3 clinical	(b) (4) ppm	AA
ZR497138PFA171	Phase 3 clinical	(b) (4) ppm	AA
ZR497138PFA181	Phase 3 clinical	(b) (4) ppm	AA
Y501S5-12-002	Registration	(b) (4)	ICP-MS
Y501S5-13-001	Registration	(b) (4)	ICP-MS
Y501S5-13-002	Registration	(b) (4)	ICP-MS

AA = Atomic absorption spectroscopy; ICP-MS = Inductively coupled plasma mass spectrometry

The exposure to (b) (4) would be about (b) (4)  $\mu\text{g}$  per day at the recommended dose of 100 mg BID. All levels were well below the proposed USP <231> limit of (b) (4) ppm for oral drug products with a maximum daily dose  $\leq 10$  g per day or the maximum exposure to (b) (4) is less than the Permitted Daily Exposure (PDE) of (b) (4)  $\mu\text{g}$  per day per the ICH Q3D guidelines (Step 2b, July 2013) for elemental impurities. (b) (4)

**Residual Solvents:**

(b) (4). The following table (from page 15 of Section 3.2.S.3.2 of the submission) shows the list of the organic solvents and their ICH Q3C limits. Residual solvent levels were well below the ICH Q3C limits and are acceptable.

**Table 3.2.S.3.2-5 Organic Solvents Used in the Synthesis of Eluxadoline**

(b) (4)

## 2.6 Proposed Clinical Population and Dosing Regimen

Eluxadoline is indicated for the treatment of diarrhea and abdominal pain in men and women with diarrhea predominant irritable bowel syndrome (IBS-d). The recommended dosage is 100 mg twice daily (BID).

## 2.7 Regulatory Background

The following are the regulatory milestones.

- IND 79,214 was submitted on November 21, 2007
- Type C meeting [End of Phase 1 (EOP1)]: March 16, 2010
- Fast Track designation was granted on January 19, 2011
- Type B meeting [End of Phase 2 (EOP2)]: September 27, 2011
- Type B meeting (Pre-NDA meeting): April 22, 2014

## 3 Studies Submitted

### 3.1 Studies Reviewed

The following table shows the list of studies reviewed.

STUDY TITLE	REPORT NO.	PAGE
<b>PHARMACOLOGY</b>		18
<b>PHARMACOKINETICS/ADME</b>		43
<b>ABSORPTION</b>		43
Pharmacokinetics in Hepatic Portal and Jugular Vein Catheterized Male Sprague Dawley Rats after Oral Administration	DD07389	43
Pharmacokinetics in Female Dogs after Intravenous and Oral Administration	DD07393	43
Pharmacokinetics in the Mouse	DD07397	44
Pharmacokinetics Following a Single Intravenous or Oral Administration to Male and Female Rats	FK10138	45

Pharmacokinetics in Male and Female Rhesus Monkeys Following a Single Intravenous Bolus Administration	FK10141	46
Pharmacokinetics in Male and Female Cynomolgus Monkeys Following a Single Oral Gavage or Intravenous Bolus Administration in a Crossover Design	FK10142	47
Bioavailability and Pharmacokinetics in Male and Female Cynomolgus Monkeys Following a Single Oral, Subcutaneous, and Intravenous Bolus Administration	FK5721	48
Pharmacokinetics Following Administration of a Single Intravenous Dose Rhesus Monkeys	FK5863	50
Pharmacokinetics in Male Dogs Following a Single Intravenous Bolus Administration	FK5947	51
Pharmacokinetics Following a Single Intravenous, Subcutaneous or Oral Administration to Male and Female Mice	FK6179	52
Pharmacokinetics Following a Single Intravenous, Subcutaneous or Oral Administration to Male and Female Rats	FK6180	53
Evaluation of Transport Mechanisms in MDCKII-MDR1, MDCKII-MRP2 and MDCKII, Using Expressed Cells	FK6635	54
Uptake Transporter Inhibition in Solute Carrier Family	OPT-2012-063	55
Uptake Transporter Substrate in Solute Carrier Family	OPT-2012-064	56
<b>DISTRIBUTION</b>		57
Tissue Distribution in Mice	ADME04-199	57
Plasma Kinetics, Tissue Distribution, Metabolism and Excretion in Male Sprague-Dawley Rats After Single Oral and Subcutaneous Dose Administration	FK5756	58
Binding to the Proteins of Mouse, Rat, Rabbit, Dog, Monkey and Human Plasma	FK6315	59
Tissue Distribution as Studied by Whole-Body Autoradiography in the Pigmented Male Rat After a Single Oral Dose	FK6706	60
Tissue Distribution and Placental Transfer as Studied by Whole-Body Autoradiography in the Pregnant Rat after a Single Subcutaneous Dose	FK6707	63
Solubility, Metabolism, Permeability, Protein Binding and Red Blood Cell Binding	RWJ-P01	65
<b>METABOLISM</b>		66
In Vivo Metabolism in Rats and Monkeys	FK5858	66
In Vivo Metabolism in Dead Female Rhesus Monkey from Discovery Study of Abuse Liability	FK5865	68
The In Vitro Stability Studies of Acyl Glucuronide	FK5944	70
<b>EXCRETION</b>		71
The Biliary Excretion in Male SPF Sprague-Dawley Rats After a Single Oral Administration	FK6432	71
<b>TOXICOLOGY</b>		71
<b>Acute</b>		71
<b>Mouse</b>		71
Oral and Intraperitoneal	TOX7687	71
<b>Rat</b>		72
Oral and Intraperitoneal	TOX7688	72
<b>Subacute/Subchronic/Chronic</b>		73
<b>Mouse</b>		73
Oral, 28-Day	1808-001	74
Oral, 13-Week	1808-006	76
<b>Rat</b>		81
Intravenous Dose-Range Finding Toxicity Study in Rats	1808-013	81
2-Week Intravenous Toxicity Study In Rats With A 2-Week Recovery Period	1808-014	82
Investigative Oral Toxicity Evaluation in Rats	TOX6746	91
Five-Day Range Finding Oral and Subcutaneous Toxicity Study in Rats	TOX7310	88
4-Week Oral and Oral/Subcutaneous Toxicity Study of JNJ-27018966-AAA in Rats	TOX7686	95
13-Week Oral and Subcutaneous Toxicity Study in Rats	TOX8677	104
26-Week Oral Toxicity Study In Rats With A 4-Week Recovery Period	1808-007	112
2-Week Oral Dose Range-Finding Toxicity Study In Juvenile Rats	1808-017	122

4-Week Oral Toxicity Study with a 4-Week Recovery In Juvenile Rats	1808-018	121
<b>Monkey</b>		128
2-Week Intravenous Toxicity Study In Cynomolgus Monkeys With A 2-Week Recovery Period	1808-012	128
An Intravenous Dose Range-Finding Toxicity Study in Cynomolgus Monkeys	1808-015	129
An Investigative Oral Toxicity Evaluation in Monkeys	TOX6747	140
7-Day Dose Range-Finding Oral/Subcutaneous Toxicity and Toxicokinetic Study in Cynomolgus Monkeys	TOX7383	141
28-Day Oral/Subcutaneous Toxicity Study in Cynomolgus Monkeys	TOX8103	142
13-Week Oral and Subcutaneous Toxicity Study in Cynomolgus Monkeys	TOX8661	148
9-Month Oral Toxicity Study In Monkeys With A 4-Week Recovery	1808-004	154
<b>GENOTOXICITY</b>		166
Ames Test	AC34AZ503 (b) (4)	166
Chromosome Aberration Test	AC34AZ341	174
Mouse Lymphoma Assay	TOX7767	175
In Vitro Mutagenicity Testing in the Bacterial/Microsomal Activation Assay	TOX7777	171
Rat Bone Marrow Micronucleus Test	TOX7789	181
<b>CARCINOGENICITY</b>		187
104-Week Oral Carcinogenicity Study In Mice	1808-009	187
104-Week Oral Carcinogenicity Study In Rats	1808-008	187
<b>REPRODUCTIVE TOXICITY</b>		227
<b>Rat</b>		227
Fertility and Early Embryonic Development	1808-003	227
Pilot Oral/Subcutaneous Embryofetal Developmental	TOX8260	234
Oral/Subcutaneous Embryofetal Developmental	TOX8398	232
Oral Pre- and Post-natal Development	1808-019	247
<b>Rabbit</b>		240
Pilot Oral/Subcutaneous Embryofetal Development	TOX8261	241
Oral/Subcutaneous Embryofetal Development	TOX8376	240
<b>SPECIAL TOXICOLOGY</b>		258
Neutral Red Uptake Phototoxicity Assay	20046999	258
Murine Local Lymph Node Assay	TOX8122	259
In Vitro Bovine Corneal Opacity-Permeability Eye Irritation Test	TOX8226	261

### 3.2 Studies Not Reviewed

The following analytical method validation and abuse potential studies were not reviewed. Abuse potential studies will be reviewed by the Controlled Substance Staff (CSS).

- Validation of the LC-MS/MS Method in Rabbit Plasma (Study BA1139)
- Validation of the LC-MS/MS Method in Monkey Plasma (Study BA914)
- Validation of the LC-MS/MS Method in Rat Plasma (Study TOX7686a)
- Quantitation Method in CD-1 Mouse Plasma via HPLC with MS/MS Detection (Study XGV2)
- Addendum to Quantitation Method in CD-1 Mouse Plasma via HPLC with MS/MS Detection (Study XGV3)
- Abuse liability assessment in Rhesus monkeys (DD07334)
- Acute withdrawal assessment in mice (DD07370)
- Discriminative Stimulus and Positive Reinforcing Effects in Rhesus Monkeys (DD07374)

### 3.3 Previous Reviews Referenced

Pharmacology reviews of IND 79,214 were incorporated in appropriate sections of this review.

## 4 Pharmacology

### 4.1 Primary Pharmacology

#### In Vitro:

#### In Vitro Pharmacology of JNJ-27018966: Evaluation of Kappa Opioid Receptor Activity in the Isolated Guinea Pig Proximal Colon Preparation (DD07352)

In this study, the effects of JNJ-27018966 on inhibition of electrical stimulus-evoked contraction of the circular muscle in isolated guinea pig proximal colon were evaluated. This tissue preparation was used as a bioassay to determine the kappa opioid receptor (KOR) activity of the test article. The activities of JNJ-27018966 and KOR agonist, ICI 204,448 were evaluated in the presence of a selective  $\mu$ OR antagonist, naloxonazine (1  $\mu$ M). The maximum efficacy of JNJ-27018966 at 100  $\mu$ M was comparable to ICI 204,448 at 1  $\mu$ M. In this study, JNJ-27018966 was approximately 208-fold less potent than ICI 204,448, with  $EC_{50}$  values of 1.6  $\mu$ M and 7.7 nM, respectively.

#### In Vitro Pharmacology of JNJ-27018966: Evaluation of Mu Opioid Receptor Activity in the Isolated Guinea Pig Ileum Preparation (DD07354)

The primary objective of this study was to examine the activity of JNJ-27018966 at the  $\mu$ OR (MOR) in the electrically stimulated guinea pig ileum. A secondary objective was to determine the activity of a delta opioid receptor (DOR) antagonist in this preparation, alone and in combination with an MOR agonist. JNJ-27018966 inhibited electrical field stimulus (EFS)-evoked contractions of isolated guinea pig ileal preparations in a concentration-dependent and naloxone-reversible manner. The inhibitory effects of JNJ-27018966 were similar to those of the reference compound, selective MOR antagonist D-Ala<sup>2</sup>, N-MePhe<sup>4</sup>, Gly<sup>5</sup>-ol-enkephalin (DAMGO). The  $EC_{50}$  values of JNJ-27018966 and DAMGO were 1.06 nM and 1.89 nM, respectively. The selective delta opioid receptor antagonist, naltrindole, enhanced EFS-evoked ileal contractions and functionally opposed the MOR-mediated inhibition by DAMGO.

#### In Vitro Pharmacology: Delta Opioid Receptor Bioassay-Study of JNJ-27018966, (b) (4)

The objective of this study was to examine the agonist and antagonist activities of JNJ-27018966 at the  $\delta$  opioid receptor in the hamster vas deferens. Two other compounds (b) (4) were also tested in this assay, which are not relevant to this application and were not discussed here. The reference compound (D-

Pen<sup>2</sup>,D-Pen<sup>5</sup>-enkephalin (DPDPE) induced a concentration-dependent inhibition of the twitch contraction amplitude, which was reversed by the antagonist naltrindole. In the DPDPE treated tissues, JNJ-27018966 produced a concentration-dependent recovery of the twitch contraction amplitude. The results indicated that JNJ-27018966 caused an antagonistic activity at the  $\delta$  opioid receptors. The following table (from page 13 of the report) shows the results of the bioassay.

**Table 1 - 1**

**Effects of the compounds tested for agonist and antagonist activities  
at the  $\delta$  opioid receptors in the hamster vas deferens**

Test for agonist activity

Compounds	Control response to DPDPE (1.0E-07 M)	Responses to increasing concentrations of the compounds				+ naltrindole (3.0E-08 M)
		1.0E-09 M	1.0E-08 M	1.0E-07 M	1.0E-06 M	1.0E-06 M
<b>JNJ-27018966</b>	- 75	0	0	0	0	not tested
(b) (4)						
		1.0E-08 M	3.0E-08 M	1.0E-07 M	1.0E-07 M	1.0E-07 M
<b>DPDPE</b>	- 81	- 18	- 50	- 85	- 10	- 10

Test for antagonist activity

Compounds	Control response to DPDPE (1.0E-07 M)	Responses to DPDPE (1.0E-07 M) in the presence of increasing concentrations of the compounds				
		1.0E-09 M	1.0E-08 M	1.0E-07 M	1.0E-06 M	1.0E-05 M
<b>JNJ-27018966</b>	- 78	- 73	- 62	- 46	- 30	- 20
(b) (4)						
		3.0E-09 M	1.0E-08 M	3.0E-08 M	1.0E-08 M	3.0E-08 M
<b>naltrindole</b>	- 87	- 69	- 39	- 8	- 8	- 8

The results are expressed as a percent variation of the control twitch contraction amplitude (mean values; n=2). The signs - and + indicate a decrease and an increase, respectively.

In Vitro Pharmacology: Kappa Opioid Receptor-Study of JNJ-27018966,

(b) (4)

The objective of this study was to examine  $\kappa$  opioid receptor ( $\kappa$ OR) binding of JNJ-27018966, (b) (4) in the in vitro receptor binding assay using guinea pig cerebellum. Two other compounds ( (b) (4) ) were also tested in this assay, which are not relevant to this application and were not discussed here. The reference compound was U50488. The following tables (from page 13 and 18 of the report) show the results of the bioassay.

**Table 1 - 1****IC<sub>50</sub> Determination: Summary Results**

Assay (b) (4)	Client Compound I.D.	IC <sub>50</sub> (M)	K <sub>i</sub> (M)	n <sub>H</sub>
$\kappa$ (KOP) 870469-1	JNJ-27018966	1.6E-07	5.5E-08	0.6 (b) (4)

**Table 1 - 3****Reference Compound Data**

Assay Reference Compound	IC <sub>50</sub> (M)	K <sub>i</sub> (M)	n <sub>H</sub>
$\kappa$ (KOP) U 50488	3.4E-09	1.1E-09	0.6

Primary In Vitro Pharmacology of JNJ-27018966: Human Mu and Delta Opioid Receptor Binding (DD07371)

JNJ-27018966 was shown to exhibit high affinity binding to the rat brain delta and mu opioid receptors (DOR and MOR) with K<sub>i</sub> values of 4.3 nM and 0.59 nM, respectively. This study was conducted to determine the affinity of JNJ-27018966 for the human DOR and MOR in competition radioligand binding assays. In this study, the inhibition by JNJ-27018966 of the DOR antagonist [<sup>3</sup>H]naltrindole, and the MOR agonist [<sup>3</sup>H]DAMGO to the human DOR and MOR were examined. Human MOR binding utilized DAMGO and a commercially available recombinant human MOR expressed in Chinese hamster ovary (CHO) cells. Human delta DOR binding utilized naltrindole and endogenously expressed receptors in human SK-N-BE(2) cells. JNJ-27018966 competitively inhibited binding at MOR and DOR with respective K<sub>i</sub> values of 1.78 nM and 674 nM, respectively. These affinities of JNJ-27018966 were comparable to those of the reference compounds naloxone (K<sub>i</sub> = 1.3 nM) in the MOR binding assay and DPDPE (K<sub>i</sub>

= 621 nM) in the DOR binding assay. The following table (from page 12 of the report) shows the results of the binding assay.

Table 1: Summary of JNJ-27018966 Opioid Binding Studies

	Mu K <sub>i</sub> (nM)	Delta K <sub>i</sub> (nM)
27018966-AAC-22876175	1.7	367
27018966-AAC-22893045	2.5	509
		508
		1042
27018966-AAC-23673867	1.2	494
		1398
27018966-AAA-29744820	1.7	402
Average of all studies ± SEM	1.78 ± 0.31	674 ± 159
J&JPRD Laboratory Notebooks		(b) (4)
(b) (4)		

Table 2: A Direct Comparison of JNJ-27018966 and Reference Compounds in the Human Delta Opioid Receptor Binding Assay

Compound	K <sub>i</sub> (nM)
JNJ-27018966-AAC-23673867	494
JNJ-27018966-AAC-22876175	367
naltriben mesylate	1.2
Naloxone	214
DPDPE	621
J&JPRD Laboratory Notebook	(b) (4)

Table 3: JNJ-27018966 and Reference Compounds in the Human Mu Opioid Receptor Binding Assay

Compound	K <sub>i</sub> (nM)
JNJ-27018966-AAC-23673867	1.2
JNJ-27018966-AAC-22893045	2.5
Loperamide	10.1
Naloxone	1.3
Naltrindole	20
J&JPRD Laboratory Notebooks	(b) (4)

### Primary In Vitro Pharmacology of JNJ-27018966: Delta and Mu Opioid Receptor Binding and Function (DD07373)

This study evaluated the affinities of JNJ-27018966 for the delta opioid receptor (DOR) and mu opioid receptor (MOR) in competition radioligand binding assays using rat brain membranes. [<sup>3</sup>H]DPDPE and [<sup>3</sup>H]DAMGO were used as radioligands for DOR and MOR, respectively. In addition, JNJ-27018966 was evaluated in DOR and MOR functional assays measuring agonist stimulated [<sup>35</sup>S]GTPγS binding, respectively. Two selective DOR agonists (SNC-80 and DPDPE) and one selective MOR agonist (DAMGO) were included as reference compounds. The DOR functional assay was

performed using NG108-15 cell membranes, and the MOR functional study was performed using CHO cells transfected with MOR.

JNJ-27018966 exhibited high affinity binding to both the DOR and MOR, with  $K_i$  values of 4.3 nM and 0.59 nM, respectively. In the DOR functional study, JNJ-27018966 did not stimulate [ $^{35}$ S]GTP $\gamma$ S binding up to 10  $\mu$ M. In contrast, JNJ-27018966 (10  $\mu$ M) completely inhibited the [ $^{35}$ S]GTP $\gamma$ S binding stimulated by DOR agonist, SNC 80 (1  $\mu$ M). In the MOR functional study, JNJ-27018966 behaved as a MOR agonist, with an  $EC_{50}$  value of 0.96 nM and a relative intrinsic efficacy ( $\alpha$ ) of 0.86. Overall, the results suggest that JNJ-27018966 is an agonist at MOR and an antagonist at DOR. The following tables (from page 13 of the report) show the results of this study.

Table 1: Binding Affinities of JNJ-27018966 and SNC-80 to Delta and Mu Opioid Receptors

Compound	$K_i$ (nM)	
	DOR	MOR
JNJ-27018966	$4.4 \pm 3.3$ (n=4)	$1.05 \pm 0.29$ (n=3)
SNC-80	0.42	7620

J&JPRD Laboratory Notebooks: (b) (4)

Table 2: Potency and Relative Efficacy of JNJ-27018966, SNC-80 and DPDPE in a Delta Opioid Receptor Functional Assay

Compound	$EC_{50}$ (nM)	$\alpha$
JNJ-27018966	>10000	0
SNC-80	39	1
DPDPE	38	0.62

(b) (4)

Table 3: Potency and Relative Efficacy of JNJ-27018966 and DAMGO in a Mu Opioid Receptor Functional Assay

Compound	$EC_{50}$ (nM)	$\alpha$
JNJ-27018966	0.96	0.86
DAMGO	20.6	1

(b) (4)

## **In Vivo:**

### **Effects on Upper Gastrointestinal Motility in Mice (DD07335)**

The effects of JNJ-27018966 on motility of the upper GI tract were tested in CD-1 mice following oral (5, 10, 25, 50, 100, and 250 mg/kg) or IV (0.05, 0.10, 0.25, 0.50, 1.0 and 5.0 mg/kg) administration. JNJ-27018966 did not prevent gastric emptying at doses up to 250 mg/kg, PO and 5 mg/kg, IV. JNJ-27018966 inhibited upper GI tract transit in a dose related manner, with the onset of action of 45 minutes. The minimum effective oral dose was 25 mg/kg, and the minimum effective IV dose was 0.05 mg/kg. The onset of

action of JNJ-27018966 occurred one hour earlier than that of loperamide, and the peak effect occurred two hours earlier. The duration of action of JNJ-27018966 was shorter than that of loperamide, with upper GI tract transit returning to control levels within three hours after a 30 mg/kg, PO dose, while mice administered loperamide, 3 mg/kg, PO had significantly inhibited upper GI motility five hours later. However, the potency of JNJ-27018966 ( $ED_{50} \sim 40$  mg/kg, PO) was less than that of loperamide ( $ED_{50} \sim 2$  mg/kg, PO). The  $ED_{50}$  for IV administration of JNJ-27018966 was 1.0 mg/kg.

#### Evaluation of the Effects of JNJ-27018966 on Altered Gastrointestinal Motility in a Mouse Model of Post-Inflammatory IBS (DD07351)

The effects of JNJ-27018966 on the accelerated upper GI tract motility in a chronic mouse model of post-inflammatory (PI)-IBS were examined following oral administration. In this PI-IBS mouse model, an acute colitis was induced by intracolonic administration of allyl isothiocyanate (AITC, oil of mustard) 28 day prior to evaluation of upper GI transit. Upper GI transit was significantly accelerated in PI-IBS mice (116% of non-PI-IBS control mice). JNJ-27018966 (10 mg/kg, PO) showed a dose-related (10-100 mg/kg, PO) inhibitory effect on upper GI tract transit in PI-IBS mice ( $ED_{50} = 45.7$  mg/kg).

#### Effects on Upper Gastrointestinal Motility in Rats (DD07353)

The effects of JNJ-27018966 on motility of the upper GI tract were evaluated in rats following oral administration at 5, 10, 25, 50 and 100 mg/kg. JNJ-27018966 inhibited upper GI tract transit in a dose-related manner, with an  $ED_{50}$  value of 25 mg/kg. However, JNJ-27018966 did not prevent gastric emptying at any of the tested doses. The minimum effective oral dose in fed rats was 50 mg/kg, and the minimum effective dose in fasted rats was 25 mg/kg, indicating a food effect. In fed rats, the maximum inhibition (64% of controls) was observed at 50 mg/kg.

#### Evaluation of the Effects of JNJ-27018966 on Stress-Induced Altered Gastrointestinal Motility and Defecation in Mice (DD07356)

The objectives of these studies were to determine the effects on JNJ-27018966 on the altered motility in models of mild (novel environment) and moderate (simple restraint) stress in mice. In this study, the effects of orally administered JNJ-27018966 (5, 10, 25, 50 and 100 mg/kg) on GI motility and defecation were studied in CD-1 male mice that were subjected to mild or moderate stress.

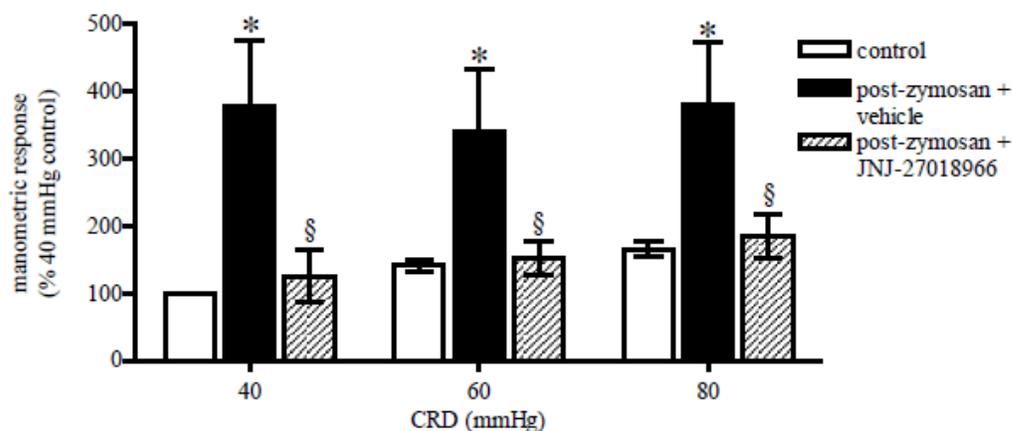
JNJ-27018966 (5-100 mg/kg) inhibited the transit of a marker from stomach to cecum in a dose related manner. Similar results were obtained with loperamide (0.1, 1, 2.5, 5 and 10 mg/kg), used as a reference compound. Neither drugs caused gastroparesis at any of the tested doses. The transit time of a marker from stomach to fecal excretion was significantly enhanced by restraint stress, and JNJ-27018966 reduced the transit time in a dose related manner (5-100 mg/kg), without preventing excretion of the marker during the six-hour test period. Loperamide (0.1-10 mg/kg) had similar effects but prevented

excretion of the marker at the highest dose. JNJ-27018966 also normalized the increased fecal output observed during the first hour in mild stress over the entire dose range tested (5-100 mg/kg), whereas loperamide (0.1-10 mg/kg) had no effect at the lowest dose and prevented fecal output at the highest dose. In case of three hours of restraint stress, JNJ-27018966 (5-250 mg/kg) normalized fecal output. Loperamide (0.1-10 mg/kg) reversed the effects of restraint stress on fecal output at 5 mg/kg but had no effect at the lower doses and significantly inhibited fecal output at 10 mg/kg. Overall, these data suggested that JNJ-27018966 has an inhibitory effect on upper GI transit. JNJ-27018966 appeared to normalize lower GI transit and defecation, which were increased under conditions of mild and moderate stress.

#### In Vivo Pharmacology of JNJ-27018966: Evaluation of Effects in a Rat Model of Visceral Hyperalgesia (DD07378)

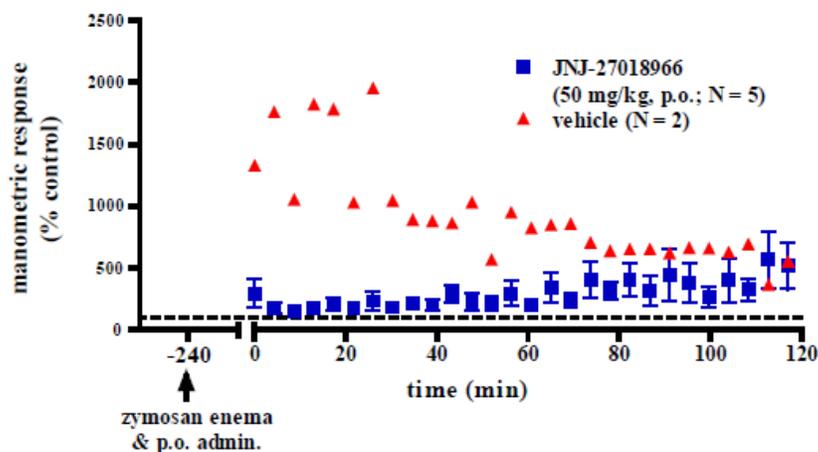
In this study, JNJ-27018966 was tested in a rat model of visceral hyperalgesia in which a barostat-controlled colorectal balloon was inflated to evoke a pseudoaffective behavior that consisted of anterior abdominal wall contraction. Following intracolonic administration of 2.5% zymosan, the responses to the same colorectal balloon stimuli were enhanced (i.e., hyperalgesic effect). Both intraperitoneal (IP) and oral doses of JNJ-27018966 reversed the hyperalgesic response as shown in the following figures (from page 18-19 of Section 2.6.2.2.3.3 of the submission). JNJ-27018966 reduced the response to control levels within 10 minutes of IP administration, which persisted throughout the 2-hour duration of the experiment. Following oral dose, JNJ-27018966 reduced the hyperalgesic response at 4 hours postdose, which persisted for approximately 30 minutes.

**Figure 2.6.2.2-5 Parenteral Administration of Eluxadoline Reversed Post Zymosan Hyperalgesia to Colorectal Distension in Rats**



\* = \* = P < 0.001 vs control; § = P > 0.05 vs control and < 0.01 vs vehicle-treated rats

**Figure 2.6.2.2-6 Oral Administration of Eluxadoline Reversed Post-Zymosan Hyperalgesia to Colorectal Distension in Rats**



## 4.2 Secondary Pharmacology

### In Vitro:

#### In Vitro Pharmacology Study of JNJ-27018966-AAC Using Abuse Receptor Panel (Report No.100006176)

In this in vitro receptor binding study, three concentrations of JNJ-27018966-AAC (b) (4) version of JNJ-27018966, (b) (4) were tested (1.0E-07 M, 1.0E-06 M, 1.0E-05 M). Results showing an inhibition (or stimulation) greater than 50% were considered to represent significant effects of the test compounds. Results showing an inhibition (or stimulation) between 25% and 50% were indicative of weak to moderate effects. Results showing an inhibition (or stimulation) lower than 25% were not considered significant and mostly attributable to variability of the signal around the control level.

Based on the above criteria, eluxadoline did not inhibit or stimulate receptors associated with abuse potential, including cannabinoid receptors (CB1 and CB2), N-methyl-D-aspartate (NMDA), nicotinic (N) neuronal  $\alpha_4\beta_2$ , N neuronal  $\alpha_7$ , or N muscle-type. The following table (from page 7 of the report) shows the results of this receptor binding assays.

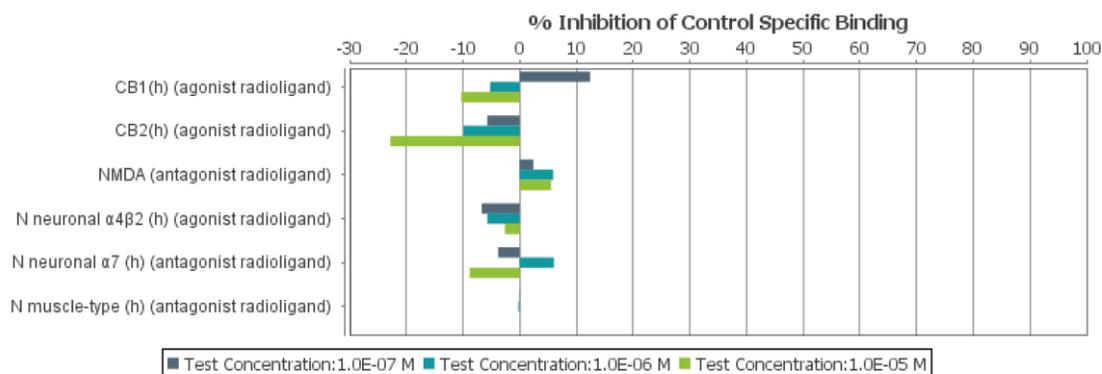


Figure 1. Histogram for JNJ-27018966-AAC

(b) (4) I.D.	Compound	Client Compound I.D.	Test Concentration	% Inhibition of Control Specific Binding			Flags	
				1 <sup>st</sup>	2 <sup>nd</sup>	Mean	1 <sup>st</sup>	2 <sup>nd</sup>
<b>CB<sub>1</sub> (h) (agonist radioligand)</b>								
100006176-1		JNJ-27018966-AAC	1.0E-07 M	7.8	17.0	12.4		
100006176-1		JNJ-27018966-AAC	1.0E-06 M	-19.1	8.8	-5.2		
100006176-1		JNJ-27018966-AAC	1.0E-05 M	-11.9	-8.7	-10.3		
<b>CB<sub>2</sub> (h) (agonist radioligand)</b>								
100006176-1		JNJ-27018966-AAC	1.0E-07 M	-7.3	-4.0	-5.7		
100006176-1		JNJ-27018966-AAC	1.0E-06 M	-10.7	-9.2	-9.9		
100006176-1		JNJ-27018966-AAC	1.0E-05 M	-14.9	-30.7	-22.8		
<b>NMDA (antagonist radioligand)</b>								
100006176-1		JNJ-27018966-AAC	1.0E-07 M	1.8	2.9	2.4		
100006176-1		JNJ-27018966-AAC	1.0E-06 M	3.6	8.1	5.9		
100006176-1		JNJ-27018966-AAC	1.0E-05 M	8.5	2.5	5.5		
<b>N neuronal <math>\alpha 4\beta 2</math> (h) (agonist radioligand)</b>								
100006176-1		JNJ-27018966-AAC	1.0E-07 M	-6.7	44.1	-6.7		∅
100006176-1		JNJ-27018966-AAC	1.0E-06 M	-2.5	-8.8	-5.7		
100006176-1		JNJ-27018966-AAC	1.0E-05 M	-1.9	-3.2	-2.6		
<b>N neuronal <math>\alpha 7</math> (h) (antagonist radioligand)</b>								
100006176-1		JNJ-27018966-AAC	1.0E-07 M	-3.8	-3.8	-3.8		
100006176-1		JNJ-27018966-AAC	1.0E-06 M	10.2	1.9	6.0		
100006176-1		JNJ-27018966-AAC	1.0E-05 M	-9.2	-8.4	-8.8		
<b>N muscle-type (h) (antagonist radioligand)</b>								
100006176-1		JNJ-27018966-AAC	1.0E-07 M	-4.5	4.3	-0.1		
100006176-1		JNJ-27018966-AAC	1.0E-06 M	0.1	-0.5	-0.2		
100006176-1		JNJ-27018966-AAC	1.0E-05 M	7.9	-8.0	0.0		

∅: That replicate was excluded from the calculation

### Muscarinic Acetylcholine Receptor Subtype 1 Activity of JNJ-27018966 (DD07355)

The purpose of this study was to examine the pharmacological activity of JNJ-27018966 on human muscarinic acetylcholine receptor subtype 1 (m1). JNJ-27018966 was tested to determine whether it behaved as an agonist or an antagonist of the receptor using human m1-transfected cells. In this study, CHOK1 cell lines that stably express the human m1 receptor were used. For the agonist screen, JNJ-27018966 was added to the plates at various concentrations, and fluorescence was observed. For the antagonist screen, JNJ-27018966 was added 1 minute prior to the addition of 1  $\mu$ M carbachol

(CCH), a muscarinic agonist. The results suggested that JNJ-27018966 has neither significant agonist nor antagonist activity at human m1 receptors.

### In Vitro Receptor Binding Study with JNJ-27018966 (DD07362)

This study was conducted to examine the effects of JNJ-27018966 in various in vitro receptor [histamine 2(H2), muscarinic 1 (M1), 5-hydroxytryptamine 6 (5-HT6), somatostatin (SST) and calcium-activated potassium channels (SK<sup>+</sup>Ca<sup>2+</sup>)] binding assays.

Because of low inhibition at the highest tested concentration (100 µM), Ki values could not be determined for the 5-HT6 (< 25%), somatostatin (< 25%), or H2 (< 50%) receptors, nor for the SK<sup>+</sup>Ca<sup>2+</sup> channel (< 25%) except for the human muscarinic M1 receptor (Ki = 3.1 µM). The following table (from page 9 of the report) shows the results of this assay.

**Table 1 - 1**

#### **IC<sub>50</sub> Determination: Summary Results**

Assay (b)(4) Compound I.D.	Client Compound I.D.	IC <sub>50</sub> (M)	K <sub>i</sub> (M)	n <sub>H</sub>	Flags
H <sub>2</sub>					
870419-1	JNJ-27018966				> 1.0E-04
M <sub>1</sub> (h)					
870419-1	JNJ-27018966	3.7E-06	3.1E-06	0.6	
5-HT <sub>6</sub> (h)					
870419-1	JNJ-27018966				N.C.
sst (non-selective)					
870419-1	JNJ-27018966				N.C.
SK <sup>+</sup> <sub>Ca</sub> channel					
870419-1	JNJ-27018966				N.C.

> Conc. Above the highest test concentration. IC50 value is above the highest tested concentration. Dose response curve has an inhibitory shape with less than 50 % inhibition at the highest tested concentration

N.C. Not calculable. IC50 value is not calculable because of less than 25% inhibition at the highest tested concentration.

### In Vitro Receptor Binding Study with JNJ-27018966 (DD07380)

In these receptors binding assays, a 50 receptor/ion channel binding screen was used. JNJ-27018966 (10 µM) inhibited the binding of control ligands (≥30% inhibition) to four non-opioid receptors: histamine H2 (32%), muscarinic M1 (62%), serotonin 5-HT6 (31%) and somatostatin (31%); and the calcium-dependent potassium channel, SK<sup>+</sup>Ca<sup>2+</sup> (48%).

### In Vitro Receptor Binding Study with JNJ-40453855 (DD07434)

One of the in vivo metabolites (identified as M2) of JNJ-27018966 is formed by hydrolysis of the 3,5-dimethyl-benzamide moiety to a carboxyl group. The M2 metabolite of eluxadoline (JNJ-40453855) was synthesized and subjected to the same 50 receptor/ion channel binding screen as the parent compound and was found to inhibit (>50% inhibition at 10  $\mu$ M) the binding of control ligands only to  $\mu$ OR (99%),  $\delta$ OR (96%), and  $\kappa$ OR (58%).

### Primary In Vitro Pharmacology of JNJ-40453855: Delta and Mu Opioid Receptor Binding and Function (DD07435)

The affinities of JNJ-40453855, the M2 metabolite of JNJ-279018966, for the delta opioid receptor (DOR) and mu opioid receptor (MOR) were evaluated in competition radioligand binding assays using [ $^3$ H]naltrindole and [ $^3$ H]DAMGO as radioligands for DOR and MOR, respectively. In addition, the test compound was evaluated in DOR and MOR functional assays of agonist-stimulated [ $^{35}$ S]GTP $\gamma$ S binding. The DOR binding and functional studies were performed using membranes from NG108-15 cells. The MOR binding assay was performed on rat brain membranes, while the MOR functional study was performed on membranes from Chinese hamster ovary (CHO) cells that had been transfected with the human MOR.

JNJ-40453855 showed affinity to both the DOR and MOR, with  $K_i$  values of 407 nM and 153 nM, respectively. In the DOR functional study, JNJ-40453855 did not stimulate [ $^{35}$ S]GTP $\gamma$ S binding at concentrations up to 10  $\mu$ M. In the MOR functional study, JNJ-40453855 was a weak agonist with an  $EC_{50}$  value of 2.7  $\mu$ M and a relative intrinsic efficacy ( $\alpha$ ) of 0.91 compared to DAMGO.

Overall, the results indicate that the metabolite M2 has lower affinity for  $\delta$ OR ( $K_i$ , 407 nM) and  $\mu$ OR ( $K_i$ , 153 nM) than the parent compound ( $K_i$ , 4.4 nM and 1.05 nM, respectively). It is to be mentioned here that, human studies did not identify any metabolite in the plasma and only one metabolite, a glucuronidated metabolite has been identified in human urine.

### In Vivo:

#### JNJ-27018966 von Frey Study (Furiex-001)

Opioids are known to cause spasm of the sphincter of oddi. A rat pancreatitis study was conducted to examine the potential for JNJ-27018966 to exacerbate the pain seen with that model. In this study, rats were placed into von Frey chambers for a 30-minute habituation period and then tested for either a positive or negative reaction to the von Frey filament (10 times; 3 different filament sizes tested). A positive reaction was recorded if the animal displayed any of the following: a strong pull of abdominal region away from the filament, licking/scratching of the abdominal region or vocalization. Pancreatitis was induced by dibutyltin dichloride (8 mg/kg, IV) and rats were allowed to

recover for three days. On the fourth day, response to the filaments was recorded at different times post oral doses of eluxadoline (32 mg/kg, PO, n = 8), loperamide (32 mg/kg, PO, n = 7), morphine (32 mg/kg, PO, n = 8) or vehicle (0.5% hydroxypropyl methylcellulose, PO, n = 8). Overall, the results of this study demonstrated that administration of JNJ-27018966 did not alter the percent response rate to von Frey stimulation compared to vehicle controls.

### 4.3 Safety Pharmacology

#### **Central Nervous System (CNS)**

##### **Effects on the Rat Central Nervous System as Assessed by the General Observational Test Battery (DD07345)**

The goal of this non-GLP study was to examine the CNS effects of JNJ-27018966 in Sprague-Dawley (SD) rats using modified Irwin test. In this study, JNJ-27018966 was evaluated for possible effects on behavior, physiology and other CNS effects following oral (gavage) administration at 30 and 300 mg/kg. Vehicle was 0.5% methylcellulose. Animals were observed 24 hours postdose and were maintained for 14 days for any delayed signs. Motor activity, musculature, reflexes, and excitation were evaluated at 1 and 4 hours postdose.

JNJ-27018966 did not produce any significant CNS effects in this study at the tested doses. There were no visible signs of CNS toxicity during a subsequent 2-week observation period.

##### **Antinociceptive Evaluation of JNJ-27018966 and (b) (4) in the Mouse Hot Plate Test (DD07369)**

This review is restricted to JNJ-27018966. This study was conducted to determine whether JNJ-27018966 (b) (4) when administered by routes other than oral route, exhibits behavioral effects that are consistent with a mechanism of action (MOA) involving mu opioid receptors (MOR). In addition, potency ratios for different routes of administration were determined to evaluate potential liabilities related to abuse (e.g., MOR-like agonist effects upon IV administration). For SC time course studies (10 and 60 mg/kg), mice were randomly assigned to groups (n = 10 mice/group); latencies and observable behavioral effects were evaluated 30, 60 and 180 min postdose. For PO time course studies (1000 mg/kg), mice were randomly assigned to groups (n = 5 mice/group); latencies were re-evaluated at 15, 30, 60, 120 and 180 min postdose. For IV time course studies (0.3, 1.0 and 3.0 mg/kg), mice were randomly assigned to groups (n = 5 mice/group); latencies were evaluated at 5, 15, 30, 60, 120 and 180 min postdose. For IV and PO potency studies, mice were assigned to groups (n = 5-8 mice/group); latencies were evaluated at 15 min after IV dose.

JNJ-27018966 showed behavioral effects, such as rigidity and Straub tail, consistent with MOR agonists following SC administration but not after PO administration. In addition, JNJ-27028966 produced antinociceptive effects under conditions (48°C hot plate) consistent with other MOR agonists (morphine and loperamide). However, there were differences between the IV and PO potencies. After IV administration, ED<sub>50</sub> value for increasing hot plate latency was 1.5 mg/kg. In comparison, no change in hot plate latency was observed at 1000 mg/kg PO dose. Overall, these results indicated that JNJ-27018966 has minimal MOR agonist-like activity when administered by the oral route, the intended route of administration.

#### Modified Irwin Test of JNJ-27018966-AAA in Male Rats (TOX7689)

The purpose of this study was to assess the effects of JNJ-27018966-AAA (b) (4) form of JNJ-27018966) on general and neurobehavioral activities when administered as a single oral (gavage) dose to male rats. In this study, twenty male rats (n = 5 rats/group) were administered a single oral dose of vehicle (0.5% hypromellose) or 500, 1000, or 2000 mg/kg of JNJ-27018966. Neurobehavioral activity was assessed using a modified Irwin's method once during predose and at 1, 2, 4, 6, and 24 hours postdose. All rats were examined again on Day 8. Other assessments included mortality, clinical observations, body weight, and body temperature.

There was no mortality or treatment related effects on body weight or body temperature at any dose. There were no treatment related clinical signs at 500 mg/kg. At 1000 and 2000 mg/kg, decreased activity was observed in all rats. Miosis was noted in 3 of 5 rats at 1000 mg/kg and all rats at 2000 mg/kg. At 24-hour postdose, these signs were no longer present. No significant treatment related neurobehavioral or clinical signs were noted on Day 8.

#### Effects of JNJ-27018966-AAC on Behavioral Parameters in Instrumented, Awake Dogs (CPF1246)

In this study, the potential behavioral effects of JNJ-27018966-AAC were examined. Awake and chronically instrumented Beagle dogs (n = 2) were treated at 0.04, 0.08, 0.16 and 0.32 mg/kg IV (infusion) doses over a period of 15 min at 60-min time intervals. In addition, lower doses of JNJ-27018966-AAC (0.001, 0.003, 0.01 and 0.03 mg/kg) were administered to two other dogs according to the same protocol.

Behavioral changes observed at all lower doses (0.001 to 0.03 mg/kg) included licking, retching and vomiting. These signs were also observed at higher doses (0.04 to 0.32 mg/kg). However, at 0.32 mg/kg, sedation and heavy breathing was seen in both dogs and in one dog ptosis was observed and in other dog salivation, vomiting (foam) and exteriorization of the tongue were noted.

#### Cardiovascular System

##### In Vitro:

Effects of JNJ-27018966-AAC on the Membrane K<sup>+</sup> current (IKr) in HERG-Transfected HEK293 cells Compared to Astemizole (Delta Opioid antagonist/Mu agonist) (CPF1226)

This study was conducted using the whole-cell voltage clamp technique to determine the possible effects of JNJ-27018966-AAC (b) (4) on the IKr-like membrane potassium (K<sup>+</sup>) current in a human embryonic kidney cell line (HEK293) transfected with the human *ether-à-gogo*-related gene (HERG). Astemizole was used as a positive control. JNJ-27018966-AAC was tested at three concentrations (10<sup>-7</sup> M, 3 x 10<sup>-7</sup> M and 3 x 10<sup>-6</sup> M) and the reference compound, astemizole, was also tested at three concentrations (3 x 10<sup>-9</sup> M, 10<sup>-8</sup> M and 3 x 10<sup>-8</sup> M).

The results showed that, compared to the solvent, JNJ-27018966-AAC had no significant effect on the IKr current at any of the tested concentrations (10<sup>-7</sup> M: 6.0% decrease in current, versus 3.8% with solvent; 3 x 10<sup>-7</sup> M: 12.8%, versus 8.8% with solvent; 3 x 10<sup>-6</sup> M: 17.5%, versus 10.0% with solvent). The reference compound astemizole inhibited the HERG current at nanomolar concentrations. The following table (from page 18 of the report) shows the results of the hERG assay.

Table 1: Inhibition of HERG-mediated K<sup>+</sup> current in HEK293 cells by increasing concentrations of cumulatively-applied JNJ-27018966-AAC-23189446 compared to astemizole (JNJ-120432-AAA-9559529)

	Conc. (M)	Mean inhibition (%) ± SEM	
		Test drug	Solvent control
<b>JNJ-27018966-AAC</b>	1 x 10 <sup>-7</sup>	6.0 ± 3.7 (n = 4)	3.8 ± 0.8 (n = 4)
	3 x 10 <sup>-7</sup>	12.8 ± 4.9 (n = 4)	8.8 ± 2.8 (n = 4)
	3 x 10 <sup>-6*</sup>	17.5 ± 5.6 (n = 4)	10.0 ± 1.5 (n = 4)
<b>Astemizole</b>	3 x 10 <sup>-9</sup>	50.8 ± 7.3 (n = 4)	
	1 x 10 <sup>-8</sup>	83.5 ± 6.2 (n = 4)	
	3 x 10 <sup>-8</sup>	96.5 ± 1.5 (n = 4)	

\* NB: Analysis revealed a recovery above the 66% cut off value.

n = the number of cells tested

Electrophysiological Evaluation of JNJ-27018966-AAC in Isolated Rabbit Purkinje Fibers in Conditions of a Normal Standard Rhythm, Bradycardia and Tachycardia (CPF1238)

In this study, potential electrophysiological effects of JNJ-27018966-AAC was examined in isolated rabbit Purkinje fibers, in conditions of a normal rhythm (stimulation rate of 1 Hz), bradycardia (0.2 Hz) and tachycardia (2 Hz). JNJ-27018966-AAC was tested at four increasing concentrations of 1 x 10<sup>-8</sup> M, 1 x 10<sup>-7</sup> M, 1 x 10<sup>-6</sup> M and 1 x 10<sup>-5</sup> M (15

min for each concentration, except for the last concentration for 25 min; n = 7) or solvent (n = 7) was continuously infused into the superfusion medium. The Purkinje fibers were stimulated at a normal rhythm (1 Hz) for 60 min, then the electrical stimulation rate was reduced to 0.2 Hz and increased to 2 Hz for 5 min each (total contact time of compound or solvent = 70 min).

Relative to solvent control, JNJ-27018966-AAC had no significant or physiologically relevant effect on the amplitude of the action potential (AAP), duration of the action potential at 40%, 50% and 90% repolarization (APD<sub>40</sub>, APD<sub>50</sub> and APD<sub>90</sub>), triangulation of the action potential, V<sub>max</sub> and the resting membrane potential (RMP) in conditions of a normal standard rhythm (1 Hz), and at 1 x 10<sup>-5</sup> M in conditions of bradycardia (0.2 Hz) and tachycardia (2 Hz). In addition, JNJ-27018966-AAC did not elicit early after depolarizations (EADs) at any of the tested concentrations.

#### Effects of JNJ-27018966 in the Isolated, Spontaneously Beating Right Atrium of the Guinea-Pig (DD07347)

The purpose of this in vitro study was to evaluate the effects of JNJ-27018966 on contractile function of the isolated guinea pig right atrium. The drug was tested at 1, 3 and 10 μM concentrations.

JNJ-27018966 at 1, 3 and 10 μM caused concentration dependent decreases in contractile force, time to peak (TTP) and rate of rise (ROR), and decreases in contractile rate that were similar to the vehicle group (Table 1). There were no significant effects on effective refractory frequency (ERF). The following table (from page 9 of the report) shows the results of this study.

	concentration	force	rate	TTP	ROR	ERF hz
JNJ-27018966	baseline <sup>a</sup>	1039 ± 49	125 ± 2	65 ± 1	43 ± 2	
1 μM	% change	-15 ± 4	-6 ± 1	-3 ± 1	-3 ± 3	
3 μM	% change	-27 ± 3	-11 ± 1	-3 ± 1	-7 ± 2	
10 μM	% change	-37 ± 3	-14 ± 1	-9 ± 2	-9 ± 2	11.0 ± 0.6
vehicle <sup>b</sup>	baseline <sup>a</sup>	827 ± 153	143 ± 5	60 ± 3	39 ± 1	
0.01 %	% change	-14 ± 1	-3 ± 1	-2 ± 1	-4 ± 1	
0.03 %	% change	-26 ± 1	-6 ± 2	-5 ± 0	-6 ± 1	
0.1 %	% change	-32 ± 1	-8 ± 2	-7 ± 1	-8 ± 1	10.0 ± 0.0

<sup>a</sup> Units for baseline values: force (mg); rate (beats/min); TTP (ms); ROR (mg/ms)

<sup>b</sup> vehicle concentrations indicated as %DMSO in 50 mL tissue bath

TTP, time to peak; ROR, rate of rise; ERF, effective refractory frequency

Values are mean ± S.E for n=3 in the vehicle and JNJ-27018966 groups

### Effects of JNJ-27018966-AAC on the Isolated, Spontaneously Beating Right Atrium of the Guinea-Pig (EDMS-PSDB-6412461)

In this study, the effects of JNJ-27018966-AAC were examined using the isolated, spontaneously beating right atrium of the guinea pig. The drug was tested at concentrations of  $10^{-6}$  M,  $3 \times 10^{-6}$  M and  $10^{-5}$  M.

JNJ-27018966-AAC did not affect the rate or force of contraction of the Guinea pig heart relative to control. However, JNJ-27018966-AAC reduced the frequency of electrical stimulation at  $10^{-5}$  M concentration. JNJ-27018966-AAC did not affect the rate and force of contraction. However, at the relatively high concentration ( $10^{-5}$  M), the drug reduced the frequency of electrical stimulation. The results are shown in the table below (from page 14 of the report).

Table 1: Isolated right atrium of the guinea-pig; screening results of JNJ-27018966-AAC (data presented as median values of n = 3 preparations)

Concentration (M)	Rate of contraction (median % of baseline value)			Force of contraction (median % of baseline value)			Effective refractory frequency (Hz)
	$10^{-6}$	$3 \times 10^{-6}$	$10^{-5}$	$10^{-6}$	$3 \times 10^{-6}$	$10^{-5}$	$10^{-5}$
JNJ-27018966-AAC	99	97	97 #	93	87	82 #	10 #
95% Prediction interval *	95 - 103	93 - 106	94 - 110	80 - 95	68 - 91	61 - 90	11 - 15

\* Ranges in this row represent Fligner-Wolfe 95% prediction intervals for the median of three future experiments; Data based on n = 505 time- and volume-matched solvent control experiments.

# Organ bath analysis revealed an actual exposure of 87% of the  $10^{-5}$  M target concentration (expressed relative to stock solution).

### In Vivo:

#### Effects of JNJ-27018966-AAC on Cardio-Hemodynamic, Cardioelectrophysiological and Pulmonary Parameters in Anesthetized Guinea-Pigs (CPF1211)

In the present study, the potential cardio-hemodynamic, cardio-electrophysiological and pulmonary effects of JNJ-27018966-AAC were examined in anesthetized guinea-pigs. In this study, the electrocardiogram (ECG), heart rate, mean arterial blood pressure and pulmonary inflation pressure were measured in two groups of experiments. In the first group (n = 7), increasing doses of JNJ-27018966-AAC (0.16, 0.32, 0.64, 1.25, 2.5 and 5 mg/kg) were administered intravenously (IV) over a period of 5 min at 15-min intervals. In the second group (n = 7), corresponding volumes of solvent were administered according to the same protocol.

JNJ-27018966 (0.16 to 5 mg/kg) had no significant, relevant or consistent effect on the duration of the PQ and QRS intervals, pulmonary inflation pressure, and induced no changes in ECG morphology. However, JNJ-27018966-AAC at doses of 0.16 to 1.25 mg/kg IV increased mean arterial blood pressure. The drug increased heart rate and

decreased the duration of the QT and QTc (Bazett) intervals at a dose of 0.16, and up to 5 mg/kg. Dofetilide (0.02 mg/kg IV over 1 min), positive control, decreased heart rate and prolonged the QT and QTc (Bazett) intervals. The following tables (from pages 28-29 of the report) show the results of this study.

Table 2: Effects of JNJ-27018966-AAC, at the end of each infusion, on heart rate (HR), mean arterial blood pressure (BPm), ECG parameters and on pulmonary inflation pressure in anesthetized guinea-pigs (data are expressed as actual values).

Parameters	JNJ-27018966-AAC i.v.; n = 7						
	0 min	5 min	20 min	35 min	50 min	65 min	80 min
	Baseline	0.16 mg/kg	0.32 mg/kg	0.64 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg
HR (b/min)	211 197/250	240 203/271	236 220/262	233 224/254	232 226/252	241 222/263	239 211/265
BPm (kPa)	3.8 3.5/4.5	6.4 5.1/7.5	5.5 4.7/5.9	5.1 4.7/5.4	5.2 4.7/5.5	5.3 4.0/5.5	4.5 3.9/5.4
PQ (ms)	59 53/75	57 52/71	54 52/68	55 53/71	57 51/69	57 52/68	58 51/73
QRS (ms)	44 44/48	45 44/47	45 44/48	45 42/46	46 41/49	45 41/46	46 42/47
QT (ms)	212 167/224	190 155/222	196 167/203	191 172/207	190 173/202	190 167/206	187 167/210
QTcB (ms)	398 341/406	379 330/407	385 347/393	375 353/402	370 357/398	375 350/395	370 350/400
Pip (kPa)	1.9 1.3/2.5	1.9 1.3/2.5	2.0 1.3/2.8	2.1 1.3/3.1	2.0 1.3/2.7	2.1 1.3/3.6	2.6 1.4/6.9

Values are median (min/max).

Table 3: Effects of solvent, at the end of each infusion, expressed as percentage changes relative to the pre-administration values, on heart rate (HR), mean arterial blood pressure (BPm), ECG parameters and on pulmonary inflation pressure (Pip) in anesthetized guinea-pigs.

Parameters	Solvent i.v.; n = 7						
	0 min	5 min	20 min	35 min	50 min	65 min	80 min
	0.16 mg/kg eq	0.32 mg/kg eq	0.64 mg/kg eq	1.25 mg/kg eq	2.5 mg/kg eq	5 mg/kg eq	
HR		0 -3/18	-1 -6/8	-3 -11/4	-4 -13/6	-5 -13/5	-4 -12/4
BPm		3 -5/51	3 -3/29	3 -7/31	5 -14/32	13 -21/50	18 -5/68
PQ		2 -8/6	0 -7/5	0 -8/7	0 -7/11	-2 -7/10	-2 -7/10
QRS		2 -9/7	4 -4/5	2 0/7	4 -9/17	4 -2/13	5 -2/24
QT		1 -14/8	7 -7/11	6 -2/15	8 -3/20	6 -4/16	6 -2/14
QTcB		1 -6/4	4 -3/6	4 -1/8	5 1/10	6 -2/7	4 0/9
Pip		0 0/27	0 0/13	6 -5/33	16 0/43	16 5/67	24 0/62

Values are median (min/max).

Table 4: Effects of JNJ-27018966-AAC, at the end of each infusion, expressed as percentage changes relative to the pre-administration values, on heart rate (HR), mean arterial blood pressure (BPm), ECG parameters and on pulmonary inflation pressure (Pip) in anesthetized guinea-pigs.

Parameters	JNJ-27018966-AAC i.v.; n = 7						
	0 min	5 min	20 min	35 min	50 min	65 min	80 min
		0.16 mg/kg	0.32 mg/kg	0.64 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg
HR		8 -2/28	12 0/24	8 -5/20	9 -5/19	11 -1/24	8 0/25
BPm		50 43/97	27 24/55	27 16/51	31 18/57	25 8/51	22 -13/37
PQ		-5 -12/0	-8 -10/5	-5 -10/4	-3 -14/5	-5 -12/0	-3 -14/4
QRS		0 -2/5	0 -6/9	0 -6/5	2 -7/11	-2 -9/2	0 -5/5
QT		-8 -23/8	-10 -17/5	-10 -14/11	-7 -15/12	-11 -17/7	-9 -17/4
QTcB		-3 -13/7	-4 -8/4	-5 -8/8	-4 -9/8	-5 -8/5	-5 -10/4
Pip		0 0/5	5 -6/13	11 -6/27	5 -17/32	11 0/44	40 4/188

Values are median (min/max).

### Cardio-Hemodynamic, Cardio-Electrophysiological and Pulmonary/Respiratory Effects of JNJ-27018966-AAC in Mechanically Ventilated Anesthetized Dogs (CPF1330)

This study was conducted to examine the potential effects of JNJ-27018966-AAC on cardio-hemodynamic, cardio-electrophysiological, and pulmonary parameters in mechanically ventilated, anesthetized dogs. Dogs (n = 4; 1 female, 3 male) were treated at increasing IV doses of 0.003 to 1 mg/kg (total dose = 1.443 mg/kg,  $C_{max}$  = 4200 ng/mL) by infusion over 5 min at 30-min intervals and control animals received the vehicle [5% hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD), n = 2/sex]. Dofetilide (0.05 mg/kg IV), a class III antiarrhythmic agent, was used as a reference compound.

JNJ-27018966-AAC did not show significant or relevant effects on the cardio-hemodynamic, cardio-electrophysiological and respiratory parameters, except for a slight tendency to decrease aortic blood pressure, compared to the respective predose baselines, and heart rate at 0.3 mg/kg and 1 mg/kg, respectively. JNJ-27018966-AAC did not induce overt ventricular and supraventricular arrhythmias on the ECG, or early after depolarizations (EADs) and delayed after depolarizations (DADs) on the right ventricular monophasic action potential (MAP) signal. Dofetilide at 0.05 mg/kg IV induced the expected changes in the repolarization parameters.

### Effects of JNJ-27018966 on Arterial Pressure, Heart Rate and Electrocardiogram in Anesthetized Guinea Pigs (DD07346)

In this study, JNJ-27018966 was evaluated in an anesthetized guinea pig model for potential hemodynamic and electrocardiographic effects. Each dose of JNJ-27018966 was infused intravenously over 5 minutes. Cumulative doses of JNJ-27018966 (0.1, 0.3,

1, 3 and 10 mg/kg) or equivalent volumes of vehicle (30% ethanol: propylene glycol in 5% dextrose in water) were administered incrementally as five-minute IV infusions at 0, 20, 40, 60 and 80 minutes to anesthetized guinea pigs (n = 3 for test drug and n = 14 for the vehicle).

JNJ-27018966 had no effect on the heart rate up to 10 mg/kg. There were transient increases in mean arterial pressure particularly at the lower doses but similar effects were also noted in the vehicle group. Overall, JNJ-27018966 had no significant effect on QT interval, QTc, PR interval or QRS duration up to 10 mg/kg.

#### Evaluation of Cardiovascular Function by Telemetry Following a Single Subcutaneous Injection to Conscious Monkeys (TOX8159)

In this study, five Cynomolgus monkeys (two males and three females) implanted with telemetric devices were allocated to a single group using a cross-over design. Animals were treated with JNJ-27018966-AAA by SC injection at 5, 15 and 30 mg/kg doses for 2 consecutive days. Cardiovascular parameters were measured on the first day of each dose-level at the following time-points: before and 15, 30, 60, 90, 120, 180, 240, 300, 360, 480, 600, 1200 minutes postdose. At these time-points, heart rate (HR), diastolic, systolic and mean arterial pressures (DAP, SAP and MAP), and PQ, QRS and QT intervals were measured including corrected QTs e.g., Bazett (QTb) and Fridericia (QTf). On the second day of each dose-level, blood samples were collected for the determination of plasma levels of the drug before dosing and 30 and 120 minutes after dosing.

All arterial pressure parameters (systolic, diastolic and mean) were significantly decreased from 30 to 300 minutes postdose at all dose-levels. There were no treatment related effects on heart rate, PQ and QRS durations. However, QT interval including QTb and QTf was prolonged (QTb: 106-112% of control) at all doses. The following tables (from pages 42 and 43 of the report) show the mean values of ECG parameters.

Table 2: Mean values of cardiac electrophysiology parameters

Time min	Vehicle				
	PQ ms	QRS ms	QT ms	QTb ms	QTf ms
0	<b>89 ± 8</b>	<b>41 ± 5</b>	<b>193 ± 49</b>	<b>311 ± 37</b>	<b>264 ± 41</b>
15	83 ± 7	40 ± 7	176 ± 37	322 ± 36	263 ± 37
30	88 ± 7	39 ± 5	180 ± 36	306 ± 35	256 ± 34
60	89 ± 8	40 ± 6	187 ± 38	304 ± 30	258 ± 32
90	86 ± 10	41 ± 4	201 ± 55	315 ± 32	270 ± 41
120	93 ± 10	41 ± 6	193 ± 35	316 ± 17	267 ± 23
180	95 ± 6	42 ± 4	208 ± 29	332 ± 36	284 ± 33
240	90 ± 8	43 ± 7	198 ± 31	319 ± 24	272 ± 28
300	90 ± 10	42 ± 6	193 ± 24	313 ± 23	266 ± 22
360	89 ± 8	43 ± 6	200 ± 27	314 ± 9	269 ± 16
480	94 ± 7	42 ± 7	209 ± 36	330 ± 25	283 ± 30
600	90 ± 4	42 ± 5	235 ± 13	343 ± 23	302 ± 17
1200	95 ± 14	42 ± 6	249 ± 22	351 ± 12	313 ± 11

Time min	JNJ-27018966-AAA 5 mg/kg				
	PQ ms	QRS ms	QT ms	QTb ms	QTf ms
0	<b>85 ± 6</b>	<b>42 ± 5</b>	<b>190 ± 38</b>	<b>328 ± 48</b>	<b>273 ± 44</b>
15	79 ± 9	40 ± 5	181 ± 33	330 ± 33	270 ± 33
30	86 ± 9	41 ± 6	201 ± 36	334 ± 44	282 ± 41
60	88 ± 4	41 ± 5	207 ± 33	324 ± 34	279 ± 34
90	85 ± 3	44 ± 7	211 ± 28	344 ± 23	292 ± 24
120	87 ± 4	41 ± 5	212 ± 32	338 ± 34	289 ± 32
180	88 ± 5	42 ± 4	209 ± 28	330 ± 31	283 ± 30
240	88 ± 7	44 ± 6	205 ± 33	337 ± 39	285 ± 37
300	90 ± 10	43 ± 6	204 ± 20	337 ± 27	285 ± 23
360	90 ± 8	41 ± 6	203 ± 26	322 ± 43	276 ± 36
480	91 ± 5	42 ± 6	228 ± 30	330 ± 30	292 ± 30
600	93 ± 5	42 ± 6	259 ± 11	342 ± 18	312 ± 14
1200	94 ± 10	44 ± 7	265 ± 36	356 ± 16	322 ± 23

Table 2: Mean values of cardiac electrophysiology parameters (continued)

Time min	JNJ-27018966-AAA 15 mg/kg				
	PQ ms	QRS ms	QT ms	QTb ms	QTf ms
0	<b>86 ± 8</b>	<b>44 ± 7</b>	<b>194 ± 11</b>	<b>328 ± 29</b>	<b>275 ± 21</b>
15	83 ± 5	39 ± 3	187 ± 22	327 ± 37	271 ± 29
30	85 ± 9	41 ± 7	199 ± 21	333 ± 40	280 ± 30
60	87 ± 6	41 ± 6	211 ± 31	341 ± 36	291 ± 32
90	90 ± 3	43 ± 6	226 ± 17	343 ± 17	298 ± 16
120	86 ± 5	42 ± 7	211 ± 29	341 ± 33	290 ± 31
180	92 ± 7	42 ± 5	222 ± 26	345 ± 27	298 ± 26
240	89 ± 7	42 ± 5	217 ± 9	336 ± 20	290 ± 15
300	91 ± 10	44 ± 8	202 ± 8	316 ± 28	272 ± 20
360	89 ± 13	45 ± 3	206 ± 19	329 ± 22	281 ± 20
480	89 ± 10	43 ± 6	214 ± 6	332 ± 13	287 ± 10
600	92 ± 5	44 ± 8	257 ± 12	343 ± 24	311 ± 19
1200	93 ± 13	44 ± 7	234 ± 57	327 ± 37	292 ± 45

\*:p&lt;0.05 vs. Vehicle

Time min	JNJ-27018966-AAA 30 mg/kg				
	PQ ms	QRS ms	QT ms	QTb ms	QTf ms
0	<b>86 ± 7</b>	<b>41 ± 6</b>	<b>189 ± 16</b>	<b>324 ± 41</b>	<b>270 ± 30</b>
15	86 ± 7	42 ± 4	185 ± 25	321 ± 44	267 ± 36
30	88 ± 7	41 ± 5	202 ± 24	328 ± 39	279 ± 33
60	87 ± 3	43 ± 4	218 ± 25	341 ± 37	293 ± 32
90	86 ± 5	42 ± 6	209 ± 38	340 ± 47	289 ± 44
120	84 ± 6	42 ± 6	207 ± 13	331 ± 42	283 ± 28
180	86 ± 5	41 ± 5	208 ± 32	331 ± 37	283 ± 34
240	86 ± 5	41 ± 3	210 ± 27	335 ± 34	287 ± 30
300	93 ± 5	41 ± 6	212 ± 22	331 ± 31	286 ± 27
360	93 ± 8	42 ± 8	210 ± 23	317 ± 33	276 ± 29
480	93 ± 11	43 ± 5	216 ± 48	322 ± 39	281 ± 43
600	94 ± 10	46 ± 7	256 ± 44	339 ± 29	308 ± 34
1200	99 ± 7	44 ± 4	247 ± 29	345 ± 38	308 ± 33

Systemic exposure to JNJ-27018966 increased in a dose-related manner. There were no apparent gender differences in exposure. The following table (from page 8 of the report) shows plasma concentrations in male and female monkeys.

Mean JNJ-27018966 plasma concentrations (ng/mL) in male and female monkeys (N=2) 0, 0.5, and 2 hours after receiving the second of two daily subcutaneous doses of JNJ-27018966-AAA (0, 5, 15, or 30 mg/kg) (TOX8159)

Gender	Dose (mg/kg)	Time (h)		
		0	0.5	2
Male	0	0.00	0.00	0.00
	5	0.00	2210	914
	15	0.89	5780	3060
	30	1.51	12300	5080
Female	0	0.00	0.00	0.00
	5	0.00	1640	1010
	15	0.00	4910	2830
	30	1.84	10500	5080

### **Respiratory System**

#### **Pulmonary Evaluation of Intravenously Administered JNJ-27018966-AAC in Male and Female Sprague-Dawley Rats (1808-016)**

This study was conducted to evaluate the potential effects of a single bolus IV administration of JNJ-27018966-AAC on pulmonary function in freely moving rats. Three treatment groups of eight male and eight female Sprague Dawley (SD) rats were treated with the test article at 5, 10, and 20 mg/kg. An additional group of eight male and eight female rats served as control animals and were administered the vehicle (40% hydroxypropyl-beta-cyclodextrin (HP $\beta$ CD) in water. The vehicle or test article was administered to all groups via IV (bolus) injection once, at a dose volume of 1 mL/kg. Pulmonary function (respiratory rate, tidal volume, minute volume, inspiration time, inspiratory pause, expiration time, and expiratory pause) was monitored for at least 1 hour prior to dosing and for at least 3.5 hours postdose.

Rats showed changes in breathing following treatment with JNJ-27018966-AAC consistent with its pharmacological activity as a mu-agonist and delta opioid antagonist. Changes in respiratory rates and group mean minute volumes appeared to be caused by significant changes in the patterns of breathing in these rats. Rats took longer to inhale and exhale each breath and took longer pauses between shifting from inhalation to exhalation as is typical of mu opioids. Treatment related clinical signs in both sexes included labored or “difficulty in breathing”, which was observed during the periods of maximum changes in the following breath parameters: mean respiratory rate, mean

inspiration times, mean inspiratory pause, mean expiration time, and mean expiratory pauses. Female rats were more sensitive to these changes when compared to male rats. Three out of eight female rats and one out of eight male rats needed to be rescued by naloxone blockade shortly following the high dose of 20 mg/kg. The following tables show the mean values for the respiratory parameters in males and females.

Male:

Dose (mg/kg)	Respiration rate (breaths/min)	Tidal Volume (mL)	Minute volume (mL/min)	Inspiration Time (Sec)	Expiration Time (Sec)	End of Inspiratory pause (msec)	End of Expiratory pause (msec)
0	119.38	1.595	179.95	0.22	0.33	8.45	20.71
5	121.49	1.574	179.85	0.23	0.32	8.34	21.40
10	119.78	1.697	187.32	0.23	0.34	8.51	19.61
20	107.00	1.782	178.39	0.25	0.37	8.46	21.92

Female:

Dose (mg/kg)	Respiration rate (breaths/min)	Tidal Volume (mL)	Minute volume (mL/min)	Inspiration Time (Sec)	Expiration Time (Sec)	End of Inspiratory pause (msec)	End of Expiratory pause (msec)
0	109.98	1.565	179.95	0.24	0.40	8.95	28.24
5	100.87	1.668	179.85	0.25	0.44	9.08	32.96
10	110.40	1.692	187.32	0.24	0.42	9.10	28.04
20	121.87	1.459	178.39	0.22	0.36	9.72	19.45

#### Evaluation of Respiratory Function Following a Single Subcutaneous Injection to Conscious Rats (TOX8158)

The objective of this study was to evaluate the effects of JNJ-27018966-AAA on respiratory function after a single subcutaneous (SC) administration to conscious rats. In this study, 40 female (32 main study and 8 satellites for plasma levels) SD rats were assigned to four groups and received the vehicle or test article, JNJ-27018966-AAA, by a single SC administration at 5, 15 and 30 mg/kg. The following respiratory parameters were examined: respiration rate, peak flows, enhanced pause, and minute and tidal volumes at predose and at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 minutes postdose. Blood samples were collected from two satellite animals at each dose at 30 min and 2 hours postdose for the determination of plasma levels of the test article.

There were no mortality and clinical signs. Except for tidal volumes, all the measured respiratory parameters increased at all the dose-levels. Although the magnitude of the effects of the test article did not increase with the dose-level, the duration of the effects was dose-related. The animals returned to baseline levels 4 hours postdose at 5 mg/kg and 6 hours postdose at 15 mg/kg. These were still above baseline values at the end of the evaluation period (6 hours postdose) at 30 mg/kg. The following table (from page 42 of the report) shows the mean values of respiratory function parameters.

Table: 1. Mean values of respiratory function parameters

Vehicle						
Time	PIF	PEF	TV	MV	f	Penh
min	mL/s	mL/s	mL	mL	bpm	
0	5.9 ± 0.8	4.9 ± 0.6	1.1 ± 0.0	83 ± 5	78 ± 3	0.6 ± 0.1
15	7.5 ± 2.1	5.4 ± 1.0	1.2 ± 0.1	108 ± 26	92 ± 15	0.6 ± 0.1
30	7.1 ± 2.6	5.4 ± 1.0	1.1 ± 0.2	96 ± 26	88 ± 17	0.6 ± 0.3
45	4.6 ± 0.8	4.2 ± 0.5	0.9 ± 0.1	72 ± 11	79 ± 11	0.8 ± 0.1
60	5.7 ± 2.3	4.7 ± 0.9	1.0 ± 0.2	82 ± 11	82 ± 10	0.7 ± 0.3
90	5.3 ± 1.2	5.0 ± 1.2	1.1 ± 0.2	84 ± 18	79 ± 8	0.8 ± 0.2
120	4.9 ± 0.6	4.7 ± 0.4	1.0 ± 0.1	77 ± 12	79 ± 12	0.8 ± 0.3
150	5.8 ± 2.0	5.3 ± 1.4	1.0 ± 0.2	86 ± 21	83 ± 11	0.8 ± 0.3
180	5.0 ± 0.9	4.5 ± 0.4	1.0 ± 0.1	82 ± 12	81 ± 10	0.8 ± 0.3
240	4.8 ± 1.1	4.3 ± 0.5	1.0 ± 0.1	74 ± 14	76 ± 10	0.7 ± 0.1
300	5.7 ± 1.3	4.9 ± 1.0	1.1 ± 0.2	83 ± 18	79 ± 11	0.8 ± 0.3
360	5.0 ± 0.7	4.5 ± 0.9	1.0 ± 0.1	80 ± 16	80 ± 8	0.8 ± 0.2

JNJ-27018966-AAA 5 mg/kg						
Time	PIF	PEF	TV	MV	f	Penh
min	mL/s	mL/s	mL	mL	bpm	
0	5.7 ± 1.1	5.0 ± 1.0	1.0 ± 0.1	84 ± 12	83 ± 8	0.7 ± 0.2 *
15	11.1 ± 1.3 *	11.2 ± 1.7 *	1.0 ± 0.1 *	128 ± 13	134 ± 10	2.9 ± 0.8
30	5.6 ± 1.6	6.0 ± 1.5	0.8 ± 0.1 *	95 ± 13	115 ± 5	1.4 ± 0.8
45	6.6 ± 2.8	5.5 ± 1.3	0.9 ± 0.2	97 ± 27	108 ± 21	0.9 ± 0.3
60	8.0 ± 2.5	5.8 ± 1.0	1.0 ± 0.1	118 ± 21 *	121 ± 18	0.6 ± 0.1
90	8.5 ± 2.2 *	6.3 ± 0.8	1.1 ± 0.1	129 ± 17 *	125 ± 19	0.6 ± 0.1
120	8.4 ± 2.4 *	6.4 ± 1.3 *	1.0 ± 0.2	123 ± 25 *	121 ± 9	0.6 ± 0.2
150	7.8 ± 3.2	6.3 ± 1.4	1.0 ± 0.2	113 ± 27	108 ± 13	0.8 ± 0.3
180	8.1 ± 3.0	6.5 ± 1.7 *	1.1 ± 0.1	115 ± 33	109 ± 22	0.8 ± 0.3
240	5.1 ± 1.7	5.1 ± 1.0	1.0 ± 0.1	81 ± 14	85 ± 10	0.9 ± 0.2
300	4.7 ± 0.7	5.0 ± 0.8	1.0 ± 0.1	83 ± 11	88 ± 15	0.9 ± 0.3
360	5.5 ± 2.2	5.4 ± 1.4	1.0 ± 0.1	80 ± 14	81 ± 4	0.9 ± 0.5

\* : p&lt;0.05 versus Vehicle group

: p&lt;0.05 versus T0

PIF: Peak inspiratory flow

PEF: Peak expiratory flow

TV: Tidal volume

MV: Minute volume

F: Respiratory rate

PENH: Enhanced pause

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

#### Absorption

#### Pharmacokinetics of JNJ-27018966 in Hepatic Portal and Jugular Vein Catheterized Male Sprague Dawley Rats after Oral Administration (DD07389)

**Methods:** In this study, absorption and first-pass elimination of JNJ-27018966 were determined in male Sprague Dawley rats (n = 4) after an oral (gavage) dose of 10 mg/kg (10 mL/kg, vehicle = 0.5% hydroxypropyl methylcellulose).

**Results:** Hepatic portal vein concentrations of JNJ-27018966 were low ( $C_{max}$  = 72 ng/mL) after oral administration indicating limited absorption through the gastrointestinal (GI) tract. The concentrations in the jugular vein were also low, being below the limit of quantification (LOQ) in most samples, indicating extensive first-pass elimination. Overall, these results indicated poor systemic exposure following oral administration. The following table (from page 13 of the report) shows the PK parameters.

Table 4: Pharmacokinetic parameters determined for hepatic portal vein sampling after the oral administration of 10 mg/kg JNJ-27018966 to male Sprague Dawley rats.

Animal	$t_{1/2}$ (h)	$t_{max}$ (h)	$C_{max}$ (ng/mL)	$AUC_{last}$ (h*ng/mL)
Rat 1	Missing	0.1	148	150
Rat 2	Missing	0.3	36	56
Rat 3	Missing	2.0	36	82
Rat 4	Missing	2.0	69	214
N	0.00	4	4	4
Mean	Missing	1.1	72	126
SD	Missing	1.1	53	71
CV%	Missing	98	73	57

#### Pharmacokinetics of JNJ-27018966 in Female Beagle Dogs after Intravenous and Oral Administration (DD07393)

**Methods:** The pharmacokinetic (PK) properties of JNJ-27018966 were examined in female Beagle dogs (n = 3) after intravenous (2 mg/kg) and oral (10 mg/kg) administration. Blood was collected at 5, 15 and 30 min and at 1, 2, 4, 7 and 24 hours postdose.

**Results:** Plasma JNJ-27018966 concentrations decreased in a biphasic manner after IV administration. The volume of distribution was slightly higher than total body water and the clearance was high resulting in a short half-life of 0.75 hours. Absorption of JNJ-27018966 following oral administration was poor with  $C_{max}$  of 5.7 ng/mL achieved within 0.25-2 hours postdose. The terminal half-life after oral administration could not be calculated due to the paucity of the data. Bioavailability after oral administration was poor (0.2%). The following tables (from page 14 of the report) show the PK parameters after IV and oral dose.

Table 3: Pharmacokinetic Parameters after the i.v. Administration of 2 mg/kg JNJ-27018966 to Female Beagle Dogs

Animal	$t_{1/2}$ (h)	C0 (ng/mL)	AUC <sub>last</sub> (h*ng/mL)	AUC <sub>Inf</sub> (h*ng/mL)	AUC <sub>Extr</sub> (%)	Vz (L/kg)	V <sub>ss</sub> (L/kg)	CL (mL/min/kg)
Dog 4	0.47	4730	1257	1284	2.1	1.05	1.54	25.71
Dog 5	0.93	7577	2928	3576	18.1	0.75	0.61	9.32
Dog 6	0.84	2762	1342	1347	0.4	1.80	0.83	24.75
N	3	3	3	3	3	3	3	3
MEAN	0.75	5023	1842	2069	6.9	1.20	0.99	19.92
SD	0.25	2421	941	1305	9.8	0.54	0.49	9.19
CV	33.09	48	51	63	142.2	45.07	48.93	46.15

Table 4: Pharmacokinetic Parameters after the p.o. Administration of 10 mg/kg JNJ-27018966 to Female Beagle Dogs

Animal	$t_{1/2}$ (h)	$t_{max}$ (h)	$C_{max}$ (ng/mL)	AUC <sub>last</sub> (h*ng/mL)	AUC <sub>Inf</sub> (h*ng/mL)	AUC <sub>Extr</sub> (%)	F (%)
Dog 1	NA	2	5	7	NA	NA	0.1
Dog 2	NA	0.5	8	13	NA	NA	0.3
Dog 3	NA	0.25	4	1	NA	NA	0.4
N	0	3	3	3	0	0	3
Mean	NA	0.92	5.7	7	NA	NA	0.2
SD	NA	0.95	2.1	6	NA	NA	0.1
CV%	NA	103.25	36.7	83	NA	NA	57.1

### **Pharmacokinetics of JNJ-27018966 in the Mouse (DD07397)**

**Methods:** In this study, pharmacokinetic properties of JNJ-27018966 were determined in male CD-1 mice (n = 64) after IV and PO administration. Thirty-two mice were administered the drug intravenously at 3 mg/kg. Blood was collected at 5, 15, 30, 60, 120, 240, 360, 480, and 1440 minutes postdose. There were four mice for each time point. Another thirty-two mice were administered the drug at 30 mg/kg via the oral route. Blood was collected at 15, 30, 60, 120, 240, 360, 480 and 1440 minutes postdose. There were four mice used for each time point.

**Results:** JNJ-27018966 exhibited extensive distribution with high clearance and a short elimination half-life ( $t_{1/2}$ ) in mice. Oral administration resulted in very low plasma concentrations ( $C_{max} = 15$  ng/mL) and poor bioavailability (1.7%). The PK parameters are shown in the table below (from page 13 of the report).

Table 3: Pharmacokinetic Parameters after the i.v. Administration of 3 mg/kg JNJ-27018966 to Mice

	$t_{1/2}$ (h)	$C_0$ (ng/mL)	AUC <sub>last</sub> (h*ng/mL)	AUC <sub>Inf</sub> (h*ng/mL)	AUC Extr (%)	$V_z$ (L/kg)	$V_{ss}$ (L/kg)	CL (mL/min/kg)
Mean	0.79	4267	523	528	0.86	4.31	1.75	63.2

Table 4: Pharmacokinetic Parameters after the p.o. Administration of 30 mg/kg JNJ-27018966 to Mice

	$t_{1/2}$ (h)	$t_{max}$ (h)	$C_{max}$ (ng/mL)	AUC <sub>last</sub> (h*ng/mL)	AUC <sub>Inf</sub> (h*ng/mL)	AUC Extr (%)	F (%)
Mean	1.33	0.5	15	38	44	13.2	1.7

### **Pharmacokinetics of JNJ-27018966 Following a Single Intravenous or Oral Administration of JNJ-27018966 to Male and Female Rats (FK10138)**

**Methods:** This study was performed in support of a previously conducted study (FK6180) to characterize the PK of JNJ-27018966 in male and female SD rats (n = 16/sex) following a single IV administration of JNJ-27018966-AAC at 10 mg/kg or after a single oral administration of JNJ-27018966-AAA at 1000 mg/kg. In addition, the absolute bioavailability and the differences between non-fasted and fasted condition were evaluated.

**Results:** The absolute bioavailability in fasted and non-fasted rats was low and ranged from 0.04% to 0.17%. The exposure in fasted condition was comparable or higher in female than in male animals. However, in non-fasted condition, the AUC values tended to be lower in females than in males. The following tables (from page 5 of the report) show the PK parameters following IV and oral administrations.

Treatment Gender Status	IV			
	Female		Male	
	Fasted	Fed	Fasted	Fed
C <sub>0</sub> (ng/ml)	52300	61100	38500	33800
t <sub>1/2</sub> , 2-4h (h)	<u>0.9</u>	0.6	0.9	<i>14.0<sup>1)</sup></i>
Cl (l/h/kg)	2.56	2.41	2.66	3.03
V <sub>dz</sub> (l/kg)	15.0	6.15	3.38	7.16
V <sub>dss</sub> (l/kg)	0.689	1.60	0.460	1.41
AUC <sub>0-24 h</sub> (ng.h/ml)	3980	<b>4180</b>	3780	<u>6500</u>
AUC <sub>0-inf</sub> (ng.h/ml)	3990	5360	3780	<u>6550</u>

**Bold:** n=3

Underline: n= 2

*Italic:* n= 1

<sup>1)</sup>t<sub>1/2</sub>, 8-24 h

Treatment Gender Status	PO			
	Female		Male	
	Fasted	Fed	Fasted	Fed
C <sub>max</sub> (ng/ml)	38.6	65.6	25.9	235
T <sub>max</sub> (h)	0.8	0.6	0.6	3.0
t <sub>1/2</sub> , 8-24 h (h)	<b>9.6</b>	<b>10.2</b>	<b>6.4</b>	<b>6.2</b>
AUC <sub>0-24 h</sub> (ng.h/ml)	297	237	202	858
AUC <sub>0-inf</sub> (ng.h/ml)	<b>424</b>	<b>295</b>	<b>161</b>	<b>1123</b>

**Bold:** n=3

### **Pharmacokinetics of JNJ-27018966 in Male and Female Rhesus Monkeys Following a Single Intravenous Bolus Administration (FK10141)**

**Methods:** In this study, three male and three female Rhesus monkeys were treated with JNJ-27018966 intravenously at 3.2 mg/kg (0.32 mL/kg). Blood samples were collected at pre-dose and at 0.033, 0.083, 0.25, 0.50, 1, 2, 4, and 8 hours postdose.

**Results:** In males and females, the elimination was rapid based on terminal half-life values of 0.655 and 0.643 hours, respectively. The volume of distribution (V<sub>dss</sub>) values were 285 and 323 mL/kg in males and females, respectively, which were approximately 41 and 47% of total body water (693 mL/kg) in the monkey, indicating that JNJ-27018966 was not widely distributed outside the plasma. There were no apparent gender differences in PK parameters. The summary of PK parameters is shown in the table (from page 14 of the report) below.

Table SD2: Summary of Individual and Mean Plasma Pharmacokinetic Parameters of JNJ-27018966 in Male and Female Monkeys (N = 3) Following Administration of a Single Intravenous Dose (3.2 mg/kg) of JNJ-27018966-AAC (FK10141)

Gender	Subject ID	C <sub>0</sub> (ng/mL)	AUC <sub>0-∞</sub> (ng•h/mL)	t <sub>½</sub> (h)	CL (mL/h•kg)	Vd <sub>ss</sub> (mL/kg)
Male	MKY 301	40800	2970	0.675	1080	249
	MKY 302	26800	1440	0.646	2230	352
	MKY 303	36800	1780	0.643	1800	254
	<b>Mean</b>	<b>34800</b>	<b>2060</b>	<b>0.655</b>	<b>1700</b>	<b>285</b>
	<b>SD</b>	<b>7220</b>	<b>807</b>	<b>0.0176</b>	<b>582</b>	<b>58.3</b>
Female	MKY 304	25900	1610	0.735	1990	377
	MKY 305	28000	1590	0.602	2010	342
	MKY 306	37500	2770	0.592	1160	250
	<b>Mean</b>	<b>30500</b>	<b>1990</b>	<b>0.643</b>	<b>1720</b>	<b>323</b>
	<b>SD</b>	<b>6210</b>	<b>674</b>	<b>0.0797</b>	<b>487</b>	<b>65.7</b>

### **Pharmacokinetics of JNJ-27018966 in Male and Female Cynomolgus Monkeys Following a Single Oral Gavage or Intravenous Bolus Administration in a Crossover Design (FK10142)**

**Methods:** The objectives of this study were to characterize the PK parameters and oral bioavailability of JNJ-27018966 in male and female Cynomolgus monkeys following a single IV (10 mg/kg) dose of JNJ-27018966-AAC (b)(4) and a single oral dose (200 mg/kg) of JNJ-27018966-AAA (b)(4). In this study, four male and four female Cynomolgus monkeys were used. The animals were fasted prior to dose administration. The IV (10 mg/kg, 1 mL/kg) formulation (in saline solution) was administered on Day 1 and the oral formulation (0.5% methylcellulose in deionized water suspension) was administered following a 7-day washout period (200 mg/kg, 5 mL/kg). Blood samples were collected at predose and at 0.083 (IV only), 0.25 (IV only), 0.50, 1, 2, 4, 8 and 24 hours postdose.

**Results:** Following a single IV dose (10 mg/kg) of JNJ-27018966-AAC in male and female monkeys, the terminal half-life values were 4.07 and 3.40 hours, respectively. The Vd<sub>ss</sub> values were 316 and 402 mL/kg in males and females, respectively, which were approximately 46 and 58% of total body water (693 mL/kg) in male and female monkeys, respectively, indicating that JNJ-27018966 was not widely distributed outside the plasma.

Following a single oral dose (200 mg/kg) of JNJ-27018966-AAA in males and females, the absorption was slow with mean t<sub>max</sub> values of 7.75 (males) and 7.13 (females) hours. The oral bioavailability was 0.134% in males and 0.126% in females. There were

no apparent gender differences. The following table (from page 17 of the report) shows the PK parameters.

Table SD2: Summary of Individual and Mean Plasma Pharmacokinetic Parameters of JNJ-27018966 in Male and Female Monkeys (N = 4) Following Administration of Single Intravenous (10 mg/kg) or Oral (200 mg/kg) Doses of JNJ-27018966-AAC (Intravenous) or JNJ-27018966-AAA (Oral) (FK10142)

Gender	Route	Dose (mg/kg)	Subject ID	C <sub>max</sub> <sup>a</sup> (ng/mL)	t <sub>max</sub> (h)	AUC <sub>0-∞</sub> (ng•h/mL)	AUC <sub>0-24h</sub> (ng•h/mL)	t <sub>1/2</sub> (h)	CL/F (mL/h•kg)	Vd <sub>ss</sub> (mL/kg)	F (%)
Male	intravenous	10	MKY 201	90400		12000	12000	5.11	833	332	
			MKY 202	85800		9460	9460	5.29	1060	333	
			MKY 203	93200		10400	10400	4.74	959	320	
			MKY 204	81300		10300	10300	1.13	971	278	
			<b>Mean</b>	<b>87700</b>		<b>10500</b>	<b>10500</b>	<b>4.07</b>	<b>955</b>	<b>316</b>	
	<b>SD</b>	<b>5230</b>		<b>1060</b>	<b>1050</b>	<b>1.97</b>	<b>91.9</b>	<b>25.8</b>			
Male	oral	200	MKY 201	26.1	2.00	NC	397	NC	NC		0.165
			MKY 202	25.1	1.00	NC	221	NC	NC		0.117
			MKY 203	37.0	4.00	NC	395	NC	NC		0.190
			MKY 204	8.61	24.0	NC	128	NC	NC		0.062
			<b>Mean</b>	<b>24.2</b>	<b>7.75</b>	<b>NC</b>	<b>285</b>	<b>NA</b>	<b>NA</b>		<b>0.134</b>
<b>SD</b>	<b>11.7</b>	<b>10.9</b>	<b>NC</b>	<b>133</b>	<b>NA</b>	<b>NA</b>		<b>0.056</b>			
Female	intravenous	10	MKY 205	54600		5490	5480	1.33	1820	391	
			MKY 206	92200		13100	13100	5.52	762	296	
			MKY 207	34900		6440	6430	1.27	1550	592	
			MKY 208	75400		10800	10800	5.48	922	328	
			<b>Mean</b>	<b>64300</b>		<b>8970</b>	<b>8960</b>	<b>3.40</b>	<b>1270</b>	<b>402</b>	
<b>SD</b>	<b>24900</b>		<b>3620</b>	<b>3610</b>	<b>2.43</b>	<b>505</b>	<b>133</b>				
Female	oral	200	MKY 205	18.0	2.00	NC	265	NC	NC		0.241
			MKY 206	17.2	2.00	NC	149	NC	NC		0.057
			MKY 207	11.9	24.0	NC	215	NC	NC		0.167
			MKY 208	8.17	0.500	222 <sup>b</sup>	84.2	28.6	901000		0.039
			<b>Mean</b>	<b>13.8</b>	<b>7.13</b>	<b>222<sup>b</sup></b>	<b>179</b>	<b>28.6<sup>c</sup></b>	<b>901000<sup>c</sup></b>		<b>0.126</b>
<b>SD</b>	<b>4.64</b>	<b>11.3</b>	<b>NA</b>	<b>78.8</b>	<b>NA</b>	<b>NA</b>		<b>0.095</b>			

<sup>a</sup>: C<sub>0</sub> for i.v. dose

<sup>b</sup>: individual value >25% extrapolated of the total AUC

<sup>c</sup>: individual value

NC: Not calculable due to plasma concentration profile

NA: Not applicable

### **Bioavailability and Pharmacokinetics of JNJ-27018966 in Male and Female Cynomolgus Monkeys Following a Single Oral, Subcutaneous, and Intravenous Bolus Administration (FK5721)**

**Methods:** Originally, the objectives of this study were to characterize the PK parameters of JNJ-27018966 in male and female Cynomolgus monkeys following a single IV bolus (1 mg/kg), SC (1 mg/kg) or PO (gavage, 5 mg/kg), and to determine the absolute bioavailability of the oral and SC doses. However, due to the limited concentration levels detected which were above the lower limit of quantitation (LOQ)

following the oral 5 mg/kg dose, the study protocol was amended, and an additional 200 mg/kg oral dose was added to the study. In this study, three male and three female Cynomolgus monkeys were used. Each fasted monkey received a single IV dose (1 mg/kg) of JNJ-27018966-AAA (in 10% HP- $\beta$ -CD, 1 mL/kg). Blood samples were collected at pre dose and at 0.08, 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose. Following a one-week wash-out period, the same fasted monkeys received a single SC dose (1 mg/kg) of JNJ-27018966-AAA (in 10% HP- $\beta$ -CD, 1 mL/kg). Blood samples were collected at predose and at 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose. Following a three-week wash-out period, the same fasted monkeys received a single oral dose (5 mg/kg) of JNJ-27018966-AAA (0.5% Methocel, 1 mL/kg). Blood samples were collected at predose and at 0.5, 1, 2, 4, and 8 hours post dose. At least 6 months later, the same fasted monkeys received a single oral dose (200 mg/kg) of JNJ-27018966-AAA (0.5% Methocel, 1 mL/kg). Blood samples were collected at predose and at 0.5, 1, 2, 4, 8, and 24 hours postdose.

**Results:** Following a single IV dose (1 mg/kg) of JNJ-27018966-AAA, elimination was rapid based on mean terminal half-life values of 1.43 and 0.67 hours in males and females, respectively. The mean  $V_{d_{ss}}$  values for males and females were 341 and 273 mL/kg, which were less than the volume of total body water (693 mL/kg), indicating that JNJ-27018966 was not widely distributed outside the plasma.

Following a single SC dose of 1 mg/kg, the mean  $t_{max}$  value was 0.42 hours for both sexes. Elimination was rapid based on mean terminal half-life values of 1.58 and 0.97 hours in males and females, respectively. The calculated SC bioavailability was approximately 171% and 174% in female and male monkeys, respectively.

Following a single oral dose (200 mg/kg) of JNJ-27018966-AAA in male and female monkeys, the mean  $t_{max}$  values were 3.33 and 4.0 hours for male and female monkeys, respectively. Elimination appeared to be slow based on mean terminal half-life values of 18.7 and 39.1 hours in males and females, respectively. The estimated oral bioavailability values were approximately 0.35% and 0.16% in female and male monkeys, respectively. The following table (from page 23 of the report) shows the summary of mean PK parameters following a single IV, PO and SC dose in monkeys.

Table SD6: Summary of Mean (SD) Plasma Pharmacokinetic Parameters for JNJ-27018966 in Male and Female Monkeys Following a Single Intravenous (1 mg/kg), Single Oral (200 mg/kg) or a Single Subcutaneous Dose (1 mg/kg) of JNJ-27018966-AAA (FK5721)

Route	Dose (mg/kg)	Gender	C <sub>max</sub> <sup>a</sup> (ng/mL)	t <sub>max</sub> (h)	AUC <sub>0-∞</sub> (ng•h/mL)	t <sub>1/2</sub> (h)	CL/F (mL/h•kg)	Vd <sub>B</sub> (mL/kg)	F (%)
IV	1	Female	6977 (3819)	NA	911 (318)	0.67 (0.21)	1182 (365)	273 (119)	NA
		Male	6215 (717)	NA	1078 (102)	1.43 (0.22)	934 (92.0)	341 (27.0)	NA
SC	1	Female	1220 (479)	0.42 (0.14)	1430 (97.0)	0.97 (0.31)	702 (46.0)	NA	171 (61.7)
		Male	837 (116)	0.42 (0.14)	1862 (168)	1.58 (0.98)	540 (50.0)	NA	174 (26.8)
Oral	200	Female	30.3 (10.0)	4.00 (3.46)	557 (373)	39.1 (55.2)	496000 (331000)	NA	0.35 (0.33)
		Male	25.2 (12.9)	3.33 (1.15)	351 (155)	18.7 (13.4)	670000 (346000)	NA	0.16 (0.06)

<sup>a</sup> C<sub>0</sub> i.v. dose

NA = not applicable

### **Pharmacokinetics of JNJ-27018966 Following Administration of a Single Intravenous Dose of JNJ-27018966-AAC to Rhesus Monkeys (FK5863)**

**Methods:** The objective of this study was to characterize the PK parameters of a single IV dose (3.2, 10, 17.8, or 56 mg/kg) of JNJ-27018966-AAC in male and female Rhesus monkeys. Four non-fasted male and female monkeys (2/sex) were used in this study. Monkeys received a single IV dose (3.2, 10, 17.8, or 56 mg/kg) by injection of JNJ-27018966-AAC in sterile saline. The dose volumes were 0.32, 0.1, 0.178, or 0.28 mL/kg for the 3.2, 10, 17.8, or 56 mg/kg doses, respectively. Blood samples were collected at 0.08, 0.5, and 2 hours postdose.

**Results:** JNJ-27018966 appeared to undergo rapid elimination following IV administration as evidenced by mean half-life values ranging from 0.28 to 0.37 hours. The mean C<sub>max</sub> and mean AUC increased in a dose-related manner. No gender differences were apparent in the PK parameters. Low mean volume of distribution values suggested that JNJ-27018966 was not widely distributed outside the plasma. Mean plasma pharmacokinetic parameters are summarized in the table (from page 5 of the report) below.

FK5863 **Summary of Mean Pharmacokinetic Parameters for JNJ-27018966 in Male and Female Monkeys (N = 2) following a Single Intravenous (3.2, 10, 17.8, or 56 mg/kg) Dose of JNJ-27018966-AAC (FK5863)**

Test Article: JNJ-27018966-AAC

Study No.	FK5863		FK5863		FK5863		FK5863	
Species	Rhesus Monkey		Rhesus Monkey		Rhesus Monkey		Rhesus Monkey	
Feeding Condition	Fed		Fed		Fed		Fed	
Vehicle/Formulation	Purified water		Purified water		Purified water		Purified water	
Route	Intravenous		Intravenous		Intravenous		Intravenous	
Gender (M/F)/Number of Animals	M: 2	F: 2						
Dose (mg/kg)	3.2	3.2	10	10	17.8	17.8	56	56
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C <sub>0</sub> (ng/mL)	28737	21722	146854	103886	204853	403498	528436	791233
AUC (ng × h/mL)	7864	5376	34013	23316	65920	85868	476470	688660
t <sub>1/2</sub> (h)	0.30	0.29	0.37	0.28	0.32	0.31	0.32	0.28
Clearance (mL/h•kg)	442	635	321	429	276	215	119	83.5
Vd <sub>ss</sub> (mL/kg)	98.3	120	91.7	70.0	71.0	36.9	14.6	8.57

### **Pharmacokinetics of JNJ-27018966 in Male Dogs Following a Single Intravenous Bolus Administration (FK5947)**

**Methods:** The objective of this study was to characterize the PK parameters of JNJ-27018966 in male dogs (n = 4) following a single IV bolus dose of JNJ-27018966-AAA. In this study, each dog received a single IV (0.2 mg/kg) dose of JNJ-27018966-AAA. Blood samples were collected at predose and at 0.08, 0.25, 0.5, 1, 2, 4, and 6 hours postdose.

**Results:** Following IV administration, mean half-life value was 0.45 hour. The mean volume of distribution was 1180 mL/kg. This volume exceeded the total body water in the dog (606 mL/kg) suggesting that JNJ-27018966 was widely distributed outside the plasma. The following table (from page 4 of the report) shows the PK parameters.

FK5947 **Summary of Mean Pharmacokinetics Parameters for JNJ-27018966 Plasma Concentrations in Male Dogs (N = 4) Following a Single Bolus Intravenous (0.2 mg/kg) Dose of JNJ-27018966-AAA (FK5947)**

Test Article: JNJ-27018966-AAA

Study No.	FK5947
Species	Dog
Feeding Condition	Fasted
Vehicle/Formulation	5% HPbCD in 5% D5W
Route	Intravenous
Gender (M/F)/Number of Animals	M: 4
Dose (mg/kg)	0.2
Sample (whole blood, plasma, serum, etc.)	Plasma
Analyte	JNJ-27018966
Assay	LC-MS/MS
Pharmacokinetic Parameters	
C <sub>0</sub> (ng/mL)	267
AUC 0-∞ (ng × h/mL)	61.6
t <sub>1/2</sub> (h)	0.450
Clearance (mL/h•kg)	3260
Volume of Distribution (mL/kg)	1180
Additional Information	
HPbCD	: Hydroxypropyl-β-Cyclodextrin
D5W	: 5% Dextrose in water

**Pharmacokinetics of JNJ-27018966 Following a Single Intravenous, Subcutaneous or Oral Administration of JNJ-27018966-AAA to Male and Female Mice (FK6179)**

**Methods:** The objectives of this study were to characterize the PK parameters of JNJ-27018966 in male and female mice following single oral (1000 mg/kg), SC (1 mg/kg) or IV (1 mg/kg) doses of JNJ-27018966-AAA, and to determine the absolute bioavailability of the oral and SC doses. One hundred and fifty mice (3/sex/group/time point) were used in this study. Mice (n = 3/sex/group/time point) received a single oral dose of 1000 mg/kg (10 mL/kg, formulated in 0.5% Methocel), a single SC dose of 1 mg/kg (1 mL/kg, formulated in 10% HP- $\beta$ -CD) or a single IV dose of 1 mg/kg (1 mL/kg, 10% HP- $\beta$ -CD). Blood samples were collected at the following time points: predose, (0.08, IV only), 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose.

**Results:** Following a single IV dose (1 mg/kg) of JNJ-27018966-AAA, elimination was rapid based on terminal half-life values of 0.243 and 0.216 hours in males and females, respectively. The  $V_{dss}$  values for males and females were 663 and 727 mL/kg, respectively, which were similar to the volume of total body water (725 mL/kg).

Following a single SC dose (1 mg/kg) of JNJ-27018966-AAA in male and female mice, the distribution was rapid based on a mean  $t_{max}$  value of 0.25 hours for both sexes. Elimination was rapid based on mean terminal half-life values of 0.296 and 0.552 hours in males and females, respectively. The SC bioavailability was approximately 104% and 135% in male and female mice, respectively.

Following a single oral dose (1000 mg/kg) of JNJ-27018966-AAA in male and female mice, the absorption rate was moderate based on  $t_{max}$  values of 0.50 hours for both sexes. In males, elimination appeared to be slow based on a terminal half-life value of 27.3h while the elimination was moderate in female mice based on a terminal half-life value of 5.82 hours. The estimated oral bioavailability values were approximately 0.18% and 0.15% in male and female mice, respectively.

The following table (from page 6 of the report) shows the PK data.

**Summary of Mean Pharmacokinetic Parameters for JNJ-27018966 in Male and Female Mice (N = 3/Time point/Sex/Dose Group) Following a Single Oral (1000 mg/kg), Subcutaneous (1mg/kg) or a Intravenous (1 mg/kg) Dose of JNJ-27018966-AAA (FK6179)**

Test Article: JNJ-27018966

Study No.	FK6179		FK6179		FK6179	
Species	Mouse		Mouse		Mouse	
Feeding Condition	Fasted		Fasted		Fasted	
Vehicle/Formulation	0.5% Methocel <sup>®</sup>		10% HP-β-CD		10% HP-β-CD	
Route	p.o		s.c		i.v.	
Gender (M/F)/Number of Animals	M: 3/timepoint	E: 3/timepoint	M: 3/timepoint	E: 3/timepoint	M: 3/timepoint	E: 3/timepoint
Dose (mg/kg)	1000	1000	1	1	1	1
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966
<b>Assay</b>						
<b>Pharmacokinetic Parameters</b>						
C <sub>max</sub> (ng/mL)	27.6	119	369	302	<sup>a</sup> 1763	<sup>a</sup> 1493
t <sub>max</sub> (h)	0.500	0.500	0.250	0.250	NA	NA
AUC 0-∞ (ng×h/mL)	435	302	247	269	238	200
t <sub>1/2</sub> (h)	27.3	5.82	0.296	0.552	0.243	0.216
Clearance/F (ml/h•kg)	2300796	3314364	4057	3711	4203	5012
Volume of Distribution (ml/kg)	NA	NA	NA	NA	663	727
Bioavailability (%)	0.18	0.15	104	135	NA	NA

<sup>a</sup> C<sub>0</sub> (ng/mL)

NA: Not applicable

**Pharmacokinetics of JNJ-27018966 Following a Single Intravenous, Subcutaneous or Oral Administration of JNJ-27018966 to Male and Female Rats (FK6180)**

**Methods:** The objectives of this study were to characterize the PK parameters of JNJ-27018966 in male and female rats following a single IV (1 mg/kg), SC (1 mg/kg) or oral (1000 mg/kg) administration, and to determine the absolute bioavailability after oral and SC doses. Twenty four rats (4/sex/group) were used in this study. Group 1 rats received a single IV dose of 1 mg/kg of JNJ-27018966-AAA. Group 2 rats received a single SC dose of 1 mg/kg of JNJ-27018966-AAA and the Group 3 rats received a single oral dose of 1000 mg/kg of JNJ-27018966-AAA. Blood samples were collected at the following time points: predose (0.0), 0.08 (IV only), 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose.

**Results:** Following a single IV dose (1 mg/kg), elimination was rapid based on mean terminal half-life values of 1.01 and 0.557 hours in males and females, respectively. The mean V<sub>dss</sub> value for male rats was 901 mL/kg which is greater than the volume of total body water indicating that JNJ-27018966 was distributed outside the plasma. In male rats, the mean V<sub>dss</sub> value was 603 mL/kg, which is slightly less than the volume of total body water (670 mL/kg), indicating that JNJ-27018966 was not widely distributed outside the plasma.

Following a single SC dose (1 mg/kg), the distribution was rapid based on mean t<sub>max</sub> values of 0.667 and 0.250 hours for male and female rats, respectively. Elimination was slow in males based on a mean terminal half-life value of 9.55 hours. However, elimination was fast in females based on a mean terminal half-life value of 1.21 hours. The SC bioavailability was approximately 165% and 125% in male and female rats, respectively.

Following a single oral dose (1000 mg/kg), the absorption rate was fast based on a mean  $t_{max}$  value of 1.88 hours for both sexes. Elimination was slow based on mean terminal half-life values of 6.66 and 13.9 hours in males and females, respectively. The estimated oral bioavailabilities were approximately 0.066% and 0.063% in female and male rats, respectively.

The following table (from page 6 of the report) shows the PK data.

FK6180

Pharmacokinetics: Absorption after a Single IV Dose, Single Oral, and a Single Subcutaneous Dose

Test Article: JNJ-27018966

Study No.	FK6180		FK6180		FK6180	
Species	Rat		Rat		Rat	
Vehicle/Formulation	10 % HP-β-CD		10 % HP-β-CD		0.5 % Methocellulose	
Route	IV		Subcutaneous		Oral	
Gender (M/F)/Number of Animals	M/4	F/4	M/4	F/4	M/4	F/4
Feeding Condition	Fasted	Fasted	Fasted	Fasted	Fasted	Fasted
Dose (mg/kg)	1	1	1	1	1000	1000
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966	JNJ-27018966
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
$C_{max}$ (ng/mL)	<sup>a</sup> 2110	<sup>a</sup> 2910	213	246	33.5	25.2
$t_{max}$ (h)	NA	NA	0.667	0.250	1.88	1.88
AUC <sub>(0-∞)</sub> (ng •h/mL)	365	380	603	476	240	238
$t_{1/2}$ (h)	1.01	0.557	9.55	1.21	6.66	13.9
Clearance (mL/h kg)	2800	2710	1700	2220	5350000	4720000
Volume of Distribution (mL/kg)	901	603	NA	NA	NA	NA
F (%)	NA	NA	165	125	0.066	0.063
<sup>a</sup> $C_0$ (ng/mL)						

NA: not applicable

### Transport Mechanisms of JNJ-27018966 in MDCKII-MDR1, MDCKII-MRP2 and MDCKII, Using Expressed Cells (FK6635)

**Methods:** Three membrane proteins have been identified as important drug efflux transporters in humans: P-glycoprotein or P-gp (encoded by the MDR1 gene) and several member of the multidrug resistance-associated protein (MRP) family, namely MRP1 and MRP2. This study was conducted to examine whether JNJ-27018966 is a substrate and/or inhibitor of human P-gp or MRP2, using human MDR1- and MRP2-transfected MDCKII cell lines. In this study, [<sup>14</sup>C]JNJ-27018966 (specific activity = 53 mCi/mmol) was used. The transport study was conducted in the presence of [<sup>3</sup>H] digoxin (0.05 μM) or [<sup>14</sup>C] etoposide (1.0 μM), marker substrates for P-gp and MRP2, respectively, and in the presence of [<sup>14</sup>C]mannitol (10 μM), a paracellular marker used as an indicator of the tightness of the junctions of the cells. Transport was measured in both the absorptive and secretive directions i.e., apical to basolateral (A→B) and basolateral to apical (B→A), respectively.

The inhibitory effect of JNJ-27018966 at two concentrations (1 and 100 μM) on the transport of [<sup>3</sup>H] digoxin (0.05 μM) was determined in MDR1-MDCKII cells. Cyclosporin (10 μM) or GF-120918 (1 μM) specific inhibitors of MDR1 were used as positive controls. In addition, transport of [<sup>14</sup>C]JNJ-27018966 (5 μM) in the presence of cyclosporine (10 μM) and GF-120918 (1 μM) was determined to assess MDR1

substrate specificity. Similarly, the inhibitory effect of JNJ-27018966 (1 and 100  $\mu\text{M}$ ) on the transport of [ $^{14}\text{C}$ ] etoposide (1  $\mu\text{M}$ ) was determined in MRP2 MDCKII cells. Two following known inhibitors of MRP2 were used as positive controls: sulfobromophthalein (SBPL, 100  $\mu\text{M}$ ) and MK-571 (100  $\mu\text{M}$ ). In addition, transport of [ $^{14}\text{C}$ ]JNJ-27018966 (5  $\mu\text{M}$ ) in the presence of SBPL (100  $\mu\text{M}$ ) and MK-571 (100  $\mu\text{M}$ ), was determined to assess MRP2 substrate specificity. Cells were preincubated with the various inhibitors for 25 min before initiation of the transport studies.

**Results:** Permeability of JNJ-27018966 in the absorptive direction ( $3.91 \times 10^{-7}$  cm/sec) was not significantly different than the impermeable paracellular marker mannitol ( $2.96 \times 10^{-7}$  cm/sec). The results suggested that the test compound had very low passive permeability. The permeability values of JNJ-27018966 in the A-B and B-A directions were only slightly higher than either mannitol or atenolol, indicating that the drug did not cross MDCKII membranes significantly. Overall, due to limited cellular permeability, the ability of JNJ-27018966 to interact with P-gp and MRP2, as substrates or inhibitors, could not be assessed in the MDCKII cell systems. The following table (from page 10 of the report) shows the results.

Table SD1: Recovery and Transport of [ $^{14}\text{C}$ ] JNJ-2701899 in MDCKII Cells

Substrate	Secretive Transport (B-A)		Absorptive Transport (A-B)	
	Recovery (%)	Permeability (cm/sec $\times 10^{-7}$ )	Recovery (%)	Permeability (cm/sec $\times 10^{-7}$ )
[ $^{14}\text{C}$ ] JNJ-27018966 (5.0 $\mu\text{M}$ )	97.4	3.60 $\pm$ 0.29	96.3	3.91 $\pm$ 0.46
[ $^3\text{H}$ ] Atenolol (5.0 $\mu\text{M}$ )	98.6	2.80 $\pm$ 0.26	99.1	3.32 $\pm$ 0.59
[ $^3\text{H}$ ] Propranolol (5.0 $\mu\text{M}$ )	89.1	170 $\pm$ 7.52	82.2	179 $\pm$ 6.24
[ $^{14}\text{C}$ ] Mannitol (10 $\mu\text{M}$ )	98.5	2.04 $\pm$ 0.20	98.0	2.96 $\pm$ 0.19

ND = Not determined

### **Assessment of JNJ-27018966 as a Potential Inhibitor of Human Pgp, BCRP, BSEP, MRP2, OAT1, OAT3, OCT1, OCT2, OATP1B1 and OATP1B3-Mediated Transport (OPT-2012-063)**

**Methods:** The purpose of this study was to determine the potential of JNJ-27018966 to inhibit the transport of substrate by P-gp, BCRP (Breast Cancer Resistance Protein), BSEP (Bile Salt Export Pump), MRP2 (Multidrug Resistance-associated Protein 2), OCT1 (Organic Cation Transporter 1), OCT2 (Organic Cation Transporter 2), OAT1 (Organic Anion Transporter 1), OAT3 (Organic Anion Transporter 3), OATP1B3 (Organic Anion Transporting Polypeptide 1B3). The transport of substrate in the presence of the vehicle control (0.5% DMSO) was compared to the uptake in the presence of 400 ng/mL of JNJ-27018966 or a reference inhibitor.

**Results:** JNJ-27018966 did not significantly inhibit BCRP, BSEP, MRP2, OCT1, OCT2, OAT1, OAT3, OATP1B3-mediated transport of probe substrates. Compared to the vehicle control, JNJ-27018966 inhibited the transport of probe substrates of OATP1B1 and P-gp with respective inhibitions of 32.6% and 6.25%.

**Assessment of JNJ-27018966 as a Potential Substrate of Human P-gp, BCRP, BSEP, MRP2, OAT1, OAT3, OCT1, OCT2, OATP1B1 and OATP1B3-Mediated Transport (OPT-2012-064)**

**Methods:** The purpose of this study was to determine whether JNJ-27018966 is transported by P-gp, BCRP, BSEP, MRP2, OAT1, OAT3, OCT1, OCT2, OATP1B1 or OATP1B3. In this study, JNJ 27018966 was studied at three concentrations (4, 40 and 400 ng/mL).

**Results:** No statistically significant differences of JNJ-27018966 uptake were observed between OAT1, OCT1, OCT2 or OATP1B3 transfected and mock transfected cells at any of the concentrations studied. The results indicated that JNJ-27018966 was not transported by OAT1, OCT1, OCT2 or OATP1B3.

The OAT3 and OATP1B1 dependent uptakes of JNJ-27018966 were observed at the 400 ng/mL concentration, indicating JNJ 27018966 was transported by OAT3 and OATP1B1 only at the highest concentration.

The P-gp dependent efflux ratios were determined to be 1.89, 2.29 and 1.63 at 4, 40 and 400 ng/mL, respectively, which were not significantly above the efflux substrate cutoff of 2.0, indicating that JNJ-27018966 was not transported by P-gp.

The BCRP dependent efflux ratios were determined to be 2.04, 1.36 and 1.57 at 4, 40 and 400 ng/mL, respectively, which were not significantly above the efflux substrate cutoff of 2.0, indicating that JNJ-27018966 was not transported by BCRP.

The BSEP dependent vesicular accumulation of JNJ-27018966 was observed at the 400 ng/mL concentration, indicating that JNJ-27018966 was transported by BSEP at the highest concentration.

The MRP2 dependent vesicular accumulation of JNJ-27018966 was observed at all three concentrations, indicating that JNJ-27018966 was a substrate of MRP2 under the experimental conditions.

Overall, the results indicated that JNJ-27018966 was not transported by OAT1, OCT1, OCT2 or OATP1B3, was transported by OAT3 and OATP1B1 (at highest concentration), and was not transported by P-gp or BCRP. However, JNJ-27018966 was transported by BSEP (at the highest concentration) and was a substrate of MRP2.

## Distribution

### Tissue Distribution in Mice (ADME04-199)

**Methods:** In this study, mice were treated with JNJ-27018966-AAC at 3 mg/kg IV, 30 mg/kg PO, and 300 mg/kg PO (N = 3/dose). For oral gavage dosing, uniform suspensions of the test article at 3 mg/mL and 30 mg/mL concentration in 0.5% hydroxypropyl methylcellulose were used at a dose volume of 10 mL/kg. For IV dosing, the drug was dissolved in 10% Solutol (1.5 mg/mL) and was administered at a dose volume of 2 mL/kg. Mice were sacrificed for tissue harvesting at 30 min and 120 min postdose. The following tissues were collected: brain, liver, kidney, stomach, small intestine, and distal colon.

**Results:** JNJ-27018966 was found in higher amounts in the stomach and intestine following oral dosing. The concentration of JNJ-27018966 in each of the tissues at 30 and at 120 min postdose by oral or IV routes is shown in the following table (from page 2 of the report).

Table 1. Tissue distribution of JNJ-27018966 following oral or intravenous administration in mice.

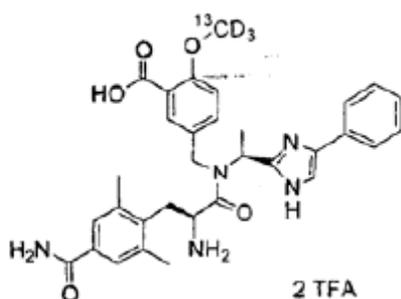
Dose;Route;Time	Plasma	Brain	Kidney	Liver	Stomach	Intestine	Colon
<b>LLOQ</b>	<b>14.5 ng/mL</b>	<b>6.2 ng/g</b>	<b>5.6 ng/g</b>	<b>5.9 ng/g</b>	<b>6.1 ng/g</b>	<b>5.8 ng/g</b>	<b>6.0 ng/g</b>
<b>30 mg/kg oral 30 min</b>	BLOQ	11.621	610.686	2,136.035	24,960.319	706.277	1,010.025
	31.104	BLOQ	229.919	1,319.470	13,375.054	650.449	249.288
	97.300	BLOQ	819.641	793.664	45,605.046	1,459.950	2,014.581
	<b>42.8 ± 28.7</b>	<b>3.9 ± 3.9</b>	<b>553.4 ± 172.6</b>	<b>1,416.4 ± 390</b>	<b>27,980.1 ± 9,4256</b>	<b>938.9 ± 261</b>	<b>1,091.3 ± 511</b>
<b>30 mg/kg oral 120 min</b>	BLOQ	BLOQ	175.914	332.345	4,330.745	9903.143	389.426
	BLOQ	BLOQ	418.366	74.627	3,934.255	6768.705	217.158
	BLOQ	BLOQ	52.751	127.036	6,567.611	9179.321	137.518
	<b>0.00</b>	<b>0.00</b>	<b>215.7 ± 107.4</b>	<b>178.0 ± 78.6</b>	<b>4,944.2 ± 819.7</b>	<b>8,617.1 ± 948</b>	<b>248.0 ± 74.3</b>
<b>300 mg/kg oral 30 min</b>	40.674	46.713	55.258	861.569	45,839.294	779.195	301.014
	32.357	15.951	1,900.191	1,108.008	201,292.895	3,365.724	2,804.941
	80.779	13.786	2,093.309	5,076.215	206,797.046	319.015	3,861.451
	<b>51.3 ± 14.9</b>	<b>25.5 ± 10.6</b>	<b>1,349.6 ± 650</b>	<b>2,348.6 ± 1,366</b>	<b>151,309.8 ± 52,759</b>	<b>1,488 ± 948</b>	<b>2,322.5 ± 1056</b>
<b>300 mg/kg oral 120 min</b>	35.775	15.609	144.924	663.096	32,421.515	2,656.713	657.855
	75.083	22.901	312.977	2,720.972	53,656.078	64,635.214	2,273.895
	61.638	22.673	264.213	1,964.222	42,601.290	1,220.689	456.761
	<b>57.5 ± 11.5</b>	<b>20.4 ± 2.4</b>	<b>240.7 ± 49.9</b>	<b>1,782.8 ± 601</b>	<b>42,893.0 ± 6131.6</b>	<b>22,837.5 ± 20,903</b>	<b>1,129.5 ± 575</b>
<b>3 mg/kg i.v. 30 min</b>	280.050	BLOQ	432.493	522.160	8,584.129	290.874	267.289
	198.815	BLOQ	878.773	298.165	741.482	159.052	147.772
	430.215	BLOQ	971.629	10,221.475	1,159.620	442.406	286.430
	<b>303 ± 67.8</b>	<b>0.00</b>	<b>760.9 ± 166</b>	<b>3,681 ± 3,271</b>	<b>3,495.1 ± 2,547.4</b>	<b>297.4 ± 81.9</b>	<b>233.8 ± 43.4</b>
<b>3 mg/kg i.v. 120 min</b>	28.597	BLOQ	86.932	91.717	180.585	432.949	629.827
	34.978	50.473	33.611	BLOQ	766.206	1,095.703	150.051
	28.939	BLOQ	56.397	BLOQ	48.764	2,834.450	194.257
	<b>30.8 ± 2.1</b>	<b>16.8 ± 16.8</b>	<b>58.9 ± 15.4</b>	<b>30.6 ± 30.6</b>	<b>331.8 ± 220.5</b>	<b>1,454.4 ± 716</b>	<b>324.7 ± 153</b>

Individual values for each mouse are listed; Means ± s.e.m appear in bold; N=3;  
A value of zero was used in calculating means when BLOQ

### **Plasma Kinetics, Tissue Distribution, Metabolism and Excretion of <sup>14</sup>C-JNJ-27018966 in Male Sprague-Dawley Rats After Single Oral and Subcutaneous Administration (FK5756)**

**Methods:** The purpose of the present study was to examine plasma kinetics, tissue distribution, metabolism and excretion of JNJ-27018966 in the male Sprague-Dawley rats after single oral and subcutaneous administration of <sup>14</sup>C-JNJ-27018966 (specific activity = 82.2 μCi/mg) at 50 and 25 mg/kg, respectively. Blood samples were collected from groups of three animals at 1, 3, 8, 24, 48 and 96 hours postdose. The following tissues were collected from three animals per time point: liver, brain, kidney, muscle, pancreas, stomach, small intestine, and large intestine without the cecum. Urine was collected from male rats at the following intervals: 0-4 h, 4-8 h, 8-24 h, 24-48 h, 48-72 h, and 72-96 h postdose. Feces were collected at 0-24 h and subsequent 24 h intervals up to 96 h postdose. Concentrations of total radioactivity (TR) were measured in various samples using a liquid scintillation counting (LSC). The following figure (from page 118 of the report) shows the position of radiolabel.

Structure and Position of Label:



**Results:** Concentrations of total radioactivity (TR) after SC dosing were higher than after PO dosing, except for the stomach. Brain TR concentrations were below the lower limit of quantification (LLOQ) after both PO and SC dosing. In general, higher tissue concentrations of TR were observed in the GI tract. The AUC<sub>0-t</sub> values of TR in the blood, plasma and tissues were higher after SC dosing compared to PO dosing, except for the stomach and the proximal and distal part of the large intestine, in which AUC<sub>0-t</sub> values were higher after PO dosing. Highest TR was observed in tissues of the GI tract and ranged from 16 to 525 μg eq.h/g for both routes of administration. Mean blood/plasma ratios of TR were about 0.6 at 1, 3 and 8 h after SC administration and could not be calculated after PO administration because of blood concentrations being below the LLOQ. After PO dosing about 0.5% of radioactivity was recovered in the urine and the major part of the radioactivity was excreted in the feces (97%). After SC dosing urinary excretion amounted to 7.3% of the administered dose and the excretion in feces amounted to 90%. The following table (from page 32 of Section 2.6.4 of the submission) shows the results.

**Table 2.6.4.4-1 AUC Values ( $\mu\text{g eq.h/g}$  of TR) and TR Concentration ( $\mu\text{g eq./g}$ ) in Blood, Plasma and Tissues in Male Sprague-Dawley Rats (n=3) Following Single Oral and Subcutaneous Dose Administration of  $^{14}\text{C}$ -Eluxadoline at 50 and 25 mg/kg, Respectively**

	AUC		TR (1 hour)		TR (3 hours)		TR (8 hours)	
	Oral	SC	Oral	SC	Oral	SC	Oral	SC
Blood	NC <sup>a</sup>	4.35 <sup>c</sup>	BLOQ <sup>f</sup>	1.15	BLOQ <sup>g</sup>	1.04	BLOQ	0.0456
Plasma	0.210 <sup>d</sup>	8.29 <sup>b</sup>	0.101	1.88	0.0615 <sup>f</sup>	1.81	BLOQ	0.0785
Liver	0.697 <sup>d</sup>	29.7 <sup>c</sup>	0.280	6.96	0.277 <sup>f</sup>	7.10	BLOQ	0.458
Brain	NC	NC	BLOQ	BLOQ	BLOQ	0.135 <sup>f</sup>	BLOQ <sup>f</sup>	BLOQ
Kidney	NC	181 <sup>b</sup>	BLOQ <sup>f</sup>	67.6	BLOQ <sup>f</sup>	28.7	BLOQ	1.52
Muscle	NC	0.471 <sup>d</sup>	BLOQ	0.203 <sup>f</sup>	BLOQ	0.168 <sup>f</sup>	BLOQ <sup>f</sup>	BLOQ
Pancreas	NC	1.23 <sup>d</sup>	BLOQ <sup>f</sup>	0.422 <sup>f</sup>	BLOQ	0.599	BLOQ	BLOQ
Stomach	278 <sup>c</sup>	74.1 <sup>b</sup>	74.7	38.6	59.4	4.50	4.67	0.578
Small Intestine								
Duodenum	102 <sup>c</sup>	418 <sup>c</sup>	21.9	265	22.1	38.5	2.80 <sup>f</sup>	0.935
(excl. duodenum)	525 <sup>c</sup>	498 <sup>e</sup>	79.9	8.15	137	57.2	13.7	41.3
Large Intestine Proximal	369 <sup>b</sup>	40.3 <sup>e</sup>	0.904	1.58	0.928	0.853	38.6	1.26 <sup>f</sup>
Large Intestine Distal	58.5 <sup>b</sup>	16.3 <sup>b</sup>	BLOQ <sup>f</sup>	1.75	0.992	0.737	3.46	BLOQ <sup>f</sup>

<sup>a</sup> NC : not calculated

<sup>b</sup> AUC<sub>0-24 h</sub>

<sup>c</sup> AUC<sub>0-8 h</sub>

<sup>d</sup> AUC<sub>0-3 h</sub>

<sup>e</sup> AUC<sub>0-48 h</sub>

<sup>f</sup> median value

<sup>g</sup> n=2

BLOQ = below limit of quantification; SC = subcutaneous; TR = total radioactivity

### **Binding of JNJ-27018966 to the Proteins of Mouse, Rat, Rabbit, Dog, Monkey and Human Plasma (FK6315)**

**Methods:** In this study, the binding of JNJ-27018966 to the proteins of rat, dog, mouse, monkey, and human plasma were determined. The plasma samples were spiked with JNJ-27018966-AAA at concentrations of 200, and 2000 ng/mL. Plasma protein binding was assessed using rapid equilibrium dialysis (RED) techniques.

**Results:** JNJ-27018966 was moderately bound to the plasma protein for all species, and the observed binding and the mean percent bound ranged from 68.47% (dog) to 87.79% (mouse). There were no concentration-dependent changes in protein binding in the plasma of all tested species. The rank order of observed plasma protein binding for all tested species tested was mouse > monkey > human > rabbit > rat > dog. Protein binding data for all species studied are shown below (from page 3 of the report).

Species Plasma Matrix	Plasma Concentration	
	200 ng/mL	2000 ng/mL
	Mean % Bound <sup>a</sup> (SD)	
Mouse	87.44 (0.53)	87.79 (0.55)
Rat	77.37 (2.96)	76.45 (1.28)
Rabbit	80.67 (2.52)	80.46 (1.09)
Dog	71.46 (1.73)	68.47 (3.17)
Monkey	85.92 (1.79)	85.73 (1.65)
Human	80.85 (1.49)	81.04 (1.79)

<sup>a</sup> N=4 observations per concentration for each species

### **Tissue distribution of <sup>14</sup>C-JNJ-27018966 by Whole Body Autoradiography in the Pigmented Male Rat (FK6706)**

**Methods:** This study was conducted to examine the tissue distribution of the total JNJ-27018966-related radioactivity in male pigmented Long Evans rats (n = 5), after single oral (gavage, 5 mL/kg) administration of <sup>14</sup>C-JNJ-27018966 (specific activity = 1.96 GBq/mmol) at 50 mg/kg using whole-body autoradiography (WBA) technique. Pigmented rat was used to include the examination of the distribution in melanin-rich tissues. The position of radiolabel was not provided. Blood and tissue samples were collected at 3, 7, 24, 48 and 168 h postdose. Blood radioactivity was determined using LSC.

**Results:** Limited distribution was observed in the non-pigmented tissues. Total radioactivity (TR) concentrations were below the LLOQ (0.083 µg/g) at all the time points for bone, bone marrow, brain, fat brown, fat white, harderian gland, lung, mammary gland, muscle, esophagus, salivary gland, skin white, testicle, thyroid and salivary gland. In some tissues, TR concentrations could only be quantified at one time point e.g., at 3 h for the heart, preputial gland and prostate and a 24 h in the rectum. In other tissues, TR levels were quantifiable up to 7 h only e.g., in the adrenal gland, kidney cortex, kidney medulla, pancreas, spleen and stomach tissue. In most non-pigmented tissues, TR levels declined at a similar or faster rate than that in the blood, except in the liver and urinary bladder, which showed 27% and 10%, respectively, of the maximum radioactivity value at 24 h post-dose. TR levels in the pigmented tissues were only observed in the pigmented tissue of the eye (uveal tract and eyeball), which were measurable (~ 24% of the maximum radioactivity) up to 168 hour post-dose. In contrast, TR concentrations in the pigmented skin and brain meninges were below the LLOQ. The following table (from page 9 of the report) shows the results.

**Table 6-1: Concentrations of total radioactivity (TR) after a single oral dose of <sup>14</sup>C-JNJ-27018966 at 50 mg/kg.**

Study No	FK6706						
Species	Long Evans Rat						
Feeding Condition	fed						
Vehicle/Formulation	0.5% Methocel suspension						
Route	PO (gavage)						
Gender (M/F)/Number of Animals	M/5						
Dose (mg/kg)	50 mg /kg						
Radionuclide	<sup>14</sup> C-JNJ-27018966						
Specific activity	0.234 MBq/mg						
Sampling times	3, 7, 24, 48, 168 h						
TR concentrations <sup>a)</sup>	Concentration (µg-eq. of [ <sup>14</sup> C]- JNJ-27018966/g <sup>c)</sup> )					C <sub>max</sub>	T <sub>max</sub>
Tissue or organ	3 h	7 h	24 h	48 h	168 h	(µg-eq./g <sup>c)</sup> )	(h)
Blood	0.092	<0.08	<0.08	<0.08	<0.08	0.092	3
Blood_LSC <sup>b)</sup>	0.067	0.037	<0.012	<0.012	<0.012	0.067	3
Plasma_LSC <sup>b)</sup>	0.092	0.051	<0.010	<0.010	<0.010	0.092	3
Adrenal gland	0.156	0.091	<0.08	<0.08	<0.08	0.156	3
Bone	<0.08	<0.08	<0.08	<0.08	<0.08	N.R. <sup>d)</sup>	N.R
Bone Marrow	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Brain	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Brain Meninges	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Eyeball_LSC <sup>b)</sup>	0.146	0.146	0.095	0.067	0.033	0.146	3-7
Fat Brown (Hib. gl.)	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Fat White	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Harderian gland	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Heart (myometrium)	0.095	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Kidney cortex	0.347	0.151	<0.08	<0.08	<0.08	0.347	3
Kidney medulla	0.361	0.124	<0.08	<0.08	<0.08	0.361	3
Liver	0.369	0.217	0.101	<0.08	<0.08	0.369	3
Lung	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Mammary gland	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Muscle	<0.08	<0.08	<0.08	<0.08	<0.08	N.R.	N.R
Pancreas	0.160	0.169	<0.08	<0.08	<0.08	0.169	3-7
Preputial gland	0.104	<0.08	<0.08	<0.08	<0.08	0.104	3
Prostate gland	0.134	<0.08	<0.08	<0.08	<0.08	0.134	3
Rectum <sup>e)</sup>	<0.08	<0.08	0.811	<0.08	<0.08	0.811	24
Salivary gland	<0.08	<0.08	<0.08	<0.08	<0.08	N.R	N.R
Skin, Pigmented	<0.08	<0.08	<0.08	<0.08	<0.08	N.R	N.R
Skin, White	<0.08	<0.08	<0.08	<0.08	<0.08	N.R	N.R
Spleen	0.646	0.094	<0.08	<0.08	<0.08	0.646	3
Stomach	0.960	0.417	<0.08	<0.08	<0.08	0.960	3
Testicle	<0.08	<0.08	<0.08	<0.08	<0.08	N.R	N.R
Thyroid	<0.08	<0.08	<0.08	<0.08	<0.08	N.R	N.R
Urinary bladder	2.90	0.668	0.287	<0.08	<0.08	2.90	3
Uveal Tract	1.49	2.10	1.52	0.952	0.504	2.1	7

**Additional Information:**

- a) Total radioactivity concentrations determined by radioluminography (RLG).  
b) Total radioactivity concentrations determined by liquid scintillation counting (LSC).  
c) Concentration expressed in µg-eq./ml for plasma.  
d) N.R. no result, due to insufficient data  
e) Rectum tissue TR concentration only at 24 h post-dose.

**Table 6-2: AUC-values and AUC tissue to blood ratios of total radioactivity (TR) after a single oral dose of <sup>14</sup>C-JNJ-27018966 at 50 mg/kg.**

Study No	FK6706			
Species	Long Evans Rat			
Feeding Condition	fed			
Vehicle/Formulation	0.5% Methocel suspension			
Route	PO (gavage)			
Gender (M/F)/Number of Animals	M/5			
Dose (mg/kg)	50 mg/kg			
Radionuclide	<sup>14</sup> C-JNJ-27018966			
Specific activity	0.234 MBq/mg			
Sampling times	3, 7, 24, 48, 168 h			
Tissue or organ	T <sub>last</sub> (h)	AUC <sub>last</sub> (µg-eq. h/g or ml <sup>c</sup> )	AUC <sub>0-7h</sub> (µg-eq. h/g or ml <sup>c</sup> )	AUC <sub>0-7h</sub> (µg-eq. h/g or ml <sup>c</sup> )
Blood	3	N.R.	N.R.	N.R.
Blood_LSC <sup>a)</sup>	7	0.309	0.309	1.00
Plasma_LSC <sup>a)</sup>	7	0.424	0.424	1.37
Adrenal gland	7	0.729	0.729	2.36
Bone	N.R. <sup>b)</sup>	N.R.	N.R.	N.R.
Bone Marrow	N.R.	N.R.	N.R.	N.R.
Brain	N.R.	N.R.	N.R.	N.R.
Brain Meninges	N.R.	N.R.	N.R.	N.R.
Eyeball_LSC <sup>a)</sup>	168	10.8	0.805	2.61
Fat Brown (Hib. gl.)	N.R.	N.R.	N.R.	N.R.
Fat White	N.R.	N.R.	N.R.	N.R.
Harderian gland	N.R.	N.R.	N.R.	N.R.
Heart (myometrium)	3	N.R.	N.R.	N.R.
Kidney cortex	7	1.52	1.52	4.92
Kidney medulla	7	1.51	1.51	4.89
Liver	24	4.43	1.73	5.60
Lung	N.R.	N.R.	N.R.	N.R.
Mammary gland	N.R.	N.R.	N.R.	N.R.
Muscle	N.R.	N.R.	N.R.	N.R.
Oesophagus	N.R.	N.R.	N.R.	N.R.
Pancreas	7	0.897	0.897	2.90
Preputial gland	3	N.R.	N.R.	N.R.
Prostate gland	3	N.R.	N.R.	N.R.
Rectum	24	N.R.	N.R.	2.68
Salivary gland	N.R.	N.R.	N.R.	N.R.
Skin, Pigmented	N.R.	N.R.	N.R.	N.R.
Skin, White	N.R.	N.R.	N.R.	N.R.
Spleen	7	2.45	2.45	7.93
Stomach	7	4.19	4.19	13.6
Testicle	N.R.	N.R.	N.R.	N.R.
Thyroid	N.R.	N.R.	N.R.	N.R.
Urinary bladder	24	19.6	11.5	37.2
Uveal Tract	168	157	9.43	30.5

**Additional Information:**

<sup>a)</sup> Total radioactivity concentrations determined by liquid scintillation counting (LSC).

<sup>b)</sup> N.R. no result

<sup>c)</sup> Ratio AUC<sub>0-7h</sub> calculated with the blood AUC<sub>0-7h</sub> value determined by LSC

**Tissue Distribution and Placental Transfer of <sup>14</sup>C-JNJ-27018966 Using Whole-Body Autoradiography in the Pregnant Rat (FK6707)**

**Methods:** The purpose of the present study was to examine the blood, tissue distribution and placental transfer of JNJ-27018966-related radioactivity in pregnant SD rats (n = 4) after a single SC dose of <sup>14</sup>C-JNJ-27018966 (specific activity = 1.96 GBq/mmol) at 25 mg/kg using whole body autoradiography (WBA). The position of radiolabel was not provided. Blood and tissue samples were collected at 1, 3, 7, and 24 h postdose. Blood radioactivity was determined using LSC.

**Results:** Concentrations of radioactivity were below the LLOQ of 0.142 µg eq./g at all the time points for the brain, fetus and fetal liver. In the blood, plasma and the other tissues, maximum concentrations were reached at 3 h and total radioactivity (TR) declined rapidly to below the LLOQ by 24 h post-dose. However, in the kidney, vagina and uterus, TR concentrations declined at a slower rate, and TR was still measurable at 24 h postdose. Plasma TR concentrations were 1.7 times higher than those of corresponding blood samples. In the non-reproductive organs, the highest AUC<sub>0-7h</sub> values of TR were observed in the kidney cortex, kidney medulla and liver with exposure levels of about 4, 3 and 2 times higher than that in the blood, respectively. The TR in the other tissues was lower than in the blood. In reproductive organs, the highest tissue to blood AUC<sub>0-7h</sub> ratios of 1.6 was observed in the uterine epithelium and lumen. In other reproductive organs (uterus, vagina, placenta, ovary and mammary gland), AUC<sub>0-7h</sub> values of TR were lower than those in the blood. Concentrations in the whole fetus and fetal liver were below the LLOQ levels. Exposures to the brain and fetal tissues were very limited due to the restriction of the blood brain and placental barriers, respectively. The following tables (from pages 10 and 11 of the report) show the results.

**Table 6-1: Concentrations of total radioactivity (TR) after a single subcutaneous dose of <sup>14</sup>C-JNJ-27018966 at 25 mg/kg.**

Study No	FK6707					
Species	Sprague-Dawley pregnant rat					
Feeding Condition	fed					
Vehicle/Formulation	10 % HPBCD					
Route	SC (subcutaneous))					
Gender (M/F)/Number of Animals	F/4					
Dose (mg/kg)	25 mg /kg					
Radionuclide	<sup>14</sup> C-JNJ-27018966					
Specific activity	0.200 MBq/mg					
Sampling times	1, 3, 7 and 24 h					
TR concentrations <sup>a)</sup>	Concentration (µg-eq. of [ <sup>14</sup> C]- JNJ-27018966/g <sup>c)</sup> )				C <sub>max</sub>	T <sub>max</sub>
Tissue or organ	1 h	3 h	7 h	24 h	(µg-eq./g <sup>c)</sup>	(h)
Blood (cardiac)	2.52	3.45	0.566	< 0.142	3.45	3
Blood_LSC <sup>b)</sup>	1.46	2.15	0.25	< 0.014	2.15	3
Plasma <sup>b)</sup>	2.39	3.57	0.423	0.015	3.57	3
Adrenal gland	1.00	1.61	0.372	< 0.142	1.61	3
Brain	< 0.142	< 0.142	< 0.142	< 0.142		
Fat, Brown	0.848	1.55	0.234	< 0.142	1.55	3
Fat, White	< 0.142	0.415	< 0.142	< 0.142	0.415	3
Fetal Liver	< 0.142	< 0.142	< 0.142	< 0.142		
Fetus	< 0.142	< 0.142	< 0.142	< 0.142		
Heart	0.713	0.860	0.15	< 0.142	0.86	3
Kidney cortex	9.98	12.5	2.78	0.380	12.5	3
Kidney medulla	7.00	9.46	2.18	< 0.142	9.46	3
Liver	5.35	6.22	1.6	< 0.142	6.22	3
Lung	2.09	2.58	0.58	< 0.142	2.58	3
Mammary gland	0.557	0.761	0.224	< 0.142	0.761	3
Muscle	0.193	0.236	< 0.142	< 0.142	0.236	3
Ovary	0.559	0.975	0.213	< 0.142	0.975	3
Pancreas	0.924	0.874	0.243	< 0.142	0.924	1
Placenta	0.748	1.419	0.240	< 0.142	1.42	3
Spleen	0.469	0.679	0.244	< 0.142	0.679	3
Uterine epithelium and lumen	1.67	5.25	2.94	2.27	5.25	3
Uterus	1.15	1.95	0.258	< 0.142	1.95	3
Vagina	0.934	1.49	0.214	0.271	1.49	3

**Additional Information:**

- a) Total radioactivity concentrations determined by radioluminography (RLG).  
b) Total radioactivity concentrations determined by liquid scintillation counting (LSC).  
c) Concentration expressed in µg-eq./ml for plasma.

**Table 6-2: AUC-values and AUC tissue to blood ratios of total radioactivity (TR) after a single subcutaneous dose of <sup>14</sup>C-JNJ-27018966 at 25 mg/kg.**

Study No	FK6707			
Species	Sprague-Dawley pregnant rat			
Feeding Condition	fed			
Vehicle/Formulation	10 % HPBCD			
Route	SC (subcutaneous))			
Gender (M/F)/Number of Animals	F/4			
Dose (mg/kg)	25 mg /kg			
Radionuclide	<sup>14</sup> C-JNJ-27018966			
Specific activity	0.200 MBq/mg			
Sampling times	1, 3, 7 and 24 h			
Tissue or organ	Tlast (h)	AUClast (µg-eq. h/g or ml)	AUC <sub>0-7h</sub>	AUC <sub>0-7h</sub> (µg-eq. h/g or ml <sup>c</sup> )
Blood (cardiac)	7	15.2	15.2	1.00
Blood_LSC <sup>a)</sup>	7	9.15	9.15	0.60
Plasma <sup>a)</sup>	24	18.9	15.1	0.99
Adrenal gland	7	7.06	7.06	0.46
Brain	N.R. <sup>b)</sup>	N.R.	N.R.	N.R.
Fat, Brown	7	6.39	6.39	0.42
Fat, White	3	0.415	N.R.	N.R.
Fetal Liver	N.R.	N.R.	N.R.	N.R.
Fetus	N.R.	N.R.	N.R.	N.R.
Heart	7	3.95	3.95	0.26
Kidney cortex	24	84.8	57.9	3.80
Kidney medulla	7	43.3	43.3	2.84
Liver	7	29.9	29.9	1.96
Lung	7	12.0	12.0	0.79
Mammary gland	7	3.57	3.57	0.23
Muscle	3	0.526	N.R.	13.7 <sup>d)</sup>
Ovary	7	4.19	4.19	0.27
Pancreas	7	4.49	4.49	0.29
Placenta	7	5.86	5.86	0.38
Spleen	7	3.23	3.23	0.21
Uterine (epithelium and lumen)	24	68.3	24.1	1.58
Uterus	7	8.08	8.08	0.53
Vagina	24	10.4	6.30	0.41

**Additional Information:**

<sup>a)</sup> Total radioactivity concentrations determined by liquid scintillation counting (LSC).

<sup>b)</sup> N.R. no result

<sup>c)</sup> Ratio AUC<sub>0-7h</sub> calculated with the blood AUC<sub>0-7h</sub> value determined by Blood (cardiac) RLG.

<sup>d)</sup> Ratio AUC<sub>0-3h</sub> calculated with the blood AUC<sub>0-3h</sub> value [7.22 µg eq.h/g] determined by Blood (cardiac) RLG

**Solubility, Metabolism, Permeability, Protein Binding and Red Blood Cell Binding (RWJ-P01)**

**Methods:** This study was conducted to determine the following: the bi-directional permeability of JNJ-27018966 using Caco-2 monolayer cells, the extent of protein binding using equilibrium dialysis and the extent of red blood cell (RBC) binding.

**Results:** JNJ-27018966 showed low absorption potential and no significant efflux in Caco-2 cells. In the equilibrium dialysis study, JNJ-27018966 showed moderate protein binding with human plasma (82%) and moderate binding in human serum albumin (HAS, 66%) and low binding with  $\alpha$ 1-acid glycoprotein (AGP, 13.8%). JNJ-27018966 showed negligible partitioning into red blood cells. The following table (from page 7 of the report) shows the protein binding data.

Table 6.1 Protein Binding via Equilibrium Dialysis

Test Article Identification	Submitter/ Team	Matrix	Donor			Receiver			Average %Recovery	Average %Bound
			Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3		
27018966-23086098	(b) (6)	Human Plasma	7.20	7.68	7.70	1.24	1.42	1.35	88.6	82.2
		HSA	4.99	5.08	5.10	1.69	1.75	1.79	68.0	65.5
		AGP	4.30	5.02	4.85	3.66	4.43	4.13	88.0	13.8

## Metabolism

### In Vivo Metabolism of JNJ-27018966 in Rats and Monkeys (FK5858)

**Methods:** In vivo metabolism of JNJ-27018966 was examined in the plasma, urine and fecal samples from JNJ-27018966 treated SD rats (n = 4, 2/sex, oral/SC dose of 500/15 mg/kg/day) and Cynomolgus monkeys (n = 2, 1/sex, oral/SC dose of 100/25 mg/kg/day). Following a single oral/SC dose of JNJ-27018966 (500/15 mg/kg/day), blood samples were collected at 1 and 4 hours postdose. Urine and feces were collected at approximately 0-8 and 8-24 hours postdose. Following a single oral/SC dose of JNJ-27018966 (100/25 mg/kg; 5-mL/kg for oral and 2 mL/kg for SC), blood samples were collected at 45 minutes postdose on Day 2. Urine and feces were collected from 0 to 8 hours and continued until approximately 24 hours post-dose on Day 2.

### Results:

**Plasma:** The unchanged drug was the major component in the plasma and accounted for 95-99% of total drug-derived components in both sexes in both species. Metabolite M2, from hydrolysis of 3,5-dimethyl benzamide moiety, was the only metabolite identified in the rat plasma and accounted for 1% in both sexes. Metabolite M2 was also detected in the monkey plasma and accounted for 3% and 2% in male and female monkey plasma, respectively. The acyl glucuronide (metabolite M11) was only detected in the plasma samples from monkeys (male: 2% and female: <1%). There was no qualitative gender difference in the plasma metabolite profiles of JNJ-27018966 in rats and monkeys.

**Urine:** The unchanged drug was the major component in the rat and monkey urine in both sexes (from 94% to 99%). The following metabolites were present at  $\geq 1\%$  in the urine of monkeys: M2, M11 and M12 (monooxygenation of 1,2,3-trimethyl-benzene moiety). Metabolites M2 and M11 were detected in all urine samples from rats and monkeys and the acyl glucuronide metabolite (M11) was present in greater amount in females for both rats and monkeys (female rats 3% and female monkeys 4%).

**Feces:** The unchanged drug was the major drug-derived components in the rat and monkey fecal samples (from 95% to 98%). The metabolites M2 and M12 were identified in the rat and monkey fecal samples from both sexes and greater amount of M2 was detected in female monkey feces (4%). Only M4 from monooxygenation of 2-ethyl-4-phenyl-1-H-imidazole moiety was identified in both male and female rat fecal samples.

Overall, the unchanged drug was the major component in systemic circulation in both species and genders. The unchanged drug was also the major component in the urine and fecal samples from rats and monkeys. There was no apparent qualitative gender difference in metabolism of JNJ-27018966 in rats and monkeys. However, there was qualitative species difference in metabolism, with M7 and M16 specifically formed in monkeys but not in rats. All the metabolites generated by the human hepatocytes were also seen in the rat and monkey. The in vivo metabolic pathways for rats and monkeys consisted of oxygenation (carbon and nitrogen), further oxidation to keto, amide hydrolysis, and acyl glucuronidation. The following table (from page 29 of the report) shows JNJ-27018966 and its metabolites in the plasma, urine and fecal samples from both sexes of rats and monkeys.

Compounds	Rats						Monkeys					
	1 Hour Plasma		0-8 Hour Urine		8-24 Hour Feces		45 Min Plasma		0-8 Hour Urine		8-24 Hour Feces	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Unchanged drug	99	99	99	95	97	98	95	97	94	94	97	95
Carboxy (M2)	1	1	<1	1	1	<1	3	2	1	<1	1	4
Monooxygenated (M4)	ND	ND	ND	ND	<1	<1	ND	ND	<1	<1	ND	ND
Amide hydrolysis (M7)	ND	ND	ND	ND	ND	ND	ND	ND	<1	<1	ND	ND
Acyl glucuronide (M11)	ND	ND	<1	3	ND	ND	2	<1	1	4	ND	ND
Monooxygenated (M12)	ND	ND	ND	ND	1	<1	ND	ND	3	<1	1	1
Hydroxamic acid (M16)	ND	ND	ND	ND	ND	ND	ND	ND	ND	<1	ND	ND
Carbonyl (M15)	ND	ND	ND	<1	ND	ND	ND	ND	<1	<1	ND	ND

ND = not detected

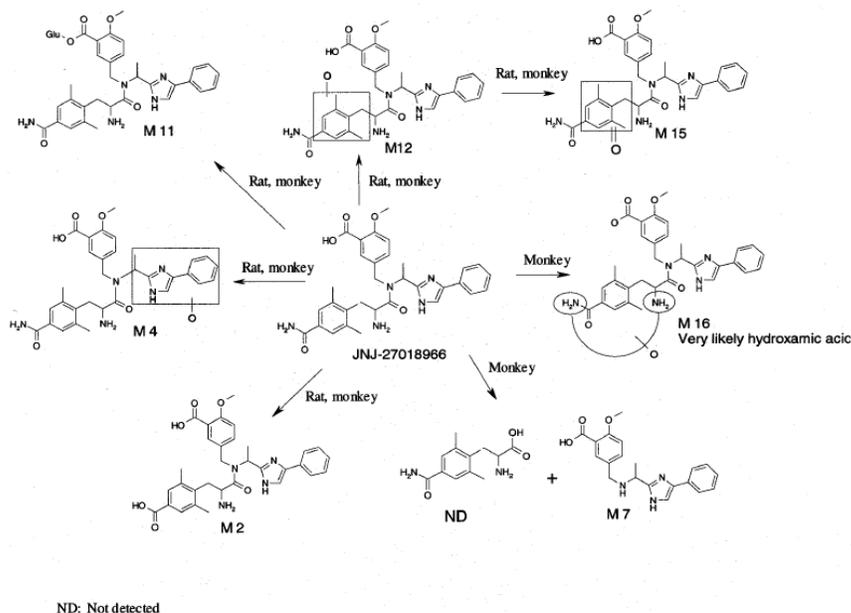
Urine (8-24hr) and fecal (0-8hr) samples were also analyzed but only data corresponding to urine (0-8hr) and fecal (8-24hr) samples are tabulated

The percent of each component is estimated from integrated peak areas in reconstructed ion chromatograms with assumption of equal ionization efficiencies and no suppression of ionization by co-eluting components from matrix. Data is only for qualitative use only.

The possible metabolic pathways of JNJ-27018966 in rats and monkeys are shown in the following figure (from page 8 of the report).

Study No. FK5858

## Proposed In Vivo Metabolic Pathways of JNJ-27018966 in Rats and Monkeys



### In Vivo Metabolism of JNJ-27018966 in Dead Female Rhesus Monkey from Discovery Study of Abuse Liability (FK5865)

**Methods:** In vivo metabolism of JNJ-27018966 was examined in the brain, liver, blood, cerebral spinal fluid (CSF), urine and bile from a dead female Rhesus monkey from the discovery study (study number was not mentioned) of potential abuse liability of JNJ-27018966. Portions of liver and brain were removed in addition to CSF, aqueous humor, blood, bile and urine.

**Results:** The unchanged drug was the major component in most monkey tissues and accounted for 46%, 62%, 67%, and 50% of total drug-derived components in the liver, CSF, urine, and bile, respectively, and relatively lower in the brain (24%) and blood (25%). The major metabolic pathway of JNJ-27018966 in the monkey brain, liver, blood and bile was glucuronidation of methoxy-benzoic acid moiety to form acyl glucuronide metabolite M11. Hydrolysis of the 3,5-dimethyl-benzamide moiety to form 3,5-dimethylbenzoic acid metabolite M2 was the major drug-derived component in the CSF. Monooxygenated metabolite (M12) from the addition of oxygen to 1,2,3-trimethylbenzene moiety was the major metabolite in the monkey urine. M4, another monooxygenated metabolite from addition of oxygen to 2-ethyl-4-phenyl-1-H-imidazole moiety, was detected in all tissue samples from the monkey. M12 and M4 could further undergo glucuronidation of the methoxy-benzoic acid moiety to form monooxygenated acyl glucuronide metabolites M8 and M6, respectively. M9 and M3 were formed due to further oxygenation of the 3,5-dimethylbenzene group and 2-ethyl-4-phenyl-1-H-imidazole moiety of M12 respectively, to form dioxygenated metabolites. In addition, M1 was identified as glucuronide of M2 and the glucuronidation occurred on the methoxy

benzoic acid moiety. M15 was a keto metabolite from further oxidation of monooxygenated metabolite M12.

In summary, all the metabolites generated by the human hepatocytes were also identified in the dead female Rhesus monkey. The major metabolic pathway of JNJ 27018966 in the dead female Rhesus monkey was direct glucuronidation of the methoxy-benzoic acid moiety to form acyl glucuronides.

The following table (from page 33 of the report) shows the JNJ-27018966 and its metabolites in various tissues from the dead female Rhesus monkey.

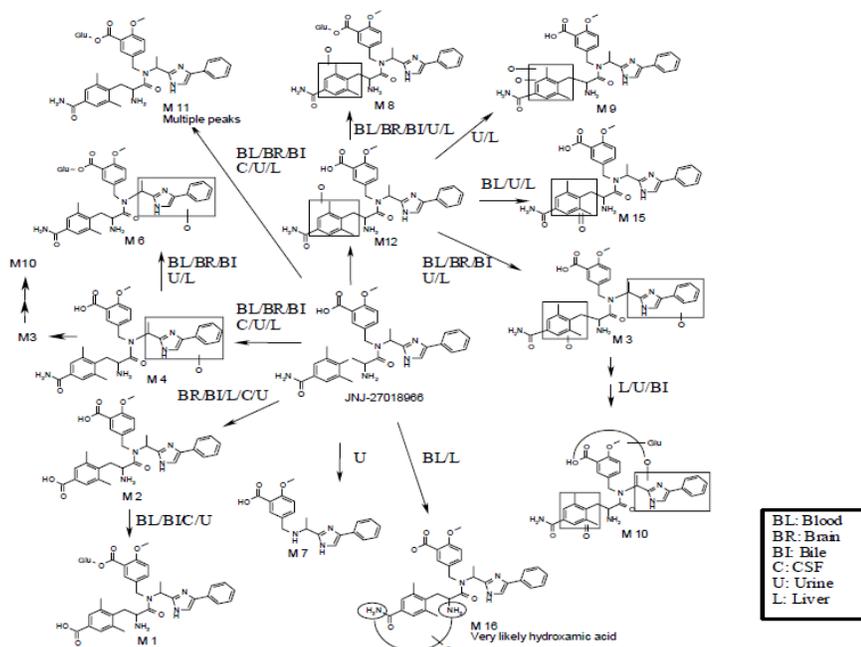
Test Article: JNJ-27018966

Metabolites of JNJ-27018966 identified in biological matrices from dead female rhesus monkey						
Metabolite	Brain	Liver	Blood	CSF	Urine	Bile
Unchanged drug	24	46	25	62	67	50
Glucuronide of M2 (M1)	ND	ND	3	2	<1	<1
Carboxy (M2)	4	7	ND	20	11	12
Dioxygenated (M3)	<1	<1	<1	ND	<1	<1
Monooxygenated (M4)	1	2	6	1	2	6
Glucuronide of M4 (M6)	3	<1	<1	ND	<1	<1
Glucuronide of M12 (M8)	3	ND	5	ND	<1	1
Dioxygenated (M9)	ND	1	ND	ND	<1	1
Glucuronide of M13 (M10)	ND	<1	ND	ND	ND	<1
Acyl glucuronide (M11)	59	33	55	4	7	19
Monooxygenated (M12)	4	7	6	12	12	10
Carbonyl (M15)	ND	<1	<1	ND	<1	ND
Hydroxamic acid (M16)	ND	<1	<1	ND	ND	ND

ND = not detected  
 The percent of each component is estimated from integrated peak areas in reconstructed ion chromatograms with assumption of equal ionization efficiencies and no suppression of ionization by co-eluting components from matrix. Data is for qualitative use only.

The possible metabolic pathway is shown below (from page 8 of the report).

Proposed in vivo metabolic pathways of JNJ-27018966 in rhesus monkey



### **In Vitro Stability Studies of Acyl Glucuronide of JNJ-27018966 (FK5944)**

Acyl glucuronide of JNJ-27018966 was identified as the major metabolite in rats and monkeys. Acyl glucuronides are unstable metabolites that can undergo both spontaneous hydrolysis and intramolecular acyl migration at physiological conditions. This study was conducted to assess the stability of acyl glucuronide under physiological conditions and to identify the primary configuration of the acyl glucuronide in the monkey urine samples. Another objective was to investigate the metabolism of JNJ-27018966 by human intestinal microsomes.

In vitro biosynthesis of acyl glucuronide: JNJ-27018966 (10  $\mu$ M final concentration) was incubated for 1 hour at 37°C with human liver microsomes. The reaction was stopped by addition of acetonitrile and then centrifuged at 3000 rpm for 10 minutes.

Hydrolysis 1-O- $\beta$ -acyl glucuronide with  $\beta$ -glucuronidase: Monkey urine was added to buffered  $\beta$ -glucuronidase solution, and subsequently incubated for 1 and 4 hours at 37°C. The reaction was terminated by acetonitrile. The resultant supernatant from centrifugation was evaporated to dryness followed by reconstitution, and then filtration and the filtrate was used for liquid chromatography/mass spectrometry (LC/MS) analysis.

1-O- $\beta$ -Acyl Glucuronide Chemical Degradation Half-Life Determination: Monkey urine (pooled 0-8 hour samples from oral/SC dose at 100/25 mg/kg of JNJ-27018966) was incubated in phosphate buffer at 37°C. Aliquots were withdrawn at 0, 2, 4, 8, 48 and 72 hours and processed for sample preparation as described above. These samples were analyzed for the determination of chemical degradation half-life of the 1-O- $\beta$ -acyl glucuronide of JNJ-27018966.

In Vitro Metabolism by Human Intestinal Microsomes: JNJ-27018966 (10  $\mu$ M final concentration) was incubated for 1 hour at 37°C with human intestinal microsomes in potassium phosphate buffer. The reaction was stopped by the addition of acetonitrile and then centrifuged. The resultant supernatant from centrifugation was used for sample preparation as described above.

**Results:** The primary acyl glucuronide in the monkey urine was 1-O- $\beta$ -acyl glucuronide from the hydrolysis with  $\beta$ -glucuronidase. In the monkey urine, the degradation half-life of 1-O- $\beta$ -acyl glucuronide was determined to be 4.3 hours. JNJ-27018966 was metabolized by human intestinal microsomal systems to acyl glucuronide (M11), M4 (monooxygenation) and M2 (amide hydrolysis). The acyl glucuronide was the major metabolite detected in the human intestinal microsomal system.

## **Excretion**

### **Biliary Excretion of <sup>14</sup>C-JNJ-27018966 in Male Sprague-Dawley Rats after a Single Oral Administration (FK6432)**

**Methods:** The objective of this study was to examine the formation and to isolate the acyl-glucuronide M11 metabolite. In this study, rats were treated orally with <sup>14</sup>C-JNJ-27018966 at 500 mg/kg (single dose). Bile was collected at 48 h post-dose.

**Results:** Analysis of the bile showed that M11 was not present and therefore could not be isolated from the bile. The study was stopped and classified as "Terminated study". The following table (from page 1 of the memo, raw data) shows an average of 1.70% of the dose was recovered from the bile collected during the 48-hours post-dose.

Table 1: The biliary excretion of <sup>14</sup>C-JNJ-27018966 in male SPF Sprague-Dawley rats after a single oral administration of <sup>14</sup>C-JNJ-27018966 at 500 mg/kg

Specimen	% administered dose recovered
Rat 1	(b) (4)
Rat 2	(b) (4)
Rat 3	(b) (4)
Rat 4	(b) (4)

## **5.2 Toxicokinetics**

Included in the reviews of the individual toxicology study reports

## **6 General Toxicology**

### **6.1 Single-Dose Toxicity**

#### **Acute Oral and Intraperitoneal Toxicity Study of JNJ-27018966-AAA in Mice (Study TOX7687)**

Report No.	Testing Laboratory	Species & Route	Date Started	Date Completed	Batch No.
TOX7687	Johnson & Johnson, Raritan, NJ	CD-1 mouse, Oral, Intraperitoneal (IP)	3/13/2006	5/16/2007	JNJ-27018966-AAA, (b) (4) form of JNJ-27018966 (Batch No: 30205959)

**GLP Compliance:** Statements of compliance with GLP regulations and the quality assurance unit (QAU) were included.

**Methods:** Fifty mice (5/sex/group) were administered a single oral dose of vehicle (0.5% hypromellose solution) or JNJ-27018966 at 250, 500, 1000, or 2000 mg/kg. Another fifty mice (5/sex/group) were administered a single intraperitoneal (IP) dose of the same vehicle or JNJ-27018966 at doses of 62.5, 125, 250, or 500 mg/kg. The mice were observed for up to 14 days after dosing. Assessments included mortality, clinical observations, body weight, and gross pathology.

## **Results:**

### **Oral Administration**

There were no treatment related effects on body weight or gross pathology findings at necropsy at any dose. Although there were no deaths at 250, 500, or 2000 mg/kg, three mice (1 female and 2 males) at 1000 mg/kg died on Days 1, 2, and 3, respectively. Treatment related clinical signs included (at 1000 mg/kg) in mice died on study included decreased activity, decreased rate and/or depth of respiration, urine-stained/unkempt coat, hunched posture, body cold to touch, loss of righting reflex, ptosis, prostration, and absence of feces. Of those 1000-mg/kg mice that survived to scheduled necropsy one mouse showed signs of urine-stained/unkempt coat and distended abdomen, while the other 6 mice exhibited no clinical signs of toxicity. The maximum non-lethal oral dose of JNJ-27018966 was 500 mg/kg.

### **Intraperitoneal Administration**

There were no deaths or treatment related gross pathology findings after IP administration at any dose. There were no treatment related clinical signs at 62.5 mg/kg. At 125 and 250 mg/kg, distended abdomen was observed. On Days 1 and 2, CNS signs included decreased activity at doses  $\geq$  125 mg/kg and increased activity with or without circling at doses  $\geq$  250 mg/kg. Urine-stained/unkempt coat and hunched posture were considered to be treatment related at doses  $\geq$  250 mg/kg. Most of these signs were observed on Days 1 and 2 and dissipated during the remaining days of the study. There was no significant treatment related effect on mean body weight, but mean body weight gain was lower for female mice at 125 and 250 mg/kg, and for males and females at 500 mg/kg. The maximum non-lethal IP dose of JNJ-27018966 was 500 mg/kg.

### **Acute Oral and Intraperitoneal Toxicity Study of JNJ-27018966-AAA in Rats (Study TOX7688)**

Report No.	Testing Laboratory	Species & Route	Date Started	Date Completed	Batch No.

TOX7688	Johnson & Johnson, Raritan, NJ	SD rats, Oral, Intraperitoneal (IP)	3/13/2006	8/1/2007	JNJ-27018966-AAA, (b) (4) form of JNJ-27018966 (Batch No: 30205959)
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**GLP Compliance:** Statements of compliance with GLP regulations and the quality assurance unit (QAU) were included.

**Methods:** Fifty rats (5/sex/group) were administered a single oral (gavage) dose of vehicle (0.5% hypromellose solution) or JNJ-27018966 at 250, 500, 1000, or 2000 mg/kg. Another fifty rats (5/sex/group) were treated a single intraperitoneal (IP) dose of the same vehicle or JNJ-27018966 at doses of 31.25, 62.5, 125, or 250 mg/kg. The mice were observed for up to 14 days after dosing. Assessments included mortality, clinical observations, body weight, and gross pathology.

### **Results:**

#### **Oral Administration**

There were no treatment related mortalities. No treatment related clinical signs were observed at  $\leq 1000$  mg/kg. At 2000 mg/kg, treatment related clinical signs included decreased feces in females. There were no treatment related gross pathology findings at any dose level. The maximum non-lethal oral dose of JNJ-27018966 was 2000 mg/kg in both sexes.

#### **Intraperitoneal Administration**

No mortalities were observed at doses  $\leq 62.5$  mg/kg. At 125 mg/kg, 1 male was found dead on Day 1. At 250 mg/kg, 2 males and 2 females were found dead on Days 1 to 2. Clinical signs at 31.25 mg/kg were limited to decreased feces. At  $\geq 62.5$  mg/kg, ataxia, decreased activity, and mydriasis were seen. At doses  $\geq 125$  mg/kg, prostration, decreased feces, watery ocular discharge, decreased depth and rate of respiration, pallor, and unkempt coat (males) were observed. At 250 mg/kg, clinical signs included cold-to-touch and absent feces. In decedent animals, there were treatment related necropsy findings at  $\leq 62.5$  mg/kg. Abdominal adhesions were seen in one animal at 250 mg/kg that was found dead on Day 2. At scheduled necropsy on Day 15, treatment related finding was abdominal adhesions in one rat at 125 mg/kg. The maximum non-lethal IP dose was 62.5 mg/kg in males and 125 mg/kg in females.

## **6.2 Repeat-Dose Toxicity**

### **Mouse**

**Study title: 28-Day Oral Range-Finding Toxicity and Toxicokinetic Study in Mice**

Study no.: 1808-001  
 Study report location: EDR 4.2.3.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: December 14, 2009  
 GLP compliance: Non-GLP  
 QA statement: No  
 Drug, lot #, and % purity: JNJ-27018966-AAA, Lot No. 21298-99A, 94.6%

**Methods:** This is a non-GLP dose ranging study and was conducted in two phases (Phase A and TK Phase). In Phase A, three treatment groups of 15 male and 15 female CD-1 mice were administered the test article at 500, 1000, and 2000 mg/kg/day. One additional group of 15 animals per sex served as the control and received the vehicle, 0.5% hydroxypropyl methylcellulose (HPMC) in water. The vehicle or the test article was administered to all groups via oral gavage, once daily for 28 consecutive days, at a dose volume of 5 mL/kg. In the TK phase of the study, four groups of 39 mice per sex per group were administered the test article once daily for 6 consecutive days via oral gavage at 500, 1000, 2000, and 3000 mg/kg/day (5, 5, 5, and 7.5 mL/kg, respectively). The following table (from page 12 of the report) shows the study design.

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
<b>Phase A<sup>a</sup></b>			
1	0	15	15
2	500	15	15
3	1000	15	15
4	2000	15	15
<b>TK Phase<sup>b</sup></b>			
5	500	36 + 3 <sup>c</sup>	36 + 3 <sup>c</sup>
6	1000	36 + 3 <sup>c</sup>	36 + 3 <sup>c</sup>
7	2000	36 + 3 <sup>c</sup>	36 + 3 <sup>c</sup>
8	3000 <sup>d</sup>	36 + 3 <sup>c</sup>	36 + 3 <sup>c</sup>

<sup>a</sup>Once daily dosing for 28 consecutive days.  
<sup>b</sup>Once daily dosing for 6 consecutive days.  
<sup>c</sup>Additional animals for use as possible replacements.  
<sup>d</sup>400 mg/mL concentration administered at a dose volume of 7.5 mL/kg.

Mortality was observed twice daily. Clinical signs were observed once daily for the first 7 days and once weekly thereafter for Phase A animals, and once daily for animals in the TK phase. Body weights and food consumption were recorded once daily for the first 7 days and once weekly thereafter for Phase A animals, and once daily for animals in the TK Phase. Blood and urine samples for clinical pathology evaluations were collected from Phase A animals prior to the scheduled terminal necropsy. Blood samples for TK analysis were collected from TK Phase animals at 0.5, 1, 2, 4, 8, and 24 hours postdose on Days 1 and 6. For Phase A, necropsy examinations were performed and organ

(brain, gastrointestinal tract, ovary, testis, heart, kidney and liver) weights were recorded.

**Results:** All animals in Phase A survived to terminal necropsy with the exception of one male at 500 mg/kg/day, which was found dead on Day 7 and two males at 2000 mg/kg/day were found dead on Days 23 and 11. During the TK phase, one male at 2000 mg/kg/day was found dead on Day 4 and one female at 3000 mg/kg/day was euthanized in extremis on Day 3. There were no significant treatment related clinical signs. There were no significant treatment related effects on body weight or food consumption, clinical pathology, organ weight, or macroscopic findings.

On Day 1, there was no apparent relationship between dose and  $C_{max}$ . On Day 6,  $C_{max}$  increased with increasing dose. There was no apparent drug accumulation after once daily dosing for 6 days. There was an apparent sex difference in exposure. On Day 6, females showed higher total exposures than males at all doses. Terminal half-lives ranged from about 3 to 8 hours. The following tables (from page 254 and 255 of the report) show the TK parameters.

**Table 1.1 Toxicokinetic Parameters for JNJ-27018966-AAA in Mice after Oral Administration of JNJ-27018966-AAA on Days 1 and 6 (Excluding Outliers)**

Day	Dose (mg/kg/day)	$C_{max}$ (ng/mL)	$T_{max}$ (hr)	AUC(0-t) (ng*hr/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose ((ng*hr/mL)/(mg/kg))	AUC(0-inf) (ng*hr/mL)	Alpha (1/hr)	Beta (1/hr)	T1/2alpha (hr)	T1/2beta (hr)	AR
1	500	76.50	1.00	173.02	212.78	0.43	-	-	-	-	-	-
	1000	42.40	4.00	191.98	295.98	0.30	-	-	-	-	-	-
	2000	54.80	1.00	152.68	255.88	0.13	-	-	-	-	-	-
	3000	59.40	1.00	326.40	326.40	0.11	336.08	-	0.1399	-	4.96	-
6	500	11.72	0.50	63.88	114.92	0.23	-	-	-	-	-	0.54
	1000	28.50	0.50	86.93	150.41	0.15	-	-	-	-	-	0.51
	2000	37.15	4.00	194.39	309.99	0.15	-	-	-	-	-	1.21
	3000	36.85	0.50	129.20	247.20	0.08	-	-	-	-	-	0.76

(-) Indicates not applicable or could not be calculated.

TK analysis was carried out based on median of 3 samples/gender at each timepoint. Samples from following timepoints: Day 1: 4 hr in Group 1 (males), 1 hr in Group 2 (females), 4 hr in Group 3 (females) and 8 hr in Group 4 (females); Day 6: 4 hr in Group 1 (females) and 1 hr in Group 2 (females) were excluded due to extreme outliers.

**Table 1.2 Toxicokinetic Parameters for JNJ-27018966-AAA in Mice by Sex after Oral Administration of JNJ-27018966-AAA on Days 1 and 6 (Excluding Outliers)**

Day	Dose (mg/kg/day)	Sex	Cmax (ng/mL)	Tmax (hr)	AUC(0-t) (ng*hr/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose ((ng*hr/mL)/(mg/kg))	AUC(0-inf) (ng*hr/mL)	Alpha (1/hr)	Beta (1/hr)	T1/2alpha (hr)	T1/2beta (hr)	AR
1	500	Female	122.00	1.00	207.71	245.95	0.42	-	-	-	-	-	-
		Male	23.90	1.00	88.76	130.04	0.18	-	-	-	-	-	-
	1000	Female	78.60	4.00	286.25	341.93	0.29	-	-	-	-	-	-
		Male	32.60	0.50	100.00	212.80	0.10	-	-	-	-	-	-
2000	Female	Female	76.20	0.50	178.60	290.60	0.09	325.36	-	0.0954	-	7.27	-
		Male	85.70	1.00	278.60	278.60	0.14	300.81	-	0.0932	-	7.44	-
	3000	Female	58.20	1.00	308.61	308.61	0.10	-	-	-	-	-	-
		Male	64.80	0.50	335.60	335.60	0.11	344.71	-	0.1426	-	4.86	-
6	500	Female	26.70	0.50	80.22	138.06	0.16	-	-	-	-	-	0.56
		Male	9.08	0.50	55.62	94.42	0.11	109.22	-	0.0905	-	7.66	0.73
	1000	Female	139.00	0.50	215.63	306.03	0.22	-	-	-	-	-	0.90
		Male	21.90	0.50	77.22	106.50	0.08	91.83	-	0.2506	-	2.77	0.50
	2000	Female	91.80	0.50	319.90	427.10	0.16	-	-	-	-	-	1.47
		Male	23.10	1.00	113.43	237.43	0.06	-	-	-	-	-	0.85
	3000	Female	52.40	4.00	393.37	393.37	0.13	-	-	-	-	-	1.27
		Male	94.20	2.00	216.40	318.00	0.07	-	-	-	-	-	0.95

(-) Indicates not applicable or could not be calculated.

TK analysis was carried out based on median of 3 samples/gender at each timepoint. Samples from following timepoints: Day 1: 4 hr in Group 1 (males), 1 hr in Group 2 (females), 4 hr in Group 3 (females) and 8 hr in Group 4 (females); Day 6: 4 hr in Group 1 (females) and 1 hr in Group 2 (females) were excluded due to extreme outliers.

**Summary:** In a 28-day oral (gavage) dose ranging study in CD-1 mice, animals were treated with JNJ-27018966 at 500, 1000 and 2000 mg/kg/day. One Phase A male at 500 mg/kg/day died on Day 7 and two males at 2000 mg/kg/day were found dead on Days 23 and 11. There were no significant treatment related effects on body weight or food consumption, clinical pathology, organ weight, or macroscopic findings.

### **13-Week Oral Toxicity Study in Mice (Study No. 1808-006)**

The review of the above study is incorporated below from pharmacology review of IND 79,214 dated March 17, 2011.

**Study title: 13-Week Oral Toxicity Study of JNJ-27018966-AAA in Mice (Draft Report)**

Study no.:	1808-006
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 29, 2010
GLP compliance:	Yes. GLP statement was not signed
QA statement:	Yes. QAU statement was not signed
Drug, Batch #, and % purity:	JNJ-27018966-AAA, 0020297431. Purity data not provided.

**Key Study Findings:**

- There were no mortalities in the main study animals. However, three TK animals died.
- Body weight and food consumption were not affected.
- The target organ could not be identified in the absence of any significant organ toxicity or histopathology findings.
- The NOAEL appeared to be 1500 mg/kg/day.

**Methods:**

Doses:	500, 1000, and 1500 mg/kg/day, PO
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% hydroxypropyl Methylcellulose (HPMC, high viscosity)
Species/Strain:	CD-1 mice
Number/Sex/Group:	15
Age:	6 weeks
Weight:	Male: 28.0-36.0 g; female: 21.9-29.4 g
Satellite groups:	Toxicokinetics (Groups 6, 7, 8, 9, 10)
Study design:	Please see the table below
Deviation from study protocol:	Protocol deviations had no impact on the results and interpretations of the study

The following table (from page 14 of the study report) shows the study design.

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
<b>Main Study Groups</b>			
1	0	15	15
2	500	15	15
3	1000	15	15
4	1500	15	15
5 <sup>a</sup>	3000	15	15
<b>Toxicokinetic Groups</b>			
6	500	52	52
7	1000	52	52
8	1500	52	52
9 <sup>a</sup>	3000	52	52
10 <sup>a</sup>	0	15	15

<sup>a</sup>Animals were randomized and assigned to study but not administered the vehicle or JNJ-27018966-AAA formulations (b) (4); removed from study on Day 8 and maintained in the animal room for use as possible replacements until Day 44. Data pertaining to animals in Groups 5, 9, and 10 are not reported but are maintained in the study file.

**Basis of Dose Selection:** The dose levels were selected based on the results from previous studies including (b) (4) Study Number 1808-001 entitled, "JNJ-27018966-AAA: A 28 Day Oral Range-Finding Toxicity and Toxicokinetic Study in Mice". However, the sponsor did not submit the report of this study or provide any details.

### **Observations and Results:**

**Mortality:** Mortality was checked twice daily. All main study animals survived to the scheduled terminal or recovery necropsies. Three TK animals died during the study including one male at 500 mg/kg/day that was euthanized *in extremis* on Day 1, one male at 500 mg/kg/day that was found dead on Day 62, and one female at 1000 mg/kg/day that was found dead on Day 54. Necropsies were not performed on these three TK animals therefore, cause of death was not determined.

**Clinical Signs:** Clinical signs were observed once weekly. There were no significant treatment-related clinical signs.

**Body Weights:** Body weights were recorded on a weekly basis. The mean initial (Week -1) and final (Week 13) body weights of control males were 32.05 and 38.93 g, respectively. The mean initial (Week -1) and final (Week 13) body weights of control females were 25.45 and 30.49 g,

respectively. There was no significant treatment-related effect on body weight. The following table (from page 23 of the report) shows the body weight data.

Summary of Group Mean Body Weights; kg (Groups 1 through 4 at Termination)				
Dose Level (mg/kg/day)	Male		Female	
	Week 13	(%) Difference from Control	Week 13	(%) Difference from Control
0	38.9	NA	30.5	NA
500	40.0	+2.8	31.5	+3.3
1000	38.4	-1.3	30.9	+1.3
1500	38.2	-1.8	31.1	+2.0

NA – Not applicable

**Food Consumption:** Food consumption was recorded for main study animals on a weekly basis. The mean initial (Week 1) and final (Week 13) food consumption of control males were 7.38 and 5.38 g/animal/day, respectively. The mean initial (Week -1) and final (Week 13) food consumption of control females were 5.64 and 4.95 g/animal/day, respectively. Food consumption was increased in treated animals as shown in the following table (from page 23 of the study report).

Average Food Consumption; g/animal/day				
Dose Level (mg/kg/day)	Male (Weeks 1 to 13)		Female (Weeks 1 to 13)	
	Mean	(%) Difference from Control	Mean	(%) Difference from Control
0	6.4	NA	5.6	NA
500	6.7	+4.7	6.0	+7.1
1000	6.4	NA	6.4	+14.3
1500	6.8	+6.3	6.2	+10.7

NA – Not Applicable

**Ophthalmoscopy:** Ophthalmoscopic examinations were conducted on all main study animals prior to the scheduled terminal necropsy. Two females (animal numbers 2342 and 2343) at 1500 mg/kg/day had retinal atrophy in the left eye.

**Hematology:** Hematology was conducted prior to scheduled necropsy. There were no test article-related effects on hematology parameters.

**Clinical Chemistry:** Clinical chemistry was conducted prior to scheduled necropsy. There were no test article-related effects on clinical chemistry parameters.

**Urinalysis:** Urine was collected prior to scheduled necropsy. There were no test article-related effects on urinalysis parameters.

**Gross Pathology:** Necropsy examinations were performed on all animals at the scheduled terminal necropsy (Day 92 or 93). There were no test article-related macroscopic findings in this study.

**Organ Weights:** The following table (from page 515 of the report) shows the list of organs and tissues for organ weights. There were no significant treatment-related effects on organ weights.

The following list constitutes the full complement of organs and tissues:

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- Adrenal (2)*	- Lacrimal gland, exorbital (2)
- Aorta	- Larynx
- Bone with marrow [femur]	- Liver [collected whole; 2 examined]*
- Bone with marrow [sternum]	- Lung with bronchi [collected whole; all lobes examined]*
- Bone marrow smear [2 collected] <sup>a</sup>	- Lymph nodes: mandibular and mesenteric
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*	- Mammary gland [process females only]
- Epididymis (2)	- Pancreas
- Eye including optic nerve (2)	- Pituitary*
- Gallbladder	- Prostate* and seminal vesicle (2)*
- GALT [gut associated lymphoid tissue]	- Salivary gland, mandibular/sublingual [2 collected; 1 examined] <sup>a,b</sup>
- Gastrointestinal tract:	- Salivary gland, parotid [2 collected; 1 examined]
esophagus	- Sciatic nerve
stomach [glandular and nonglandular]	- Skeletal muscle, biceps femoris
duodenum	- Skin
jejunum	- Spinal cord [cervical, thoracic, and lumbar]
ileum	- Spleen*
cecum	- Thymus*
colon	- Thyroid/parathyroid (2)*
rectum	- Tongue
- Gonads:	- Trachea
ovary (2)* with oviduct (2)*	- Ureters (2)
testis (2)*	- Urinary bladder
- Gross lesions	- Uterus [both horns]/Cervix*
- Heart*	- Vagina
- Joint, tibiofemoral	
- Kidney (2)*	

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<sup>a</sup>Bone marrow smears were collected at the scheduled necropsy and held.

<sup>b</sup>The combined weight of the right mandibular/sublingual salivary gland was obtained.

\*Weighed organ

(2) Paired organ

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**Histopathology:** Histopathological examinations were conducted on tissues listed above from animals at 0 and 1500 mg/kg/day. There were no significant treatment-related microscopic findings.

**Adequate Battery:** Yes

**Peer Review:** No

**Histological Findings:** None

**Special Evaluation:** None

**Toxicokinetics:** Blood samples were collected from eight cohorts of three TK animals per sex per group for TK analysis. Samples were collected at 0.5, 1, 2, 3, 4, 8, 12, and 24 hours postdose on Days 1 and 90. The TK report and the results were not provided.

**Dosing Formulation Analysis:** Dosing formulations prepared for the study were evaluated for homogeneity and concentration. The Sponsor stated that the test article formulations were stable under refrigerated conditions for at least 13 days at the concentrations used in study.

## **Rat:**

### **Study title: Intravenous Dose Range-Finding Toxicity Study in Rats**

Study no.:	1808-013
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 4, 2012
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	JNJ-27018966-AAA, 29807904, 98.6%

**Methods:** In this study, animals (n = 15/sex/dose) were treated with JNJ-27018966-AAC by IV bolus injection at 5, 10, 20, and 40 mg/kg/day for 7 days (1 mL/kg). One additional group of 15 animals per sex was used as the control and received the vehicle, 40% hydroxy propyl beta cyclodextrin (HP $\beta$ CD) in water. The 40 mg/kg dose was administered to three animals per sex once on Day 1 and was subsequently eliminated due to adverse clinical signs from the study design. The three animals per sex that received 40 mg/kg remained on study through the terminal necropsy.

Mortality was observed twice daily. Clinical signs were observed three times daily. Body weights were recorded on Days -1, 1, and 7. Food consumption was recorded on a weekly basis. Blood and urine samples for clinical pathology were collected from all animals prior to the scheduled terminal necropsy. At study termination, necropsy examinations were performed and organ (adrenal, brain, heart, kidney, liver, lung, pancreas, spleen, thymus and thyroid/parathyroid) weights were recorded.

**Results:** All animals survived to the terminal necropsy following 7 days of treatment. Three animals per sex at 40 mg/kg/day received the Day 1 dose only. These three animals per sex remained on study through study termination. The remaining 12 animals per sex at 40 mg/kg/day were removed from study and did not receive the test article. At 40 mg/kg, clinical signs were observed at approximately 10 minutes postdose, which included ataxia, shallow breathing, decreased activity, dilated pupil (one male), and rigid body in both sexes. Intervention was required at this dose and naloxone (0.04 mg/mL) was administered to two males and one female (animal numbers 5001, 5002, and 5502). In males at 20 mg/kg, treatment related effects in the eyes (drying of the cornea) were observed. At 10 and 20 mg/kg/day in both sexes, treatment related clinical signs included decreased activity, rigid body, and shallow breathing (20 mg/kg/day only). These findings were transient and were resolved within 2 hours postdose. There were no treatment related effects on body weight, food consumption, hematology, coagulation, clinical chemistry, or urinalysis parameters. Treatment related macroscopic observations included minimal to mild cloudy areas on the corneas of two males at 20 mg/kg/day and a mild abrasion/scab on the cornea of one male at 20 mg/kg/day. Abrasion/scab on the tail was seen in one male at 5 mg/kg/day, three at 10 mg/kg/day, and two at 20 mg/kg/day. Abrasion/scab on the tail was seen in one female at 20 mg/kg/day and a nodule was noted on the tail of one female at 40 mg/kg/day. Black, blue, or grey discoloration of the tail was also seen in an occasional female at 0 and 5 mg/kg/day. In males, mean adrenal gland weights were higher at 40 mg/kg/day (0.065 g) than controls (0.051 g). In males, mean lung weights at 40 mg/kg/day (1.652 g) were also higher than controls (1.229 g).

**Study title: 2-Week Intravenous Toxicity Study in Rats with a 2-Week Recovery Period**

Study no.:	1808-014
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 26, 2012
Date of study completion:	October 30, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	JNJ-27018966-AAC, Batch No. 00563229, 98.6%

**Key Study Findings:**

- In a 2-week IV study in rats, animals were treated with JNJ-27018966-AAC at 5, 10, and 20 mg/kg/day.
- There was no mortality.
- Treatment related clinical signs included decreased activity, prostration, impaired (or lost) righting reflex, rigid body, low carriage, stereotypy, rapid, shallow, and/or slow breathing, cloudy eyes, and dilated pupils. The above clinical findings were related to eluxadoline treatment. However, these observations were expected

from this class of drug and considered to be non-adverse due to its relative short duration of appearance, the overall general health of the animals throughout the dosing period, and the lack of these findings during the recovery period.

- Minimal to moderate renal tubular vacuolation, minimal alveolar histiocytosis and minimal urothelial vacuolation was seen in all groups including the control group. These microscopic findings in the kidney were considered secondary to the vehicle cyclodextrin [Gould S, and Scott RC, 2005, 2-Hydroxylpropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD): a toxicology review. Food Chem Toxicol, 43:1451-9] and were not considered test article-related.
- The NOAEL was considered as 20 mg/kg/day.

### Methods:

Doses:	5, 10, 20 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Intravenous
Dose volume:	1 mL/kg
Formulation/Vehicle:	40% Hydroxyl propyl beta cyclodextrin (HP $\beta$ CD) in water
Species/Strain:	SD rats
Number/Sex/Group:	15-20/sex/group
Age:	6 Weeks
Weight:	Male: 193-223; Female: 155-184 g
Satellite groups:	Yes (Groups 5, 6, 7)
Unique study design:	Study design is shown below
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study

The following table (from page 15 of the report) shows the study design.

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
<b>Main Study Groups</b>			
1	0 <sup>a</sup>	20 <sup>b</sup>	20 <sup>b</sup>
2	5	15	15
3	10	15	15
4	20	20 <sup>b</sup>	20 <sup>b</sup>
<b>Toxicokinetic Groups</b>			
5	5	12 + 1 <sup>c</sup>	12 + 1 <sup>c</sup>
6	10	12 + 1 <sup>c</sup>	12 + 1 <sup>c</sup>
7	20	12 + 1 <sup>c</sup>	12 + 1 <sup>c</sup>
<sup>a</sup> Administered vehicle only. <sup>b</sup> The last five animals per sex were evaluated during the 2-week recovery period. <sup>c</sup> Additional animals assigned to study for use as possible replacements.			

**Observations:**

**Mortality:** Mortality was observed twice daily.

**Clinical Signs:** Clinical signs were observed three times daily.

**Functional Observational Battery (FOB) Evaluations:** FOB evaluations were conducted on main study animals at predose, at 0.5 hours postdose on Day 1, and once during the last week of the recovery period. The observations included evaluation of activity and arousal, posture, rearing, bizarre behavior, clonic and tonic movements, gait, mobility, stereotypy, righting reflex, response to stimulus (approach, click, tail pinch, and touch), palpebral closure, pupil response, piloerection, exophthalmus, lacrimation, salivation, and respiration.

**Body Weights:** Body weights were recorded once weekly.

**Food Consumption:** Food consumption was recorded on a weekly basis.

**Ophthalmoscopy:** Ophthalmoscopic examinations were conducted on all animals at pretest and main study animals prior to the scheduled terminal and recovery necropsies.

**Hematology:** Hematology was conducted at necropsy.

**Clinical Chemistry:** Clinical chemistry was conducted at necropsy.

Urinalysis: Urine samples collected from one female at 0 mg/kg/day and two females at 10 mg/kg/day prior to necropsy.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 1253 of the report) organs were weighed from all main study animals.

- |   |   |
|---|---|
| - Adrenal (2)*  | - Larynx  |
| - Aorta   | - Liver [3 sections collected; 2 examined]*                                     |
| - Bone with marrow [femur]                              | - Lung with bronchi [collected whole; 2 sections examined]*                     |
| - Bone with marrow [sternum]                            | - Lymph nodes: mandibular [2 collected; 1 examined] and mesenteric              |
| - Bone marrow smear [2 collected] <sup>a</sup>          | - Mammary gland [process females only]  |
| - Brain [cerebrum, midbrain, cerebellum, medulla/pons]* | - Pancreas  |
| - Epididymis (2)  | - Pituitary*  |
| - Eye including optic nerve (2)                         | - Prostate* and seminal vesicle (2)*  |
| - GALT [gut associated lymphoid tissue]                 | - Salivary gland, mandibular/sublingual [2 collected; 1 examined]* <sup>b</sup> |
| - Gastrointestinal tract:                               | - Salivary gland, parotid [2 collected; 1 examined]                             |
| esophagus   | - Sciatic nerve   |
| stomach [glandular and nonglandular]                    | - Skeletal muscle, biceps femoris   |
| duodenum  | - Skin  |
| jejunum   | - Spinal cord [cervical, thoracic, and lumbar]                                  |
| ileum   | - Spleen*   |
| cecum   | - Thymus*   |
| colon   | - Thyroid/parathyroid (2)*  |
| rectum  | - Tongue  |
| - Gonads:   | - Trachea   |
| ovary (2)* with oviduct (2)*                            | - Ureter (2)  |
| testis (2)*   | - Urinary bladder   |
| - Gross lesions   | - Uterus [both horns]/Cervix*   |
| - Heart*  | - Vagina  |
| - Injection site, tail                                  |   |
| - Joint, tibiofemoral                                   |   |
| - Kidney (2)*   |   |
| - Lacrimal gland, exorbital (2)                         |   |

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<sup>a</sup>Bone marrow smears were collected at necropsy and held.

<sup>b</sup>The combined weight of the right mandibular/sublingual salivary gland was obtained.

(2) Paired organ

\*Organ weighed

Histopathology: The above table shows the list of organs or tissues collected for histopathology.

Toxicokinetics: Blood samples were collected from three TK animals per sex per group at 2, 5, 10 and 30 minutes and at 1, 2, 4, 8, 12, and 24 hours postdose on Days 1 and 14.

Dosing Solution Analysis: Dosing solutions were analyzed for concentration and homogeneity.

**Results:**

Mortality: There was no treatment related mortality.

Clinical Signs: Treatment related clinical signs included decreased activity, prostration, impaired (or lost) righting reflex, rigid body, low carriage, stereotypy, rapid, shallow, and/or slow breathing, cloudy eyes, and dilated pupils. Due to the above clinical signs, intervention was performed as needed by 0.1 mg/kg of Naloxone (SC or IV). Most of these findings were seen in both sexes at 10 and 20 mg/kg/day. Although related to JNJ-27018966-AAC administration, these observations were expected from this class of drug and were considered to be non-adverse based on the relative short duration of appearance, the overall general health of the animals throughout the dosing period, and the lack of findings observed during the recovery period.

Functional Observational Battery (FOB) Evaluations:

In males at all dose levels, mean forelimb grip strength and mean hind limb splay measurements were significantly decreased on Day 1. In males at 10 mg/kg/day on Day 1, significant treatment related findings were observed pertaining to general arousal, handling reactivity, stereotypy, tail pinch response, touch response. In males at 20 mg/kg/day, significant treatment related findings were observed pertaining to general arousal, handling reactivity, posture, gait, mobility, righting reflex, stereotypy, respiration, tail pinch response, touch response, and thermal response.

In females at all dose levels, general arousal, tail pinch response, and hind limb splay measurements were significantly decreased on Day 1. In females at 5 mg/kg/day on Day 1, treatment related significant findings included ease of removal and handling reactivity. In females at 10 mg/kg/day on Day 1, significant treatment related findings included decreased forelimb grip strength. In females at 20 mg/kg/day on Day 1, significant treatment related findings were observed pertaining to ease of removal, posture, gait, mobility, righting reflex, stereotypy, respiration, approach response, touch response, forelimb grip strength, hind limb grip strength, and thermal response.

Overall, the FOB findings were related to the treatment. However, these observations were expected from this class of drug and were considered to be non-adverse due to its relative short duration of appearance, the overall general health of the animals throughout the dosing period, and the lack of these findings during the recovery period.

Body Weights: The mean initial (Day -2) and final (Day 14) weights of control males were 208 and 315 g, respectively. The mean initial (Day -2) and final (Day 14) weights of control females were 169 and 218 g, respectively. In males, final body weights were 98%, 96% and 95% of control at 5, 10 and 20 mg/kg/day, respectively. In females, final

body weights were 103%, 102% and 103% of control at 5, 10 and 20 mg/kg/day, respectively. There were no significant treatment related effects.

Food Consumption: The mean initial (Week 1) and final (Week 2) food consumption of control males were 25.86 and 28.37 g/animal/day, respectively. The mean initial (Week 1) and final (Week 2) food consumption of control females were 19.28 and 20.26 g/animal/day, respectively. In males, final food consumption values were 93%, 96% and 93% of control at 5, 10 and 20 mg/kg/day, respectively. In females, final food consumption values were 93%, 96% and 93% of control at 5, 10 and 20 mg/kg/day, respectively. There were no significant treatment related effects.

Ophthalmoscopy: There were no significant treatment related effects.

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: There were no significant treatment related effects.

Organ Weights: There was a statistically significant decrease in the spleen weights in males at 10 and 20 mg/kg/day. Due to low magnitude, lack of microscopic correlates, and lack of similar finding in females, this change was considered incidental and not test article-related.

Histopathology: Microscopic findings at the injection site in either sex included minimal to mild subacute/chronic inflammation, minimal to mild hemorrhage, minimal vascular degeneration/regeneration, minimal to mild vascular necrosis, minimal to mild ulcer or erosion of the epidermis, minimal to mild epidermal exudate and minimal foreign material (hair/keratin). One or more of these findings were present in both the control group and treatment groups at similar incidence and/or severity. Hence, these findings were considered secondary to the injection procedure and not test article related. At recovery necropsy, there was complete resolution of vascular necrosis, vascular degeneration/regeneration, hemorrhage and erosion/ulcer. There was a decrease in the incidence and severity of subacute/chronic inflammation and epidermal exudate with a single incidence of foreign material (hair/keratin). Minimal to moderate renal tubular vacuolation, minimal alveolar histiocytosis and minimal urothelial vacuolation was seen in all groups including the control group. However, there was no evidence of dose response at terminal and recovery necropsies. Per the Sponsor, these microscopic findings were considered secondary to the vehicle cyclodextrin [Gould S, and Scott RC, 2005, 2-Hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD): a toxicology review. Food Chem Toxicol, 43:1451-9] and were not considered test article-related.

Toxicokinetics: Plasma concentrations of JNJ-27018966-AAC increased in a less than dose proportional manner from 5 to 10 mg/kg; however, this increased in a slightly

greater than dose proportional manner from 10 to 20 mg/kg on both Days 1 and 14. Generally, the exposure ( $AUC_{0-24h}$ ) increased greater than dose proportionally from 5 to 20 mg/kg on both Day 1 and Day 14. The  $AUC_{0-24h}$  for 10 mg/kg dose increased in a slightly less than proportional manner on Day 14. Terminal half-lives at 20 mg/kg ranged from 1.11 to 2.41 hours. There was no apparent gender difference in exposure. There was no apparent accumulation of JNJ-27018966. The following table (from page 1205 of the report) shows the TK parameters.

**Table 1.1 Toxicokinetic Parameters for JNJ-27018966 in Rats After Daily IV Administration of 5, 10 and 20 mg/kg of JNJ-27018966-AAC at Day 1 and Day 14**

Day	Dose (mg/kg/d)	C0 (ng/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose (ng*hr/mL)/(mg/kg/d)	AUC(0-t) (ng*hr/mL)	AUC(0-inf) (ng*hr/mL)	T1/2 (hr)	Lambda-z (1/hr)	CL or CLss (L/hr/kg)
1	5	22351.81	1412.60	282.52	1377.60	.	.	.	.
	10	39086.36	3468.83	346.88	3465.02	.	.	.	.
	20	98549.89	15644.43	782.22	15641.85	15642.54	1.11	0.6249	1.28
14	5	34140.22	1761.12	352.22	1759.96	.	.	.	2.84
	10	44472.96	3044.07	304.41	3042.91	.	.	.	3.29
	20	151671.53	8543.72	427.19	8543.72	8544.30	2.41	0.2873	2.34

CL=Dose/AUC(0-inf) on Day 1. Total body clearance  
 CLss=Dose/AUC(0-24) only on Day 14. Total body clearance at steady state  
 (.) Indicates not applicable or could not be calculated.

**Dosing Solution Analysis:** Dosing solution analysis results revealed that dose formulations contained 96% to 100% of the respective targeted concentrations of 5.0, 10.0, and 20.0 mg/mL and met the protocol requirement for solution formulations ( $\pm$  10% of nominal concentration). No test article was detected in the analyzed vehicle administered to the control group.

### Study title: Five-Day Range Finding Oral and Subcutaneous Toxicity Study of JNJ-27018966-AAA in Rats

Study no.: Study TOX7310  
 Study report location: EDR 4.2.3.2  
 Conducting laboratory and location: Johnson & Johnson, Raritan, NJ  
 Date of study initiation: October 5, 2005  
 GLP compliance: Non-GLP  
 QA statement: No  
 Drug, lot #, and % purity: JNJ-27018966-AAA, 29807904, Not provided

**Methods:** In this study, rats (5/sex/group) were administered vehicles (0.5% hypromellose solution for oral; 10% hydroxypropyl-beta-cyclodextrin for SC) or JNJ 27018966-AAA at 500, 1000, or 2000 mg/kg/day orally, or 500/5, 500/15, or 2000/5 mg/kg/day PO/SC for 5 days. An additional 2 rats/sex/group were used for toxicokinetics. Following parameters were examined: mortality, clinical signs, body weight, hematology, serum chemistry, gross pathology and histopathology. Blood samples were collected for TK analysis on Days 1 and 5 from TK animals at 0 (predose), 0.5, 1, 2, 4, 8, and 24 hours postdose.

**Results:** There was no mortality. Clinical signs were observed at the injection site (primarily at the high dose), which included edema, erythema, and scab formation. Bruise, ulceration, or necrosis was also observed at the injection site. There were no significant treatment related effects on body weight, hematology or serum chemistry. Gross necropsy findings included slight cecal distention at  $\geq 500$  mg/kg/day. In addition, slight food retention in the stomach was noted sporadically in several treated animals. Histopathology findings (tissue necrosis of primarily skeletal muscle of the subcutis, hemorrhage, and/or edema) were limited to the injection site reactions.

Oral absorption of JNJ-27018966 was generally rapid (mean  $t_{max}$  values ranged from 0.50 to 2.25 hours), except for one rat at 2000 mg/kg ( $t_{max}$  of 24 hr on Day 5). Elimination rate was generally more rapid following PO/SC dosing (mean  $t_{1/2}$  range: 0.87 to 3.10 hours at 500/5 and 500/15 mg/kg/day) than after oral dosing alone (mean  $t_{1/2}$  range: 3.48 to 11.3 hours at 500 and 1000 mg/kg/day). Mean  $t_{1/2}$  values were longer (up to 19.8 hr) at 2000/5 mg/kg/day and (up to 23.1 hr) at 2000 mg/kg/day. No drug accumulation was observed after the PO/SC route; however, slight accumulation was observed after oral administration. There were no apparent gender related differences in exposure to JNJ-27018966 following the oral only route of administration. However, exposures in males were generally higher than those in females following PO/SC routes of administration. The following tables (from pages 6-9 of the TK report) show the TK parameters.

TOX7310 Pharmacokinetics: Absorption after a Single Oral Dose

Test Article: JNJ-27018966-AAA

Study No.	TOX7310		TOX7310		TOX7310	
	Rat		Rat		Rat	
Species	Rat		Rat		Rat	
Feeding Condition	fed		fed		fed	
Vehicle/Formulation	0.5% hypromellose		0.5% hypromellose		0.5% hypromellose	
Route	Oral		Oral		Oral	
Gender (M/F)/Number of Animals	M:2	F:2	M:2	F:2	M:2	F:2
Dose (mg/kg)	500		1000		2000	
Sample	plasma		plasma		plasma	
Analyte	JNJ-27018966		JNJ-27018966		JNJ-27018966	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters						
$C_{max}$ (ng/mL)	11.5	13.8	24.0	40.1	43.4	86.2
$t_{max}$ (h)	2.25	2.00	1.50	0.75	0.50	0.50
AUC <sub>0-∞</sub> (ng × h/mL)	147	187	367	226	210	NA
$t_{1/2}$ (h)	7.92	11.3	9.28	8.15	3.48	NA
CL/F (mL/h × kg)	4333260	2715985	2765485	4925427	9545686	NA

NA = Not available

TOX7310

**Pharmacokinetics: Absorption after a Single Oral/Subcutaneous Dose**

Test Article: JNJ-27018966-AAA

Study No.	TOX7310		TOX7310		TOX7310	
Species	Rat		Rat		Rat	
Feeding Condition	fed		fed		fed	
Vehicle/Formulation	0.5% hypromellose/10% HPBCD		0.5% hypromellose/10% HPBCD		0.5% hypromellose/10% HPBCD	
Route	Oral/SC		Oral/SC		Oral/SC	
Gender (M/F)/Number of Animals	M:2	E:2	M:2	E:2	M:2	E:2
Dose (mg/kg)	500/5		500/15		2000/5	
Sample	plasma		plasma		plasma	
Analyte	JNJ-27018966		JNJ-27018966		JNJ-27018966	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS	
<b>Pharmacokinetic Parameters</b>						
C <sub>max</sub> (ng/mL)	1100	744	3080	3275	950	816
t <sub>max</sub> (h)	2.00	1.00	2.00	2.00	0.75	0.75
AUC <sub>0-∞</sub> (ng × h/mL)	4730	3469	14851	12959	4483	3479
t <sub>1/2</sub> (h)	2.43	1.42	2.27	3.07	2.42	19.8
CL/F (mL/h x kg)	106430	144961	40936	39652	484114	574803

TOX7310

**Pharmacokinetics: Absorption after a Multiple Oral Doses**

Test Article: JNJ-27018966-AAA

Study No.	TOX7310		TOX7310		TOX7310	
Species	Rat		Rat		Rat	
Feeding Condition	fed		fed		fed	
Vehicle/Formulation	0.5% hypromellose		0.5% hypromellose		0.5% hypromellose	
Route	Oral		Oral		Oral	
Gender (M/F)/Number of Animals	M:2	E:2	M:2	E:2	M:2	E:2
Dose (mg/kg/day)	500		1000		2000	
Duration of Dosing	5		5		5	
Sample	plasma		plasma		plasma	
Analyte	JNJ-27018966		JNJ-27018966		JNJ-27018966	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS	
<b>Pharmacokinetic Parameters</b>						
C <sub>max</sub> (ng/mL)	23.6	20.2	143	79.8	120	148
t <sub>max</sub> (h)	0.75	0.75	0.25	0.75	13.0	1.28
AUC <sub>0-24</sub> (ng × h/mL)	190	158	471	533	675	445
t <sub>1/2</sub> (h)	8.21	10.1	4.66	8.76	23.1	4.06
CL/F (mL/h x kg)	2671459	3186138	2153393	2409432	3128738	4498339

TOX7310

**Pharmacokinetics: Absorption after a Multiple Oral/Subcutaneous Doses**

Test Article: JNJ-27018966-AAA

Study No.	TOX7310		TOX7310		TOX7310	
Species	Rat		Rat		Rat	
Feeding Condition	fed		fed		fed	
Vehicle/Formulation	0.5% hypromellose/10% HPBCD		0.5% hypromellose/10% HPBCD		0.5% hypromellose/10% HPBCD	
Route	Oral/SC		Oral/SC		Oral/SC	
Gender (M/F)/Number of Animals	M:2	E:2	M:2	E:2	M:2	E:2
Dose (mg/kg/day)	500/5		500/15		2000/5	
Duration of Dosing	5		5		5	
Sample	plasma		plasma		plasma	
Analyte	JNJ-27018966		JNJ-27018966		JNJ-27018966	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS	
<b>Pharmacokinetic Parameters</b>						
C <sub>max</sub> (ng/mL)	967	695	3415	2870	1601	709
t <sub>max</sub> (h)	0.75	1.00	1.25	1.50	1.25	0.50
AUC <sub>0-24</sub> (ng × h/mL)	3148	2661	14843	8364	4828	2211
t <sub>1/2</sub> (h)	2.53	1.57	3.10	2.04	0.87	NA
CL/F (mL/h x kg)	164086	189358	34546	62482	419125	910687

NA = Not available

**Study title: Oral Dose Ranging Study of JNJ-27018966 in Rats (TOX6746)**

Study no.: TOX6746  
Study report location: EDR 4.2.3.2  
Conducting laboratory and location: Johnson & Johnson, Raritan, NJ  
Date of study initiation: August 24, 2004  
GLP compliance: Non-GLP  
QA statement: No  
Drug, lot #, and % purity: JNJ-27018966-AAC, 23726500, purity data not provided

**Methods:** This is a non-GLP exploratory study and was conducted in two phases. In the single dose escalation (SDE) phase, 35 male SD rats were assigned to one of 7 groups. The first two groups were vehicle control and administered either 0.5% methocel orally (Group 1) or 0.9% saline subcutaneously (Group 2). The remaining five groups received JNJ-27018966-AAC at either 500 or 2000 mg/kg orally or 10, 100, or 465 mg/kg subcutaneously during the SDE phase. Based on the results of the SDE phase, 57 male rats were administered JNJ-27018966-AAC during a 5-day repeat dose (RD) phase at 0 (0.5% methocel), 0 (0.9% saline), 500 (oral), 5, 25, and 75 (SC) mg/kg/day. During each phase of this study, first five rats per group were assessed for toxicology parameters. In the SDE phase, the same rats were used for the assessment of toxicology and pharmacokinetic (PK) parameters. During the repeat dose phase, 3 rats per group were assigned to assess PK parameters (5, 25, 75, and 500 mg/kg/day) and the last three male rats in the saline vehicle, 5, 25, 75, and 500 mg/kg/day groups were used for gene expression analysis. Rats assigned for gene expression analysis only, were dosed on one day only. During both phases of this study, in-life and clinical pathology parameters were evaluated. During the RD phase, five sites (one per day) were used for SC injections starting at Site 1 (left scapular region) through Site 5 (left posterior region). At the end of each phase, a gross necropsy was performed and microscopic evaluations of selected organs were also conducted after the RD phase only. Blood samples were collected for TK analysis (SDE phase: 0.3 and 3 hours postdose; RD phase: on Day 1 and 5 at 0.5, 1, 3, 8, and 24 hours postdose). The following table (from page 17 of the report) shows the study design.

SDE Phase:

Group	1	2	3	4	5	6	7
	Rat Numbers (TOX6746)						
Males <sup>a</sup>	1001- 1005 Vehicle (0.5% Methocel)	2001- 2005 Vehicle (Saline)	3001- 3005	4001- 4005	5001- 5005 JNJ-27018966	6001- 6005	7001- 7005
Route	Oral	SC	Oral		SC		
Dosage <sup>b</sup> (mg/kg)	0	0	500	2000	10	100	465
Concentration <sup>b</sup> (mg/mL)	0	0	50	200	2	20	93
Dosage Volume (mL/kg)	10	5	10	10	5	5	5

<sup>a</sup> All rats in each dosage group will be also be assessed for drug exposure, clinical pathology parameters, and gross necropsy.

<sup>b</sup> Calculated based on the (b) (4)

SC = subcutaneous

RD phase:

Group	1	2	3	4	5	6
	Rat Numbers (TOX6746)					
Males <sup>a</sup>	1001-1005 Vehicle (0.5% Methocel)	2001-2008 Vehicle (Saline)	3001-3011	4001-4011 INJ-27018966	5001-5011	6001-6011
Route	Oral	SC	Oral		SC	
Toxicology – Males	1001-1005	2001-2005	3001-3005	4001-4005	5001-5005	6001-6005
Dosage <sup>b</sup> (mg/kg/day)	0	0	500	5	25	75
Concentration <sup>b</sup> (mg/mL)	0	0	50	1	5	15
Dosage Volume (mL/kg)	10	5	10	5	5	5
On Study: Clinical	5	5	5	5	5	5
Pathology Necropsy	5	5	5	5	5	5
Group	1 (Vehicle)	2 (Vehicle)	3	4 (Low)	5 (Mid)	6 (High)
	Rat Numbers (Study TOX6746)					
PK – Males	NA	NA	3006-3008	4006-4008	5006-5008	6006-6008
Dosage <sup>b</sup> (mg/kg/day)	NA	NA	500	5	25	75
Concentration <sup>b</sup> (mg/mL)	NA	NA	50	1	5	15
Dosage Volume (mL/kg)	NA	NA	10	5	5	5
On Study:	NA	NA	3	3	3	3
Gene Analysis – Males	1 (Vehicle)	2 (Vehicle)	3	4 (Low)	5 (Mid)	6 (High)
	Rat Numbers (Study TOX6746)					
	NA	2006-2008	3009-3011	4009-4011	5009-5011	6009-6011
Dosage <sup>a</sup> (mg/kg/day)	NA	0	500	5	25	75
Concentration (mg/mL)	NA	0	50	1	5	15
Volume (mL/kg/day)	NA	5	10	5	5	5
Males/group	NA	3	3	3	3	3

<sup>a</sup> All rats in each dosage group will be also be assessed for drug exposure, clinical pathology parameters, and gross necropsy.

<sup>b</sup> Calculated based on the (b) (4)  
SC = subcutaneous

**Results:**

**SDE Phase:** Treatment related clinical signs included catalepsy, decreased activity, and ataxia at 4 hours postdose at 100 and 465 mg/kg. After SC treatment, serum chemistry changes included elevations in transaminase levels at 100 and 465 mg/kg, as well as marked increase in urea nitrogen and creatinine levels at the high dose. At 100 mg/kg SC, mildly higher (2.03x) ALT and moderately higher (3.23x) AST values, while at 465 mg/kg SC moderately higher mean ALT (4.80x) and AST (8.47) values were observed when compared to control. At 465 mg/kg SC, markedly higher (4.79x) urea nitrogen value and a minimally higher (1.33x) creatinine value were observed. Histopathological

changes after SC dose were seen at the injection site (degeneration and necrosis of the epidermis, hair follicle epithelium, sebaceous glands and subcutaneous skeletal myocytes, with associated chronic inflammation).

RD Phase: At 25 and 75 mg/kg/day SC, treatment related clinical signs included bruising, erythema, eschar formation, scabbing, and/or thickening of the injection site, with an increased severity and/or incidence at the high dose. Exophthalmia, was observed in 2 and 3 rats administered 25 and 75 mg/kg/day, respectively, with catalepsy (4 of 5 rats), decreased activity (2 of 5 rats), and watery ocular discharge (1 of 5 rats), being observed at 75 mg/kg/day only. Male rats at 25 mg/kg/day SC had minimally higher (1.35x) mean alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values. However, at 75 mg/kg/day SC, minimally higher (1.49x) mean ALT and mildly higher (1.88x) mean AST values were observed compared to control. At 75 mg/kg/day SC, minimally higher (1.33x) mean urea nitrogen value was observed. Histopathological changes included chronic inflammation at the injection site of all rats at 25 and 75 mg/kg/day and in two rats at 5 mg/kg/day. Other injection site lesions seen frequently at the two highest doses included degeneration/necrosis of the epidermis, hair follicle epithelium, and sebaceous glands; sebaceous gland loss; periadnexal fibrosis; degeneration/necrosis of subcuticular skeletal myocytes; and serocellular crusts. In addition, epidermal ulceration was seen in three rats at 75 mg/kg/day and one rat at 25 mg/kg/day. No injection site lesions were noted in the saline control rats. Other histopathological changes occurred at a higher incidence or severity in the treated animals compared to the control were seen in the pancreas [minimal to slight/mild degeneration and necrosis of pancreatic acinar cells in two Group 6 animals, one Group 3 (500 mg/kg/day PO) animal, and one Group 1 control animal], spleen (minimal splenic lymphoid necrosis in two Group 6 and two Group 3 animals), liver (one Group 3 animal had a gross liver lesion of a dark red area that correlated histologically with a focally extensive area of mild hemorrhage and moderate hepatocyte necrosis), and epididymis (minimal to slight/mild increased debris in epididymal tubules in one Group 6, one Group 5 and one Group 3 animal; the affected Group 3 animal also had minimal epididymal hypospernnia and minimal testicular degeneration). Relationship of these lesions in the epididymis, testis, liver, spleen and pancreas with treatment is unknown in the absence of a dose response.

JNJ-27018966 had minimal effects on gene expression in the rat liver by both routes of administration. There were no gene expression changes of biological relevance.

Following single SC dose, mean  $t_{max}$  values ranged from 0.67 to 2.17 hours. Elimination was rapid with mean terminal half-life values of 1.02-2.83 hours. Following multiple SC doses, the  $C_{max}$  values increased but not dose proportionally. Following multiple oral doses,  $C_{max}$  values increased. The following table (from page 71 of the report) shows the TK parameters.

**Table 4:** Summary of Mean (SD) Plasma Pharmacokinetic Parameters for JNJ-27018966 in Male Rats (N=3) Following a Single or 5 Daily Subcutaneous Doses (5, 25, and 75 mg/kg) or Oral Doses (500 mg/kg/day) of JNJ-27018966-AAC (TOX6746)

Subcutaneous						
Period (Day)	Dose (mg/kg)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (0-∞) <sup>a</sup> (ng•h/mL)	t <sub>1/2</sub> (h)	CL/F (mL/h•kg)
1	5	515 (8.50)	0.67 (0.29)	967 <sup>b</sup> (160)	1.02 <sup>b</sup> (NA)	5243 <sup>b</sup> (NA)
1	25	1307 (266)	0.67 (0.29)	5705 (1198)	1.81 (0.68)	4533 (1077)
1	75	2863 (185)	2.17 (1.44)	24142 (3693)	2.83 (0.38)	3153 (451)
5	5	459 (131)	5.67 (4.04)	5558 (681)	6.47 <sup>c</sup> (NA)	761 <sup>c</sup> (NA)
5	25	776 (210)	0.83 (0.29)	3040 (531)	1.90 <sup>b</sup> (NA)	6791 <sup>b</sup> (NA)
5	75	1867 (816)	1.50 (1.32)	8792 (3989)	2.58 <sup>c</sup> (NA)	8727 <sup>c</sup> (NA)
Oral						
1	500	2.65 (0.52)	1.33 (1.44)	NC NA	NC NA	NC NA
5	500	127 (62.9)	8.00 (0.00)	1339 (671)	NC (NA)	NC (NA)

<sup>a</sup>: AUC (0 – 24 h) for Day 5.<sup>b</sup>: N=2. Unable to calculate for one rats.<sup>c</sup>: N=1. Unable to calculate for two rat.

NA: Not Applicable

NC: Not Calculated.

**Study title: Four-Week Oral and Oral/Subcutaneous Toxicity Study of JNJ-27018966-AAA in Rats (Study TOX7686)**

Study no.: TOX7686  
 Study report location: EDR 4.2.3.2  
 Conducting laboratory and location: Johnson & Johnson, Raritan, NJ  
 Date of study initiation: March 13, 2006  
 Date of study completion: August 15, 2007  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAA ( (b) (4) form of JNJ-27018966), Batch No. 30205959, 98%

**Key Study Findings:**

- In a 4-week oral (PO) and oral/subcutaneous (PO/SC) toxicity study in rats, animals were administered JNJ-27018966-AAA at 200 or 1000 mg/kg/day PO, or 200/5 or 1000/5 mg/kg/day PO/SC for 4 weeks.
- No significant treatment related microscopic changes were observed following oral doses. Following PO/SC treatment, histopathological changes were seen at the injection site (inflammation and ulceration of the subcutaneous tissue). In addition, minimal to mild vacuolation of proximal tubules observed in kidneys from control and 200/5 and 1000/5 mg/kg PO/SC. Since vacuolation was present in kidneys from vehicle control and treated rats, and there was no clinical pathology findings related to the kidney, and hydroxypropyl- $\beta$ -cyclodextrin has been reported to cause vacuolation of proximal renal tubular cells, these findings were not considered to be related to the test article.
- The NOAELs for this study were determined to be 1000 mg/kg by oral administration and 1000/5 mg/kg by oral/SC administration.

**Methods:** The purpose of this study was to assess the toxicity of JNJ-27018966-AAA (b) (4) form of JNJ-27018966) when administered orally (gavage) alone or in combination with subcutaneous administration to optimize.

Doses: Oral: 200 or 1000 mg/kg/day; PO/SC: 200/5 or 1000/5 mg/kg/day

Frequency of dosing: Daily

Route of administration: Oran and SC

Dose volume: Oral : 10 ml/kg; SC: 1 ml/kg

Formulation/Vehicle: 0.5% Hypromellose for oral dosing or 10% hydroxypropyl- $\beta$ -cyclodextrin for SC dosing

Species/Strain: SD rats

Number/Sex/Group: 10/sex/group

Age: 8 Weeks

Weight: 158-304 g

Satellite groups: TK (4/sex/treatment group and 3/sex/control)

Unique study design: Study design is shown below

Deviation from study protocol: Protocol deviations did not affect the validity or integrity of the study

The following table (from page 16 of the report) shows the study design.

Group	1 <sup>a</sup> (Vehicle)	2 <sup>b</sup> (Vehicle)	3	4	5	6
Rat Numbers (TOX7686)						
Males <sup>c</sup>	1001-1010	2001-2010	3001-3010	4001-4010	5001-5010	6001-6010
Females <sup>c</sup>	1501-1510	2501-2510	3501-3510	4501-4510	5501-5510	6501-6510
JNJ-27018966						
Dose <sup>d</sup> (mg/kg)	0	0/0	200	200/5	1000	1000/5
Concentration <sup>d</sup> (mg/mL)	0	0/0	20	20/5	100	100/5
Dose Volume (mL/kg)	10	10/1	10	10/1	10	10/1
Group (Toxicokinetics)	7 <sup>a</sup> (Vehicle)	8 <sup>b</sup> (Vehicle)	9	10	11	12
Rat Numbers (TOX7686)						
Males <sup>c</sup>	7001-7003	8001-8003	9001-9004	10001-10004	11001-11004	12001-12004
Females <sup>c</sup>	7501-7503	8501-8503	9501-9504	10501-10504	11501-11504	12501-12504
JNJ-27018966						
Dose <sup>d</sup> (mg/kg)	0	0/0	200	200/5	1000	1000/5
Concentration <sup>d</sup> (mg/mL)	0	0/0	20	20/5	100	100/5
Dose Volume (mL/kg)	10	10/1	10	10/1	10	10/1

<sup>a</sup> Rats were dosed orally with 0.5% hypromellose (PO)

<sup>b</sup> Rats were dosed orally with 0.5% hypromellose and subcutaneously with 10% HP-β-CD(PO/SC)

<sup>c</sup> Groups 1,3,5,7,9, and 11 were dosed orally (PO). Groups 2,4,6,8,10, and 12 were dosed orally and subcutaneously (PO/SC)

<sup>d</sup> Calculated as the (b) (4)

## **Observations:**

**Mortality:** Mortality was observed once daily.

**Clinical Signs:** Clinical signs were observed once daily.

**Body Weights:** Body weights were recorded once weekly.

**Food Consumption:** Food consumption was recorded on a weekly basis.

**Ophthalmoscopy:** Ophthalmoscopic examinations were conducted on all animals at Weeks -2, and 4.

**Hematology:** Hematology was conducted at necropsy.

**Clinical Chemistry:** Clinical chemistry was conducted at necropsy.

**Urinalysis:** Urine samples collected during Week 5.

**Gross Pathology:** Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 20 of the report) organs were weighed from all main study animals.

**2.6.3. Tissue List**

Collect	Weigh	Fix	Microscopic Examination
Adrenal gland (both)	X	F	X
Aorta, thoracic		F	X
Bone and bone marrow, sternum		F	X
Bone and bone marrow, stifle joint including distal femur and proximal tibia		F	X
Brain	X	F	X
Cervix		F	X
Coagulating gland (both)		F	X
Epididymis (both)		Bouin's	X
Esophagus		F	X
Eye (both) with optic nerve(s)		F	X
Harderian gland (both)		F	X
Heart	X	F	X
Injection site(s)*		F	X
Kidney (both)	X	F	X
Large intestine (cecum, colon, rectum)		F	X
Larynx		F	X
Liver	X	F	X
Lung	X	F	X
Lymph node(s), mesenteric		F	X
Lymph node, popliteal (both)		F	X
Mammary gland(s), inguinal		F	X
Nose		F	
Ovary (both)	X	F	X
Oviduct (both)		F	X
Pancreas		F	X
Parathyroid gland(s)		F	X
Peyer's patch(es)		F	X
Pituitary gland	X (fixed)	F	X
Prostate		F	X
Salivary glands, parotid, mandibular, and sublingual (unilateral)		F	X
Sciatic nerve (both)		F	X
Seminal vesicles		F	X
Skeletal muscle, quadriceps femoris		F	X
Skin, inguinal		F	X
Small intestine (duodenum, jejunum, ileum)		F	X
Spinal cord (cervical, thoracic, and lumbar)		F	X
Spleen	X	F	X
Stomach		F	X
Testis (both)	X	Bouin's	X
Thymus	X	F	X
Thyroid gland (both)	X (fixed)	F	X
Tongue		F	X
Trachea		F	X
Ureter(s)		F	X
Urinary bladder		F	X
Uterus		F	X
Vagina		F	X

F = fixed in 10% neutral buffered formalin

Bouin's = fixed in Bouin's solution at scheduled necropsy

\* Skin tissue from all injection sites were collected, but only the tissue from the site of the last injection was examined microscopically.

Histopathology: The above listed tissues from all controls (Group 1, dosed orally and Group 2, dosed orally and subcutaneously), rats at 1000 mg/kg (Group 5, dosed orally), and rats at 1000/5 mg/kg (Group 6, dosed orally at 1000 mg/kg and subcutaneously at 5 mg/kg) were collected for histopathology.

Toxicokinetics: Blood samples were collected from satellite animals (Groups 7 through 12) on Days 1 and 28. Control Groups 7 and 8 consisted of three animals per sex per group, while Groups 9 through 12 consisted of 4 animals per sex per group. Samples were collected from control group animals at 0 hours (predose), and at 1, 4 and 8 hours postdose. Blood samples were collected from animals in Groups 9 through 12 at 0 hours (predose), and at 0.5, 1, 2, 4, 8 and 24 hours postdose.

Dosing Solution Analysis: Concentration, uniformity (oral suspensions only), and stability of the formulated test article were verified.

## **Results**:

Mortality: There were no treatment related deaths at any oral dose. At 200 mg/kg PO, one female died on Day 31. The death was not considered related to the test article. There were no treatment related deaths at any PO/SC dose. In the PO/SC group, one control female died subsequent to the blood collection procedure on Day 31.

Clinical Signs: There were no treatment related clinical signs following oral treatment. Following PO/SC administration, treatment related clinical signs at both doses included increased activity (one female at 200/5 mg/kg/day on Day 24 and one male and five females at 1000/5 mg/kg/day on Days 14 and/or 24). Other treatment related clinical signs were seen at the injection sites and included edema (males only), erythema, scab formation and scaling.

## **Body Weights**:

Oral: The mean initial (Week -2) and final (Week 4) weights of control males were 200 and 412 g, respectively. The mean initial (Week -2) and final (Week 4) weights of control females were 166 and 247 g, respectively. In males, final body weights were 96% and 92% of control at 200 and 1000 mg/kg/day, respectively. In females, final body weights were 99% and 96% of control at 200 and 1000 mg/kg/day, respectively. There were no significant treatment related effects on body weight following oral treatment.

PO/SC: The mean initial (Week -2) and final (Week 4) weights of control males were 200 and 397 g, respectively. The mean initial (Week -2) and final (Week 4) weights of control (oral) females were 165 and 243 g, respectively. In males, final body weights were 98% and 95% of control at 200 and 1000 mg/kg/day, respectively. In females, final body weights were 100% and 105% of control at 200 and 1000 mg/kg/day, respectively. There were no significant treatment related effects on body weight following PO/SC treatment.

Food Consumption:

Oral: The mean initial (Week -1) and final (Week 4) food consumption of control males were 25.46 and 29.11 g/animal/day, respectively. The mean initial (Week -1) and final (Week 4) food consumption of control females were 18.03 and 20.72 g/animal/day, respectively. There were no significant treatment related effects.

PO/SC: The mean initial (Week -1) and final (Week 4) food consumption of control males were 25.70 and 27.96 g/animal/day, respectively. The mean initial (Week -1) and final (Week 4) food consumption of control females were 18.59 and 21.24 g/animal/day, respectively. There were no significant treatment related effects.

Ophthalmoscopy: There were no significant treatment related effects.

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: There were no significant treatment related effects.

Organ Weights: No significant treatment related effects were observed. In males, the mean absolute and relative liver weights at 1000 mg/kg PO were significantly decreased when compared with controls (absolute: 10.04 g vs 11.92 g in control; relative: mean % brain weight of 477.5 vs 573.4 in control). However, since the mean liver weights of females treated orally or males and females treated PO/SC, which produced higher exposures, were not decreased, the lower mean liver weights at 1000 mg/kg/day in males was considered to be incidental and unrelated to the treatment.

Following PO/SC treatment, localized red discoloration of the stomach was observed in two females at 200/5 mg/kg and one female at 1000/5 mg/kg, and these findings correlated with erosions in the glandular mucosa. However, erosions in the glandular mucosa were seen in two control females (one dosed orally and one dosed PO/SC) which suggested that the gross localized red discoloration of the stomach that correlated microscopically with erosions was probably unrelated to the test article.

In males following PO/SC treatment at 1000/5 mg/kg, absolute (1.25 g vs. 1.35 g in control) and relative (mean % brain weight: 59.32 vs 65.15 in control) heart weights were significantly decreased when compared to controls. Since a similar weight difference was not evident in females and no microscopic changes were present in male or female hearts, the lower mean heart weights were considered to be incidental and not related to treatment.

Histopathology: No significant treatment related microscopic changes were observed following oral doses. Histopathological changes were seen at the injection site

(inflammation and ulceration of the subcutaneous tissue) following PO/SC treatment, which were seen to a similar extent in both 200/5 and 1000/5 mg/kg groups. In addition, minimal to mild vacuolation of proximal tubules observed in the kidneys from control and 200/5 and 1000/5 mg/kg. Minimal cytoplasmic vacuolation of tubular epithelia was seen in kidneys from 3 male and 3 female controls (Group 2), 3 males and 4 females at 200/5 mg/kg (Group 4), and 4 males and 6 females at 1000/5 mg/kg (Group 6). Mild vacuolation of proximal tubules was noted in 1 male at 1000/5 mg/kg. Vacuoles were located in the cytoplasm of proximal tubular epithelium, but there was no evidence of degeneration in these cells. Since vacuolation was present in kidneys from vehicle control and treated rats, and there was no clinical pathology findings related to the kidney, and hydroxypropyl- $\beta$ -cyclodextrin has been reported (Gould S and Scott RC, 2005, 2-Hydroxypropyl- $\beta$ -Cyclodextrin (HP- $\beta$ -CD): A Toxicology Review. Food and Chemical Toxicology, 43:1451-1459; Frijlink HW, et al., 1991, The Effects of Cyclodextrins on the Disposition of Intravenously Injected Drugs in the Rat, Pharmaceutical Research, 8:380-384) to cause vacuolation of proximal renal tubular cells, these findings were not considered to be related to the test article.

Toxicokinetics: Following a single oral dose of JNJ-27018966-AAA, mean  $t_{max}$  values ranged from 0.500 to 2.38 hours and mean half-life values ranged from 13.0 to 23.7 hours. Systemic exposure (AUC and  $C_{max}$ ) to JNJ-27018966 appeared to be dose related. There were no clear changes in exposure with multiple dosing and no clear gender differences. The following table (from page 569 of the report) shows the TK parameters after oral dose.

Table DM9: Mean (SD) JNJ-27018966 Plasma Toxicokinetic Parameters in Male and Female Rats (N=4) Following a Single or 28 Daily Oral Doses of JNJ-27018966-AAA (200 or 1000 mg/kg/day) (TOX7686)

Period (Day)	Dose (mg/kg)	Gender	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (0-∞) <sup>a</sup> (ng•h/mL)	t <sub>1/2</sub> (h)	CL/F [mL/(h•kg)]
1	200	Male	4.48 (2.15)	2.38 (1.89)	86.5 <sup>b</sup> NA	23.7 <sup>b</sup> NA	4440000 <sup>b</sup> NA
1	200	Female	3.44 (1.78)	1.38 (1.75)	29.0 <sup>b</sup> NA	13.0 <sup>b</sup> NA	6940000 <sup>b</sup> NA
28	200	Male	2.92 (0.953)	6.75 (11.5)	12.0 (5.46)	14.1 <sup>b</sup> NA	6180000 <sup>b</sup> NA
28	200	Female	8.04 (4.31)	0.750 (0.866)	26.1 (30.7)	3.68 <sup>b</sup> NA	6530000 <sup>b</sup> NA
1	1000	Male	11.8 (7.02)	1.75 (0.500)	257 <sup>c</sup> (138)	15.5 <sup>c</sup> (6.96)	4720000 <sup>c</sup> (2400000)
1	1000	Female	185 (153)	0.500 (0.000)	856 (861)	22.6 (16.6)	2250000 (1820000)
28	1000	Male	16.8 (7.42)	6.25 (11.8)	170 (53.3)	13.3 <sup>b</sup> NA	4500000 <sup>b</sup> NA
28	1000	Female	70.9 (106)	0.875 (0.250)	138 (56.6)	11.4 (1.95)	6060000 (1410000)

<sup>a</sup>: AUC (0-24) hours on Day 28.

<sup>b</sup>: N=2. Unable to calculate for 2 rats due to plasma profile.

<sup>c</sup>: N=3. Unable to calculate for 1 rat due to plasma profile.

Following a single PO/SC dose of JNJ-27018966, AUC and C<sub>max</sub> was higher in all combination dose groups than oral dosing alone, but there was no apparent relationship between exposure and dose. Mean t<sub>max</sub> ranged from 0.50 to 1.00 hours and mean half-life ranged from 1.37 to 3.50 hours following PO/SC combination doses. Exposure following multiple combination doses did not appear to change. There were no apparent gender differences following the combination doses. The following table (from page 570 of the report) shows the TK parameters after PO/SC dose.

Table DM10: Mean (SD) JNJ-27018966 Plasma Toxicokinetic Parameters in Male and Female Rats Following a Single or 28 Daily Oral Plus Subcutaneous Doses of JNJ-27018966-AAA (200/5 or 1000/5 mg/kg/day) (TOX7686)

Period (Day)	Dose (mg/kg)	Gender	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (0-∞) <sup>a</sup> (ng•h/mL)	t <sub>1/2</sub> (h)	CL/F [mL/(h•kg)]
1	200/5	Male	706 (310)	1.00 (0.707)	1910 (788)	1.37 (1.02)	125000 (59800)
1	200/5	Female	617 (104)	0.500 (0.000)	2260 (133)	1.64 (1.07)	90900 (5140)
28	200/5	Male	649 (125)	4.00 (0.00)	3380 (358)	NC <sup>b</sup> NA	NC <sup>b</sup> NA
28	200/5	Female	372 (129)	1.25 (0.500)	2090 (264)	2.25 (0.580)	106000 (11900)
1	1000/5	Male	680 (121)	0.625 (0.250)	2060 (164)	3.36 (0.327)	490000 (37500)
1	1000/5	Female	887 (303)	0.875 (0.750)	3190 (1330)	3.50 (0.459)	348000 (105000)
28	1000/5	Male	595 (147)	3.50 (1.00)	3610 (980)	3.43 (0.732)	290000 (70800)
28	1000/5	Female	441 (106)	0.750 (0.289)	2020 (194)	3.89 (0.359)	492000 (51600)

<sup>a</sup>: AUC (0-24) hours on Day 28.

<sup>b</sup>: Unable to calculate for any rat due to plasma profile.

**Dosing Solution Analysis:** Concentration, uniformity (oral suspensions only), and stability of the formulated test article were verified. Vehicles did not contain any detectable test article above the limit of detection. The formulated test and control articles were within acceptable limits ((b) (4) % of label claim). Homogeneity of the suspensions was found to be acceptable. The stability samples met the specification ((b) (4) % of label claim).

### **13-Week Oral and Subcutaneous Toxicity Study of JNJ-27018966-AAA in Rats (Study No. TOX8677)**

The review of the above study is incorporated below from the pharmacology review of IND 79214 dated March 17, 2011.

**Study title: 13-Week Oral and Subcutaneous Toxicity Study of JNJ-27018966-AAA in Rats**

Study no.: TOX8677  
 Study report location: Raritan, NJ  
 Conducting laboratory and location: Johnson & Johnson Pharmaceutical Research & Development, L.L.C. (J&JPRD), Raritan, NJ  
 Date of study initiation: February 6, 2008  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966, 21298-90A, 95.2%

**Key Study Findings:**

- JNJ-27018966 was generally well tolerated when administered orally (up to 1000 mg/kg/day) or by a combination of oral and subcutaneous administrations (200/5 mg/kg) for 13 weeks.
- There was no treatment-related mortality. Clinical signs (erythema and edema) were noted at the site of injection for animals received the drug by SC route.
- There were no significant treatment-related effects on the body weight, food consumption, clinical chemistry, gross pathology, organ weights or histopathology parameters.
- The target organ could not be identified in the absence of any significant organ toxicity except the injection site reactions in the animals treated by SC route.
- The NOAEL appeared be 1000 mg/kg, PO

**Methods**

Doses: 200, 1000 mg/kg/day, PO; 200/5 mg/kg/day PO/SC  
 Frequency of dosing: Daily  
 Route of administration: Oral; Oral/SC  
 Dose volume: 10 mL/kg; 1 mL/kg (SC)  
 Formulation/Vehicle: 0.5% hypromellose (PO); 10% hydroxypropyl-beta-cyclodextrin (HP- $\beta$ -CD) for SC  
 Species/Strain: Sprague Dawley rats  
 Number/Sex/Group: 10  
 Age: 8 weeks  
 Weight: 163.2-278.2 grams  
 Satellite groups: Yes for TK (Groups 6, 7, 8, 9, 10)  
 Unique study design: Please see the table below  
 Deviation from study protocol: Protocol deviations had no impact on the results and interpretations of the study

The following table (from page 17 of the study report) shows the study design. The vehicle and test article formulations were administered orally by gavage or by oral gavage in combination with a subcutaneous injection once daily.

Group	1 <sup>a</sup> (Vehicle)	2 <sup>b</sup> (Vehicle)	3	4	5
Rat Numbers (TOX8677-)					
Males <sup>c</sup>	1001-1010	2001-2010	3001-3010	4001-4010	5001-5010
Females <sup>c</sup>	1501-1510	2501-2510	3501-3510	4501-4510	5501-5510
JNJ-27018966					
Dose <sup>d</sup> (mg/kg/day)	0	0	200	200/5	1000
Concentration <sup>d</sup> (mg/mL)	0	0/0	20	20/5	100
Dose Volume (mL/kg/day)	10	10/1	10	10/1	10
Group (Toxicokinetics)	6 <sup>a</sup> (Vehicle)	7 <sup>b</sup> (Vehicle)	8	9	10
Rat Numbers (TOX8677-)					
Males <sup>c</sup>	6001-6003	7001-7003	8001-8004	9001-9004	10001-10004
Females <sup>c</sup>	6501-6503	7501-7503	8501-8504	9501-9504	10501-10504
JNJ-27018966					
Dose <sup>d</sup> (mg/kg/day)	0	0	200	200/5	1000
Concentration <sup>d</sup> (mg/mL)	0	0/0	20	20/5	100
Dose Volume (mL/kg/day)	10	10/1	10	10/1	10

<sup>a</sup> Rats were dosed orally with 0.5% hypromellose (PO).  
<sup>b</sup> Rats were dosed orally with 0.5% hypromellose and subcutaneously with 10% HP-β-CD (PO/SC)  
<sup>c</sup> Groups 1, 3, 5, 6, 8, and 10 were dosed orally (PO). Groups 2, 4, 7, and 9 were dosed orally and subcutaneously (PO/SC).  
<sup>d</sup> Calculated as the <sup>(b)(4)</sup> form.

**Basis of Dose Selection:** The doses were selected based on the results of a 4-week study (TOX7686). In a 4-week oral and oral/subcutaneous toxicity study, rats were administered either the vehicle (0.5% hypromellose for oral dosing or 10% HP-β-CD for subcutaneous dosing) or JNJ-27018966 at 200 or 1000 mg/kg/day orally, or 200/5 or 1000/5 mg/kg/day administered orally/subcutaneously for 4 weeks. JNJ-27018966 was generally well tolerated when administered orally or by a combination of oral and subcutaneous administrations to rats for 4 weeks at oral doses up to 1000 mg/kg or 1000/5 mg/kg oral/SC, respectively. Slight decreases in body weight and food consumption were seen at 1000 mg/kg or 1000/5 mg/kg when compared to the control animals. In addition, slight local irritation at the site of injection was also observed when injected subcutaneously. The NOAELs for this study were determined to be 1000 mg/kg

by oral administration and 1000/5 mg/kg by oral/SC administration. In addition, significantly increased exposure of JNJ-27018966 in the animals with oral/subcutaneous administration was noted when compared to the exposure with only oral administration. Based on these results, two oral doses of 200 and 1000 mg/kg/day, and one oral/subcutaneous dose of 200/5 mg/kg/day were selected for this current 13-week toxicity study.

### **Observations and Results:**

**Mortality:** Mortality was checked once daily. There were no treatment-related deaths in the study. One male (#2003) was sacrificed in moribund condition on Day 79 due to its poor health conditions (dyspnea, tachypnea, stained urogenital region, and decreased body weight). The cause of death was attributed to gavage error.

**Clinical Signs:** Clinical signs were observed twice at predose, twice weekly through Week 3 and once weekly through Week 12. There were no significant treatment-related clinical signs in animals treated orally. In animals treated SC, clinical signs at the injection site included edema in males, skin irritation, and slight to moderate erythema, crusty skin, skin lesion, and scaling.

**Body Weights:** Body weights were recorded on a weekly basis. The mean initial (Day -1) and final (Day 90) body weights of control males were 256.03 and 499.61 g, respectively. The mean initial (Day -1) and final (Day 90) body weights of control females were 189.37 and 317.88 g, respectively. There was no test article-related effect on body weight.

**Food Consumption:** Food consumption was recorded for main study animals on a weekly basis. The mean initial (Day -1 to 6) and final (Day 83 to Day 90) food consumption of control males were 25.69 and 26.57 g/animal/day, respectively. The mean initial (Day -1 to 6) and final (Day 83 to Day 90) food consumption of control females were 20.02 and 20.12 g/animal/day, respectively. There were no significant treatment-related effects on food consumption.

**Ophthalmoscopy:** Ophthalmoscopic examinations were conducted once predose and during Week 12. There were no treatment-related ophthalmoscopic findings at any dose.

**Hematology:** Hematology was conducted prior to scheduled necropsy. There were no test article-related effects on hematology parameters.

**Clinical Chemistry:** Clinical chemistry was conducted prior to scheduled necropsy. There were no test article-related effects on clinical chemistry parameters.

**Urinalysis:** Urine was collected prior to scheduled necropsy. There were no test article-related effects on urinalysis parameters.

**Gross Pathology:** Necropsy examinations were performed on all animals at the scheduled terminal necropsy. There were no test article-related macroscopic findings in this study.

**Organ Weights:** Organ weights were recorded for all animals at the scheduled terminal necropsy. The following table (from page 22 of the report) shows the list of organs and tissues that were weighed.

**2.6.3. Tissue List**

Collect	Weigh	Fix	Microscopic Examination
Adrenal gland (both)	X	F	X
Aorta, thoracic		F	X
Bone and bone marrow, sternum		F	X
Bone and bone marrow, stifle joint including distal femur and proximal tibia		F	X
Brain	X	F	X
Cervix		F	X
Coagulating gland (both)		F	X
Epididymis (both)		MD	X
Esophagus		F	X
Eye (both) with optic nerve(s)		F	X
Harderian gland (both)		F	X
Heart	X	F	X
Injection sites 1, 2, 3, and 4		F	X <sup>a</sup>
Kidney (both)	X	F	X
Large intestine (cecum, colon, rectum)		F	X
Larynx		F	X
Liver	X	F	X
Lung	X	F	X
Lymph node(s), mesenteric		F	X
Lymph node, popliteal (both)		F	X
Mammary gland(s), inguinal		F	X
Nose		F	
Ovary (both)	X	F	X
Oviduct (both)		F	X
Pancreas		F	X
Parathyroid gland(s)		F	X
Peyer's patch(es)		F	X
Pituitary gland	X (fixed)	F	X
Prostate		F	X
Salivary glands, parotid, mandibular, and sublingual (unilateral)		F	X
Sciatic nerve (both)		F	X
Seminal vesicles		F	X
Skeletal muscle, quadriceps femoris		F	X
Skin, inguinal		F	X
Small intestine (duodenum, jejunum, ileum)		F	X
Spinal cord (cervical, thoracic, and lumbar)		F	X
Spleen	X	F	X
Stomach		F	X
Testis (both)	X	MD	X
Thymus	X	F	X
Thyroid gland (both)	X (fixed)	F	X
Tongue		F	X
Trachea		F	X
Ureter(s)		F	X
Urinary bladder		F	X
Uterus		F	X
Vagina		F	X

F = fixed in 10% neutral buffered formalin

MD = fixed in modified Davidson's solution at scheduled necropsy

<sup>a</sup> Microscopic evaluation was performed on the last site injected.

X = completed

There were no significant treatment-related effects on organ weights. One male (2003) was sacrificed moribund on day 79. At necropsy the esophagus was found ruptured and fluid was present in the thoracic cavity. Treatment-related findings at necropsy were limited to the injection site. The 200/5 mg/kg (Group 4) male rats had more prominent tissue reaction at the injection site (red discoloration and scab formation) compared to the females at the same dose.

**Histopathology:** Histopathological examinations were conducted on tissues listed above from animals from control and high dose (PO, 1000 mg/kg/day and PO/SC, 200/5 mg/kg/day).

**Adequate Battery:** Yes

**Peer Review:** No

**Histological Findings:** Treatment-related microscopic findings were seen at the injection sites in the 200/5-mg/kg (Group 4) males and females, which included scab formation, acanthosis, and degeneration of the subcutaneous muscle, multifocal to diffuse infiltrations of chronic inflammatory cells and hemorrhage in a loose connective tissue stroma. One male (2003) was sacrificed moribund on Day 79. The microscopic findings were consistent with a gavage accident and consisted of an esophagus with chronic inflammation, chronic pleuritis and chronic pericarditis.

**Toxicokinetics:** Blood samples were collected on Day 0 and Week 13 (Day 90) in satellite groups of rats (4/sex/group) at 200, 200/5, or 1000 mg/kg at 0.5, 1, 2, 4, 8, and 24 hours postdose. Two additional satellite groups of rats (3/sex) were dosed with the vehicle for oral or SC administration and plasma samples were analyzed for the presence of JNJ-27018966. For each interval, control samples were collected predose and at 1, 4, and 8 hours postdose.

Following a single oral dose of JNJ-27018966, the mean  $T_{max}$  values ranged from 0.625 to 1.50 hours, and mean  $T_{1/2}$  values ranged from 6.24 to 30.9 hours. Systemic exposure (AUC and  $C_{max}$ ) increased in a dose-related manner. There were no apparent changes in exposure with multiple administrations and there were no apparent gender differences.

Following a single oral plus SC dose of JNJ-27018966 (200 mg/kg and 5 mg/kg), mean  $T_{max}$  values ranged from 0.750 to 1.38 hours, and mean half-life values ranged from 7.18 to 14.7 hours. Systemic exposure (AUC and  $C_{max}$ ) to JNJ-27018966 was higher than exposure for the 200 mg/kg, PO. There were no clear gender-related differences in exposure, and no clear changes in exposure with multiple administrations.

The mean TK parameters are shown in the following table (from page 778 of the study report).

Table SD6: Individual and Mean (SD) JNJ-27018966 Plasma Toxicokinetic Parameters in Female Rats Following a Single or 91 Daily Oral/Subcutaneous Doses of JNJ-27018966-AAA (200/0, 200/5, and 1000/0mg/kg/day) (TOX8677)

Day	Dose (mg/kg)	Subject	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (0-∞) <sup>a</sup> (ng·h/mL)	t <sub>1/2</sub> (h)	CL/F (mL/h/kg)
0	200/0	RAT8501	11.0	2.00	320 <sup>b</sup>	67.5	625000
		RAT8502	13.1	1.00	182 <sup>b</sup>	33.9	1100000
		RAT8503	217	1.00	481	12.1	416000
		RAT8504	17.9	2.00	252	10.1	795000
		Mean	64.8	1.50	309	30.9	734000
		SD	102	0.577	128	26.7	289000
	200/5	RAT9501	775	1.00	2170	14.8	94500
		RAT9502	1280	0.500	2620	5.43	78300
		RAT9503	3210	1.00	8090	8.72	25300
		RAT9504	1180	0.500	2390	29.7	85600
		Mean	1610	0.750	3820	14.7	70900
		SD	1090	0.289	2850	10.8	31100
	1000/0	RAT10501	2010	0.500	4640	4.52	215000
		RAT10502	5680	1.00	23900	6.44	41800
		RAT10503	97.9	0.500	746	5.58	1340000
		RAT10504	52.0	0.500	229	8.40	4370000
Mean		1960	0.625	7380	6.24	1490000	
SD		2640	0.250	11200	1.64	2000000	
90	200/0	RAT8501	4.24	0.500	39.3	27.1	1830000
		RAT8502	4.26	0.500	24.9	NC	NC
		RAT8503	13.3	2.00	50.7	25.1	2270000
		RAT8504	15.2	0.500	166	65.3	240000
		Mean	9.25	0.875	70.2	39.2	1450000
		SD	5.83	0.750	64.7	22.7	1070000
	200/5	RAT9501	709	1.00	3500	4.93	57800
		RAT9502	569	2.00	2070	0.893	103000
		RAT9503	439	1.00	2210	3.93	92300
		RAT9504	792	4.00	3250	7.29	62600
		Mean	627	2.00	2760	4.26	78900
		SD	156	1.41	723	2.65	22100
	1000/0	RAT10501	437	0.500	557	5.61	1610000
		RAT10502	682	1.00	1400	6.99	688000
		RAT10503	48.3	0.500	263	11.0	2840000
		RAT10504	16.7	0.500	179	32.1	1810000
		Mean	296	0.625	600	13.9	1740000
		SD	321	0.250	558	12.3	883000

Note: Doses listed as PO/SC doses administered.

NC: Not calculated due to plasma profile.

<sup>a</sup> AUC (0-24 h) on Day 90.

<sup>b</sup> AUC % extrapolated >25% Total AUC

**Dosing Formulation Analysis:** The sponsor verified concentration, content uniformity, and stability of the formulated test article and also verified the vehicle control article for the presence of any detectable test article. In addition, pH, density, and physical appearance of vehicle control and formulated test articles were also checked. The sponsor stated that the vehicle control and

formulated test articles were within acceptable limits for use in J&JPRD Toxicology/Pathology studies as defined by the SOPs of J&JPRD Preclinical Formulations and Analysis.

**Study title: 26-Week Oral (Gavage) Toxicity Study in Rats With a 4-Week Recovery Period**

Study no.: 1808-007  
 Study report location: EDR 4.2.3.2.  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: July 28, 2010  
 Date of study completion: July 28, 2011  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAA, 0020300266, 99.2%

**Key Study Findings:**

- In a 26-week oral (gavage) toxicity study in SD rats, JNJ-27018966-AAA was administered at 500, 1000, and 2000 mg/kg/day for 26 consecutive weeks followed by a 4 week recovery period.
- There was no treatment related mortality or clinical signs. There were no test article-related effects on body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, or urinalysis parameters or organ weights.
- There were no significant treatment related macroscopic and microscopic changes in either sex.
- The NOAEL was considered to be 2000 mg/kg/day.

**Methods:**

Doses: 500, 1000 and 2000 mg/kg/day  
 Frequency of dosing: Once daily  
 Route of administration: Oral (Gavage)  
 Dose volume: 13.33 mL/kg  
 Formulation/Vehicle: 0.5% Hydroxypropyl methylcellulose (high viscosity)  
 Species/Strain: SD rats  
 Number/Sex/Group: 20 or 15/sex/group  
 Age: 6 weeks  
 Weight: Male: 240-297 g; Female: 176-219 g  
 Satellite groups: TK (15/sex/dose)  
 Unique study design: Study design is shown below  
 Deviation from study protocol: Protocol deviations did not affect the quality or integrity of the study

The following table (from page 16 of the report) shows the study design.

<b>Group Assignments</b>			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
<b>Main Study</b>			
1	0	20 <sup>a</sup>	20 <sup>a</sup>
2	500	15	15
3	1000	20 <sup>a</sup>	20 <sup>a</sup>
4	2000	20 <sup>a</sup>	20 <sup>a</sup>
<b>Toxicokinetics</b>			
5	500	12+3 <sup>b</sup>	12+3 <sup>b</sup>
6	1000	12+3 <sup>b</sup>	12+3 <sup>b</sup>
7	2000	12+3 <sup>b</sup>	12+3 <sup>b</sup>

<sup>a</sup>The first five surviving animals were maintained for a subsequent 4-week recovery period  
<sup>b</sup>Additional animals included as possible replacements

**Basis of dose selection:** The dose levels were selected based on the results of the previous studies. However, the Applicant did not provide any details or specifics of these studies.

**Observations:**

**Mortality:** Mortality was observed twice daily.

**Clinical Signs:** Clinical signs were observed once weekly.

**Body Weights:** Body weights were recorded once weekly.

**Food Consumption:** Food consumption was recorded on a weekly basis.

**Ophthalmoscopy:** Ophthalmoscopic examinations were conducted on all animals at pretest and at terminal or recovery necropsy.

**Hematology:** Hematology was conducted at Week 13 and prior to terminal or recovery necropsy.

**Clinical Chemistry:** Clinical chemistry was conducted at Week 13 and prior to terminal or recovery necropsy.

**Urinalysis:** Urine samples collected at Week 13 and prior to terminal or recovery necropsy.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 52 of the report) organs were weighed from all main study animals.

**Organs or Tissues to be Weighed, Preserved, and Microscopically Examined**

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec <sup>a</sup>
Adrenal gland	X	X	X		
Aorta		X	X		
Bone with bone marrow, femur		X	X		
Bone with bone marrow, sternum		X	X		
Bone marrow smear <sup>b</sup>		X			
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X		
Epididymis	X	X	X		
Esophagus		X	X		
Eye (with optic nerve)		X	X		
GALT <sup>g</sup>		X	X		
Heart	X	X	X		
Joint, tibiofemoral		X	X		
Kidney	X	X	X		
Lacrimal gland, exorbital		X	X		
Large intestine, cecum		X	X		
Large intestine, colon		X	X		
Large intestine, rectum		X	X		
Larynx		X	X		
Liver	X	X	X		
Lung with bronchi	X	X	X		
Lymph node, mandibular		X	X		
Lymph node, mesenteric		X	X		
Mammary gland (process females only)		X	X		
Nerve, sciatic		X	X		
Ovary	X	X	X		
Oviducts	X	X	X		
Pancreas		X	X		
Pituitary	X	X	X		
Prostate	X	X	X		

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec <sup>g</sup>
Salivary gland, mandibular <sup>c</sup>	X	X	X		
Salivary gland, parotid		X	X		
Salivary gland, sublingual	X	X	X		
Seminal vesicles	X	X	X		
Skeletal muscle, biceps femoris		X	X		
Skin		X	X		
Small intestine, duodenum		X	X		
Small intestine, ileum		X	X		
Small intestine, jejunum		X	X		
Spinal cord, cervical		X	X		
Spinal cord, lumbar		X	X		
Spinal cord, thoracic		X	X		
Spleen	X	X	X		
Stomach, glandular		X	X		
Stomach, nonglandular		X	X		
Target Organs <sup>d</sup>		X	X	X	X
Testis	X	X	X		
Thymus	X	X	X		
Thyroid gland (with parathyroid) <sup>e</sup>	X	X	X		
Tongue		X	X		
Trachea		X	X		
Ureters		X	X		
Urinary bladder		X	X		
Uterus with cervix	X	X	X		
Vagina		X	X		
Gross lesions		X	X	X	X
Tissue masses with regional lymph node <sup>f</sup>		X	X	X	X

<sup>a</sup> Recovery

<sup>b</sup> Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).

<sup>c</sup> The combined weight of the right mandibular/sublingual salivary gland will be obtained.

Histopathology: The above listed organs/tissues from animals from Groups 1-4 were collected for histopathology.

Toxicokinetics: Blood samples were collected from three TK animals per sex per group at pretest and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours postdose on Days 1, 90, and 180.

Dosing Solution Analysis: Dosing formulations were evaluated for homogeneity and concentration. Homogeneity was tested every week and concentration analysis was conducted every 4 weeks and at Week 25.

### **Results**:

Mortality: All main study animals survived to their scheduled terminal or recovery necropsy with the exception of three animals. One male at 1000 mg/kg/day (animal number 1051) was found dead on Day 177, one female at 1000 mg/kg/day (animal number 1543) was found dead on Day 57, and one female at 2000 mg/kg/day (animal number 1558) was found dead on Day 25. Microscopic examinations revealed that all of these deaths were the result of dosing injury and not directly attributed to the test article. In addition, three TK animals were found dead and another was euthanized *in extremis* during the study. One male at 500 mg/kg/day (animal number 1089) was euthanized on Day 66, one female at 500 mg/kg/day (animal number 1584) was found dead on Day 33, one male at 2000 mg/kg/day (animal number 1120) was found dead on Day 21, and one female at 2000 mg/kg/day (animal number 1610) was found dead on Day 97. As terminal necropsies were not performed on these animals per protocol, causes of death could not be determined. The following table shows the mortality data.

<b>Dose (mg/kg/day)</b>	<b>Animal No. (Sex)</b>	<b>Day of Death</b>	<b>Cause</b>
1000	1051 (M)	177	Dosing error
1000	1543 (F)	57	Dosing error
2000	1558 (F)	25	Dosing error
<b>TK Animals</b>			
500	1089 (M)	66	Not determined
500	1584 (F)	33	Not determined
2000	1120 (M)	21	Not determined
2000	1610 (F)	97	Not determined

Clinical Signs: There were no significant treatment related clinical signs.

Body Weights: The mean initial (Week -1) and final (Week 26) body weights of control males were 271 and 607 g, respectively. The mean initial (Week -1) and final (Week 26) body weights of the control females were 196 and 370 g, respectively. There were no significant treatment related effects. Summary of body weights is shown below (from page 24 of the report).

<b>Summary of Main Study Group Mean Body Weights; g (At Termination)</b>				
Dose Level (mg/kg/day)	Male		Female	
	Week 26	(%) Difference from Control	Week 26	(%) Difference from Control
0	606.7	NA	369.8	NA
500	593.4	(-2.2)	362.3	(-2.0)
1000	601.6	(-0.8)	380.5	(+2.9)
2000	590.7	(-2.6)	384.8	(+4.1)

NA – Not applicable

<b>Summary of Group Mean Body Weights; g (End of Recovery)</b>						
Dose Level (mg/kg/day)	Male			Female		
	Week 27	Week 30	(%)	Week 27	Week 30	(%)
0	629.2	657.2	(+4.5)	422.6	444.8	(+5.3)
1000	597.6	617.8	(+3.4)	384.4	401.8	(+4.5)
2000	570.8	595.2	(+4.3)	386.0	403.4	(+4.5)

% - Percent difference from Week 27

**Food Consumption:** The mean initial (Week 1) and final (Week 26) food consumption of control males were 27.63 and 28.21 g/animal/day, respectively. The mean initial (Week -1) and final (Week 26) food consumption of control females were 20.48 and 21.76 g/animal/day, respectively. There were no significant treatment related effects. The following table (from page 24 of the report) shows the food consumption data.

<b>Average Food Consumption; g/animal/day (Weeks 1 through 26)</b>				
Dose Level (mg/kg/day)	Male		Female	
	Mean	(%) Difference from Control	Mean	(%) Difference from Control
0	28.51	NA	21.37	NA
500	27.86	(-2.3)	20.90	(-2.2)
1000	27.80	(-2.5)	21.57	(+0.9)
2000	27.54	(-3.4)	21.60	(+1.1)

NA – Not applicable

Average Recovery Food Consumption; g/animal/day (Weeks 27 through 30)				
Dose Level (mg/kg/day)	Male		Female	
	Mean	(%) Difference from Control	Mean	(%) Difference from Control
0	29.63	NA	24.45	NA
1000	27.68	(-6.6)	22.75	(-7.0)
2000	27.48	(-7.3)	23.71	(-3.0)
NA – Not applicable				

Ophthalmoscopy: One male (animal # 1058) at 2000 mg/kg/day had superficial keratitis in the left eye at termination but was not considered to be test article-related. Per the ophthalmologist's report, this observation is expected for this group of animals considering age, sex and strain. There was no obvious trend suggestive of treatment effect. There were no other findings in any animal at the terminal or recovery examinations. Overall, there was no treatment related effect.

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: There were no significant treatment related gross necropsy findings.

Organ Weights: No significant treatment related effects were observed in either sex.

Histopathology: There were no significant treatment related histopathology findings. A high incidence of minimal subacute/chronic inflammation in the lung of both sexes was observed, which was attributed to aspiration of the high viscosity vehicle. Accumulations of lymphocytes and macrophages were located at the level of the distal airways and around adjacent blood vessels.

Toxicokinetics: On Day 1,  $C_{max}$  increased with the dose in an approximately dose proportional manner. On Day 90, increase in  $C_{max}$  was less than dose proportional. On Day 180, there was no consistent trend in increase of  $C_{max}$  with the dose. On Days 1 and 90, exposure ( $AUC_{0-24h}$ ) increased dose proportionally from 500 mg/kg to 1000 mg/kg/day and less than dose proportionally from 1000 mg/kg/day to 2000 mg/kg/day. On Day 180, increase in  $AUC_{0-24h}$  was less than dose proportional from 500 mg/kg/day to 2000 mg/kg/day. In general, there was no apparent gender difference in  $C_{max}$  across all dose levels on Days 1, 90, and 180. For the AUC, males showed slightly higher values than females at all dose levels on Day 1. However, there was no consistent trend in gender difference in AUC on Days 90 and 180. There was some accumulation of

JNJ-27018966-AAA following repeated administrations for 180 days. Accumulation was higher in females than that in males at Day 90 and Day 180. Terminal half-lives ranged from 9 to 15 hours. The following tables (from page 935 and 936 of the report) show the TK data.

Table 1.1 Toxicokinetic Parameters for JNJ-27018966 in Rats After Daily Oral Administration of JNJ-27018966-AAA on Days 1, 90, and 180

Day	Dose (mg/kg/day)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose ((ng*hr/mL)/(mg/kg))	AUC(0-inf) (ng*hr/mL)	Lambda-z (1/hr)	T <sub>1/2</sub> (hr)	AR
1	500	7.43	0.50	74.91	0.15	.	.	.	.
	1000	17.56	0.25	135.47	0.14	.	.	.	.
	2000	37.78	0.25	154.39	0.08	231.99	0.0467	14.85	.
90	500	14.13	0.25	95.88	0.19	.	.	.	1.28
	1000	21.56	0.25	186.68	0.19	.	.	.	1.38
	2000	33.88	0.25	218.72	0.11	314.01	0.0563	12.32	1.42
180	500	37.78	2.00	166.27	0.33	188.03	0.0736	9.41	2.22
	1000	32.60	0.25	239.46	0.24	324.68	0.0589	11.76	1.77
	2000	67.63	0.25	273.97	0.14	331.18	0.0687	10.09	1.77

Table 1.2 Toxicokinetic Parameters for JNJ-27018966 in Rats by Sex After Daily Oral Administration of JNJ-27018966-AAA on Days 1, 90, and 180

Day	Dose (mg/kg/day)	Sex	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose ((ng*hr/mL)/(mg/kg))	AUC(0-inf) (ng*hr/mL)	Lambda-z (1/hr)	T <sub>1/2</sub> (hr)	AR
1	500	Female	8.87	0.50	57.86	0.12	.	.	.	.
		Male	8.32	1.00	91.96	0.18	.	.	.	.
	1000	Female	18.09	0.25	125.85	0.13	.	.	.	.
		Male	17.03	0.25	145.10	0.15	.	.	.	.
	2000	Female	40.37	0.25	140.24	0.07	186.03	0.0588	11.78	.
		Male	35.20	0.25	168.55	0.08	288.65	0.0379	18.30	.
90	500	Female	13.35	0.25	86.08	0.17	.	.	.	1.49
		Male	14.90	0.25	105.68	0.21	.	.	.	1.15
	1000	Female	30.97	0.50	205.15	0.21	.	.	.	1.63
		Male	27.45	0.25	168.21	0.17	.	.	.	1.16
	2000	Female	45.70	0.25	249.97	0.12	309.59	0.0767	9.04	1.78
		Male	22.07	0.25	187.46	0.09	.	.	.	1.11
180	500	Female	64.25	0.50	161.49	0.32	175.10	0.0955	7.26	2.79
		Male	46.33	2.00	171.06	0.34	208.77	0.0505	13.73	1.86
	1000	Female	45.10	0.50	268.19	0.27	.	.	.	2.13
		Male	46.00	0.25	210.73	0.21	.	.	.	1.45
	2000	Female	60.03	0.25	288.41	0.14	327.29	0.0923	7.51	2.06
		Male	75.23	0.25	259.52	0.13	335.07	0.0566	12.24	1.54

**Dosing Solution Analysis:** Top, middle, and bottom replicate samples were collected for homogeneity analysis from Week 1 formulations prepared for the low and high concentrations. The formulations were considered to be homogeneous. Analyses were conducted to confirm acceptable formulation concentrations during Weeks 1-4, 8, 12, 16, 20, 24, and 25. Results indicated that all dosing formulations were within the targeted concentration range throughout the period of use.

**Study title: 4-Week Oral Toxicity Study with JNJ-27018966-AAA in Juvenile Rats With a 4-Week Recovery Period**

Study no.: 1808-018  
 Study report location: EDR 4.2.3.2.  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: June 8, 2012  
 Date of study completion: January 18, 2013  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAA, ZR497138PFA141, 99.7%

**Key Study Findings:**

- In a 4-week oral (gavage) toxicity study in juvenile (21 days old) rats, JNJ-27018966-AAA was administered daily at 500, 750, and 1500 mg/kg/day for 4 weeks followed by a 4-week treatment free recovery period.
- There were no significant test article-related effects on mortality, clinical signs, body weight, food consumption, FOB (functional observational battery) evaluations, ophthalmology, hematology, clinical chemistry, or urinalysis parameters, organ weights and bone length measurements.
- There were no significant treatment related macroscopic and microscopic findings in either sex.
- The NOAEL was considered to be 1500 mg/kg/day.

**Methods:**

Doses: 500, 750 and 1500 mg/kg/day  
 Frequency of dosing: Once daily  
 Route of administration: Oral (Gavage)  
 Dose volume: 13.33 mL/kg  
 Formulation/Vehicle: 0.5% Hydroxypropyl methylcellulose  
 Species/Strain: SD rats  
 Number/Sex/Group: 15-20/sex/group  
 Age: 21 days  
 Weight: Male: 55.5-77.6 g; Female: 49.0-68.4 g  
 Satellite groups: TK (Groups 5-8: 37/sex/dose; Control: 7/sex)  
 Study design: Study design is shown below  
 Deviation from study protocol: Protocol deviations did not affect the quality or integrity of the study

The following table (from page 16 of the report) shows the study design.

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
Main Study			
1	0	20 <sup>a</sup>	20 <sup>a</sup>
2	500	15	15
3	750	15	15
4	1500	20 <sup>a</sup>	20 <sup>a</sup>
Toxicokinetic <sup>b</sup>			
5	0	7	7
6	500	37	37
7	750	37	37
8	1500	37	37

<sup>a</sup>Five animals were maintained for the recovery period  
<sup>b</sup>One extra animal/sex was available as replacement

**Basis of dose selection:** Dose levels were selected based on the results of a 2-week oral (gavage) dose ranging study (Study No. 1808-017, non-GLP) in juvenile rats (21 days of age). In this dose ranging study, four groups (n = 5/sex/group) of rats were administered the test article at 150, 500, 750, or 1500 mg/kg/day (10 mL/kg). One additional group (n = 5/sex) served as the control and was treated with the vehicle, 0.5% hydroxypropyl methylcellulose (HPMC) in water. There were no significant treatment related effects on mortality, body weight, food consumption, clinical signs, hematology, clinical chemistry, or urinalysis parameters, organ weights and macroscopic evaluations in either sex. Histopathology was not conducted. Based on the results of this dose ranging study, the high dose for the current study was selected as 1500 mg/kg/day.

### **Observations:**

**Mortality:** Mortality was observed twice daily.

**Clinical Signs:** Clinical signs were observed twice weekly.

**Body Weights:** Body weights were recorded twice weekly.

**Food Consumption:** Food consumption was recorded twice weekly.

**Functional Observational Battery (FOB):** Functional observational battery evaluations were conducted on all main study animals prior to initiation of dosing, 1 hour postdose on Day 1, prior to terminal necropsy, and during the last week of the recovery period. The examinations included evaluation of activity and arousal, posture, rearing, bizarre behavior, clonic and tonic movements, gait, mobility, stereotypy, righting reflex,

response to stimulus (approach, click, tail pinch, and touch), palpebral closure, pupil response, piloerection, exophthalmus, lacrimation, salivation, and respiration.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted on all animals at pretest and at terminal or recovery necropsy.

Hematology: Hematology was conducted prior to terminal or recovery necropsy.

Clinical Chemistry: Clinical chemistry was conducted prior to terminal or recovery necropsy.

Urinalysis: Urine samples collected prior to terminal or recovery necropsy.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 22 of the protocol) organs were weighed from all main study animals.

**Organs or Tissues to be Weighed, Preserved, and Microscopically Examined**

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec <sup>a</sup>
Adrenal gland	X	X	X		
Aorta		X	X		

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec <sup>a</sup>
Bone with bone marrow, femur		X	X		
Bone with bone marrow, sternum		X	X		
Bone marrow smear <sup>b</sup>		X			
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X		
Epididymis	X	X	X		
Esophagus		X	X		
Eye (with optic nerve)		X	X		
GALT <sup>g</sup>		X	X		
Heart	X	X	X		
Joint, tibiofemoral		X	X		
Kidney	X	X	X		
Lacrimal gland, exorbital		X	X		
Large intestine, cecum		X	X		
Large intestine, colon		X	X		
Large intestine, rectum		X	X		
Larynx		X	X		
Liver	X	X	X		
Lung with bronchi	X	X	X		
Lymph node, mandibular		X	X		
Lymph node, mesenteric		X	X		
Mammary gland (process females only)		X	X		
Nerve, sciatic		X	X		
Ovary	X	X	X		
Oviducts		X	X		
Pancreas		X	X		
Pituitary	X	X	X		
Prostate		X	X		
Salivary gland, mandibular <sup>c</sup>	X	X	X		
Salivary gland, parotid		X	X		

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec <sup>a</sup>
Salivary gland, sublingual		X	X		
Seminal vesicles		X	X		
Skeletal muscle, biceps femoris		X	X		
Skin		X	X		
Small intestine, duodenum		X	X		
Small intestine, ileum		X	X		
Small intestine, jejunum		X	X		
Spinal cord, cervical		X	X		
Spinal cord, lumbar		X	X		
Spinal cord, thoracic		X	X		
Spleen	X	X	X		
Stomach, glandular		X	X		
Stomach, nonglandular		X	X		
Target Organs <sup>d</sup>		X	X	X	X
Testis	X	X	X		
Thymus	X	X	X		
Thyroid gland (with parathyroid) <sup>e</sup>	X	X	X		
Tongue		X	X		
Trachea		X	X		
Ureters		X	X		
Urinary bladder		X	X		
Uterus with cervix		X	X		
Vagina		X	X		
Gross lesions		X	X	X	X
Tissue masses with regional lymph node <sup>f</sup>		X	X	X	X

<sup>a</sup>Recovery

<sup>b</sup>Bone marrow smears will be prepared only for animals necropsied at scheduled intervals.

Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).

<sup>c</sup>The combined weight of the right mandibular/sublingual salivary gland will be obtained.

<sup>d</sup>Target organs (and target organ gross lesions) will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost).

**Histopathology:** The above listed organs/tissues from animals from Groups 1-4 were collected for histopathology.

**Bone Length Measurement:** Bone lengths of the femur and tibia (right side) were measured for all surviving main study animals at the scheduled necropsies.

Toxicokinetics: Blood samples were collected from TK animals (3/sex/group) at pretest and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours postdose on Days 1 and 28.

Dosing Solution Analysis: Dosing formulations were evaluated for homogeneity and concentration. Homogeneity analysis was conducted on prepared formulations from Week 1 at 50 and 150 mg/mL concentrations. Concentrations were analyzed from samples collected from Week 1, 2, 3 and 4.

## **Results**:

Mortality: There were no test article-related deaths. One male at 500 mg/kg/day (animal number 1027) was found dead on Day 20. A cause of death could not be determined; however, the thoracic cavity contained white fluid which would support the possibility of a dosing error.

Clinical Signs: There were no significant treatment related clinical signs.

Body Weights: The mean initial (Day -4) and final (Day 29) body weights of control males were 64.1 and 302.7 g, respectively. The mean initial (Week -1) and final (Week 26) body weights of the control females were 58.2 and 207.3 g, respectively. There were no significant treatment related effects.

Food Consumption: The mean initial (Day 4) and final (Day 28) food consumption of control males were 15.23 and 28.53 g/animal/day, respectively. The mean initial (Day 4) and final (Day 28) food consumption of control females were 13.17 and 20.71 g/animal/day, respectively. There were no significant treatment related effects.

Functional Observational Battery (FOB): There was no significant treatment related FOB findings (activity/arousal, neuromuscular, sensorimotor, autonomic, and physiological measurements).

Ophthalmoscopy: There were no significant treatment effects.

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: There were no significant treatment related gross necropsy findings.

Organ Weights: There were no significant treatment related organ weight changes in both sexes. In males, increased spleen weights were observed at 1500 mg/kg/day. There were no microscopic correlates for these increases and no similar changes were seen in females. These changes were not considered to be treatment related.

**Histopathology:** There were no significant treatment related histopathology findings in either sex. One female at 1500 mg/kg/day (Animal 1241) had a malignant mammary tumor. This was considered to be a spontaneous/incidental finding, as there were no other mammary tumor findings or evidence of treatment related pre-neoplastic lesions such as mammary gland hyperplasia or atypia.

**Bone Length Measurement:** There were no test article related changes in bone length [(femur and tibia (right side))] measurements.

**Toxicokinetics:** There were no measurable concentrations of JNJ-27018966 in plasma from control rats on Day 1. However, low concentrations of JNJ-27018966 were found in the plasma of 5 (1.93, 2.00, 1.07, 2.23, and 17.3 ng/mL for animal number 1075, 1076, 1062, 1063 and 1064, respectively) of 6 control rats on Day 28. The Applicant did not provide any reason for this finding. The AUC<sub>0-24h</sub> and C<sub>max</sub> increased approximately dose proportionately between 500 and 1500 mg/kg/day on Days 1 and 28. Combined mean T<sub>max</sub> occurred between 0.250 and 12.1 hours. Systemic exposure was greater on Day 28 than on Day 1, indicating drug accumulation. Combined mean accumulation ratios for AUC<sub>0-24</sub> and C<sub>max</sub> ranged from 2.04 to 6.45 and from 13.1 to 17.5, respectively. There were no consistent gender differences in the TK parameters on Days 1 and 28. The following table (from page 966 of the report) shows the TK data.

Table 3. Toxicokinetic Parameters for JNJ-27018966 in Male and Female Juvenile Rats given Daily Oral Doses of JNJ-27018966-AAA

Day	Dose (mg/kg/day)	Sex	AUC <sub>0-24</sub> (hr•ng/mL)	AUC <sub>0-24</sub> /Dose ((hr•ng/mL)/mg/kg)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose ((ng/mL)/mg/kg)	t <sub>1/2</sub> (hr)	T <sub>max</sub> (hr)	Accumulation ratio (Day 28/Day 1)	
									AUC <sub>0-24</sub>	C <sub>max</sub>
1	500	M	148	0.296	12.1	0.0243	9.66	2.00	NA	NA
		F	165	0.330	17.5	0.0349	NC	0.500	NA	NA
		Combined	156	0.313	14.8	0.0296	9.66	1.25	NA	NA
	750	M	211	0.281	21.3	0.0284	NC	4.00	NA	NA
		F	167	0.222	17.6	0.0235	NC	0.250	NA	NA
		Combined	189	0.252	19.4	0.0259	NC	2.13	NA	NA
	1500	M	296	0.197	19.1	0.0127	NC	4.00	NA	NA
		F	377	0.252	38.7	0.0258	NC	1.00	NA	NA
		Combined	337	0.224	28.9	0.0193	NC	2.50	NA	NA
28	500	M	626	1.25	179	0.358	NC	0.250	4.24	14.7
		F	171	0.343	201	0.402	9.13	0.250	1.04	11.5
		Combined	399	0.798	190	0.380	9.13	0.250	2.64	13.1
	750	M	2100	2.80	177	0.236	NC	24.0	9.98	8.32
		F	488	0.651	420	0.560	7.48	0.250	2.93	23.8
		Combined	1300	1.73	298	0.398	7.48	12.1	6.45	16.1
	1500	M	461	0.307	363	0.242	10.0	0.250	1.56	19.0
		F	954	0.636	615	0.410	NC	1.00	2.53	15.9
		Combined	707	0.471	489	0.326	10.0	0.625	2.04	17.5

NA = not applicable.

NC = not calculated; insufficient data.

**Dosing Solution Analysis:** Homogeneity analysis results were 101% of the targeted concentrations. Concentration analysis from samples collected from Weeks 1, 2, and 4 prepared formulations (50, 75, and 150 mg/mL) showed a percent recovery ranging from 94 to 106% of the targeted concentrations. For the Week 3 samples at 50 mg/mL, the sample assayed at 16% of the expected concentration. The 75 mg/mL sample assayed at 81% of the expected concentration. The 150 mg/mL analysis sample assayed at 99% of the expected concentration for Week 3. An investigation was

conducted in regards to the Week 3, 50 and 75 mg/mL preparations; however no assignable cause for the low recoveries was determined.

## **Monkeys:**

### **Study title: 2-Week Intravenous Toxicity Study in Cynomolgus Monkeys With a 2-Week Recovery Period**

Study no.: 1808-012  
 Study report location: EDR 4.2.3.2.  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: April 26, 2012  
 Date of study completion: October 30, 2012  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAC, 00563229, 98.6%

### **Key Study Findings:**

- In a 14-Day IV (bolus injection) toxicity study in Cynomolgus monkeys, animals were treated with JNJ-27018966-AAC at 5, 10, and 20 mg/kg/day for 14 days followed by a 2-week recovery period.
- There was no significant treatment related mortality or clinical signs.
- There was a reversible slowing of the heart rate at all doses and a greater incidence of sinus bradycardia at 5 and 20 mg/kg/day when compared to the vehicle. There was no apparent dose response. Changes in the heart rate and RR interval were resolved at the recovery interval. The slowing of the heart rate was associated with prolongation of the QT interval; however, when corrected for the heart rate, QTc interval was not prolonged.
- There were no significant treatment ophthalmology, clinical pathology (hematology, clinical chemistry, or urinalysis parameters), macroscopy, organ weight and histopathology changes in either sex.
- The NOAEL was considered as 20 mg/kg/day.

### **Methods:**

Doses: 5, 10 mg/kg/day  
 Frequency of dosing: Once daily  
 Route of administration: Intravenous (bolus injection)  
 Dose volume: 1 mL/kg  
 Formulation/Vehicle: 40% Hydroxyl propyl beta cyclodextrin (HP $\beta$ CD) in water  
 Species/Strain: Cynomolgus monkey  
 Number/Sex/Group: 4-7/sex/group  
 Age: 2 year 7 months to 4 year 1 month

Weight: Male: 2.58-3.10 kg; Female: 2.58-3.06 kg  
 Satellite groups: None  
 Study design: Study design is shown below  
 Deviation from study protocol: Protocol deviations did not affect the quality or integrity of the study

The following table (from page 15 of the report) shows the study design.

<b>Group Assignments</b>			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1	0 <sup>a</sup>	7 <sup>b</sup>	7 <sup>b</sup>
2	5	4	4
3	10	4	4
4	20	7 <sup>b</sup>	7 <sup>b</sup>

<sup>a</sup> Administered vehicle only.  
<sup>b</sup> The first three animals per sex were evaluated during the 2-week recovery period.

**Basis of dose selection:** Dose levels were selected based on the results of an IV (bolus) dose ranging study (Study No. 1808-015) in Cynomolgus monkeys. In the above non-GLP dose ranging study, four treatment (n = 3/sex/dose) group Cynomolgus monkeys were administered JNJ-27018966-AAC at 5, 10, 20, and 40/30 mg/kg/day (1 mL/kg). One additional group (n = 3/sex) was used as the control and received the vehicle, 40% HPβCD, in water. Beginning on Day 2, due to the mortality of one male at 40 mg/kg/day, the dose level was reduced from 40 mg/kg/day to 30 mg/kg/day for all surviving Group 5 animals. The females at 40/30 mg/kg/day were dosed on Days 1 and 2, the males at 40 mg/kg/day were dosed for one day, and the replacement male at 30 mg/kg/day was dosed once.

Body weight was decreased at all doses. Treatment related clinical signs included decreased activity, unresponsiveness, and decreased body temperature and/or respiration rates at ≥ 20 mg/kg. Some decreased activity was also noted at 10 mg/kg, however, it did not progress to the magnitude as seen in the higher dose levels. The NOAEL was considered as 10 mg/kg/day.

### **Observations:**

**Mortality:** Mortality was observed twice daily.

**Clinical Signs:** Clinical signs were observed three times daily.

**Body Weights:** Body weights were recorded once weekly.

Food Consumption: Food consumption was observed qualitatively on a daily basis.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted on all animals at pretest and at terminal or recovery necropsy.

Electrocardiography (ECG): ECG examinations were conducted on all animals prior to treatment (Day -10), on Day 2 (predose and at 1 to 2 hours postdose), on Day 13 (predose and at 1 to 2 hours postdose), and once during the last week of the recovery period (Day 27).

Hematology: Hematology was conducted at pretest and prior to terminal or recovery necropsy.

Clinical Chemistry: Clinical chemistry was conducted at pretest and prior to terminal or recovery necropsy.

Urinalysis: Urine samples collected for 16 hours at termination and recovery.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 51 of the report) organs were weighed from all main study animals.

**Organs or Tissues to be Weighed, Preserved, and Microscopically Examined**

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Adrenal gland	X	X	X	X
Aorta		X	X	X

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Bone with bone marrow, femur		X	X	X
Bone with bone marrow, rib		X	X	X
Bone with bone marrow, sternum		X	X	X
Bone marrow smear <sup>a</sup>		X		
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X	X
Epididymis		X	X	X
Esophagus		X	X	X
Eye (with optic nerve)		X	X	X
Gallbladder		X	X	X
GALT <sup>f</sup>		X	X	X
Heart	X	X	X	X
Injection site (a representative site)		X	X	X
Joint, tibiofemoral		X	X	X
Kidney	X	X	X	X
Large intestine, cecum		X	X	X
Large intestine, colon		X	X	X
Large intestine, rectum		X	X	X
Larynx		X	X	X
Liver	X	X	X	X
Lung with bronchi	X	X	X	X
Lymph node, mandibular		X	X	X
Lymph node, mesenteric		X	X	X
Mammary gland (process females only)		X	X	X
Nerve, sciatic		X	X	X
Ovary	X	X	X	X
Oviducts		X	X	X
Pancreas		X	X	X
Pituitary	X	X	X	X
Prostate	X	X	X	X
Salivary gland, mandibular <sup>b</sup>	X	X	X	X
Salivary gland, parotid		X	X	X
Salivary gland, sublingual		X	X	X

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Seminal vesicles		X	X	X
Skeletal muscle, rectus femoris		X	X	X
Skin		X	X	X
Small intestine, duodenum		X	X	X
Small intestine, ileum		X	X	X
Small intestine, jejunum		X	X	X
Spinal cord, cervical		X	X	X
Spinal cord, lumbar		X	X	X
Spinal cord, thoracic		X	X	X
Spleen	X	X	X	X
Stomach, cardia		X	X	X
Stomach, fundus		X	X	X
Stomach, pylorus		X	X	X
Target Organs <sup>c</sup>		X	X	X
Testis	X	X	X	X
Thymus	X	X	X	X
Thyroid gland (with parathyroid) <sup>d</sup>	X	X	X	X
Tongue		X	X	X
Trachea		X	X	X
Ureters		X	X	X
Urinary bladder		X	X	X
Uterus with cervix	X	X	X	X

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Vagina		X	X	X
Gross lesions		X	X	X
Tissue masses with regional lymph node <sup>e</sup>		X	X	X
<sup>a</sup> Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost). <sup>b</sup> Only the right mandibular salivary gland will be weighed. <sup>c</sup> Target Organs (and target organ gross lesions) will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost). <sup>d</sup> Parathyroids cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged. <sup>e</sup> A regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be identified when a mass is present. <sup>f</sup> Gut Associated Lymphoid Tissue				

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec <sup>a</sup>
Salivary gland, mandibular <sup>c</sup>	X	X	X		
Salivary gland, parotid		X	X		
Salivary gland, sublingual	X	X	X		
Seminal vesicles	X	X	X		
Skeletal muscle, biceps femoris		X	X		
Skin		X	X		
Small intestine, duodenum		X	X		
Small intestine, ileum		X	X		
Small intestine, jejunum		X	X		
Spinal cord, cervical		X	X		
Spinal cord, lumbar		X	X		
Spinal cord, thoracic		X	X		
Spleen	X	X	X		
Stomach, glandular		X	X		
Stomach, nonglandular		X	X		
Target Organs <sup>d</sup>		X	X	X	X
Testis	X	X	X		
Thymus	X	X	X		
Thyroid gland (with parathyroid) <sup>e</sup>	X	X	X		
Tongue		X	X		
Trachea		X	X		
Ureters		X	X		
Urinary bladder		X	X		
Uterus with cervix	X	X	X		
Vagina		X	X		
Gross lesions		X	X	X	X
Tissue masses with regional lymph node <sup>f</sup>		X	X	X	X

<sup>a</sup> Recovery

<sup>b</sup> Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).

<sup>c</sup> The combined weight of the right mandibular/sublingual salivary gland will be obtained.

Histopathology: The above listed organs/tissues from all animals were collected for histopathology at terminal necropsy and recovery.

Toxicokinetics: Blood samples were collected from all animals at 2, 5, 10, and 30 minutes postdose and at 1, 2, 4, 8, 12, and 24 hours postdose on Days 1 and 14.

Dosing Solution Analysis: Dosing formulations were evaluated for homogeneity and concentration.

## **Results**:

Mortality: There was no treatment related mortality.

Clinical Signs: Clinical signs were observed at all doses including the control, which included red, discolored skin at the injection site. Soft feces in males and females at 10 and 20 mg/kg/day and watery feces in males and females at 20 mg/kg/day were observed throughout the dosing period. At 20 mg/kg/day, soft feces were also seen in both sexes during the recovery period. Tremors were observed in one male and two females at 20 mg/kg/day on Day 1.

Body Weights: The mean initial (Week -1) and final (Day 14) body weights of control males were 2.726 and 2.771 kg, respectively. The mean initial (Week -1) and final (Day 14) body weights of the control females were 2.736 and 2.714 kg, respectively. There were no significant treatment related effects.

Food Consumption: Food consumption was assessed qualitatively.

Ophthalmoscopy: There was no significant treatment related ophthalmic findings.

Electrocardiography (ECG): There was no significant treatment related effect on PR and QTc intervals or QRS duration. There was a reversible slowing of the heart rate (associated with prolongation of the RR interval) at the postdose intervals at all doses and a greater incidence of sinus bradycardia (average heart rate < 160 beats per minute) at 5 and 20 mg/kg/day when compared to the vehicle (Group 1: 1 animal, 1 instance, postdose; Group 2: 2 animals, 3 instances, postdose; Group 3: none; Group 4: 2 animals, 3 instances, 2 of which were postdose). The heart rate changes were statistically different from vehicle in males at the Day 2 postdose at 5 mg/kg/day and in females at the Day 2 and Day 13 postdose at 20 mg/kg/day. The slowing of the heart rate was accompanied by prolongation of the QT interval at the time points mentioned above as well as the pretest interval in males at 5 mg/kg/day; however, when corrected for the heart rate, QTc interval was not prolonged. The following tables (from page 222-227 of the report) show the summary of the ECG values. Although, there is no apparent dose response, slowing of heart rate appears to be treatment related (-13.1%, -8.23% and -15.77% of predose at 5, 10 and 20 mg/kg/day, respectively, vs. -3.39% of predose in the control). Changes in the heart rate and RR interval were resolved at the recovery interval and were not considered adverse.

**Averages**

Time Point	Group	HR (bpm)	% HR Pretest	% HR Predose	% HR Chg v. % Vehicle Chg	RR (sec)	% RR Pretest	% RR Predose	% RR Chg v. % Vehicle Chg
Pretest	1	243				0.248			
Day 2 predose	1	236	-2.88%			0.257	3.63%		
Day 2 postdose	1	236	-2.88%	0.00%		0.259	4.44%	0.78%	
Terminal predose	1	236	-2.88%			0.256	3.23%		
Terminal postdose	1	228	-6.17%	-3.39%		0.269	8.47%	5.08%	
Pretest	2	227				0.267			
Day 2 predose	2	221	-2.64%		0.24%	0.274	2.62%		-1.01%
Day 2 postdose	2	194	-14.54%	-12.22%	-11.66%	0.318	19.10%	16.06%	14.66%
Terminal predose	2	229	0.88%		3.76%	0.264	-1.12%		-4.35%
Terminal postdose	2	199	-12.33%	-13.10%	-6.16%	0.309	15.73%	17.05%	7.26%
Pretest	3	242				0.249			
Day 2 predose	3	221	-8.68%		-5.80%	0.273	9.64%		6.01%
Day 2 postdose	3	212	-12.40%	-4.07%	-9.52%	0.287	15.26%	5.13%	10.82%
Terminal predose	3	243	0.41%		3.29%	0.247	-0.80%		-4.03%
Terminal postdose	3	223	-7.85%	-8.23%	-1.68%	0.271	8.84%	9.72%	0.37%
Pretest	4	239				0.254			
Day 2 predose	4	216	-9.62%		-6.74%	0.286	12.60%		8.97%
Day 2 postdose	4	197	-17.57%	-8.80%	-14.69%	0.311	22.44%	8.74%	18.00%
Terminal predose	4	241	0.84%		3.72%	0.252	-0.79%		-4.02%
Terminal postdose	4	203	-15.06%	-15.77%	-8.89%	0.301	18.50%	19.44%	10.03%

**Averages**

Time Point	Group	PR (sec)	% PR Pretest	% PR Predose	% PR Chg v. % Vehicle Chg	QRS (sec)	% QRS Pretest	% QRS Predose	% QRS Chg v. % Vehicle Chg
Pretest	1	0.069				0.037			
Day 2 predose	1	0.068	-1.45%			0.036	-2.70%		
Day 2 postdose	1	0.066	-4.35%	-2.94%		0.036	-2.70%	0.00%	
Terminal predose	1	0.068	-1.45%			0.037	0.00%		
Terminal postdose	1	0.068	-1.45%	0.00%		0.037	0.00%	0.00%	
Pretest	2	0.070				0.039			
Day 2 predose	2	0.068	-2.86%		-1.41%	0.038	-2.56%		0.14%
Day 2 postdose	2	0.068	-2.86%	0.00%	1.49%	0.037	-5.13%	-2.63%	-2.43%
Terminal predose	2	0.069	-1.43%		0.02%	0.039	0.00%		0.00%
Terminal postdose	2	0.071	1.43%	2.90%	2.88%	0.038	-2.56%	-2.56%	-2.56%
Pretest	3	0.073				0.039			
Day 2 predose	3	0.073	0.00%		1.45%	0.037	-5.13%		-2.43%
Day 2 postdose	3	0.075	2.74%	2.74%	7.09%	0.037	-5.13%	0.00%	-2.43%
Terminal predose	3	0.072	-1.37%		0.08%	0.037	-5.13%		-5.13%
Terminal postdose	3	0.074	1.37%	2.78%	2.82%	0.038	-2.56%	2.70%	-2.56%
Pretest	4	0.070				0.039			
Day 2 predose	4	0.070	0.00%		1.45%	0.039	0.00%		2.70%
Day 2 postdose	4	0.070	0.00%	0.00%	4.35%	0.038	-2.56%	-2.56%	0.14%
Terminal predose	4	0.068	-2.86%		-1.41%	0.038	-2.56%		-2.56%
Terminal postdose	4	0.073	4.29%	7.35%	5.74%	0.037	-5.13%	-2.63%	-5.13%

**Averages**

Time Point	Group	QT (sec)	% QT Pretest	% QT Predose	% QT Chg v. % Vehicle Chg	QTc Bazett's (sec)	% QTc Pretest	% QTc Predose	% QTc Chg v. % Vehicle Chg
Pretest	1	0.160				0.321			
Day 2 predose	1	0.165	3.13%			0.325	1.25%		
Day 2 postdose	1	0.167	4.38%	1.21%		0.329	2.49%	1.23%	
Terminal predose	1	0.164	2.50%			0.324	0.93%		
Terminal postdose	1	0.166	3.75%	1.22%		0.321	0.00%	-0.93%	
Pretest	2	0.168				0.325			
Day 2 predose	2	0.175	4.17%		1.04%	0.335	3.08%		1.83%
Day 2 postdose	2	0.188	11.90%	7.43%	7.52%	0.334	2.77%	-0.30%	0.28%
Terminal predose	2	0.168	0.00%		-2.50%	0.327	0.62%		-0.31%
Terminal postdose	2	0.179	6.55%	6.55%	2.80%	0.325	0.00%	-0.61%	0.00%
Pretest	3	0.156				0.314			
Day 2 predose	3	0.169	8.33%		5.20%	0.323	2.87%		1.62%
Day 2 postdose	3	0.173	10.90%	2.37%	6.52%	0.323	2.87%	0.00%	0.38%
Terminal predose	3	0.162	3.85%		1.35%	0.325	3.50%		2.57%
Terminal postdose	3	0.169	8.33%	4.32%	4.58%	0.325	3.50%	0.00%	3.50%
Pretest	4	0.163				0.324			
Day 2 predose	4	0.176	7.98%		4.85%	0.332	2.47%		1.22%
Day 2 postdose	4	0.187	14.72%	6.25%	10.34%	0.335	3.40%	0.90%	0.91%
Terminal predose	4	0.165	1.23%		-1.27%	0.329	1.54%		0.61%
Terminal postdose	4	0.178	9.20%	7.88%	5.45%	0.326	0.62%	-0.91%	0.62%

**Averages: Recovery**

Time Point	Group	HR (bpm)	% HR Pretest	% HR Predose	% HR Chg v. % Vehicle Chg	RR (sec)	% RR Pretest	% RR Predose	% RR Chg v. % Vehicle Chg
Pretest	1	239				0.252			
Day 2 predose	1	235	-1.67%			0.256	1.59%		
Day 2 postdose	1	238	-0.42%	1.28%		0.255	1.19%	-0.39%	
Terminal predose	1	237	-0.84%			0.254	0.79%		
Terminal postdose	1	232	-2.93%	-2.11%		0.262	3.97%	3.15%	
Recovery	1	247	3.35%			0.244	-3.17%		
Pretest	4	250				0.241			
Day 2 predose	4	215	-14.00%		-12.33%	0.283	17.43%		15.84%
Day 2 postdose	4	195	-22.00%	-9.30%	-21.58%	0.311	29.05%	9.89%	27.86%
Terminal predose	4	241	-3.60%		-2.76%	0.251	4.15%		3.36%
Terminal postdose	4	203	-18.80%	-15.77%	-15.87%	0.297	23.24%	18.33%	19.27%
Recovery	4	258	3.20%		-0.15%	0.234	-2.90%		0.27%

**Averages: Recovery**

Time Point	Group	PR (sec)	% PR Pretest	% PR Predose	% PR Chg v. % Vehicle Chg	QRS (sec)	% QRS Pretest	% QRS Predose	% QRS Chg v. % Vehicle Chg
Pretest	1	0.065				0.037			
Day 2 predose	1	0.065	0.00%			0.035	-5.41%		
Day 2 postdose	1	0.064	-1.54%	-1.54%		0.036	-2.70%	2.86%	
Terminal predose	1	0.067	3.08%			0.035	-5.41%		
Terminal postdose	1	0.067	3.08%	0.00%		0.035	-5.41%	0.00%	
Recovery	1	0.067	3.08%			0.035	-5.41%		
Pretest	4	0.069				0.038			
Day 2 predose	4	0.071	2.90%		2.90%	0.039	2.63%		8.04%
Day 2 postdose	4	0.070	1.45%	-1.41%	2.99%	0.039	2.63%	0.00%	5.33%
Terminal predose	4	0.070	1.45%		-1.63%	0.038	0.00%		5.41%
Terminal postdose	4	0.073	5.80%	4.29%	2.72%	0.039	2.63%	2.63%	8.04%
Recovery	4	0.066	-4.35%		-7.43%	0.037	-2.63%		2.78%

**Averages: Recovery**

Time Point	Group	QT (sec)	% QT Pretest	% QT Predose	% QT Chg v. % Vehicle Chg	QTc Bazett's (sec)	% QTc Pretest	% QTc Predose	% QTc Chg v. % Vehicle Chg
Pretest	1	0.166				0.331			
Day 2 predose	1	0.166	0.00%			0.327	-1.21%		
Day 2 postdose	1	0.167	0.60%	0.60%		0.331	0.00%	1.22%	
Terminal predose	1	0.164	-1.20%			0.325	-1.81%		
Terminal postdose	1	0.164	-1.20%	0.00%		0.322	-2.72%	-0.92%	
Recovery	1	0.158	-4.82%			0.319	-3.63%		
Pretest	4	0.160				0.325			
Day 2 predose	4	0.176	10.00%		10.00%	0.332	2.15%		3.36%
Day 2 postdose	4	0.186	16.25%	5.68%	15.65%	0.333	2.46%	0.30%	2.46%
Terminal predose	4	0.162	1.25%		2.45%	0.324	-0.31%		1.50%
Terminal postdose	4	0.177	10.63%	9.26%	11.83%	0.325	0.00%	0.31%	2.72%
Recovery	4	0.157	-1.88%		2.94%	0.325	0.00%		3.63%

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: There were no significant treatment related gross necropsy findings.

Organ Weights: No significant treatment related effects were observed in either sex.

Histopathology: There were no significant treatment related histopathology findings. Microscopic findings were seen at the injection site, which included minimal to moderate hemorrhage, minimal to moderate subacute/chronic inflammation, minimal to mild vascular degeneration/regeneration, and minimal to mild vascular necrosis. These findings were present in both the control group and treatment groups at similar incidence and/or severity. These findings were considered secondary to the injection procedure and were not considered test article related.

**Toxicokinetics:** Plasma concentration of JNJ-27018966-AAC increased approximately dose proportionally from 5 to 20 mg/kg/day. Total exposure ( $AUC_{0-24h}$ ) increased greater than dose proportionally from 5 to 20 mg/kg/day on Days 1 and 14. Terminal half-lives ( $t_{1/2}$ ) appeared to increase with the increase in dose and ranged from 0.5 to 4 hours. Total body clearance decreased as the dose increased from 5 to 20 mg/kg on Day 1 and Day 14. Exposure was similar in both sexes at all doses on Days 1 and 14. No drug accumulation was observed at any dose after 14 days of daily treatment. The following tables (from page 651-654 of the report) show the TK data.

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily IV Administration of 5, 10 and 20 mg/kg JNJ-27018966-AAC at Day 1 and Day 14**

Day	Dose (mg/kg/d)		C0 (ng/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose (ng*hr/mL)/(mg/kg/d)	AUC(0-t) (ng*hr/mL)	AUC(0-inf) (ng*hr/mL)	T1/2 (hr)	Lambda-z (1/hr)	CL or CLss (L/hr/kg)
1	5	N	8	8	8	8	8	8	8	8
		Mean	6986.21	4795.41	959.08	4787.37	4790.76	0.63	1.1953	1.06
		SD	18545.34	642.28	128.46	639.69	640.83	0.23	0.3254	0.15
		Median	76630.37	4701.41	940.28	4693.02	4696.06	0.54	1.2857	1.06
		Minimum	40262.50	3682.75	736.55	3679.77	3681.97	0.46	0.6779	0.89
		Maximum	87112.60	5634.13	1126.83	5629.57	5632.77	1.02	1.5119	1.36
		CV %	26.5	13.4	13.4	13.4	13.4	36.1	27.2	14.3
10	10	N	8	8	8	8	7	7	7	7
		Mean	148139.53	11770.70	1177.07	11763.65	11570.82	0.90	0.8908	0.89
		SD	24018.43	1946.00	194.60	1947.35	2015.37	0.35	0.3823	0.16
		Median	148856.36	11502.77	1150.28	11491.57	11305.71	0.89	0.7773	0.88
		Minimum	113602.44	8615.93	861.59	8612.01	8614.62	0.47	0.4786	0.67
		Maximum	173996.69	14902.98	1490.30	14895.12	14897.86	1.45	1.4617	1.16
		CV %	16.2	16.5	16.5	16.6	17.4	39.1	42.9	17.7
20	20	N	14	14	14	14	11	11	11	11
		Mean	254962.40	28487.96	1424.40	28480.66	28156.96	2.50	0.3385	0.72
		SD	29380.31	3476.12	173.81	3478.24	3782.07	1.53	0.1114	0.09
		Median	247946.51	27961.08	1398.05	27961.08	26925.64	1.81	0.3839	0.74
		Minimum	206469.97	23531.46	1176.57	23522.70	23527.08	1.56	0.1228	0.56
		Maximum	295800.77	35816.00	1790.80	35816.00	35830.28	5.64	0.4434	0.85
		CV %	11.5	12.2	12.2	12.2	13.4	61.3	32.9	12.5

CL=Dose/AUC(0-inf) on Day 1, Total body clearance

CLss=Dose/AUC(0-24) only on Day 14, Total body clearance at steady state

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily IV Administration of 5, 10 and 20 mg/kg JNJ-27018966-AAC at Day 1 and Day 14 (Continued)**

Day	Dose (mg/kg/d)		Vz (L/kg)	MRT (hr)	Vss (L/kg)	Cminss (ng/mL)	Cavgss (ng/mL)	DFL (%)	AR
1	5	N	8	8	8	0	0	0	0
		Mean	0.98	0.18	0.19	-	-	-	-
		SD	0.45	0.05	0.06	-	-	-	-
		Median	0.78	0.16	0.17	-	-	-	-
		Minimum	0.73	0.11	0.13	-	-	-	-
		Maximum	2.00	0.25	0.30	-	-	-	-
	10	N	7	7	7	0	0	0	0
		Mean	1.12	0.18	0.16	-	-	-	-
		SD	0.38	0.03	0.04	-	-	-	-
		Median	1.15	0.18	0.15	-	-	-	-
		Minimum	0.61	0.13	0.13	-	-	-	-
		Maximum	1.55	0.22	0.24	-	-	-	-
	20	N	11	11	11	0	0	0	0
		Mean	2.51	0.21	0.15	-	-	-	-
		SD	1.33	0.04	0.02	-	-	-	-
		Median	1.95	0.20	0.14	-	-	-	-
		Minimum	1.47	0.16	0.12	-	-	-	-
		Maximum	5.71	0.28	0.19	-	-	-	-
		CV %	53.0	19.0	15.6	-	-	-	-

(-) Indicates not applicable or could not be calculated.

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily IV Administration of 5, 10 and 20 mg/kg JNJ-27018966-AAC at Day 1 and Day 14 (Continued)**

Day	Dose (mg/kg/d)		C0 (ng/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose (ng*hr/mL)/(mg/kg/d)	AUC(0-t) (ng*hr/mL)	AUC(0-inf) (ng*hr/mL)	T1/2 (hr)	Lambda-z (1/hr)	CL or CLss (L/hr/kg)
14	5	N	8	8	8	8	7	7	7	8
		Mean	59695.00	4221.83	844.37	4214.69	4251.93	0.57	1.2491	1.23
		SD	16403.16	851.96	170.39	849.01	912.19	0.13	0.2077	0.24
		Median	58426.03	3931.02	786.20	3925.60	3878.87	0.53	1.3045	1.27
		Minimum	33110.84	3022.26	604.45	3018.94	3020.13	0.49	0.7925	0.91
		Maximum	85678.70	5490.12	1098.02	5476.70	5481.93	0.87	1.4033	1.65
		CV %	27.5	20.2	20.2	20.1	21.5	23.4	16.6	19.7
	10	N	8	8	8	8	8	8	8	8
		Mean	107539.94	11330.65	1133.07	11324.59	11327.70	1.20	0.6070	0.94
		SD	29978.21	2588.80	258.88	2586.04	2586.83	0.31	0.1400	0.31
		Median	105180.13	12573.21	1257.32	12568.15	12570.63	1.09	0.6385	0.80
		Minimum	61908.14	6060.32	606.03	6058.04	6059.95	0.91	0.4074	0.72
		Maximum	163732.64	13948.05	1394.80	13931.91	13938.51	1.70	0.7638	1.65
		CV %	27.9	22.8	22.8	22.8	22.8	25.8	23.1	32.6
	20	N	14	14	14	14	10	10	10	14
		Mean	193884.50	28202.18	1410.11	28196.08	27451.68	3.92	0.2633	0.73
		SD	32294.00	5352.87	267.64	5353.81	4606.01	3.25	0.1221	0.13
		Median	193954.50	26316.44	1315.82	26305.04	26310.90	2.18	0.3183	0.76
		Minimum	142800.15	20931.16	1046.56	20924.20	20927.36	1.88	0.0610	0.50
		Maximum	268742.36	40033.74	2001.69	40033.74	36889.01	11.36	0.3678	0.96
		CV %	16.7	19.0	19.0	19.0	16.8	83.1	46.4	17.1

CL=Dose/AUC(0-inf) on Day 1, Total body clearance  
 CLss=Dose/AUC(0-24) only on Day 14, Total body clearance at steady state

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily IV Administration of 5, 10 and 20 mg/kg JNJ-27018966-AAC at Day 1 and Day 14 (Continued)**

Day	Dose (mg/kg/d)		Vz (L/kg)	MRT (hr)	Vss (L/kg)	Cminss (ng/mL)	Cavgss (ng/mL)	DFL (%)	AR
14	5	N	7	7	7	8	8	8	8
		Mean	1.02	0.14	0.17	0.00	175.91	17864.94	0.89
		SD	0.35	0.04	0.06	0.00	35.50	1918.63	0.18
		Median	0.94	0.13	0.17	0.00	163.79	18276.05	0.90
		Minimum	0.71	0.10	0.11	0.00	125.93	14677.17	0.68
		Maximum	1.70	0.22	0.29	0.00	228.76	20805.60	1.21
		CV %	34.1	27.4	36.2	.	20.2	10.7	20.5
	10	N	8	8	8	8	8	8	8
		Mean	1.60	0.18	0.17	0.00	472.11	14637.78	0.96
		SD	0.53	0.03	0.05	0.00	107.87	1401.22	0.18
		Median	1.45	0.17	0.16	0.00	523.88	14511.70	1.02
		Minimum	1.06	0.15	0.12	0.00	252.51	12130.73	0.60
		Maximum	2.75	0.23	0.27	0.00	581.17	16387.86	1.15
		CV %	33.4	14.4	30.0	.	22.8	9.6	19.2
	20	N	10	10	10	14	14	14	14
		Mean	4.04	0.21	0.15	0.91	1175.09	12544.24	0.99
		SD	3.09	0.04	0.03	1.20	223.04	885.57	0.14
		Median	2.56	0.20	0.15	0.52	1096.52	12643.40	0.98
		Minimum	1.53	0.17	0.11	0.00	872.13	11335.29	0.81
		Maximum	10.82	0.30	0.20	3.61	1668.07	14034.43	1.33
		CV %	76.4	18.4	17.3	131.7	19.0	7.1	13.9

(.) Indicates not applicable or could not be calculated.

**Dosing Solution Analysis:** Dosing solution analysis results revealed that JNJ-27018966-AAC dose formulations contained 96% to 100% of the respective targeted concentrations of 5.0, 10.0, and 20.0 mg/mL and met the protocol requirement for solution formulations ( $\pm 10\%$  of nominal concentration). No test article was detected in the vehicle.

### **Investigative Tolerance Study of Orally/Subcutaneously Administered JNJ-27018966 in Cynomolgus Monkeys (Study TOX6747)**

**Methods:** In this non-GLP study, Cynomolgus monkeys were administered JNJ-27018966 at single escalating PO/SC doses. In the single dose escalation (SDE) phase, animals in two treatment groups ( $n = 1/\text{sex}/\text{group}$ ) were administered the test article via oral gavage once at escalating dose levels of 10, 50, and 100 mg/kg, and via SC injection once at escalating dose levels of 2, 10, and 50 mg/kg. There was a washout period of 3 days between each dose. During the repeat-dose (RD) phase, animals in two treatment groups ( $n = 1/\text{sex}/\text{group}$ ) were administered the test article once daily for 5 consecutive days via oral gavage at 100 mg/kg and via SC injection at 50 mg/kg (two daily doses of 25 mg/kg, approximately 4 hours apart). The test article was administered at a dose volume of 5 mL/kg during both phases for oral administration, and 2 mL/kg during both phases for SC administration. Blood samples were collected for TK analysis for oral drug exposure. Blood samples were collected at predose and at 0.5, 1, 2, 4, 8, and 24 hours postdose during the SDE phase. In the RD phase, blood samples were collected at predose and at the above time points during the

first and final dose for the oral dose group and at predose and at 0.5, 4, 4.5, 6, 8, and 24 hours after the first daily dose for the SC dose group.

**Results:** There were no significant treatment related effects on survival, body weight, or clinical pathology parameters. For the oral dose group, there were no treatment related clinical signs or macroscopic findings. One male monkey at 100 mg/kg PO had enlarged kidney. This was considered incidental, as this was within normal limits microscopically. Subacute inflammation was observed at the injection sites at 50 mg/kg SC. In addition, squamous epithelial hyperplasia and epidermal exudates were seen in the female monkey at 50 mg/kg SC. TK results were not available in the study report.

### **7-Day Dose Range Finding Oral/Subcutaneous Toxicity and Toxicokinetic Study of JNJ-27018966-AAA in Cynomolgus Monkeys (Study No. TOX7383)**

**Methods:** In this non-GLP study in Cynomolgus monkeys, animals (n = 1/sex/group, four groups) JNJ-27018966-AAA was administered for 7 days, by daily oral gavage at 100, or 200 mg/kg; or by SC injection at 12.5 or 25 mg/kg combined with an oral dose of 100 mg/kg. One additional group of one animals/sex served as the control and received the vehicle, 0.5% hypromellose solution. The test article or vehicle was administered to all groups once daily for 7 consecutive days, at a dose volume of 5 mL/kg for PO doses and 2 mL/kg for SC doses. Blood samples for TK analysis were collected from all animals at predose, 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose on Days 1 and 7. At study termination, necropsy examinations were performed, organ weights were recorded, and selected tissues were microscopically examined.

**Results:** There was no mortality. There were no significant treatment related effects on body weight or organ weights. Clinical signs included few or absent feces in both sexes (100/12.5 and 100/25 mg/kg dose groups) and females (all doses). In addition, emesis and inappetence were noted during the course of the study and were considered to be potential effects of the test article. The male at 100 mg/kg (PO) had significant increases in AST (8.7 fold), ALT (2.9 fold) and creatinine kinase (19.7 fold) compared to control. In addition, mild increases were seen in AST (2.3 fold) and ALT (1.8 fold) in the male at the high dose [100/25 mg/kg (PO/SC)]. These changes were not dose related and there were no histopathological findings in the liver. In addition, one animal per sex was used. The relation to the treatment was unclear. The NOAEL was considered to be 200 mg/kg when administered by oral gavage alone and 100/25 mg/kg when administered by oral gavage and SC injection. The Applicant submitted an amended TK report dated September 6, 2011. This amended TK report stated "The monkey plasma method qualification could not be re-constructed and the sample analysis for this study was determined not to be acceptable. Thus, the toxicokinetic data analysis was not considered to be acceptable."

**Study title: 28-Day Oral/Subcutaneous Toxicity Study in Cynomolgus Monkeys**

Study no.: TOX8103  
 Study report location: EDR 4.2.3.2.  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: November 21, 2006  
 Date of study completion: October 25, 2007  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAA, 10925N, 100%

**Key Study Findings:**

- In a 28-day PO/SC toxicity study in Cynomolgus monkeys, animals were treated with JNJ-27018966-AAA at 50/0, 50/12.5, 200/0, and 200/25 mg/kg/day PO/SC for 28 days.
- There were no significant treatment related clinical signs or changes in body weight, clinical pathology, ophthalmology or ECG parameters.
- There was no significant treatment related gross or histopathology findings.
- The NOAEL was considered as 200/25 mg/kg/day PO/SC.

**Methods:**

Doses: 50/0, 50/12.5, 200/0 and 200/25 mg/kg/day  
 (Oral/Subcutaneous)  
 Frequency of dosing: Once daily  
 Route of administration: Oral (nasogastric) and subcutaneous  
 Dose volume: 5 mL/kg (oral); 2 mL/kg (SC)  
 Formulation/Vehicle: PO: 0.5% Hydroxypropyl methylcellulose  
 (hypromellose) in water; SC: 10% Hydroxypropyl  
 $\beta$  cyclodextrin (HPBCD) solution  
 Species/Strain: Cynomolgus monkey  
 Number/Sex/Group: 3/sex/group  
 Age: 2 years 2 months to 3 years 1 month  
 Weight: Males: 1.98 to 2.68 kg; Females: 1.94 to 2.37 kg  
 Satellite groups: None  
 Unique study design: Study design is shown below  
 Deviation from study protocol: Protocol deviations did not affect the quality or  
 integrity of the study

The following table (from page 21 of the report) shows the study design.

**3.1.4. Study Groups**

Group	1	2	3	4	5
		Monkey Numbers			
Males	101-103	107-109	113-115	119-121	125-127
Females	104-106	110-112	116-118	122-124	128-130
		JNJ-27018966-AAA			
Dosage <sup>a</sup> (mg/kg/day)	0/0	50/0	50/12.5	200/0	200/25
Concentration <sup>a</sup> (mg/mL)	0/0	10.0/0	10.0/6.25	40.0/0	40.0/12.5
Dosage Volume <sup>a</sup> (mL/kg)	5/2	5/2	5/2	5/2	5/2

<sup>a</sup>Presented as PO/SC formulations.

**Basis of dose selection:** The dose levels were selected based on the results of the previous tolerance study (TOX6747) and a 7-day dose range-finding study (TOX7383) in monkeys. In the tolerance study, monkeys were administered JNJ-27018966 orally at 100 mg/kg or subcutaneously at 50 mg/kg (two daily doses of 25 mg/kg). There were no treatment related findings after oral administration. However, subacute inflammation at the injection site (in two animals) and squamous epithelial hyperplasia and epidermal exudates (in one animal) were observed following SC administration. In the dose range-finding study, monkeys were administered JNJ-27018966 at the following doses (PO/SC): 100/0, 200/0, 100/12.5, and 100/25 mg/kg/day. There were no significant treatment related findings. Based on the above findings, following doses were selected for the current study: 50/0, 50/12.5, 200/0, and 200/25 mg/kg (oral/subcutaneous).

**Observations:**

**Mortality:** Mortality was observed twice daily.

**Clinical Signs:** Clinical signs were observed once weekly.

**Body Weights:** Body weights were recorded once weekly.

**Food Consumption:** Food consumption was observed qualitatively on a daily basis.

**Ophthalmoscopy:** Ophthalmoscopic examinations were conducted on all animals at pretest and on Week 4.

**Electrocardiography (ECG):** ECG was conducted on all animals prior to treatment and on Week 4.

**Hematology:** Hematology was conducted at pretest and at termination.

**Clinical Chemistry:** Clinical chemistry was conducted at pretest and at termination.

Urinalysis: Urine samples collected at prestudy and at termination.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 505-506 of the report) organs were weighed from all main study animals.

**Organs or Tissues to be Weighed, Preserved, and Microscopically Examined**

<b>Tissue</b>	<b>Organ Weight Taken</b>	<b>Collected and Preserved</b>	<b>Microscopic Examination</b>
Adrenal gland	X	X	X
Aorta		X	X
Bone with bone marrow, femur		X	X
Bone with bone marrow, rib		X	X
Bone with bone marrow, sternum		X	X
Bone marrow smear <sup>a</sup>		X	
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X
Epididymis	X	X	X
Esophagus		X	X
Eye (with optic nerve)		X	X
Gallbladder		X	X
Heart	X	X	X
Injection Site		X	X
Joint, tibiofemoral		X	X
Kidney	X	X	X
Large intestine, cecum		X	X
Large intestine, colon		X	X
Large intestine, rectum		X	X
Larynx		X	X
Liver	X	X	X
Lung with bronchi	X	X	X
Lymph node, mandibular		X	X
Lymph node, mesenteric		X	X
Mammary gland (process females only)		X	X
Nerve, sciatic		X	X
Ovary	X	X	X
Oviducts		X	X
Pancreas		X	X

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination
Peyer's patch		X	X
Pituitary	X	X	X
Prostate		X	X
Salivary gland, mandibular <sup>b</sup>	X	X	X
Salivary gland, parotid		X	X
Salivary gland, sublingual		X	X
Seminal vesicles		X	X
Skeletal muscle, rectus femoris		X	X
Skin		X	X
Small intestine, duodenum		X	X
Small intestine, ileum		X	X
Small intestine, jejunum		X	X
Spinal cord, cervical		X	X
Spinal cord, lumbar		X	X
Spinal cord, thoracic		X	X
Spleen	X	X	X
Stomach, cardia		X	X
Stomach, fundus		X	X
Stomach, pylorus		X	X
Target Organs <sup>c</sup>		X	X
Testis	X	X	X
Thymus	X	X	X
Thyroid gland (with parathyroid) <sup>d</sup>	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus with cervix		X	X
Vagina		X	X
Gross lesions		X	X
Tissue masses with regional lymph node <sup>e</sup>		X	X

Histopathology: The organs/tissues listed in the above table from all animals were collected for histopathology at terminal necropsy.

Toxicokinetics: Blood samples were collected from all animals at the following time points on Days 1 and 28: predose, 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose.

Dosing Solution Analysis: Dosing formulations were examined for homogeneity, concentration and stability. Duplicate samples were collected from each vehicle formulation. Five samples (one from the top, three from the middle, and one from the bottom of the batch) of the oral test article formulations were collected for each concentration. Three samples of the subcutaneous test article formulations were collected for each concentration.

## **Results**:

Mortality: One female at 200/25 (PO/SC) mg/kg/day (animal number 130) was euthanized *in extremis* on Day 28 due to physical trauma (strangulation) incurred when the neck/collar of this animal had been wedged between the perch and cage. There were no other mortalities.

Clinical Signs: Treatment related clinical signs included emesis at all doses (not dose related).

Body Weights: The mean initial (Week -1) and final (Week 4) body weights of control males were 2.243 and 2.193 kg, respectively. The mean initial (Week -1) and final (Week 4) body weights of the control females were 2.177 and 2.107 kg, respectively. There were no significant treatment related effects.

Food Consumption: Food consumption was observed qualitatively.

Ophthalmoscopy: There was no treatment related ophthalmic findings.

ECG: There were no significant treatment related ECG findings.

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: There were no significant treatment related gross necropsy findings.

Organ Weights: No significant treatment related effects were observed in either sex.

Histopathology: There were no significant treatment related histopathology findings.

Toxicokinetics: Per the TK report amendment dated December 14, 2009, both the monkey plasma method validation and study sample analysis for this study were determined not to be valid. The toxicokinetic data analysis was not considered to be

acceptable. Section 3 (Conclusions) of the original TK report was removed in the amended TK report.

Dosing Solution Analysis: Dosing solutions were found to be homogeneous and stable. Formulation samples analyzed for concentration were verified to be 102.4 to 109% of theoretical. Formulations were homogeneous, with %RSD (relative standard deviation) values ranging from 0 to 1.6%. Stability samples stored refrigerated (2-8 °C) for 107 days/25 hours ranged from 96.6-108.8% of theoretical.

**Study title: 13-Week Oral and Subcutaneous Toxicity Study in Cynomolgus Monkeys**

Study no.:	TOX8661
Study report location:	EDR 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 24, 2008
Date of study completion:	February 5, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	JNJ-27018966-AAA, 21298-90A, 95.2%

**Key Study Findings:**

- In a 13-week PO/SC toxicity study in Cynomolgus monkeys, animals were treated with JNJ-27018966-AAA at 50/0, 200/0, and 200/25 mg/kg/day PO/SC for 13 weeks.
- There were no significant treatment related clinical signs or changes in body weight, clinical pathology, ophthalmology, or ECG parameters.
- There was no significant treatment related gross or histopathology findings.
- The NOAEL was considered as 200/25 mg/kg/day PO/SC.

**Methods:**

Doses:	50, 200 mg/kg/day (Oral); 25 mg/kg/day (SC)
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage) and subcutaneous
Dose volume:	5 mL/kg (oral); 2 mL/kg (SC)
Formulation/Vehicle:	0.5% Hydroxylpropyl methylcellulose (hypromellose) in water
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	4/sex/group
Age:	Male: 2.3 to 3.1 years; Female: 2.7 to 3.8 years
Weight:	Male: 2.1-2.3 kg; Female: 2.3-2.7 kg
Satellite groups:	None
Unique study design:	Study design is shown below
Deviation from study protocol:	Protocol deviations did not affect the quality or

## integrity of the study

The following table (from page 5 of the report) shows the study design.

Group No.	Number of Males/Females	Route of Administration	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)	Number Necropsied (Males/Females) Day 93
1	4/4	Nasogastric	0	5	0	4/4
		Subcutaneous	0	2	0	
2	4/4	Nasogastric	50	5	10	4/4
		Subcutaneous	0	2	0	
3	4/4 <sup>a</sup>	Nasogastric	200	5	40	2/4 <sup>a</sup>
		Subcutaneous	0	2	0	
4	4/4	Nasogastric	200	5	40	4/4
		Subcutaneous	25	2	12.5	

<sup>a</sup> Two Group 3 males (Animals 3001 and 3003) were euthanized moribund on Days 29 and 62, respectively.

**Basis of dose selection:** The dose levels were selected based on the results of the 28-day study (Study No. TOX8103) in Cynomolgus monkeys with JNJ-27018966-AAA at 50/0, 50/12.5, 200/0, or 200/25 mg/kg/day PO/SC. In this study, there were no significant treatment related findings at any dose, and the NOAEL was determined to be 200/25 mg/kg/day. Based on the results of the above study, following doses were selected for the current study: 50/0, 50/12.5, 200/0, or 200/25 mg/kg/day PO/SC. It appears that higher doses should have been tested, as there were no significant findings at any of the tested doses.

### **Observations:**

**Mortality:** Mortality was observed twice daily.

**Clinical Signs:** Clinical signs were observed once daily.

**Body Weights:** Body weights were recorded once weekly.

**Food Consumption:** Food consumption was observed qualitatively on a daily basis.

**Ophthalmoscopy:** Ophthalmoscopic examinations were conducted on all animals at pretest and on Day 91.

**Electrocardiography (ECG):** ECG was conducted on all animals prior to treatment and on Day 91.

**Hematology:** Hematology was conducted at pretest and on Days 31 and 91.

**Clinical Chemistry:** Clinical chemistry was conducted at pretest and on Days 31 and 91.

Urinalysis: Urine samples collected at prestudy and on Day 91.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 26 of the report) organs were weighed from all main study animals.

<b>Organs Weighed</b>	
Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary	Spleen
Testes	Thymus
Thyroid with parathyroids	

Histopathology: The organs/tissues listed below (from page 26-27 of the report) from all animals were collected for histopathology at terminal necropsy.

<b>Tissues Collected</b>	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (parotid, sublingual, mandibular)	Epididymides
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ureters
Small Intestine	Ovaries
Duodenum	Oviducts
Jejunum	Uterus
Ileum with Peyer's patch	Cervix
Large Intestine	Vagina

Tissues Collected	
Cecum	Endocrine
Colon	Adrenals
Rectum	Pituitary
Pancreas	Thyroid/Parathyroids <sup>a</sup>
Liver	Skin/Musculoskeletal
Gallbladder	Skin/Mammary Gland
Respiratory	Bone (femoral head)
Larynx	Bone (7th rib)
Trachea	Skeletal Muscle (psoas and diaphragm)
Lung	Nervous/Special Sense
Lymphoid/Hematopoietic	Eyes with Optic Nerve
Bone Marrow (sternum)	Lacrimal glands
Thymus	Sciatic Nerve
Spleen	Brain
Lymph Nodes	Spinal Cord (thoracic)
Popliteal lymph nodes	Other
Axillary node	Animal Number Tattoo
Mesenteric	Gross Lesions
	Injection Site <sup>b</sup>

<sup>a</sup> The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

<sup>b</sup> Skin demarked by tattoo or other means of identification.

**Toxicokinetics:** Blood samples were collected at the following time points on Days 1 and 91: predose, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours postdose from the control and all three dose groups.

**Dosing Solution Analysis:** Dosing formulations were evaluated for homogeneity, concentration and stability. Samples were collected from the top, middle, and bottom from each concentration on the first day of preparation for homogeneity and concentration analyses. On the last day of preparation, samples were collected from the middle of each concentration for analysis of test article concentration.

## **Results:**

**Mortality:** Two males (animal no. 3001 died on Day 29; animal no. 3003 died on Day 62) at 200/0 PO/SC dose died early due to gavage error. On Study Day 29, Animal 3001 experienced difficulty in breathing soon after dosing and progressively deteriorated to the point where euthanasia was elected. Gross necropsy findings in this animal included pale discoloration of the liver (glycogen accumulation), abnormal yellow contents in the jejunum (no correlating microscopic observation), edema of the gallbladder and injection site (confirmed microscopically), red discoloration of the lungs (congestion) and thymus (hemorrhage), and a small amount of white powdery material in the larynx (no

correlating microscopic observation). On Study Day 62, animal no. 3003 had emesis after dosing and subsequently had severe respiratory distress and was euthanized in a moribund condition. Gross necropsy observations in this animal included injection site edema, white foam in the trachea, and red discoloration (congestion) in the lungs. Mild edema was microscopically observed in the lungs and the death of this animal was attributed to a gavage error. There were no other mortalities during the study.

Clinical Signs: No significant treatment related clinical signs were observed.

Body Weights: The mean initial (Day -2) and final (Day 91) body weights of control males were 2.18 and 2.45 kg, respectively. The mean initial (Day -2) and final (Day 91) body weights of the control females were 2.35 and 2.60 kg, respectively. There were no significant treatment related effects.

Food Consumption: Food consumption was not affected by the treatment. Data were not provided.

Ophthalmoscopy: There was no treatment related effect upon ophthalmic findings.

ECG: There were no significant treatment related ECG findings.

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: There were no significant treatment related gross necropsy findings.

Organ Weights: No significant treatment related effects were observed in either sex.

Histopathology: There were no significant treatment related histopathology findings. Microscopic observations at the injection sites (irrespective of dose group) included fibrosis, necrosis and infiltration of histiocytic cells (with or without hemorrhage, granulomas, and mononuclear/mixed cellular infiltrates) and were attributed to the repeated trauma following SC injection.

Toxicokinetics: Following a single oral dose of JNJ-27018966, mean  $t_{max}$  values ranged from 4.13 to 5.25 hours, and elimination was slow based on mean half-life values ranging from 13.4 to 63.0 hours. Systemic exposures (AUC and  $C_{max}$ ) to JNJ-27018966 increased in a dose related manner and were lower than exposures for the PO/SC groups. There were no clear gender differences in exposures and there was no apparent drug accumulation following multiple doses.

Following a single oral plus subcutaneous dose of JNJ-27018966, mean  $t_{max}$  values ranged from 0.375 to 0.438 hours, and elimination was slow based on mean half-life values ranging from 9.01 to 9.23 hours. Systemic exposure (AUC and  $C_{max}$ ) to JNJ-

27018966 was higher than exposure for the groups dosed with only an oral dose. There were no clear gender related differences in exposure. There were no clear changes in exposure following multiple doses. The table (from page 497 of the report) shows the TK parameters.

Table SD7: Mean (SD) JNJ-27018966 Plasma Toxicokinetic Parameters in Male and Female Monkeys Following a Single or 91 Daily Oral/Subcutaneous Doses of JNJ-27018966-AAA (50/0, 200/0, and 200/25mg/kg/day) (TOX8661)

Day	Sex	Dose <sup>a</sup> (mg/kg)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (0-∞) (ng·h/mL)	AUC (0-24 h) (ng·h/mL)	t <sub>1/2</sub> (h)	CL/F (mL/h·kg)
1	Male	50/0	6.54 (2.95)	5.25 (3.40)	NA <sup>b</sup> NA <sup>b</sup>	64.0 (23.5)	16.1 (3.61)	376000 (756000)
		200/0	25.6 (26.2)	4.63 (5.12)	348 NA <sup>c</sup>	229 (67.7)	63.0 (105)	491000 (278000)
		200/25	13700 (1870)	0.438 (0.125)	16400 (1450)	16300 (1460)	9.23 (4.76)	13800 (1210)
91	Male	50/0	5.89 (3.31)	3.00 (1.15)	NR NA	66.2 (55.3)	24.3 (18.3)	374000 (209000)
		200/0 <sup>c</sup>	14.4 NA	0.500 NA	NR NA	160 NA	795 NA	410000 NA
		200/25	27700 (10200)	0.250 (0.00)	NR NA	23600 (5090)	8.50 (1.72)	9030 (1490)
1	Female	50/0	6.96 (4.32)	4.13 (3.07)	NA <sup>c</sup> NA <sup>c</sup>	83.2 (51.6)	21.1 (4.73)	355000 (141000)
		200/0	30.7 (23.0)	5.25 (3.40)	135 NA <sup>d</sup>	342 (272)	13.4 (8.37)	846000 (748000)
		200/25	12300 (5500)	0.375 (0.144)	15600 (5660)	15500 (5650)	9.01 (4.91)	15900 (5570)
91	Female	50/0	5.30 (1.80)	2.25 (1.26)	NR NA	44.3 (39.3)	4.22 (2.97)	2270000 (1390000)
		200/0	15.7 (6.98)	4.50 (5.00)	NR NA	180 (94.1)	7.76 (3.18)	1110000 (423000)
		200/25	18000 (12200)	0.313 (0.125)	NR NA	17400 (5860)	9.66 (4.94)	13800 (3900)

<sup>a</sup> Total Daily Dose (Oral/Subcutaneous)

<sup>b</sup> All values excluded from mean calculation. Due to AUC % extrapolated >25% of Total AUC.

<sup>c</sup> N=2

NA: Not Applicable

NR: Not Reported. AUC 0-∞ is only reported for Day 1.

**Dosing Solution Analysis:** Dosing solutions met the criteria (concentrations to be (b) (4) % of label claim). The samples also met the specification for homogeneity. The stability samples met the specification.

**Study title: 9-Month Oral Toxicity Study in Cynomolgus Monkeys with 4-Week Recovery Period**

Study no.: 1808-004  
Study report location: EDR 4.2.3.2.  
Conducting laboratory and location: (b) (4)  
Date of study initiation: April 26, 2010  
Date of study completion: August 1, 2011  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: JNJ-27018966-AAA, 0020291528, 98.5%

**Key Study Findings:**

- In a 9-month oral (gavage) study in Cynomolgus monkeys, animals were treated with JNJ-27018966-AAA at 50, 100, and 200 mg/kg/day.
- Two females at 200 mg/kg/day and one control female were sacrificed *in extremis* due to poor health conditions as a result of gavage errors.
- Chorioretinitis was observed in one male at 100 mg/kg and in two males at 200 mg/kg/day at the terminal examination and in one female at 200 mg/kg/day at pretest, terminal and recovery examinations. The relation to the treatment is not clear as this was seen in one eye of each male animal and one female had chorioretinitis in both eyes at the pretest. Chorioretinitis is often caused by cytomegalovirus infection, which is seen in macaques.
- Sinus tachycardia was seen at 100 and 200 mg/kg/day including control. These findings do not appear to be treatment related in the absence of a dose response, its occurrence in control animals and common background occurrences in monkeys.
- Organ weight changes were seen in males (pituitary, prostate, testes, and epididymis) and females (thyroid/parathyroid and thymus). The relation to the treatment is unclear in the absence of a clear dose response and absence of histopathological findings in these organs.
- Treatment related microscopic changes were seen in the thymus at 200 mg/kg/day in both sexes. Two of four males at 200 mg/kg/day and one of three females at 200 mg/kg/day had minimal to mild generalized lymphoid depletion of the thymus. These findings were resolved during recovery and were not considered adverse.
- The NOAEL was considered to be 200 mg/kg/day.

**Methods:**

Doses: 50, 100, 200 mg/kg/day  
Frequency of dosing: Once daily  
Route of administration: Oral (gavage)  
Dose volume: 5 mL/kg  
Formulation/Vehicle: 0.5% Hydroxylpropyl methylcellulose (Hypromellose) in water

Species/Strain: Cynomolgus monkey (*Macaca fascicularis*)  
 Number/Sex/Group: 4-7/sex/group  
 Age: Approximately 2 year 4 months and 3 year 10 months of age  
 Weight: Male: 2.15-2.95 kg; Female: 2.20-2.95 kg  
 Satellite groups: None  
 Unique study design: Study design is shown below  
 Deviation from study protocol: Protocol deviations did not affect the quality or integrity of the study

The following table (from page 16 of the report) shows the study design.

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1	0	7 <sup>a</sup>	7 <sup>a</sup>
2	50	4	4
3	100	4	4
4	200	7 <sup>a</sup>	7 <sup>a</sup>

<sup>a</sup>The first three surviving animals/sex were maintained on study for a 4 week recovery period

**Basis of dose selection:** Dose levels were selected based on the results of the 3-month study (Study No. TOX8661) in Cynomolgus monkeys with JNJ-27018966-AAA at 50/0, 200/0, and 200/25 mg/kg/day PO/SC for 13 weeks. There were no significant treatment related clinical signs or changes in body weight, clinical pathology, ophthalmology, or ECG parameters. There was no significant treatment related gross or histopathology findings. The NOAEL was considered as 200/25 mg/kg/day PO/SC. Based on this, the high dose for the current study was selected as 200 mg/kg/day. The high dose selection may not be appropriate, as the highest tested dose in the above 3-month study did not produce any significant toxicity.

**Observations:**

**Mortality:** Mortality was observed twice daily.

**Clinical Signs:** Clinical signs were observed once weekly.

**Body Weights:** Body weights were recorded once weekly.

**Food Consumption:** Food consumption was not recorded.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted on all animals prior to terminal or recovery necropsies.

Electrocardiography (ECG): ECG was conducted on all animals prior to treatment and on Week 39 and prior to recovery necropsy.

Hematology: Hematology was conducted at pretest and on Days 91 and 181 and prior to terminal or recovery necropsies.

Clinical Chemistry: Clinical chemistry was conducted at pretest and on Days 91 and 181 and prior to terminal or recovery necropsies.

Urinalysis: Urine samples collected at pretest and on Days 91 and 181 and prior to terminal or recovery necropsies.

Gross Pathology: Gross pathology was conducted at terminal and recovery necropsies.

Organ Weights: The following (from page 1565 of the report) organs were weighed from all main study animals.

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- Adrenal (2)*	- Liver [3 sections collected; 2 examined]*
- Aorta	- Lung with bronchi [collected whole; 2 sections examined]*
- Bone with marrow [femur]	- Lymph nodes: mandibular, mesenteric, and regional where applicable
- Bone with marrow [rib]	- Mammary gland [process females only]
- Bone with marrow [sternum]	- Pancreas
- Bone marrow smear [2 collected] <sup>a</sup>	- Pituitary*
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*	- Prostate* and seminal vesicle (2)*
- Epididymis (2)*	- Salivary gland, mandibular [2 collected; 1 examined]* <sup>b</sup>
- Eye including optic nerve (2)	- Salivary gland, parotid [2 collected; 1 examined]
- Gallbladder	- Salivary gland, sublingual [2 collected; 1 examined]
- GALT [gut associated lymphoid tissue]	- Sciatic nerve
- Gastrointestinal tract:	- Skeletal muscle, rectus femoris
esophagus	- Skin
stomach [cardia, fundus, and pylorus]	- Spinal cord [cervical, thoracic, and lumbar]
duodenum	- Spleen*
jejunum	- Thymus*
ileum	- Thyroid/parathyroid (2)*
cecum	- Tissue masses
colon	- Tongue
rectum	- Trachea
- Gonads:	- Ureters (2)
ovary (2)* with oviduct (2)	- Urinary bladder
testis (2)*	- Uterus/Cervix*
- Gross lesions	- Vagina
- Heart*	
- Joint, tibiofemoral	
- Kidney (2)*	
- Larynx	

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<sup>a</sup>Bone marrow smears were collected at the scheduled necropsy and held.

<sup>b</sup>Only the right mandibular salivary gland weight was obtained.

(2) Paired organ

\*Weighed organ

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**Histopathology:** The organs/tissues listed in the above table from all animals were collected for histopathology at terminal necropsy.

**Toxicokinetics:** Blood samples were collected from all animals at predose, and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours postdose on Days 1, 92, 182, and 269.

**Dosing Solution Analysis:** Dosing formulations were evaluated for homogeneity and concentration. Top, middle, and bottom replicate samples were collected from the Week 1 formulations prepared for the low and high concentrations. Analyses were also conducted during Weeks 1-4, 8, 12, 16, 20, 24, 28, 32, and 36. In addition, Groups 2, 3, and 4 samples were analyzed during Week 34 and Groups 1 and 4 were analyzed during Week 40.

**Results:**

**Mortality:** All animals survived to the terminal or recovery necropsies with the exception of one female at 200 mg/kg/day (animal no. 144), which was euthanized *in extremis* on Day 130. Microscopic findings in this animal included severe subacute inflammation of the esophagus and minimal edema, mild hemorrhage, and minimal inflammation of the mediastinum. The cause of death was attributed to an apparent dosing injury. A control female (animal no. 127) was replaced on Day 29 due to respiratory problems possibly resulting from gavage related injury. A female at 200 mg/kg/day (animal no. 121) was replaced on Day 22. Clinical signs in this animal included dehydration and decreased activity, and this animal had decreased body weight, and low body temperature during the first 3 weeks of the study. The relation to the treatment was not clear, however, a possible gavage error was thought to have contributed to poor health.

**Clinical Signs:** No significant treatment related clinical signs were observed.

**Body Weights:** The mean initial (Week -1) and final (Week 39) body weights of control males were 2.521 and 3.757 kg, respectively. The mean initial (Week -1) and final (Week 39) body weights of the control females were 2.371 and 3.056 kg, respectively. Final body weights were decreased in males. However, there were no significant treatment related effects on body weights in females. In males, final body weights were 86%, 85%, 81% of control at 50, 100 and 200 mg/kg/day, respectively. In females, final body weights were 104%, 105%, 100% of control at 50, 100 and 200 mg/kg/day, respectively. Summary of body weights is shown below (from page 25 of the report).

<b>Summary of Group Mean Body Weights in Kilograms</b>						
<b>Treatment Period</b>						
Dose Level (mg /kg/day)	Male			Female		
	Pretest	Week 39	(%)	Pretest	Week 39	(%)
0	2.521	3.757	(+49.0)	2.371	3.056	(+28.9)
50	2.488	3.245	(+30.4)	2.438	3.175	(+30.2)
100	2.450	3.218	(+31.3)	2.438	3.215	(+31.9)
200	2.507	3.041	(+21.3)	2.342	3.067	(+31.0)
% - Percent difference from pretest						

<b>Summary of Group Mean Body Weights in Kilograms</b>						
<b>Recovery Period</b>						
Dose Level (mg /kg/day)	Male			Female		
	Week 40	Week 43	(%)	Week 40	Week 43	(%)
0	3.867	3.950	(+2.1)	2.967	2.917	(-1.7)
200	3.050	3.150	(+3.2)	2.933	2.967	(+1.2)
% - Percent difference from Week 40						

Ophthalmoscopy: Chorioretinitis was observed in three males [animal number 112 at 100 mg/kg/day (right eye), and animal numbers 139 (left eye) and 140 (right eye) at 200 mg/kg/day] at the terminal examination and in one female [animal number 122 (both eyes) at 200 mg/kg/day] at the pretest, terminal and recovery examinations. The relation to the treatment is not clear as this was seen in one eye of each male animal and the female had chorioretinitis in both eyes at the pretest. Since the above males were sacrificed at the terminal necropsy per protocol, status of recovery of chorioretinitis in these male animals could not be confirmed. Chorioretinitis (inflammation of the choroid and retina) is often caused by cytomegalovirus infection, which is seen in macaques.

ECG: Sinus tachycardia (average heart rate > 270 beats per minute for a single interval) occurred in 12 animals at 17 intervals. The incidence of sinus tachycardia did not exhibit a dose related effect (0 mg/kg/day: 6 animals, nine instances, two of which were postdose; 50 mg/kg/day: none; 100 mg/kg/day: 4 animals, four instances, one of which was postdose; 200 mg/kg/day: 2 animals, four instances, one of which was postdose). Spontaneous cardiac arrhythmias are known to occur in monkeys (Chui RW, et al., Comprehensive Analysis of Cardiac Arrhythmias in Telemetered Cynomolgus Monkeys Over a 6 Month Period, J Pharmacol Toxicol Methods, 66:84-91). These findings do not appear to be treatment related in the absence of a dose response, its occurrence in control animals and common background occurrences.

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: There were no significant treatment related gross necropsy findings.

Organ Weights: Treatment related organ weight changes were seen in the pituitary gland and reproductive organs in males and thyroid/parathyroid gland and thymus in females.

Males: Pituitary gland weights were increased compared to controls in a non-dose-related manner at all doses. Pituitary gland weight increases showed a trend of recovery through the recovery period. There was a non-dose-related decrease in prostate weights compared to controls at all doses, non-dose-related decrease in testicular weights at 100 mg/kg/day and 200 mg/kg/day, and decrease in epididymal weights at 100 mg/kg/day. These changes persisted through the recovery period, with testicular, epididymal, and prostate weights all decreased at 200 mg/kg/day recovery animals. The relation to the treatment is not clear in the absence of clear dose response. In addition, there were no histopathological findings in these organs.

Females: Thyroid/parathyroid gland weights were increased, which persisted through the recovery period. There were no microscopic correlates. Thymus

weights were decreased at 200 mg/kg/day at the end of the dosing period and increased in this group at the end of the recovery period. The decreased thymus weights were correlated with minimal thymic lymphoid depletion in one of the 200 mg/kg/day terminal females. However, there were no microscopic correlates seen for the increased thymic weights seen at recovery. The relation to the treatment is not clear in the absence of any histopathological correlate or clear dose response.

Specific organ weight changes are shown in the table (from page 29 of the report) below.

<b>Potential Test Article-related Organ Weight Changes - Terminal Male (Percent change relative to control)</b>			
<b>Dose level: mg/kg/day</b>	50	100	200
<b>Number Examined</b>	4	4	4
Body weight (kg)	↓12.1	↓13.8	↓16.4
Epididymides (g)	↑11.1	↓28.4	↓7.9
Epididymides/BWt%	↑20.3	↓25.0	↑3.1
Epididymides/BrWt ratio	↑14.8	↓22.8	↓2.7
Pituitary gland (g)	↑13.0	↓13.0	↑26.1
Pituitary gland/BWt%	↑30.8	↑7.7	↑53.9
Pituitary gland/BrWt ratio	↑16.7	0.0	↑33.3
Prostate gland (g)	↓17.4	↓35.6	↓32.9
Prostate gland/BWt%	↓3.6	↓25.6	↓20.2
Prostate gland/BrWt ratio	↓15.7	↓31.3	↓30.1
Testes (g)	↓1.6	↓36.7	↓16.7
Testes/BWt%	↑6.1	↓33.4	↓7.5
Testes/BrWt ratio	↑1.5	↓31.1	↓12.7
BWt- Body Weight BrWt- Brain Weight		↑- Increased ↓- Decreased	

<b>Potential Test Article-related Organ Weight Changes - Terminal Female (Percent change relative to control)</b>			
<b>Dose level: mg/kg/day</b>	50	100	200
<b>Number Examined</b>	4	4	3
Thymus (g)	↑1.7	↓1.0	↓39.4
Thymus/BWt%	↓4.0	↓5.0	↓43.7
Thymus/BrWt ratio	↑0.7	↑7.1	↓39.7
Thyroid/parathyroid gland (g)	↑8.3	↑7.1	↑36.0
Thyroid/parathyroid gland/BWt%	↑9.1	↑8.3	↑31.1
Thyroid/parathyroid gland/BrWt ratio	↑14.0	↑21.1	↑42.1
BWt- Body Weight BrWt- Brain Weight		↑- Increased ↓- Decreased	

<b>Potential Test Article-related Organ Weight Changes - Recovery</b>	
<b>Male (Percent change relative to control)</b>	
<b>Dose level: mg/kg/day</b>	200
<b>Number Examined</b>	3
<b>Body weight (kg)</b>	↓20.9
<b>Epididymides (g)</b>	↓32.8
<b>Epididymides/BWt%</b>	↓13.7
<b>Epididymides/BrWt ratio</b>	↓26.9
<b>Pituitary gland (g)</b>	↓8.6
<b>Pituitary gland/BWt%</b>	↑12.5
<b>Pituitary gland/BrWt ratio</b>	0.0
<b>Prostate gland (g)</b>	↓59.8
<b>Prostate gland/BWt%</b>	↓47.9
<b>Prostate gland/BrWt ratio</b>	↓57.0
<b>Testes (g)</b>	↓38.0
<b>Testes/BWt%</b>	↓16.9
<b>Testes/BrWt ratio</b>	↓30.2
BWt- Body Weight	↑ - Increased
BrWt- Brain Weight	↓ - Decreased

<b>Potential Test Article-related Organ Weight Changes - Recovery</b>	
<b>Female (Percent change relative to control)</b>	
<b>Dose level: mg/kg/day</b>	200
<b>Number Examined</b>	3
<b>Thymus (g)</b>	↑96.1
<b>Thymus/BWt%</b>	↑97.3
<b>Thymus/BrWt ratio</b>	↑85.5
<b>Thyroid/parathyroid gland (g)</b>	↑40.1
<b>Thyroid/parathyroid gland/BWt%</b>	↑40.1
<b>Thyroid/parathyroid gland/BrWt ratio</b>	↑29.9
BWt- Body Weight	↑ - Increased
BrWt- Brain Weight	

**Histopathology:** Treatment related microscopic findings were seen in the thymus at 200 mg/kg/day in both sexes. Two of four males at 200 mg/kg/day and one of three females at 200 mg/kg/day had minimal to mild generalized lymphoid depletion of the thymus. These findings were resolved during recovery and were not considered adverse.

**Toxicokinetics:** On Day 1,  $T_{max}$  values were 24 hours, 4 hours, and 7 hours at 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively. There was an apparent accumulation of JNJ-27018966 after repeated administration. Exposure ( $AUC_{0-24h}$ ) appeared to increase less than dose proportionally. The  $C_{max}$  values increased dose proportionally from 50 to 100

mg/kg/day. There were no consistent gender differences in the TK parameters. The following table (from page 1466 of the report) shows the TK parameters.

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily Oral Administration of JNJ-27018966-AAA on Days 1, 90, 180, and 270**

Day	Dose (mg/kg/day)		Cmax1 (ng/mL)	Cmax2 (ng/mL)	Tmax (hr)	AUC(0-t) (ng*hr/mL)	AUC(0-12) (ng*hr/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose (ng*hr/mL)/(mg/kg)	AR
1	50	N	8	8	8	8	8	8	8	-
		Mean	7.09	8.42	15.75	85.45	37.31	85.45	1.71	-
		SD	2.34	3.22	11.42	45.17	15.04	45.17	0.90	-
		Min	4.01	5.12	1.00	41.51	22.96	41.51	0.83	-
		Median	6.84	7.17	24.00	70.16	32.54	70.16	1.40	-
		Max	11.90	13.80	24.00	169.91	69.17	169.91	3.40	-
		CV %	33	38	73	53	40	53	53	-
100	100	N	8	8	8	8	8	8	8	-
		Mean	16.00	16.89	8.88	168.00	94.35	168.00	1.68	-
		SD	8.26	7.50	9.39	65.50	34.75	65.50	0.65	-
		Min	4.56	8.38	3.00	89.44	32.42	89.44	0.89	-
		Median	15.70	16.40	4.00	167.53	107.31	167.53	1.68	-
		Max	33.10	33.10	24.00	259.10	130.24	259.10	2.59	-
		CV %	52	44	106	39	37	39	39	-
200	200	N	14	14	14	14	14	14	14	-
		Mean	11.44	13.04	11.79	139.94	72.81	139.94	0.70	-
		SD	8.18	8.60	9.63	66.48	38.42	66.48	0.33	-
		Min	3.79	3.79	2.00	40.03	20.35	40.03	0.20	-
		Median	9.59	10.50	7.00	137.18	61.29	137.18	0.69	-
		Max	35.10	35.10	24.00	251.73	153.43	251.73	1.26	-
		CV %	72	66	82	48	53	48	48	-

(-) Indicates could not be calculated.

Cmax1: Maximum concentration from 0-12 hours; Cmax2: Maximum concentration from 0-24 hours; and Tmax: Time to reach Cmax2.

Lambda-z and associated parameters are reported for Day 270 only.

Actual sampling days were Days 1, 92, 182, and 269.

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily Oral Administration of JNJ-27018966-AAA on Days 1, 90, 180, and 270 (Continued)**

Day	Dose (mg/kg/day)		Cmax1 (ng/mL)	Cmax2 (ng/mL)	Tmax (hr)	AUC(0-t) (ng*hr/mL)	AUC(0-12) (ng*hr/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose (ng*hr/mL)/(mg/kg)	AR
90	50	N	8	8	8	8	8	8	8	8
		Mean	10.21	10.54	4.88	143.18	75.02	143.18	2.86	1.97
		SD	3.47	3.93	7.86	53.26	28.69	53.26	1.07	0.90
		Min	5.30	5.30	0.00	78.45	45.86	78.45	1.57	0.69
		Median	10.65	10.65	2.50	128.05	68.27	128.05	2.56	1.97
		Max	14.00	16.10	24.00	233.32	121.38	233.32	4.67	3.10
100	100	N	8	8	8	8	8	8	8	8
		Mean	21.99	22.15	8.13	269.79	135.66	269.79	2.70	1.78
		SD	13.42	13.54	7.61	177.72	65.09	177.72	1.78	1.19
		Min	10.10	10.10	2.00	122.92	73.42	122.92	1.23	0.65
		Median	16.65	16.65	5.00	197.00	116.73	197.00	1.97	1.33
		Max	45.60	45.60	24.00	606.75	258.15	606.75	6.07	3.99
200	200	N	14	14	14	14	14	14	14	14
		Mean	32.12	32.28	4.38	325.38	187.19	325.38	1.63	3.06
		SD	34.16	34.10	5.94	203.77	114.91	203.77	1.02	3.28
		Min	9.01	9.01	0.00	75.94	52.30	75.94	0.38	0.83
		Median	20.75	21.90	3.00	245.98	150.55	245.98	1.23	2.03
		Max	145.00	145.00	24.00	852.36	510.96	852.36	4.26	13.48
		CV %	106	106	136	63	61	63	63	107

Cmax1: Maximum concentration from 0-12 hours; Cmax2: Maximum concentration from 0-24 hours; and Tmax: Time to reach Cmax2.  
Lambda-z and associated parameters are reported for Day 270 only.  
Actual sampling days were Days 1, 92, 182, and 269.

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily Oral Administration of JNJ-27018966-AAA on Days 1, 90, 180, and 270 (Continued)**

Day	Dose (mg/kg/day)		Cmax1 (ng/mL)	Cmax2 (ng/mL)	Tmax (hr)	AUC(0-t) (ng*hr/mL)	AUC(0-12) (ng*hr/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose (ng*hr/mL)/(mg/kg)	AR
180	50	N	8	8	8	8	8	8	8	8
		Mean	10.55	10.55	7.00	153.75	79.33	153.75	3.08	1.91
		SD	6.04	6.04	3.96	96.61	45.98	96.61	1.93	0.76
		Min	4.25	4.25	2.00	65.31	30.15	65.31	1.31	0.75
		Median	8.79	8.79	8.00	126.74	74.84	126.74	2.53	1.78
		Max	21.80	21.80	12.00	370.39	179.59	370.39	7.41	2.90
100	100	N	8	8	8	8	8	8	8	8
		Mean	23.07	23.07	7.25	215.13	125.30	215.13	2.15	1.39
		SD	25.70	25.70	4.37	119.76	73.43	119.76	1.20	0.72
		Min	8.11	8.11	2.00	106.56	67.68	106.56	1.07	0.54
		Median	14.55	14.55	7.00	173.33	101.48	173.33	1.73	1.35
		Max	85.70	85.70	12.00	413.35	291.43	413.35	4.13	2.48
200	200	N	13	13	13	13	13	13	13	13
		Mean	45.24	47.02	6.02	486.81	287.43	486.81	2.43	4.66
		SD	51.00	50.77	6.58	409.73	294.53	409.73	2.05	5.44
		Min	10.30	10.30	0.25	156.45	80.07	156.45	0.78	1.34
		Median	27.50	27.50	4.00	332.26	173.57	332.26	1.66	2.63
		Max	177.00	177.00	24.00	1357.33	1055.83	1357.33	6.79	21.29
		CV %	113	108	109	84	102	84	84	117

Cmax1: Maximum concentration from 0-12 hours; Cmax2: Maximum concentration from 0-24 hours; and Tmax: Time to reach Cmax2.  
Lambda-z and associated parameters are reported for Day 270 only.  
Actual sampling days were Days 1, 92, 182, and 269.

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily Oral Administration of JNJ-27018966-AAA on Days 1, 90, 180, and 270 (Continued)**

Day	Dose (mg/kg/day)		Cmax1 (ng/mL)	Cmax2 (ng/mL)	Tmax (hr)	AUC(0-t) (ng*hr/mL)	AUC(0-12) (ng*hr/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-24)/Dose (ng*hr/mL)/(mg/kg)	AR
270	50	N	8	8	8	8	8	8	8	8
		Mean	10.31	10.31	3.66	126.19	69.61	126.19	2.52	1.70
		SD	5.35	5.35	2.88	45.19	23.19	45.19	0.90	0.80
		Min	6.80	6.80	0.00	75.46	43.12	75.46	1.51	0.85
		Median	8.50	8.50	3.50	111.66	73.17	111.66	2.23	1.42
		Max	22.90	22.90	8.00	193.55	112.37	193.55	3.87	3.42
		CV %	52	52	79	36	33	36	36	47
100	100	N	8	8	8	8	8	8	8	8
		Mean	19.37	19.54	6.31	189.31	107.78	189.31	1.89	1.26
		SD	16.27	16.11	8.12	105.15	55.10	105.15	1.05	0.78
		Min	5.86	7.19	0.25	106.10	52.36	106.10	1.06	0.51
		Median	12.00	12.00	3.00	130.29	81.71	130.29	1.30	1.08
		Max	54.10	54.10	24.00	358.12	193.07	358.12	3.58	2.67
		CV %	84	82	129	56	51	56	56	62
200	200	N	13	13	13	13	13	13	13	13
		Mean	28.05	28.54	6.73	330.73	189.45	330.73	1.65	3.44
		SD	19.67	19.37	7.81	211.88	118.80	211.88	1.06	3.80
		Min	10.10	10.20	0.50	109.24	65.98	109.24	0.55	0.83
		Median	26.30	26.30	4.00	284.91	175.20	284.91	1.42	1.86
		Max	88.20	88.20	24.00	911.66	531.86	911.66	4.56	14.42
		CV %	70	68	116	64	63	64	64	111

Cmax1: Maximum concentration from 0-12 hours; Cmax2: Maximum concentration from 0-24 hours; and Tmax: Time to reach Cmax2.  
 Lambda-z and associated parameters are reported for Day 270 only.  
 Actual sampling days were Days 1, 92, 182, and 269.

**Table 8.1 Summary of Toxicokinetic Parameters for JNJ-27018966 in Cynomolgus Monkeys After Daily Oral Administration of JNJ-27018966-AAA on Days 1, 90, 180, and 270 (Continued)**

Day	Dose (mg/kg/day)		Lambda-z (1/hr)	T1/2 (hr)	CL/F (L/hr/kg)	Vz/F (L/kg)
270	50	N	2	2	8	2
		Mean	0.0819	8.51	440.21	4428.85
		SD	0.0088	0.92	145.58	2032.80
		Min	0.0757	7.86	258.33	2991.45
		Median	0.0819	8.51	447.81	4428.85
		Max	0.0882	9.16	662.65	5866.25
		CV %	11	11	33	46
100	100	N	3	3	8	3
		Mean	0.0683	11.54	662.74	12221.98
		SD	0.0271	5.37	284.43	9268.43
		Min	0.0393	7.45	279.24	4178.14
		Median	0.0725	9.56	771.24	10130.30
		Max	0.0930	17.62	942.50	22357.51
		CV %	40	47	43	76
200	200	N	2	2	13	2
		Mean	0.0854	10.29	816.75	11884.40
		SD	0.0556	6.69	449.82	817.93
		Min	0.0461	5.56	219.38	11306.04
		Median	0.0854	10.29	701.98	11884.40
		Max	0.1248	15.02	1830.89	12462.76
		CV %	65	65	55	7

Cmax1: Maximum concentration from 0-12 hours; Cmax2: Maximum concentration from 0-24 hours; and Tmax: Time to reach Cmax2.  
 Lambda-z and associated parameters are reported for Day 270 only.  
 Actual sampling days were Days 1, 92, 182, and 269.

Dosing Solution Analysis: The average recovery for both the low and high concentrations was within the targeted range and the formulations were considered to be homogeneous. All dosing formulations were within the targeted concentration range throughout the period of use.

## 7 Genetic Toxicology

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

**Study title: Ames test with JNJ-27018966-AAA**

Study no.: AC34AZ503 (b) (4)  
Study report location: EDR 4.2.3.3.1  
Conducting laboratory and location: (b) (4)  
Date of study initiation: January 4, 2010  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: JNJ-27018966-AAA, 31746876, 94.6%

**Key Study Findings**: Negative

**Methods**: Plate incorporation method

Strains: *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvrA*  
Concentrations in definitive study: 50, 150, 500, 1500 and 5000 µg per plate in the presence and absence of S9 (metabolic activation, Aroclor 1254-induced rat liver S9). All dose levels of test article, vehicle control and positive controls were plated in triplicate.  
Basis of concentration selection: Solubility of the test article  
Negative control: Dimethyl sulfoxide (DMSO)  
Positive control: Shown below  
Formulation/Vehicle: DMSO  
Incubation & sampling time: 48-72 hours

The positive controls are shown below (from page 8 of the report).

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>			10	
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 (Alfa Aesar) Lot No. G24R025 Exp. Date 10-Feb-2010 CAS No. 26628-22-8 Purity 99.8%	1.0
TA1537			9-aminoacridine (Sigma Aldrich Chemical Co.) Lot No. 106F06682 Exp. Date 30-Oct-2010 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:** All criteria for a valid study were met as described in the protocol.

Criteria for positive results are as follows:

- Dose-related increase in mean revertants per plate for at least one tester strain over a minimum of two increasing concentrations of test article.
- For tester strains TA1535 and TA1537, the results were considered positive if the increase in mean revertants was  $\geq 3.0$ -times the mean vehicle control value.
- For tester strains TA98, TA100 and WP2 *uvrA*, the results were considered positive if the increase in mean revertants was  $\geq 2.0$ -times the mean vehicle control value.

**Results:** The results of the assay indicated that, under the conditions of this study, JNJ-27018966-AAA did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of S9. The following tables (from page 22-25) show the results.

Table 3  
Confirmatory Mutagenicity Assay without S9 activation

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	JNJ-27018966-AAA	5000 µg	22	8	1.5	14 <sup>A</sup> , 23 <sup>A</sup> , 29 <sup>A</sup>
		1500 µg	16	11	1.1	17 <sup>A</sup> , 5 <sup>A</sup> , 26 <sup>A</sup>
		500 µg	15	3	1.0	18 <sup>A</sup> , 15 <sup>A</sup> , 13 <sup>A</sup>
		150 µg	14	4	0.9	13 <sup>A</sup> , 19 <sup>A</sup> , 11 <sup>A</sup>
		50 µg	11	7	0.7	11 <sup>A</sup> , 18 <sup>A</sup> , 5 <sup>A</sup>
		DMSO	50 µL	15	5	
	TA100	JNJ-27018966-AAA	5000 µg	95	12	0.9
1500 µg			110	12	1.0	106 <sup>A</sup> , 124 <sup>A</sup> , 101 <sup>A</sup>
500 µg			102	7	0.9	110 <sup>A</sup> , 99 <sup>A</sup> , 96 <sup>A</sup>
150 µg			118	15	1.1	112 <sup>A</sup> , 135 <sup>A</sup> , 107 <sup>A</sup>
50 µg			106	30	1.0	80 <sup>A</sup> , 98 <sup>A</sup> , 139 <sup>A</sup>
DMSO		50 µL	108	2		108 <sup>A</sup> , 106 <sup>A</sup> , 110 <sup>A</sup>
TA1535	JNJ-27018966-AAA	5000 µg	16	3	0.7	19 <sup>A</sup> , 17 <sup>A</sup> , 13 <sup>A</sup>
		1500 µg	13	3	0.6	10 <sup>A</sup> , 14 <sup>A</sup> , 15 <sup>A</sup>
		500 µg	21	7	0.9	23 <sup>A</sup> , 13 <sup>A</sup> , 26 <sup>A</sup>
		150 µg	14	1	0.6	15 <sup>A</sup> , 15 <sup>A</sup> , 13 <sup>A</sup>
		50 µg	18	5	0.8	18 <sup>A</sup> , 23 <sup>A</sup> , 14 <sup>A</sup>
	DMSO	50 µL	23	6		17 <sup>A</sup> , 24 <sup>A</sup> , 28 <sup>A</sup>
TA1537	JNJ-27018966-AAA	5000 µg	9	1	1.8	9 <sup>A</sup> , 9 <sup>A</sup> , 10 <sup>A</sup>
		1500 µg	5	3	1.0	5 <sup>A</sup> , 3 <sup>A</sup> , 8 <sup>A</sup>
		500 µg	6	3	1.2	9 <sup>A</sup> , 4 <sup>A</sup> , 6 <sup>A</sup>
		150 µg	7	4	1.4	3 <sup>A</sup> , 10 <sup>A</sup> , 9 <sup>A</sup>
		50 µg	4	1	0.8	4 <sup>A</sup> , 3 <sup>A</sup> , 4 <sup>A</sup>
	DMSO	50 µL	5	1		5 <sup>A</sup> , 4 <sup>A</sup> , 5 <sup>A</sup>
WP2uvrA	JNJ-27018966-AAA	5000 µg	35	8	0.9	41 <sup>A</sup> , 26 <sup>A</sup> , 37 <sup>A</sup>
		1500 µg	34	3	0.8	36 <sup>A</sup> , 34 <sup>A</sup> , 31 <sup>A</sup>
		500 µg	45	7	1.1	45 <sup>A</sup> , 38 <sup>A</sup> , 51 <sup>A</sup>
		150 µg	43	13	1.0	57 <sup>A</sup> , 32 <sup>A</sup> , 40 <sup>A</sup>
		50 µg	46	7	1.1	38 <sup>A</sup> , 52 <sup>A</sup> , 47 <sup>A</sup>
	DMSO	50 µL	41	5		40 <sup>A</sup> , 47 <sup>A</sup> , 37 <sup>A</sup>

## Key to Automatic &amp; Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 3 cont.  
Confirmatory Mutagenicity Assay without S9 activation

Study Number: AC34AZ.503 (b) (4)  
Experiment: B2  
Exposure Method: Plate incorporation assay

Study Code: AC34AZ  
Date Plated: 2/2/2010  
Evaluation Period: 2/9/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	2NF	1.0 µg	144	32	9.6	120 <sup>A</sup> , 180 <sup>A</sup> , 131 <sup>A</sup>
TA100	SA	1.0 µg	599	26	5.5	622 <sup>A</sup> , 604 <sup>A</sup> , 570 <sup>A</sup>
TA1535	SA	1.0 µg	495	20	21.5	506 <sup>A</sup> , 506 <sup>A</sup> , 472 <sup>A</sup>
TA1537	9AAD	75 µg	171	95	34.2	66 <sup>A</sup> , 249 <sup>A</sup> , 199 <sup>A</sup>
WP2uvrA	MMS	1000 µg	213	16	5.2	195 <sup>A</sup> , 218 <sup>A</sup> , 226 <sup>A</sup>

Key to Positive Controls

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

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 4  
Confirmatory Mutagenicity Assay with S9 activation

Study Number: AC34AZ.503 (b) (4)

Study Code: AC34AZ

Experiment: B2

Date Plated: 2/2/2010

Exposure Method: Plate incorporation assay

Evaluation Period: 2/9/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	JNJ-27018966-AAA	5000 µg	23	10	1.2	29 <sup>A</sup> , 29 <sup>A</sup> , 11 <sup>A</sup>
		1500 µg	27	2	1.4	28 <sup>A</sup> , 24 <sup>A</sup> , 28 <sup>A</sup>
		500 µg	25	6	1.3	23 <sup>A</sup> , 20 <sup>A</sup> , 31 <sup>A</sup>
		150 µg	20	7	1.1	13 <sup>A</sup> , 26 <sup>A</sup> , 22 <sup>A</sup>
		50 µg	27	10	1.4	15 <sup>A</sup> , 33 <sup>A</sup> , 32 <sup>A</sup>
	DMSO	50 µL	19	1		20 <sup>A</sup> , 19 <sup>A</sup> , 19 <sup>A</sup>
TA100	JNJ-27018966-AAA	5000 µg	103	13	1.0	91 <sup>A</sup> , 101 <sup>A</sup> , 117 <sup>A</sup>
		1500 µg	102	12	1.0	89 <sup>A</sup> , 113 <sup>A</sup> , 103 <sup>A</sup>
		500 µg	111	21	1.0	124 <sup>A</sup> , 122 <sup>A</sup> , 87 <sup>A</sup>
		150 µg	110	26	1.0	135 <sup>A</sup> , 111 <sup>A</sup> , 84 <sup>A</sup>
		50 µg	109	5	1.0	107 <sup>A</sup> , 115 <sup>A</sup> , 105 <sup>A</sup>
	DMSO	50 µL	106	12		116 <sup>A</sup> , 110 <sup>A</sup> , 93 <sup>A</sup>
TA1535	JNJ-27018966-AAA	5000 µg	6	2	0.6	5 <sup>M</sup> , 5 <sup>M</sup> , 9 <sup>M</sup>
		1500 µg	6	1	0.6	6 <sup>M</sup> , 7 <sup>M</sup> , 5 <sup>M</sup>
		500 µg	5	3	0.5	6 <sup>M</sup> , 8 <sup>M</sup> , 2 <sup>M</sup>
		150 µg	5	3	0.5	8 <sup>M</sup> , 5 <sup>M</sup> , 3 <sup>M</sup>
		50 µg	7	3	0.7	6 <sup>M</sup> , 5 <sup>M</sup> , 10 <sup>M</sup>
	DMSO	50 µL	10	3		14 <sup>M</sup> , 8 <sup>M</sup> , 8 <sup>M</sup>
TA1537	JNJ-27018966-AAA	5000 µg	5	2	0.7	3 <sup>A</sup> , 6 <sup>A</sup> , 5 <sup>A</sup>
		1500 µg	7	2	1.0	8 <sup>A</sup> , 8 <sup>A</sup> , 5 <sup>A</sup>
		500 µg	4	1	0.6	3 <sup>A</sup> , 3 <sup>A</sup> , 5 <sup>A</sup>
		150 µg	5	1	0.7	6 <sup>A</sup> , 5 <sup>A</sup> , 5 <sup>A</sup>
		50 µg	6	3	0.9	6 <sup>A</sup> , 4 <sup>A</sup> , 9 <sup>A</sup>
	DMSO	50 µL	7	3		6 <sup>A</sup> , 10 <sup>A</sup> , 4 <sup>A</sup>
WP2uvrA	JNJ-27018966-AAA	5000 µg	42	4	0.9	47 <sup>A</sup> , 40 <sup>A</sup> , 40 <sup>A</sup>
		1500 µg	43	4	0.9	45 <sup>A</sup> , 38 <sup>A</sup> , 46 <sup>A</sup>
		500 µg	46	8	0.9	42 <sup>A</sup> , 55 <sup>A</sup> , 40 <sup>A</sup>
		150 µg	37	12	0.8	27 <sup>A</sup> , 50 <sup>A</sup> , 34 <sup>A</sup>
		50 µg	47	7	1.0	42 <sup>A</sup> , 55 <sup>A</sup> , 43 <sup>A</sup>
	DMSO	50 µL	49	8		41 <sup>A</sup> , 57 <sup>A</sup> , 48 <sup>A</sup>

Table 4 cont.  
Confirmatory Mutagenicity Assay with S9 activation

Study Number: AC34AZ.503 (b) (4) Study Code: AC34AZ  
 Experiment: B2 Date Plated: 2/2/2010  
 Exposure Method: Plate incorporation assay Evaluation Period: 2/9/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	2AA	1.0 µg	410	45	21.6	388 <sup>A</sup> , 380 <sup>A</sup> , 462 <sup>A</sup>
TA100	2AA	2.0 µg	1219	17	11.5	1223 <sup>A</sup> , 1233 <sup>A</sup> , 1200 <sup>A</sup>
TA1535	2AA	1.0 µg	80	14	8.0	64 <sup>A</sup> , 92 <sup>A</sup> , 84 <sup>A</sup>
TA1537	2AA	1.0 µg	38	13	5.4	23 <sup>A</sup> , 48 <sup>A</sup> , 43 <sup>A</sup>
WP2uvrA	2AA	10 µg	226	21	4.6	245 <sup>A</sup> , 230 <sup>A</sup> , 204 <sup>A</sup>

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

**Study title: Ames test with JNJ-27018966-AAA**

Study no.: TOX7777  
 Study report location: EDR 4.2.3.3.1  
 Conducting laboratory and location: Johnson & Johnson, Raritan, NJ  
 Date of study initiation: October 24, 2006  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAA, 30205959, 98%

**Key Study Findings: Negative**

**Methods:** Plate incorporation and preincubation method

Strains: *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvrA*

Concentrations in definitive study: 50, 250, 500, 1000, 2500 and 5000 µg per plate in the presence and absence of S9 (metabolic activation, Aroclor 1254-induced rat liver S9). All dose levels of test article, vehicle control and positive controls were plated in triplicate.

Basis of concentration selection: Solubility of the test article

Negative control: Dimethyl sulfoxide (DMSO)

Positive control: Shown below

Formulation/Vehicle: DMSO

Incubation & sampling time: 48 hours

The positive controls are shown below (from page 15 of the report).

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	2-Anthramine	2.00
TA98	-	Fenamino-sulf (Dexon)	200
TA100	+	2-Anthramine	2.00
TA100	-	Sodium azide	2.00
TA1535	+	2-Anthramine	2.00
TA1535	-	Sodium azide	2.00
TA1537	+	2-Anthramine	2.00
TA1537	-	9-Aminoacridine HCl	50.0
WP2 <i>uvrA</i>	+	2-Anthramine	25.0
WP2 <i>uvrA</i>	-	4-Nitroquinolone-N-Oxide (4-NQO)	1.00

**Study Validity:** All criteria for a valid study were met as described in the protocol.

Criteria for positive results are as follows:

- Test article was considered to be positive (mutagenic), if it induces a dose-dependent increase in the revertant frequency to at least 2-fold that observed in the appropriate concurrent vehicle control. In addition, the response should be reproducible.

**Results:** JNJ-27018966 (standard plate incorporation and pre-incubation assays, in the absence and presence of S9) was negative in the Ames test under the conditions of the assay. The following tables (from page 7 and 8) show the results.

TOX7777

<b>Report Title:</b> In Vitro Mutagenicity Testing of JNJ-27018966-AAA in the Bacterial/Microsomal Activation Assay	<b>Test Article:</b> JNJ-27018966-AAA
<b>Test for Induction of:</b> Reverse Point Mutations	<b>No. of Independent Assays:</b> 2
<b>Strains:</b> S. typhimurium TA98, TA100, TA1535, TA1537; E. coli WP2uvrA	<b>No. of Replicates:</b> 3
<b>Metabolizing System:</b> Aroclor 1254-induced rat liver S-9 mix	<b>Approximate No. of Bacteria Assayed/Dose:</b> 10 <sup>8</sup>
<b>Vehicle:</b> DMSO	<b>Date of Treatment:</b> 7 Nov 2006
<b>Treatment:</b> In Vitro	
<b>Cytotoxic Effects:</b> No	
<b>Genotoxic Effects:</b> No	

		Assay #1 (Plate Incorporation, test 1336) Revertant Colony Counts (Mean)							
Metabolic Activation	Test Article	Dose Level (µg/plate)	TA98	TA100	TA1535	TA1537	WP2uvrA		
Without Activation (-S9)	DMSO	50 µL	25.0	219.0	16.3	6.7	32.7		
		JNJ-27018966-AAA	50	27.0	220.3	22.7	8.3	28.0	
		250	24.0	187.7	21.0	8.3	28.3		
		500	26.0	177.0	25.0	7.0	29.7		
		1000	22.3	214.0	22.3	8.7	26.7		
		2500	24.3	177.0	28.7	8.3	23.0		
	5000	22.0	185.7	22.3	8.7	26.7			
	Dexon	200	2151.7 <sup>*</sup>	NT	NT	NT	NT		
		Sodium Azide	2	NT	1226.3 <sup>*</sup>	891.7 <sup>*</sup>	NT	NT	
		9-Aminoacridine HCl	50	NT	NT	NT	49.0 <sup>*</sup>	NT	
		4-NQO	1	NT	NT	NT	NT	404.7 <sup>*</sup>	
	With Activation (+S9)	DMSO	50 µL	35.3	227.3	34.0	11.0	29.3	
			JNJ-27018966-AAA	50	41.0	242.7	30.3	13.3	23.7
			250	37.0	249.0	21.7	11.0	31.0	
500			41.3	237.3	28.7	13.3	28.7		
1000			32.3	216.0	27.3	9.7	24.3		
2500			34.7	231.3	20.3	11.7	27.3		
5000		32.3	225.3	23.7	11.3	28.3			
2-Anthramine		2	337.3 <sup>*</sup>	756.3 <sup>*</sup>	121.3 <sup>*</sup>	71.7 <sup>*</sup>	NT		
		25	NT	NT	NT	NT	167.7 <sup>*</sup>		

<sup>\*</sup> These values represent at least a doubling of the negative control values for TA98, TA100, and WP2uvrA, and at least a tripling of the negative control values for TA1535 and TA1537.  
NT = Not Tested

TOX7777

<b>Report Title:</b> In Vitro Mutagenicity Testing of JNJ-27018966-AAA in the Bacterial/Microsomal Activation Assay	<b>Test Article:</b> JNJ-27018966-AAA
<b>Test for Induction of:</b> Reverse Point Mutations	<b>No. of Independent Assays:</b> 2
<b>Strains:</b> S. typhimurium TA98, TA100, TA1535, TA1537; E. coli WP2uvrA	<b>No. of Replicates:</b> 3
<b>Metabolizing System:</b> Aroclor 1254-induced rat liver S-9 mix	<b>Approximate No. of Bacteria Assayed/Dose:</b> 10 <sup>8</sup>
<b>Vehicle:</b> DMSO	<b>Date of Treatment:</b> 8 Nov 2006
<b>Treatment:</b> In Vitro	
<b>Cytotoxic Effects:</b> Yes	
<b>Genotoxic Effects:</b> No	

		Assay #2 (Preincubation, test 1337) Revertant Colony Counts (Mean)							
Metabolic Activation	Test Article	Dose Level (µg/plate)	TA98	TA100	TA1535	TA1537	WP2uvrA		
Without Activation (-S9)	DMSO	50 µL	20.7	130.7	14.0	6.7	21.0		
		JNJ-27018966-AAA	50	22.3	130.0	12.7	8.0	23.7	
		250	27.3	127.3	13.7	5.3	18.3		
		500	23.0	130.0	10.0	7.7	28.0		
		1000	19.7	135.0	16.3	7.3	27.0		
		2500	25.0	121.7	13.7	5.7	23.3		
	5000	25.0	135.3	12.3	5.7	0.0 <sup>k</sup>			
	Dexon	200	2165.3 <sup>*</sup>	NT	NT	NT	NT		
		Sodium Azide	2	NT	977.7 <sup>*</sup>	812.7 <sup>*</sup>	NT	NT	
		9-Aminoacridine HCl	50	NT	NT	NT	70.3 <sup>*</sup>	NT	
		4-NQO	1	NT	NT	NT	NT	2068.7 <sup>*</sup>	
	With Activation (+S9)	DMSO	50 µL	33.3	177.3	15.0	7.3	29.7	
			JNJ-27018966-AAA	50	27.3	151.3	13.7	9.3	20.0
			250	32.3	156.0	17.7	8.0	29.3	
500			23.7	168.3	8.3	8.7	24.0		
1000			30.0	170.7	16.3	8.0	31.7		
2500			30.3	162.7	11.7	7.7	27.0		
5000		25.0	166.0	10.7	9.7	25.3			
2-Anthramine		2	1275.3 <sup>*</sup>	1221.7 <sup>*</sup>	126.3 <sup>*</sup>	117.3 <sup>*</sup>	NT		
		25	NT	NT	NT	NT	109.0		

<sup>k</sup> Reduced Bacterial Lawn  
<sup>\*</sup> These values represent at least a doubling of the negative control values for TA98, TA100, and WP2uvrA, and at least a tripling of the negative control values for TA1535 and TA1537.  
NT = Not Tested

## 7.2 In Vitro Assays in Mammalian Cells

### Study title: In Vitro Mammalian Chromosome Aberration Test

Study no.: AC34AZ.341 (b) (4)  
Study report location: EDR 4.2.3.3.1  
Conducting laboratory and location: (b) (4)  
Date of study initiation: January 21, 2010  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: JNJ-27018966-AAA, 31746876, 94.6%

### **Key Study Findings:** Negative

### **Methods:**

Cell line: Human peripheral blood lymphocytes (HPBL)  
Concentrations in definitive study: 1250, 2500 and 5000 µg/mL  
Basis of concentration selection: Dose selection was based on precipitation of the test article in the treatment medium (the highest dose with the least precipitation and two lower doses).  
Negative control: DMSO  
Positive control: Mitomycin C (MMC) was used as the positive control in the non-activated study at final concentrations of 0.3 and 0.6 µg/mL. Cyclophosphamide (CP) was used as the positive control in the S9-activated study at final concentrations of 10 and 15 µg/mL.  
Formulation/Vehicle: DMSO  
Incubation & sampling time: Cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 20 hours post-treatment.

**Study Validity:** The assay met the criteria for a valid test. The positive and solvent controls fulfilled the requirements for a valid test.

**Results:** Under the conditions of the assay, JNJ-27018966-AAA was negative for the induction of structural and numerical chromosome aberrations in the non-activated and S9-activated test systems. The following table (from page 24 of the report) shows the summary results.

TABLE 7  
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural	Numerical (%)	Structural (%)		
DMSO	-S9	4	13.0	200	200	0.000	±0.000	0.0	0.0
JNJ-27018966-AAA									
1250	-S9	4	12.4	200	200	0.005	±0.071	0.0	0.5
2500	-S9	4	10.8	200	200	0.000	±0.000	0.0	0.0
5000 p	-S9	4	9.6	200	200	0.005	±0.071	0.0	0.5
MMC, 0.6	-S9	4	5.4	200	100	0.180	±0.386	0.0	18.0**
DMSO	+S9	4	12.6	200	200	0.000	±0.000	0.0	0.0
JNJ-27018966-AAA									
1250	+S9	4	13.0	200	200	0.000	±0.000	0.0	0.0
2500	+S9	4	12.4	200	200	0.000	±0.000	0.5	0.0
5000 p	+S9	4	11.5	200	200	0.005	±0.071	0.0	0.5
CP, 10	+S9	4	5.2	200	200	0.155	±0.402	0.0	14.0**
DMSO	-S9	20	11.1	200	200	0.000	±0.000	0.0	0.0
JNJ-27018966-AAA									
1250	-S9	20	10.6	200	200	0.000	±0.000	0.0	0.0
2500	-S9	20	10.4	200	200	0.000	±0.000	0.0	0.0
5000 p	-S9	20	6.0	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	20	5.7	200	100	0.360	±0.798	0.0	22.0**

**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:** \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; using the Fisher's Exact test.

p: Visible precipitate was observed in the treatment medium at the conclusion of the treatment period.

**Study title: In Vitro Mutagenicity Testing of JNJ-27018966-AAA using the Microwell Method of the L5178Y/tk<sup>+/-</sup> Mouse Lymphoma Assay**

Study no.: TOX7767

Study report location: EDR 4.2.3.3.1

Conducting laboratory and location: Johnson & Johnson, Raritan, NJ

Date of study initiation: November 6, 2006

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: JNJ-27018966-AAA, 30205959, 98%

**Key Study Findings: Negative**

**Methods:**

Cell line: L5178Y/tk<sup>+/-</sup> cell line  
Concentrations in definitive study: 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, and 2500 µg/mL for 3-hour treatments with S9 and a 24-hour treatment without S9  
Basis of concentration selection: Toxicity range finding study (see below)  
Negative control: DMSO  
Positive control: Methyl methanesulfonate (MMS) was used as the positive control in the absence of metabolic activation (-S9) and 3-methylcholanthrene (3-MCA) was used in the presence of metabolic activation (+S9).  
Formulation/Vehicle: DMSO  
Incubation & sampling time: 3 hours (+ S9); 24 hours (-S9)

Basis of concentration selection: Cytotoxicity

**Study Validity:** All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met. Criteria for positive results are as follows:

- The mutant frequency at one or more concentrations would be at least 2-fold greater than that of the concurrent vehicle control.
- A concentration related increase in mutant frequency for at least 3 concentrations.

**Results:**Initial Trial:

*3-hour (-)S9:* The average mutant frequency of the vehicle control cultures was (b) (4), while those of the cultures treated with JNJ-27018966 ranged from (b) (4).

*3-hour (+)S9:* The average mutant frequency of the vehicle control cultures was (b) (4) while those of the cultures treated with JNJ-27018966 ranged from (b) (4).

JNJ-27018966 was evaluated in the confirmatory trial using a 24-hour treatment without S9 and a 3-hour treatment with S9.

Confirmatory Trial:

3-hour (+)S9: The average mutant frequency of the vehicle control cultures was (b) (4), while those of the cultures treated with JNJ-27018966 ranged from (b) (4).

24-hour (-)S9: The average total suspension growth (TSG) for vehicle cultures did not satisfy minimum acceptance criteria (TSG = (b) (4)). JNJ-27018966 was subsequently evaluated in a retest trial using a 24-hour treatment without S9. The average mutant frequency of the vehicle control cultures was (b) (4) while those of the remaining cultures treated with JNJ-27018966 ranged from (b) (4).

The results from the above retest revealed that JNJ-27018966 was not evaluated to the acceptable limits of solubility in the absence of dose limiting cytotoxicity. It is to be noted here that precipitation of the test article was only observed immediately after the treatment and was not seen at the end of treatment following incubation at 37°C. A fourth trial was conducted to evaluate JNJ-27018966 up to the limit of solubility in all four previously tested conditions: 3-hour-S9, 3-hour+S9 (1% v/v, final S9), 3-hour+S9 (2% v/v, final S9) and 24-hour-S9.

#### Fourth Trial:

3-hour-S9: The average mutant frequency of the vehicle control cultures was 76.0 mutants/10<sup>6</sup>, while those of the cultures treated with JNJ-27018966 ranged from 50.7 to 87.9 mutants/10<sup>6</sup> cells.

3-hour+S9 (1% v/v, final S9): The average mutant frequency of the vehicle control cultures was 73.4 mutants/10<sup>6</sup>, while those of the cultures treated with JNJ-27018966 ranged from 43.7 to 75.5 mutants/10<sup>6</sup> cells.

3-hour+S9 (2% v/v, final S9): The average mutant frequency of the vehicle control cultures was 47.9 mutants/10<sup>6</sup> cells, while those of the cultures treated with JNJ-27018966 ranged from 40.5 to 57.0 mutants/10<sup>6</sup> cells.

24-hour-S9: The average mutant frequency of the vehicle control cultures was 34.5 mutants/10<sup>6</sup> cells, while those of the cultures treated with JNJ-27018966 ranged from 26.5 to 38.0 mutants/10<sup>6</sup> cells.

Under the treatment conditions in the fourth trial, JNJ-27018966-AAA did not induce a concentration dependent or  $\geq 2$ -fold increase in the frequency of mutants when compared to vehicle control. JNJ-27018966 was concluded to be negative in the mouse lymphoma cell (L5178Y/TK<sup>+/-</sup>) forward mutation test under the conditions of the experiment. The following tables (from page 49-52 of the report) show the results of the fourth trial.

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TOX7767

Toxicology/Pathology - Genetic Toxicology

Table SD9: 3-hour treatment, -S9 Summary Data

Spreadsheet Version Number 3.02

Trial 2D

Test: 2D1

TOX7767

Test Article	Concentration (µg/mL)	Day 0 cells/mL (10 <sup>5</sup> )	Day 1 cells/mL (10 <sup>5</sup> )	Day 2 cells/mL (10 <sup>5</sup> )	TSG <sup>1</sup>	ASG <sup>2</sup>	RSG <sup>3</sup>	Total Viable Colonies	Total Mutant Colonies	Small Mutant Colonies	Large Mutant Colonies	ABS PE <sup>4</sup>	REL PE <sup>5</sup>	RTC <sup>6</sup>	MF <sup>7</sup> (10 <sup>-6</sup> )	IMF <sup>8</sup> (10 <sup>-6</sup> )	Small MF <sup>9</sup> (10 <sup>-6</sup> )	Large MF <sup>10</sup> (10 <sup>-6</sup> )	Small Colony % <sup>11</sup>
<b>Vehicle Average</b>	<b>2% (v/v)</b>	<b>6.9</b>	<b>9.3</b>	<b>16.3</b>	<b>16.8</b>	<b>16.8</b>	<b>100.0</b>	<b>113</b>	<b>73</b>	<b>43</b>	<b>30</b>	<b>89.5</b>	<b>100.0</b>	<b>100.0</b>	<b>76.0</b>	<b>N/A</b>	<b>43.4</b>	<b>30.0</b>	<b>58.2</b>
DMSO	2% (v/v)	6.9	9.3	17.4	17.9	18.0	107.2	105	61	32	29	79.2	88.4	94.8	70.7	N/A	36.1	32.6	52.5
	2% (v/v)	6.9	9.0	16.0	16.0	16.1	95.8	118	96	61	35	95.3	106.5	102.0	95.6	N/A	58.7	32.9	63.5
JNJ-27018966	2% (v/v)	6.8	9.5	15.5	16.5	16.3	97.0	117	63	37	26	94.0	105.0	101.9	61.6	N/A	35.3	24.6	58.7
	25.0	6.8	9.3	18.3	18.9	18.7	111.0	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	500.0	7.2	9.4	12.9	13.4	14.0	83.5	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	1000.0	7.0	8.9	16.1	15.9	16.0	95.4	122	76	N/S	N/S	100.9	112.7	107.5	70.1	-5.9	N/S	N/S	N/S
	2000.0	7.0	9.9	16.1	17.7	18.0	107.0	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	2500.0	6.9	10.6	15.6	18.5	18.5	109.9	123	88	N/S	N/S	102.3	114.3	125.7	81.0	5.0	N/S	N/S	N/S
	2750.0	7.0	9.5	16.1	16.9	17.1	101.8	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	3000.0	6.9	9.6	17.3	18.5	18.6	110.4	110	57	N/S	N/S	85.1	95.1	104.9	61.2	-14.7	N/C	N/C	N/S
	3250.0	6.9	9.6	18.7	20.0	19.9	118.5	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	3500.0	6.8	10.7	18.1	21.4	21.1	125.6	102	63	N/S	N/S	75.8	84.7	106.4	76.4	0.5	N/S	N/S	N/S
	3750.0	6.7	10.1	17.6	19.8	19.4	115.1	128	61	N/S	N/S	109.9	122.7	141.3	50.9	-25.0	N/S	N/S	N/S
	4000.0 p	6.8	10.6	17.1	20.1	19.8	118.0	127	66	N/S	N/S	108.3	121.0	142.8	56.2	-19.8	N/S	N/S	N/S
	4250.0 p	6.8	9.8	19.9	21.7	21.4	127.5	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	4500.0 p	6.6	10.7	16.4	19.4	18.6	110.8	109	79	N/S	N/S	83.9	93.7	103.8	87.9	12.0	N/S	N/S	N/S
	4750.0 p	6.7	10.8	16.9	20.3	19.8	117.7	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
5000.0 p	6.7	11.5	18.9	24.2	23.4	139.1	115	51	N/S	N/S	91.4	102.1	142.0	50.7	-25.2	N/C	N/C	N/S	
MMS	10.0	6.4	9.6	15.6	16.6	15.3	90.8	82	376	279	97	55.7	62.2	56.5	949.5 *	873.5	594.6 *	165.5 *	74.2
	15.0	5.9	9.3	14.4	14.9	12.7	75.5	62	406	300	106	39.0	43.6	32.9	1564.7 *	1488.7	943.3 *	260.8 *	73.9

<p>EQUATIONS</p> <p>(b) (4)</p>	<p>FOOTNOTES</p> <ul style="list-style-type: none"> <li>* Positive response (≥2-fold increase in mutant frequency)</li> <li>p Precipitation</li> <li>s Not cloned due to sufficient number of surviving concentrations</li> </ul> <p>NOTATIONS</p> <p>--: Culture Discarded    N/C: Not Cloned    N/S: Not Scored    N/A: Not Applicable</p>
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TOX7767

Toxicology/Pathology - Genetic Toxicology

Table SD11: 3-hour treatment, +S9 Summary Data

Spreadsheet Version Number 3.02

Trial 2D

Test: 2D3

Test Article	Concentration (µg/mL)	Day 0 cells/mL (10 <sup>5</sup> )	Day 1 cells/mL (10 <sup>5</sup> )	Day 2 cells/mL (10 <sup>5</sup> )	TSG <sup>1</sup>	ASC <sup>2</sup>	RSG <sup>3</sup>	Total Viable Colonies	Total Mutant Colonies	Small Mutant Colonies	Large Mutant Colonies	ABS PE <sup>4</sup>	REL PE <sup>5</sup>	RTG <sup>6</sup>	MF <sup>7</sup> (10 <sup>-6</sup> )	IMF <sup>8</sup> (10 <sup>-6</sup> )	Small MF <sup>9</sup> (10 <sup>-6</sup> )	Large MF <sup>10</sup> (10 <sup>-6</sup> )	Small Colony % <sup>11</sup>
<b>Vehicle Average</b>	<b>2% (v/v)</b>	<b>7.0</b>	<b>14.2</b>	<b>16.0</b>	<b>25.1</b>	<b>25.1</b>	<b>100.0</b>	<b>107</b>	<b>43</b>	<b>29</b>	<b>14</b>	<b>81.9</b>	<b>100.0</b>	<b>100.0</b>	<b>47.9</b>	<b>N/A</b>	<b>32.1</b>	<b>15.1</b>	<b>67.0</b>
DMSO	2% (v/v)	7.1	13.9	15.1	23.3	23.7	94.4	110	39	26	13	85.1	103.9	98.1	41.2	N/A	27.1	13.4	66.7
	2% (v/v)	6.9	14.5	15.8	25.5	25.1	100.2	106	37	23	14	80.3	98.1	98.2	41.3	N/A	25.4	15.3	62.2
	2% (v/v)	7.0	14.1	16.9	26.5	26.4	105.4	106	54	39	15	80.3	98.1	103.4	61.3	N/A	43.6	16.4	72.2
	25.0	7.1	14.4	15.2	24.2	24.3	96.9	127	52	N/S	N/S	108.3	132.2	128.1	43.7	-4.3	N/S	N/S	N/S
	500.0	6.7	15.2	15.9	26.7	25.5	101.7	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	1000.0	7.0	13.8	12.4	19.1	19.1	76.1	127	57	N/S	N/S	108.3	132.2	100.6	48.1	0.2	N/S	N/S	N/S
	2000.0	7.0	13.9	16.2	25.0	24.9	99.4	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	2500.0	7.2	13.2	15.7	23.0	23.6	94.0	104	49	N/S	N/S	N/S	78.0	95.3	89.5	57.0	9.0	N/S	N/S
	2750.0	7.6	14.5	10.9	17.5	18.9	75.5	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	3000.0	7.1	13.5	15.2	22.7	22.8	91.0	107	51	N/S	N/S	N/S	81.5	99.5	90.5	56.9	8.9	N/S	N/S
	3250.0	7.0	13.7	15.5	23.6	23.6	94.1	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	3500.0	7.1	9.3	17.4	17.9	18.2	72.4	118	48	N/S	N/S	N/S	95.3	116.4	84.3	45.6	-2.3	N/S	N/S
	3750.0	<i>p</i>	6.7	13.7	14.0	21.3	20.3	80.7	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	4000.0	<i>p</i>	6.7	13.6	15.0	22.6	21.4	85.5	117	49	N/S	N/S	94.0	114.8	98.1	47.3	-0.6	N/S	N/S
	4250.0	<i>p</i>	6.6	13.5	15.9	24.0	22.4	89.4	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
	4500.0	<i>p</i>	7.0	13.1	15.4	22.4	22.2	88.6	119	59	N/S	N/S	96.7	118.1	104.6	55.9	7.9	N/S	N/S
4750.0	<i>p</i>	6.8	13.9	13.3	20.6	20.0	79.6	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	
5000.0	<i>p</i>	6.6	14.5	12.7	20.4	19.1	76.0	120	44	N/S	N/S	98.1	119.8	91.1	40.5	-7.4	N/S	N/S	
MMS	2.5	6.5	9.0	9.9	9.9	9.1	36.3	70	376	247	129	45.3	55.4	20.1	1166.3 *	1118.4	617.5 *	279.6 *	65.7
	4.0	6.6	7.2	7.5	6.0	5.7	22.6	55	310	254	56	33.8	41.2	9.3	1144.6 x	1096.6	861.5 *	151.5 *	81.9

EQATIONS

(b) (4)

**FOOTNOTES**

- \* Positive response (≥2-fold increase in mutant frequency)
- p* Precipitation
- s* Not cloned due to sufficient number of surviving concentrations
- x* Excluded from evaluation of mutagenicity due to excessive cytotoxicity (RTG<10%)

**NOTATIONS**

---: Culture Discarded    N/C: Not Cloned    N/S: Not Scored    N/A: Not Applicable

TOX7767

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JOHNSON & JOHNSON PHARMACEUTICAL RESEARCH & DEVELOPMENT

TOX7767

Toxicology/Pathology - Genetic Toxicology

Table SD12: 24-hour treatment, -S9 Summary Data

Spreadsheet Version Number 3.02

Trial 2D

Test: 2D4

Test Article	Concentration (µg/mL)	Day 1 cells/mL (10 <sup>5</sup> )	Day 2 cells/mL (10 <sup>5</sup> )	Day 3 cells/mL (10 <sup>5</sup> )	TSG <sup>1</sup>	ASG <sup>2</sup>	RSG <sup>3</sup>	Total Viable Colonies	Total Mutant Colonies	Small Mutant Colonies	Large Mutant Colonies	ABS PE <sup>4</sup>	REL PE <sup>5</sup>	RTG <sup>6</sup>	MF <sup>7</sup> (10 <sup>-6</sup> )	IME <sup>8</sup> (10 <sup>-6</sup> )	Small MF <sup>9</sup> (10 <sup>-6</sup> )	Large MF <sup>10</sup> (10 <sup>-6</sup> )	Small Colony % <sup>11</sup>	
Vehicle Average	1% (v/v)	18.6	7.9	13.3	35.9	N/A	100.0	98	28	20	8	71.4	100.0	100.0	34.5	N/A	24.4	9.8	71.1	
DMSO	1% (v/v)	18.3	8.0	14.4	39.2	N/A	109.1	97	27	21	6	70.4	98.5	107.4	34.1	N/A	26.4	7.4	77.8	
	1% (v/v)	18.7	7.9	12.5	34.3	N/A	95.4	101	26	17	9	74.7	104.5	99.7	30.9	N/A	20.1	10.5	65.4	
	1% (v/v)	18.6	7.7	13.0	34.3	N/A	95.5	96	30	21	9	69.3	97.0	92.6	38.6	N/A	26.8	11.4	70.0	
	JNJ-27018966	25.0	17.9	7.8	13.4	34.6	N/A	96.4	90	27	N/S	N/S	63.3	88.5	85.4	38.0	3.4	N/S	N/S	N/S
		100.0	17.4	6.9	13.3	29.7	N/A	82.6	102	23	N/S	N/S	75.8	106.0	87.6	26.9	-7.7	N/S	N/S	N/S
		500.0	15.9	5.6	12.1	20.0	N/A	55.6	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
		750.0	15.4	5.5	12.1	19.0	N/A	53.0	106	26	N/S	N/S	80.3	112.4	59.6	28.8	-5.8	N/S	N/S	N/S
		1000.0	14.4	5.0	12.6	16.5	N/A	46.1	100	29	N/S	N/S	73.6	103.0	47.4	35.1	0.6	N/S	N/S	N/S
		1125.0	14.0	5.6	11.7	16.9	N/A	47.1	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
		1250.0	14.2	5.8	12.7	19.3	N/A	53.8	112	28	N/S	N/S	87.5	122.5	65.9	28.5	-6.1	N/S	N/S	N/S
		1375.0	14.0	5.7	12.1	17.8	N/A	49.6	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
		1500.0	13.8	5.5	12.8	18.0	N/A	50.2	122	30	N/S	N/S	100.9	141.2	70.9	26.5	-8.0	N/S	N/S	N/S
		1625.0	13.2	5.5	11.8	16.0	N/A	44.4	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
		1750.0	13.6	5.4	12.4	17.0	N/A	47.2	100	22	N/S	N/S	73.6	103.0	48.7	26.5	-8.1	N/S	N/S	N/S
		1875.0	13.3	5.7	12.9	18.2	N/A	50.7	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
		2000.0	13.2	5.5	12.3	16.3	N/A	45.4	96	25	N/S	N/S	69.3	97.0	44.0	32.0	-2.5	N/S	N/S	N/S
		2250.0	12.9	5.5	11.8	15.6	N/A	43.4	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C
2500.0	14.0	6.0	10.9	16.8	N/A	46.7	116	31	N/S	N/S	92.7	129.7	60.6	29.8	-4.7	N/S	N/S	N/S		
MMS	5.0	17.2	8.1	10.8	27.9	N/A	77.6	88	177	129	48	61.3	85.8	66.6	299.4 *	264.9	206.8 *	71.0 *	72.9	
	7.5	16.4	7.4	9.7	21.8	N/A	60.7	76	187	133	54	50.4	70.5	42.8	389.5 *	354.9	260.5 *	97.7 *	71.1	

EQUATIONS

FOOTNOTES

- \* Positive response (≥2-fold increase in mutant frequency)
- s Not cloned due to sufficient number of surviving concentrations

NOTATIONS

-- Culture Discarded N/C: Not Cloned N/S: Not Scored N/A: Not Applicable

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

Study title: Bone Marrow Micronucleus Assay in Rats Dosed by Intraperitoneal Administration with JNJ-27018966-AAA

Study no: Study TOX7789  
 Study report location: EDR 4.2.3.3.2  
 Conducting laboratory and location: Johnson & Johnson, Raritan, NJ  
 Date of study initiation: November 1, 2006  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAA (b) (4) form of JNJ-27018966), Batch No.: 30205959, 98.8%

**Key Study Findings:** Negative

**Methods:**

Doses in definitive study: 31, 63 and 125 mg/kg  
Frequency of dosing: Single dose  
Route of administration: Intraperitoneal (IP)  
Dose volume: 0.62, 1.26 and 2.5 mL/kg for 31, 63 and 125 mg/kg, respectively  
Formulation/Vehicle: 0.5% Hypromellose solution  
Species/Strain: SD rats  
Number/Sex/Group: 5/sex/group  
Satellite groups: 3/sex/group for TK analysis. Blood samples were collected from satellite animals at 1 and 6 hours postdose for TK analysis.

Basis of dose selection: The high dose was selected based upon the results of the acute toxicity study where the MTD was determined to be 125 mg/kg, IP in male and female rats (TOX7688). In this acute IP toxicity study (TOX7688) with JNJ-27018966, there was one death in the males (1 of 5) and no deaths in the females at 125 mg/kg. At 250 mg/kg, 2 of 5 males and 2 of 5 females died. Based on these findings, the high dose for the current study was selected as 125 mg/kg, with the middle and low doses being 63 and 31 mg/kg, respectively, in both males and females.

Negative control: 0.5% Hypromellose solution  
Positive control: Cyclophosphamide (60 mg/kg, 10 mL/kg, oral gavage)  
Sampling: 24 and 48 hours (harvest). Five rats per sex per group were sacrificed at the appropriate time for bone marrow collection, and a total of 2000 polychromatic erythrocytes (PCEs) per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the ratio of PCEs to normochromatic erythrocytes (NCEs) in 1000 erythrocytes per animal.

The following table (from page 14 of the report) shows the study design.

3.1.6. Study Groups							
Study Groups Treatment Group	Dose (mg/kg)	Conc. (mg/mL)	Dose Volume (mL/kg)	Sampling Time (h)	No. of Animals per sex	Assigned ID	Assigned
						No. TOX7789- Males	ID No. TOX7789- Females
Vehicle Control	0	0	2.5	24	5	1001-1005	1501-1505
				48	5	1006-1010	1506-1510
JNJ-27018966 Low	31	50 <sup>b</sup>	0.62	24	5	2001-2005	2501-2505
				48	5	2006-2010	2506-2510
JNJ-27018966 Mid	63	50 <sup>b</sup>	1.26	24	5	3001-3005	3501-3505
				48	5	3006-3010	3506-3510
JNJ-27018966 High	125	50 <sup>b</sup>	2.5	24	5	4001-4005	4501-4505
				48	5	4006-4010	4506-4510
				extra <sup>a</sup>	5	4011-4015	4511-4515
Positive Control CP	60	6	10	24	5	5001-5005	5501-5505

<sup>a</sup> Extras to replace any animals in the high dose group should mortality occur.

<sup>b</sup> Concentrations stated as non-salt form

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

**Results:** Mortality was observed in males at 125 mg/kg (3 of 15 animals). JNJ-27018966 did not induce any statistically significant increases in the frequency of micronucleated PCEs at any of the doses evaluated. A statistically significant trend was observed in the males at 24-hour ( $p = 0.023$ ). This result was not considered to be biologically relevant as the means for the male 24-hour dose groups were within the laboratory's historical range. The following tables (from page 109 and 110 of the report) show the results of the assay (raw and transformed % mPCEs and ratio of PCE:NCE) and the statistical analysis results.

APPENDIX I  
Bone Marrow Micronucleus Assay in Rats Dosed by Intraperitoneal  
with JNJ-27018966-AAA (Study TOX7789)

TOX7789

Summary Statistics: Mean Values

Harvest time	SEX	drug	DOSE	Percent micronucleated PCEs	transformed Percent micronucleated PCEs	PCE:NCE	transformed PCE:NCE
24	F	Vehicle	0	0.100	0.77	1.74	1.83
24	F	JNJ-27018966-AAA	31	0.070	0.75	1.71	1.81
24	F	JNJ-27018966-AAA	63	0.100	0.77	2.19	1.93
24	F	JNJ-27018966-AAA	125	0.090	0.77	1.68	1.81
24	F	Positive Control	60	0.780	1.11	0.42	1.09
24	M	Vehicle	0	0.050	0.74	1.75	1.83
24	M	JNJ-27018966-AAA	31	0.120	0.79	1.91	1.81
24	M	JNJ-27018966-AAA	63	0.170	0.82	1.86	1.76
24	M	JNJ-27018966-AAA	125	0.150	0.81	1.99	1.87
24	M	Positive Control	60	0.770	1.10	0.42	1.12
48	F	Vehicle	0	0.070	0.75	2.70	1.93
48	F	JNJ-27018966-AAA	31	0.090	0.77	1.27	1.67
48	F	JNJ-27018966-AAA	63	0.130	0.79	1.43	1.70
48	F	JNJ-27018966-AAA	125	0.115	0.78	2.31	1.87
48	M	Vehicle	0	0.060	0.75	2.72	2.00
48	M	JNJ-27018966-AAA	31	0.130	0.79	3.11	2.03
48	M	JNJ-27018966-AAA	63	0.110	0.78	3.53	2.11
48	M	JNJ-27018966-AAA	125	0.080	0.76	2.73	1.92

APPENDIX II  
Bone Marrow Micronucleus Assay in Rats Dosed by Intraperitoneal  
with JNJ-27018966-AAA (Study TOX7789)

TOX7789

trend test

Obs	SEX	Harvest time	Data	Transformation	Parameter	Estimate	Standard Error	t-value	P-value	sig
1	F	24	count	sqrt	linear trend	0.00424237	0.08296331	0.05	0.4799	
2	F	24	ratio	arcsine	linear trend	0.07545748	0.29722442	0.25	0.5986	
3	F	48	count	sqrt	linear trend	0.10778535	0.10482893	1.03	0.1596	
4	F	48	ratio	arcsine	linear trend	-0.06686425	0.56396479	-0.12	0.4535	
5	M	24	count	sqrt	linear trend	0.22305632	0.10310885	2.16	0.0230	**
6	M	24	ratio	arcsine	linear trend	0.07226334	0.54125624	0.13	0.5523	
7	M	48	count	sqrt	linear trend	0.02675698	0.10934141	0.24	0.4049	
8	M	48	ratio	arcsine	linear trend	-0.14738923	0.52700078	-0.28	0.3917	

The following table (from page 105 of the report) shows the historical control data.

TOX789

**Micronucleus Historical Control Data**  
Rat Micronucleus - February 2006 through January 2007

Species	Rat										
Vehicle	(All)										
		PCE:NCE Ratio					%Micronucleated PCEs				
Sex	Harvest Time (24/48-hour)	Average	StdDev	Maximum	Minimum	N	Average	StdDev	Maximum	Minimum	N
Females	24	1.8264	1.4826	9.7530	0.6620	35	0.2407	0.1995	0.8500	0.0000	35
	48	1.7393	1.0510	6.7520	0.4770	35	0.2286	0.1800	0.7500	0.0000	35
Males	24	1.8159	1.0066	4.7350	0.4180	35	0.2286	0.2055	0.8000	0.0000	35
	48	1.8598	0.9869	5.0610	0.4680	35	0.1986	0.1442	0.4500	0.0000	35

Species (Mouse/Rat)	Rat										
Compound	Cyclophosphamide										
Concentration	60 mg/kg										
		PCE:NCE Ratio					%Micronucleated PCEs				
Sex	Harvest Time (24/48-hour)	Average	StdDev	Maximum	Minimum	N	Average	StdDev	Maximum	Minimum	N
Females	24	0.6307	0.3683	1.5250	0.1010	30	1.2317	0.6093	2.4500	0.2500	30
Males	24	0.5995	0.2415	1.3200	0.2500	30	2.3867	1.2498	5.3500	0.7000	30

PCE = Polychromatic erythrocyte  
NCE = Normochromatic erythrocyte  
N = Number of animals  
StdDev = Standard deviation

Systemic exposure to JNJ-27018966 in male and female rats was variable and occurred in most JNJ-27018966-treated rats. Mean plasma concentrations were dose-related, but less than dose-proportional. There were no clear gender differences in plasma concentrations of JNJ-27018966. The following tables (from page 43 and 44 of the report) show the mean plasma concentrations of JNJ-27018966.

Table SD1: Individual and Mean Plasma Concentration Data (ng/mL) for JNJ-27018966 in Male and Female Rats following Single Intraperitoneal Doses (0 [Vehicle], 31, 63, or 125 mg/kg) of JNJ-27018966-AAA (TOX7789)

Dose (mg/kg)	Male			Female			
	Subject	Time (h)		Dose (mg/kg)	Subject	Time (h)	
		1	6			1	6
0	RAT1011	0.00	0.00	0	RAT1511	0.00	0.00
	RAT1012	0.00	0.00		RAT1512	0.00	0.00
	RAT1013	0.00	0.00		RAT1513	0.00	0.00
	Mean	0.00	0.00		Mean	0.00	0.00
	SD	0.00	0.00		SD	0.00	0.00
31	RAT2011	0.00 <sup>a</sup>	0.00 <sup>a</sup>	31	RAT2511	561 <sup>a</sup>	162 <sup>a</sup>
	RAT2012	2710	4.74		RAT2512	1910	7.39
	RAT2013	2200	4.01		RAT2513	1820	16.3
	Mean	1640	2.92		Mean	1430	61.9
	SD	1440	2.55		SD	754	86.8
63	RAT3011	0.00 <sup>a</sup>	0.00 <sup>a</sup>	63	RAT3511	2.37 <sup>a</sup>	0.00 <sup>a</sup>
	RAT3012	5620	14.8		RAT3512	2840	82.7
	RAT3013	3440	14.1		RAT3513	0.00 <sup>a</sup>	0.00 <sup>a</sup>
	Mean	3020	9.63		Mean	947	27.6
	SD	2830	8.35		SD	1640	47.7
125	RAT4016	9120	187	125	RAT4516	9600	2200
	RAT4017	2.68 <sup>a</sup>	0.00 <sup>a</sup>		RAT4517	3.89 <sup>a</sup>	2.14 <sup>a</sup>
	RAT4018	13700	NS		RAT4518	3770	13.5
	Mean	7610	93.5		Mean	4460	739
	SD	6970	NA		SD	4830	1270

<sup>a</sup> Concentrations confirmed by re-assay (within 5% of original value)

NS: No Sample Collected. Rat found dead prior to 6 hour blood sample collection.

NA: Not Applicable

Table SD2: Mean (SD) JNJ-27018966 Plasma Concentrations in Male and Female Rats Following a Single Oral Dose of JNJ-27018966-AAA (0 [Vehicle], 31, 63, and 125 mg/kg) (TOX7789)

Dose (mg/kg)	Male		Dose (mg/kg)	Female	
	Time (h)			Time (h)	
	1	6		1	6
0	0.00 (0.00)	0.00 (0.00)	0	0.00 (0.00)	0.00 (0.00)
31	1640 (1440)	2.92 (2.55)	31	1430 (754)	61.9 (86.8)
63	3020 (2830)	9.63 (8.35)	63	947 (1640)	27.6 (47.7)
125	7610 (6970)	93.5 <sup>a</sup> NA	125	4460 (4830)	739 (1270)

<sup>a</sup> N=2.

NS: No Sample Collected.  
NA: Not Applicable

#### 7.4 Other Genetic Toxicity Studies

None

### 8 Carcinogenicity

#### **104-Week Oral Carcinogenicity Study in Mice (Study No. 1808-009)**

#### **104-Week Oral Carcinogenicity Study in Rats (Study No. 1808-008)**

The above study reports have been reviewed under IND 79,214 (pharmacology review of IND 79,214 dated July 31, 2014). The reviews of the above studies are incorporated below from the above pharmacology review of IND 79,214 dated July 31, 2014.

## Executive Summary

### Introduction

Eluxadoline (JNJ-27018966/JNJ-27018966-AAA) is a locally active, mixed mu-opioid receptor ( $\mu$ OR) agonist and delta-opioid receptor ( $\delta$ OR) antagonist. This drug is being developed for the treatment of diarrhea-predominant irritable bowel syndrome (IBS-d). JNJ-27018966 has low oral bioavailability in all species tested, including humans. JNJ-27018966 acts peripherally, within the gastrointestinal (GI) tract, and has demonstrated efficacy in normalizing GI transit and defecation in animal models of stress induced or post GI inflammation altered GI function. The recommended human dose is 100 mg twice daily (BID).

### Brief Discussion of Nonclinical Findings

In a 104-week oral (gavage) carcinogenicity study in CD1 mice, animals were treated with JNJ-27018966-AAA at 0, 150, 500 and 1500 mg/kg/day (10 mL/kg). The doses were selected per the ECAC recommendations (ECAC meeting minutes dated March 16, 2011, Attachment-1); high dose being selected based on the maximum feasible dose (MFD). There were no significant treatment-related effects on mortality in either sex. There were no significant treatment-related non-neoplastic findings in either sex. There were no significant tumor findings in either sex. Overall, this study appears to be negative for tumor findings in both sexes. The study conduct was considered adequate and acceptable.

In a 104-week oral (gavage) carcinogenicity study in SD rats, animals were treated with JNJ-27018966-AAA at 0, 150, 500 and 1500 mg/kg/day (10 mL/kg). The doses were selected per the ECAC recommendations (ECAC meeting minutes dated March 16, 2011, Attachment-1); high dose being selected based on the MFD. There were no significant treatment-related effects on mortality in either sex. There were no significant treatment-related non-neoplastic or neoplastic findings in males or females. The study conduct was considered adequate and acceptable.

### Recommendations

None

## Drug Information

### Drug

CAS Registry Number: 864821-90-9

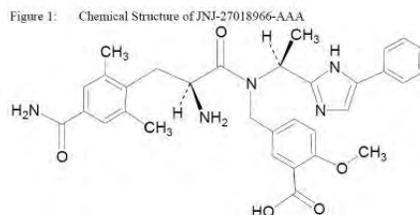
Generic Name: Eluxadoline

Code Name: JNJ-27018966/JNJ-27018966-AAA

Chemical Name: 5-[[[(2S)-2-amino-3-[4-(aminocarbonyl)-2,6-dimethylphenyl]-1-oxopropyl]][(1S)-1-(4-phenyl-1H-imidazol-2-yl)ethyl]amino]methyl]-2-methoxybenzoic acid

Molecular Formula/Molecular Weight: C<sub>32</sub>H<sub>35</sub>N<sub>5</sub>O<sub>5</sub>/569.65

Structure:



Pharmacologic class: Mu-opioid receptor agonist/delta-opioid receptor antagonist

### Proposed Clinical Population and Dosing Regimen

Eluxadoline is indicated for the treatment of patients with IBS-d.

### Studies Submitted

The sponsor submitted the following carcinogenicity study reports:

1. A 104-Week Oral (Gavage) Carcinogenicity Study in Mice (Study No. 1808-009)
2. A 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Study No. 1808-008)

### Studies Reviewed

The above listed carcinogenicity studies are reviewed here.

### Carcinogenicity

**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND  
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET  
Review of Mice Carcinogenicity Study Results**

P/T REVIEWER: Tamal K. Chakraborti, Ph.D.

DATE: July 29, 2014

IND: 79,214

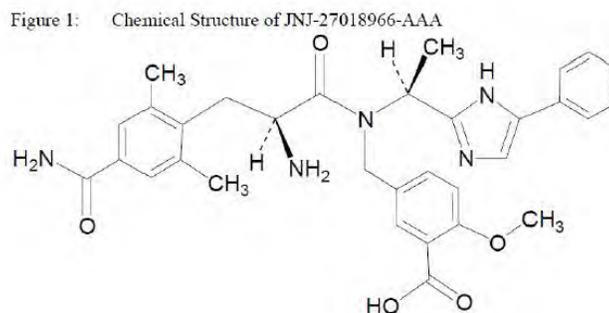
DRUG CODE#: JNJ-27018966/JNJ-27018966-AAA

CAS#: 864821-90-9

DIVISION: DGIEP

DRUG NAME: Eluxadoline

CHEMICAL STRUCTURE:



SPONSOR: Furiex Pharmaceuticals, Inc., Morrisville, NC

LABORATORY: (b) (4)

CARCINOGENICITY STUDY REPORT DATE: January 24, 2014

THERAPEUTIC CATEGORY: For the treatment of patients with IBS-d

PHARMACOLOGICAL CLASSIFICATION: Mu-opioid receptor agonist/delta-opioid receptor antagonist

MUTAGENIC/GENOTOXIC: JNJ-27018966 was negative in the Ames test, chromosomal aberration tests with mouse or human lymphocytes, and in an *in vivo* rat bone marrow micronucleus test.

**MICE CARCINOGENICITY STUDY:**

STUDY DURATION (weeks): 104

STUDY STARTING DATE: January 27, 2011

STUDY ENDING DATE: January 24, 2014

MICE STRAIN: Crl:CD1@(ICR) mice

ROUTE: Oral (Gavage)

DOSING COMMENTS: Doses were selected based on the ECAC recommendations (ECAC meeting minutes dated March 16, 2011). The Committee recommended doses of 150, 500, and 1500 mg/kg/day based on 1500 mg/kg/day being the MFD and mid- and low-dose was selected as 500 and 100 mg/kg/day, respectively, to clearly separate exposures. Doses were administered at a dose volume of 10 mL/kg.

**NUMBER OF MICE:**

- Control-1 (C1): 65/sex
- Low Dose (LD): 65/sex
- Middle Dose (MD): 65/sex
- High Dose (HD): 65/sex

**MICE DOSE LEVELS:**

- Low Dose: 150 mg/kg/day
- Middle Dose: 500 mg/kg/day
- High Dose: 1500 mg/kg/day

BASIS FOR DOSES SELECTED: The high dose of 1500 mg/kg/day was selected based on the MFD for both sexes per the ECAC recommendations. The low- and mid-dose was selected as 150 and 500 mg/kg/day, respectively, to clearly separate exposures.

PRIOR FDA DOSE CONCURRENCE: Yes.

MICE CARCINOGENICITY: Negative

MICE TUMOR FINDINGS: There were no significant tumor findings in either sex.

MICE STUDY COMMENTS: The dose selection was based on the ECAC recommendations; high dose being selected based on the MFD. There were no significant treatment-related non-neoplastic or neoplastic findings in males or females. The study conduct was considered adequate and acceptable.

**CARCINOGENICITY:****Study title:** 104-Week Oral Carcinogenicity Study in Mice**Key study findings:**

- In a 104-week oral (gavage) carcinogenicity study in CD1 mice, animals were treated with JNJ-27018966-AAA at 0, 150, 500, 1500 mg/kg/day.
- There were no significant treatment-related effects on mortality in either sex.
- There were no significant treatment-related non-neoplastic findings in males and females.
- There were no significant treatment-related neoplastic findings in male or female mice.
- The study conduct was considered adequate and acceptable.

**Study number:** 1808-009**Volume #, and page #:** EDR Section dated 4.2.3.4.1.**Conducting laboratory and location:** (b) (4)**Date of study initiation:** May 12, 2011**GLP compliance:** A statement of compliance was included.**QA report:** yes ( X ) no ( )**Drug, lot #, and % purity:** JNJ-27018966-AAA, Batch Nos. ZR497138PFA111, 0020349095, 0020349094, 0020367844.**CAC concurrence:** Yes**Study Type:** 2-year bioassay**Species/strain:** CrI:CD1@(ICR) mice**Number/sex/group; age at start of study; body weight:** 65/sex/group; approximately 6 weeks old; Males: 28.1- 36.8 g, Females: 21.9-28.1 g**Animal housing:** Animals were housed three to four per cage for 3 days upon arrival. During the 16-day acclimation period, animals were observed daily for general health and any signs of disease. Animals were individually housed in polyboxes in an environmentally controlled room. Fluorescent lighting was provided for approximately 12 hours per day. Temperature and humidity were continuously monitored, recorded, and maintained within the protocol designated ranges of 64°F to 79°F and 30% to 70%, respectively.

**Formulation/vehicle:** 0.5% hydroxypropyl methylcellulose (HPMC, high viscosity, hypromellose 2910) in purified water, USP.

**Drug stability/homogeneity:** Dosing formulations were evaluated for homogeneity and concentration. The following table (from page 16 of the report) shows dosing formulation analysis sample collection schedule.

Dosing Formulation Analysis Sample Collection <sup>a</sup>							
Sample Type	Concentration Sampled (mg/mL)	Stratum	Number of Samples per Concentration			Sample Volume (mL)	Intervals (Week)
			Collected	Analyzed	Backup		
Homogeneity Analyses <sup>b, c</sup>	15 and 150	Top	3	1	2	1.0	1
		Middle	3	1	2	1.0	
		Bottom	3	1	2	1.0	
Concentration Analyses <sup>b</sup>	0, 15, 50, and 150	Middle	3	1	2	1.0	1, 2, 3, 4

<sup>a</sup> Prior to study initiation, a test batch of JNJ-27018966-AAA was prepared at nominal concentrations of 37.6 and 150 mg/mL. Specific details pertaining to the sampling, analysis results, and disposition of the test batch are not reported but are maintained in the study data.

<sup>b</sup> The samples, including backup samples, were stored refrigerated at 2 to 8°C pending analyses or final disposition.

<sup>c</sup> Samples collected subsequent to Week 4 were collected and are reported under (b) (4) Study Number 1808-008.

### **Methods:**

Doses: 0, 150, 500, 1500 mg/kg/day

Dose volume: 10 mL/kg

Basis of dose selection: The doses of 0, 150, 500, and 1500 mg/kg/day for both sexes were selected per the ECAC recommendations. The high dose was selected based on the MFD. The low- and mid-dose was selected as 150 and 500 mg/kg/day, respectively, to clearly separate exposures.

Restriction paradigm for dietary restriction studies: N/A

Route of administration: Oral gavage

Frequency of drug administration: Once daily

Dual controls employed: No

Interim sacrifices: None

Study Design: The study design is shown in the table below (from page 17 of the study report).

<b>Group Assignments</b>			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
<b>Main Study Groups</b>			
1	0 <sup>a</sup>	65	65
2	150	65	65
3	500	65	65
4	1500	65	65
<b>Toxicokinetic Groups</b>			
5	0 <sup>a</sup>	20	20
6	150	56	56
7	500	56	56
8	1500	56	56
<b>Sentinel Animals<sup>b</sup></b>			
89	-	35	35

<sup>a</sup> Administered the vehicle formulation only.  
<sup>b</sup> Sentinel animals were not administered the vehicle or test article.

Satellite group for toxicokinetics: Yes (shown in the above table)

Deviations from original study protocol: Protocol deviations did not adversely affect either the quality or integrity of the study or the interpretation of the results.

Statistical methods:

Survival Data: Intercurrent mortality data were analyzed using the Kaplan-Meier product-limit method. An overall test comparing all groups was conducted using a log-rank test.

Tumor Data:

Sponsor's Analysis: Tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the

incidence and number of sites examined for each tumor type. Certain tumor types were combined, as determined by the pathologist (McConnell, EE, et. al., 1986, Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies, J Natl Cancer Inst, 76:283-289), prior to data analysis. The Cochran-Armitage trend test was calculated and Fisher's exact test was used to compare each treatment group with the control group. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto et al (Peto R, et al, Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments. In: Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal. Annex to Supplement 2. p. 311-426. International Agency for Research on Cancer, Lyon; 1980). Evaluation criteria (p-values of significance) were applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%) (Haseman JK, 1983, A Reexamination of False-Positive Rates for Carcinogenesis Studies. Fund Appl Toxicol, 3:334-339). The evaluation criteria (Guidance for Industry: Statistical Aspects of Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals. U.S. FDA, CDER, 2001 May) are presented in the following table (from page 23 of the report).

<b>Evaluation Criteria for Common and Rare Tumors</b>	
<b>Test for Positive Trends</b>	<b>Control-High Pair-wise Comparisons</b>
Common and rare tumors were tested at respective significance levels of 0.005 and 0.025	Common and rare tumors were tested at respective significance levels of 0.01 and 0.05

FDA Analysis: The tumor data were analyzed for dose response relationships and pair-wise comparisons between the vehicle control group with each of the treated groups were performed using the Poly-k method described in the paper of Bailer and Portier (Bailer AJ and CJ Portier, 1988, Effects of Treatment-Induced Mortality and Tumor-Induced Mortality on Tests for Carcinogenicity in Small Samples, *Biometrics*, 44, 417-431) and Bieler and Williams (Bieler, GS and RL Williams, 1993, Ratio Estimates, The Delta Method, and Quantal Response Tests for Increased Carcinogenicity, *Biometrics* 49, 793-801).

**Observations and times:**

Mortality: Mortality was checked twice daily.

Clinical Signs: Clinical signs were examined on a weekly basis.

Body weights: Body weights were recorded on Day -13 and prior to randomization (Day -3). Body weights for all surviving main study and TK animals were recorded once

weekly for Weeks 1 through 14, bi-weekly from Week 15 through Week 28, and once every 4 weeks thereafter.

Food consumption: Food consumption was recorded for surviving main study animals once weekly during Weeks 1 through 14, bi-weekly from Week 15 through Week 28, and once every 4 weeks thereafter. Food consumption was not measured and recorded for toxicokinetic (TK) animals.

Ophthalmoscopy: Ophthalmoscopy was conducted at pretest and prior to scheduled necropsy.

Clinical Pathology: Clinical pathology evaluations were conducted on all main study animals euthanized *in extremis* and on all surviving main study animals at the scheduled terminal necropsies.

Gross pathology: Gross pathology was conducted at scheduled necropsy.

Histopathology: The following (from page 1026 of the report) tissues were collected for histopathological examination from all main study animals.

- 
- 
- |  |   |
|--|---|
| <ul style="list-style-type: none"> <li>- Adrenal (2)</li> <li>- Aorta</li> <li>- Bone with marrow [femur]</li> <li>- Bone with marrow [sternum]</li> <li>- Bone marrow smear [2 collected]<sup>a</sup></li> <li>- Brain [cerebrum, midbrain, cerebellum, medulla/pons]</li> <li>- Clitoral gland (2)</li> <li>- Epididymis (2)</li> <li>- Eye including optic nerve (2)</li> <li>- Gallbladder</li> <li>- GALT [Gut Associated Lymphoid Tissue]</li> <li>- Gastrointestinal tract:             <ul style="list-style-type: none"> <li>esophagus</li> <li>stomach [glandular and nonglandular]</li> <li>duodenum</li> <li>jejunum</li> <li>ileum</li> <li>cecum</li> <li>colon</li> <li>rectum</li> </ul> </li> <li>- Gonads:             <ul style="list-style-type: none"> <li>ovary (2) with oviduct (2)</li> <li>testis (2)</li> </ul> </li> <li>- Gross lesions</li> <li>- Heart</li> <li>- Joint, tibiofemoral</li> <li>- Kidney (2)</li> <li>- Lacrimal gland, exorbital (2)</li> <li>- Larynx</li> <li>- Liver [collected whole; 2 examined]</li> </ul> | <ul style="list-style-type: none"> <li>- Lung with bronchi [collected whole; all lobes examined]</li> <li>- Lymph nodes: mandibular [2 collected; 1 examined], mesenteric, and regional where applicable</li> <li>- Mammary gland [only process females]</li> <li>- Nasal tissue [Levels A, B, C, and D]</li> <li>- Pancreas</li> <li>- Pituitary</li> <li>- Preputial gland (2)</li> <li>- Prostate and seminal vesicle (2)</li> <li>- Salivary gland, mandibular/sublingual [2 collected; 1 examined]</li> <li>- Salivary gland, parotid [2 collected; 1 examined]</li> <li>- Sciatic nerve</li> <li>- Skeletal muscle, biceps femoris</li> <li>- Skin</li> <li>- Spinal cord [cervical, thoracic, and lumbar]</li> <li>- Spleen</li> <li>- Thymus</li> <li>- Thyroid/parathyroid (2)</li> <li>- Tissue masses</li> <li>- Tongue</li> <li>- Trachea</li> <li>- Ureter (2)</li> <li>- Urinary bladder</li> <li>- Uterus [both horns]/Cervix</li> <li>- Vagina</li> <li>- Zymbal's gland (2)</li> </ul> |
|--|---|
- 
- 

<sup>a</sup>Bone marrow smears were collected at the scheduled necropsies and held.  
(2) Paired organ

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**Toxicokinetics (TK):** Blood samples were collected from TK animals for determination of the plasma concentrations of JNJ-27018966-AAA. Samples were collected from three TK control animals per sex at 1 hour post-dose on Day 91 and Day 364 and from cohorts of three JNJ-27018966-AAA-treated TK animals per sex at 0.5, 1, 3, 8, 12, and 24 hours post-dose.

### **Results:**

**Clinical signs:** There were no significant treatment-related clinical signs.

**Mortality:** There was no statistically significant dose-response for mortality and statistically significant difference in mortality in both females and males when compared

with the vehicle control group. The survival rates at scheduled sacrifice were 55%, 52%, 48% and 48% in males and 40%, 45%, 49% and 49% in females at 0, 150, 500, and 1500 mg/kg/day, respectively. All causes of death/morbidity were either commonly seen in mice of this strain and age or were considered incidental and unrelated to treatment due to the lack of a dose response and/or the lack of similar findings in both sexes. The following table (from page 1032-1035 of the report) shows the survival data.

(b) (4) Study Number 1808-009  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Mice

**Summary of Survival Estimates - MALE<sup>a</sup>**

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>0 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	2	0	65.0	1.0000	0.0000
	27-39	2	0	63.0	0.9692	0.0214
	40-52	3	0	61.0	0.9385	0.0298
	53-65	3	0	58.0	0.8923	0.0384
	66-78	6	0	55.0	0.8462	0.0448
	79-91	13	0	49.0	0.7538	0.0534
	92-104	17	19 <sup>b</sup>	26.5	0.5538	0.0617
	105	0	0	0	0	0
<u>150 mg/kg/day</u>						
	1-13	1	0	65.0	1.0000	0.0000
	14-26	0	0	64.0	0.9846	0.0153
	27-39	1	0	64.0	0.9846	0.0153
	40-52	2	0	63.0	0.9692	0.0214
	53-65	6	0	61.0	0.9385	0.0298
	66-78	7	0	55.0	0.8462	0.0448
	79-91	14	0	48.0	0.7385	0.0545
	92-104	10	24 <sup>b</sup>	22.0	0.5231	0.0620
	105	0	0	0	0	0
<u>500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	1	0	65.0	1.0000	0.0000
	27-39	2	0	64.0	0.9846	0.0153
	40-52	5	0	62.0	0.9538	0.0260
	53-65	8	0	57.0	0.8769	0.0407
	66-78	6	0	49.0	0.7538	0.0534
	79-91	12	0	43.0	0.6615	0.0587
	92-104	12	19 <sup>b</sup>	21.5	0.4769	0.0620
	105	0	0	0	0	0
<u>1500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	2	0	65.0	1.0000	0.0000
	27-39	2	0	63.0	0.9692	0.0214
	40-52	4	0	61.0	0.9385	0.0298

<sup>a</sup> Necropsy count

<sup>b</sup> No statistical significance observed

(b) (4) Study Number 1808-009  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Mice

**Summary of Survival Estimates - MALE<sup>a</sup>**

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>1500 mg/kg/day</u>	53-65	3	0	57.0	0.8769	0.0407
	66-78	12	0	54.0	0.8308	0.0465
	79-91	11	0	42.0	0.6462	0.0593
	92-104	12	19 <sup>b</sup>	21.5	0.4769	0.0620
	105	0	0	0	0	0

(b) (4) Study Number 1808-009  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Mice

**Summary of Survival Estimates - FEMALE<sup>†</sup>**

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>0 mg/kg/day</u>						
	1-13	1	0	65.0	1.0000	0.0000
	14-26	0	0	64.0	0.9846	0.0153
	27-39	3	0	64.0	0.9846	0.0153
	40-52	8	0	61.0	0.9385	0.0298
	53-65	6	0	53.0	0.8154	0.0481
	66-78	9	0	47.0	0.7231	0.0555
	79-91	12	0	38.0	0.5846	0.0611
	92-104	7	19 <sup>&amp;</sup>	16.5	0.4000	0.0608
	105	0	0	0	0	0
<u>150 mg/kg/day</u>						
	1-13	1	0	65.0	1.0000	0.0000
	14-26	1	0	64.0	0.9846	0.0153
	27-39	3	0	63.0	0.9692	0.0214
	40-52	3	0	60.0	0.9231	0.0331
	53-65	3	0	57.0	0.8769	0.0407
	66-78	12	0	54.0	0.8308	0.0465
	79-91	13	0	42.0	0.6462	0.0593
	92-104	6	23 <sup>&amp;</sup>	17.5	0.4462	0.0617
	105	0	0	0	0	0
<u>500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	0	0	65.0	1.0000	0.0000
	27-39	3	0	65.0	1.0000	0.0000
	40-52	1	0	62.0	0.9538	0.0260
	53-65	6	0	61.0	0.9385	0.0298
	66-78	10	0	55.0	0.8462	0.0448
	79-91	13	0	45.0	0.6923	0.0572
	92-104	8	24 <sup>&amp;</sup>	20.0	0.4923	0.0620
	105	0	0	0	0	0
<u>1500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	1	0	65.0	1.0000	0.0000
	27-39	5	0	64.0	0.9846	0.0153
	40-52	2	0	59.0	0.9077	0.0359

<sup>&</sup> Necropsy count

<sup>†</sup> No statistical significance observed

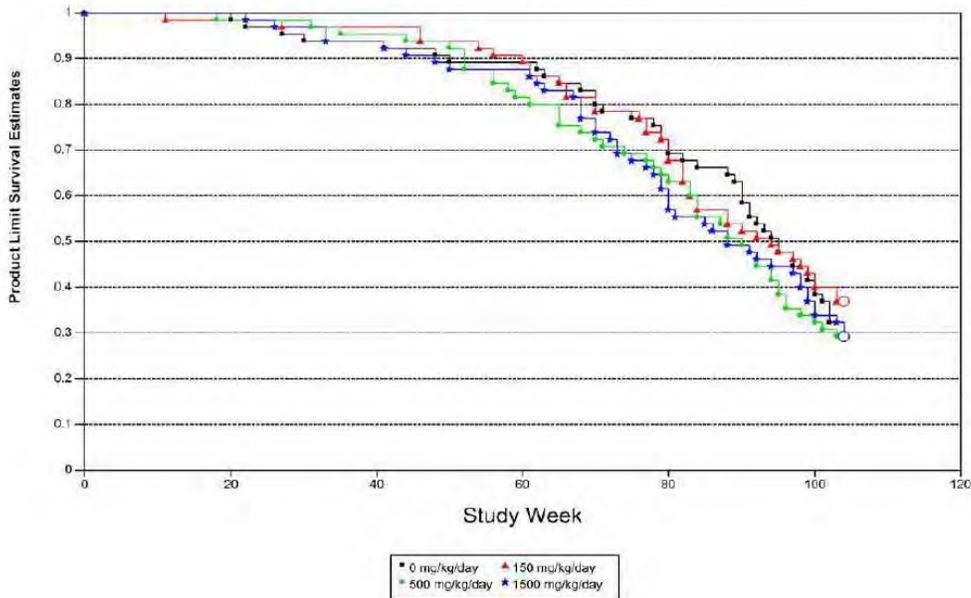
(b) (4)  
 Study Number 1808-009  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Mice

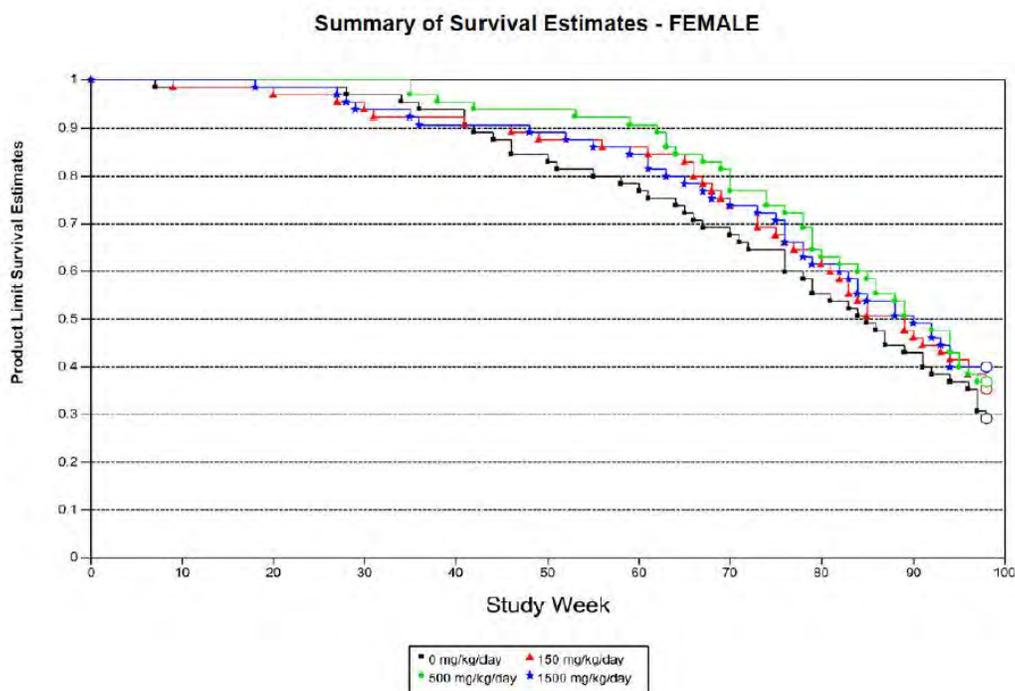
**Summary of Survival Estimates - FEMALE**

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>1500 mg/kg/day</u>	53-65	6	0	57.0	0.8769	0.0407
	66-78	10	0	51.0	0.7846	0.0510
	79-91	9	0	41.0	0.6308	0.0599
	92-104	6	26 <sup>Δ</sup>	19.0	0.4923	0.0620
	105	0	0	0	0	0

Survival curves are shown below (from page 1029 and 1030 of the report).

**Summary of Survival Estimates - MALE**





**Body weights:** The mean initial (Week 1) and final (Week 100) body weights of control males were 33.86 and 47.55 g, respectively. The mean initial (Week 1) and final (Week 96) body weights of control females were 25.64 and 37.75 g, respectively. In males, final body weights were 99.8%, 98.5%, and 94.4% of control at 150, 500 and 1500 mg/kg/day, respectively. In females, final body weights were 98.5%, 96.1% and 98.7% of control at 150, 500 and 1500 mg/kg/day, respectively. There were no significant treatment-related effects on body weights in either sex. The following tables (from page 26-27 of the report) show the absolute body weights (g) for males and females at various time points.

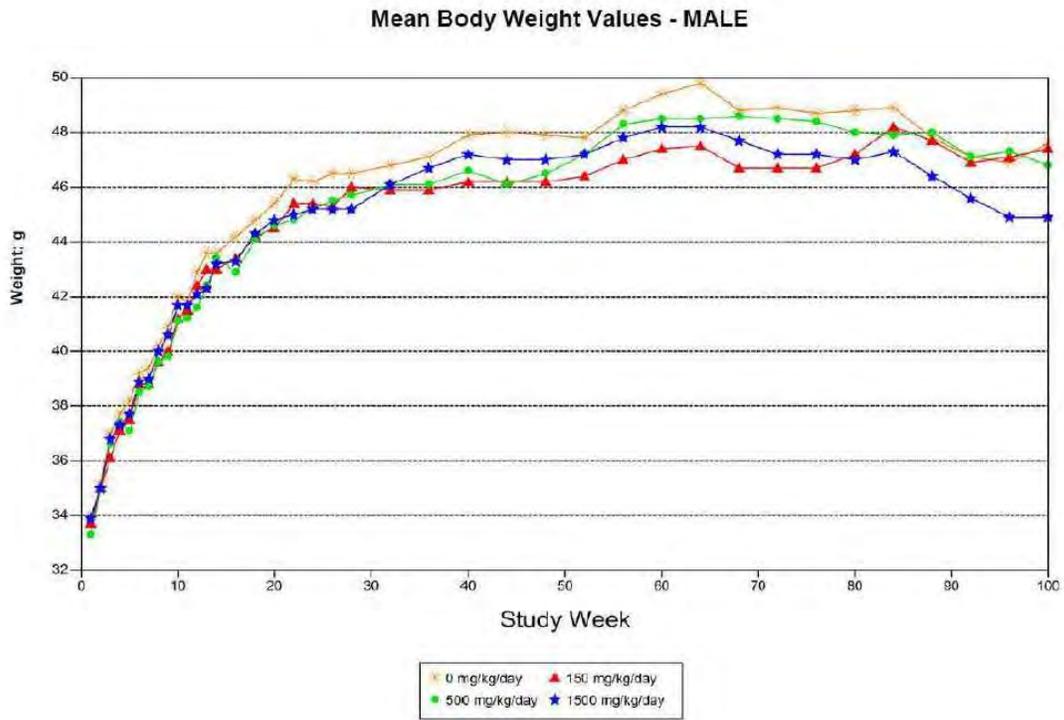
Summary of Group Mean Body Weight (g)								
Dose Level (mg/kg/day)	Main Study Males							
	Week 26	(%)	Week 52	(%)	Week 76	(%)	Week 100	(%)
0	46.5	NA	47.8	NA	48.7	NA	47.6	NA
150	45.3	(-2.6)	46.4	(-2.9)	46.7	(-4.1)	47.4	(-0.4)
500	45.5	(-2.2)	47.2	(-1.3)	48.4	(-0.6)	46.8	(-1.7)
1500	45.2	(-2.7)	47.2	(-1.3)	47.2	(-3.1)	44.9	(-5.7)

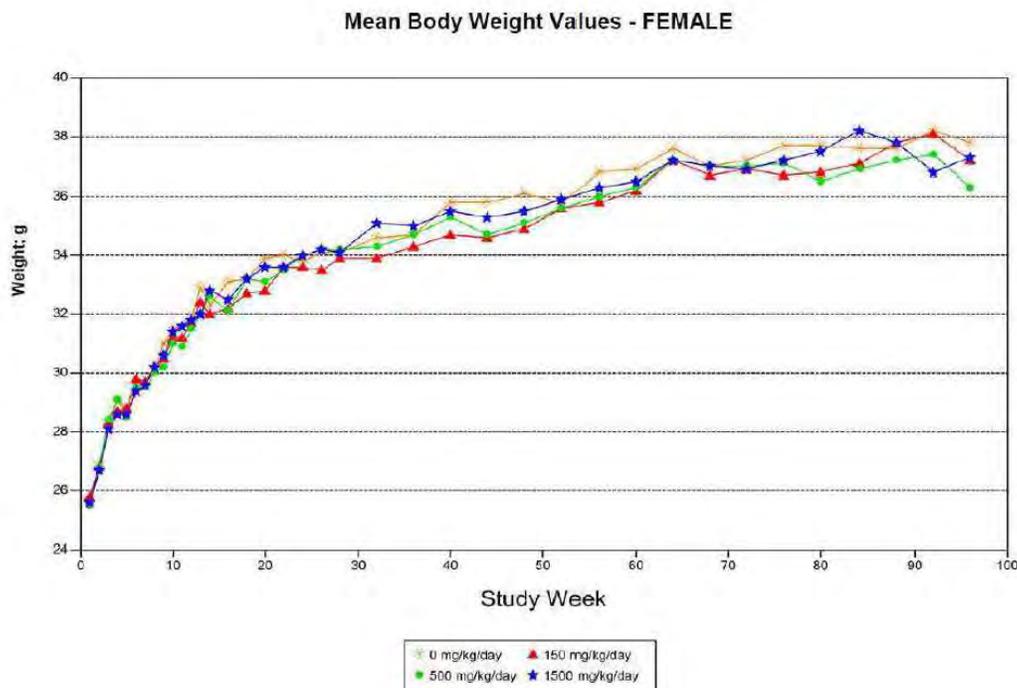
(%) - Percent difference from control  
NA – Not applicable

Summary of Group Mean Body Weight (g)								
Dose Level (mg/kg/day)	Main Study Females							
	Week 26	(%)	Week 52	(%)	Week 76	(%)	Week 96	(%)
0	34.1	NA	35.8	NA	37.7	NA	37.8	NA
150	33.5	(-1.8)	35.6	(-0.6)	36.7	(-2.7)	37.2	(-1.6)
500	34.2	(+0.3)	35.6	(-0.6)	37.1	(-1.6)	36.3	(-4.0)
1500	34.2	(+0.3)	35.9	(+0.3)	37.2	(-1.3)	37.3	(-1.3)

(%) - Percent difference from control  
NA – Not applicable

The following figures (from page 82 and 83 of the study report) show the growth curves in males and females.





**Food consumption:** The mean initial (Week 1) and final (Week 104) food consumption in control males was 6.15 and 4.88 g/animal/day, respectively. The mean initial (Week 1) and final (Week 96) food consumption in control females was 5.47 and 4.72 g/animal/day, respectively. There was no significant effect of treatment on food consumption. The following table (from page 27 of the report) shows the food consumption data.

Average Food Consumption; g/animal/day (Week 1 through Week 104) <sup>a</sup>				
Dose Level (mg/kg/day)	Male		Female	
	Mean	(%) Difference from Control	Mean	(%) Difference from Control
0	5.39	NA	5.36	NA
150	5.55	(+3.0)	5.27	(-1.7)
500	5.61	(+4.1)	5.32	(-0.7)
1500	5.44	(+0.9)	5.35	(-0.2)

<sup>a</sup>Female average food consumption values were calculated through Week 96 due to survival.  
NA – Not applicable

Ophthalmoscopy: There were no significant treatment-related effects.

Hematology: There were no significant treatment-related effects.

Gross pathology: There were no significant treatment-related effects.

Analysis of Dosing Formulations: The average concentration of the homogeneity samples collected from the formulation prepared for Weeks 1 and 8 were within  $\pm 15\%$  of the nominal concentration; precision was  $\leq 10\%$  relative standard deviation (RSD). The average concentration of samples collected from the formulations prepared for Weeks 1 through 104 and 105 were within  $\pm 15\%$  of the nominal concentration; precision was  $\leq 10\%$  RSD.

Histopathology:

Non-neoplastic: There were no significant treatment-related non-neoplastic findings in either sex.

Neoplastic: There were no significant tumor findings in males or females.

Toxicokinetics: The  $C_{max}$  of JNJ-27018966 increased less than dose proportionally from 150 to 500 mg/kg/day and more than proportionally from 500 mg/kg/day to 1500 mg/kg/day after 12 months of daily oral administration. The  $AUC_{0-24h}$  of JNJ-27018966 increased less than dose proportionally after 12 months of daily oral administration. The  $T_{max}$  of JNJ-27018966 varied from 0.5 hour to 8 hours across all dose levels following 3 and 12 months of oral dosing. Terminal half-lives for JNJ-27018966 ranged from approximately 3 to 5 hours. Sex-related difference in exposure to JNJ-27018966 was observed. Females showed higher exposures than males at 500 and 1500 mg/kg/day at 3 months and at all dose levels at 12 months. There was no apparent accumulation of JNJ-27018966 between 3 and 12 months. The following table (from page 940 of the report) shows the TK parameters.

**Table 8.1 Toxicokinetic Parameters for JNJ-27018966 in Mice After Daily Oral Administration of 150, 500 and 1500 mg/kg JNJ-27018966-AAA at 3 and 12 Months**

Month	Dose (mg/kg/day)	$C_{max}$ (ng/mL)	$T_{max}$ (hr)	$AUC_{(0-24)}$ (hr*ng/mL)	$AUC_{(0-24)}/Dose$ ((hr*ng/mL)/(mg/kg))	$T_{1/2}$ (hr)	Lambda-z (1/hr)
3	150	12.81	3.0	96.89	0.65	-	-
	500	20.97	0.5	132.99	0.27	4.37	0.1588
	1500	105.46	8.0	938.29	0.63	-	-
12	150	12.58	1.0	68.91	0.46	3.12	0.2221
	500	17.29	0.5	129.83	0.26	4.54	0.1527
	1500	74.15	1.0	325.06	0.22	3.28	0.2112

(-) Indicates not applicable or could not be calculated.

The following animals (times) were outliers at 3 months and not included in the primary analysis: Animal 6843 (3 hour), Animal 6846 (8 hour), Animal 6346 (8 hour), Animal 6349 (8 hour), Animal 6902 (8 hour).

The following animals (times) were outliers at 12 months and not included in the primary analysis: Animal 6300 (0.5 hour), Animal 6359 (0.5 hour).

**Summary of individual study findings:**

Adequacy of the carcinogenicity study and appropriateness of the test model: The dose selection was per the ECAC recommendations; high dose being selected based on the MFD. The test species is appropriate and acceptable. Overall, the study conduct was adequate and acceptable.

Evaluation of tumor findings: There were no significant tumor findings in males or females.

**Carcinogenicity Summary:** In a 104-week oral (gavage) carcinogenicity study in CD1 mice, animals were treated with JNJ-27018966-AAA at 0, 150, 500 and 1500 mg/kg/day (10 mL/kg). The doses were selected per the ECAC recommendations (ECAC meeting minutes dated March 16, 2011, Attachment-1); high dose being selected based on the MFD. There were no significant treatment-related effects on mortality in either sex. There were no significant treatment-related non-neoplastic findings in males or females. There were no significant tumor findings in either sex. Overall, this study appears to be negative for tumor findings in both sexes. The study conduct was considered adequate and acceptable.

**Carcinogenicity conclusions:**

1. The study was adequate and acceptable.
2. There were no significant drug-related neoplastic findings in male or female mice.

**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND  
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET  
Review of Rat Carcinogenicity Study Results**

P/T REVIEWER: Tamal K. Chakraborti, Ph.D.

DATE: July 29, 2014

IND: 79,214

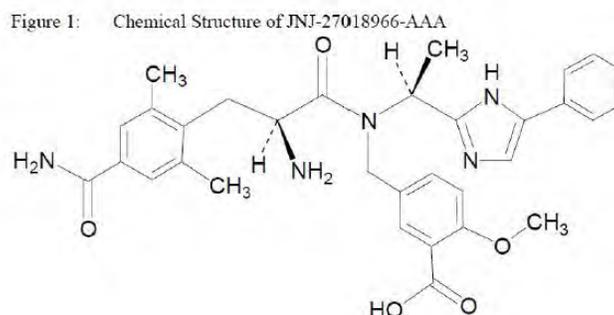
DRUG CODE#: JNJ-27018966/JNJ-27018966-AAA

CAS#: 864821-90-9

DIVISION: DGIEP

DRUG NAME: Eluxadoline

CHEMICAL STRUCTURE:



SPONSOR: Furiex Pharmaceuticals, Inc., Morrisville, NC

LABORATORY: (b) (4)

CARCINOGENICITY STUDY REPORT DATE: January 23, 2014

THERAPEUTIC CATEGORY: For the treatment of patients with IBS-d

PHARMACOLOGICAL CLASSIFICATION: Mu-opioid receptor agonist/delta-opioid receptor antagonist

MUTAGENIC/GENOTOXIC: JNJ-27018966 was negative in the Ames test, chromosomal aberration tests with mouse or human lymphocytes, and in an *in vivo* rat bone marrow micronucleus test.

**RAT CARCINOGENICITY STUDY:**

STUDY DURATION (weeks): 104

STUDY STARTING DATE: January 27, 2011

STUDY ENDING DATE: January 24, 2014

RAT STRAIN: CD<sup>®</sup> [CrI:CD<sup>®</sup>(SD)] rats

ROUTE: Oral (Gavage)

DOSING COMMENTS: Doses were selected based on the ECAC recommendations (ECAC meeting minutes dated March 16, 2011). The Committee recommended doses of 150, 500, and 1500 mg/kg/day; high dose being selected based on the MFD. The doses were administered at a dose volume of 10 mL/kg per the ECAC recommendation.

**NUMBER OF RATS:**

- Control-1 (C1): 65/sex
- Low Dose (LD): 65/sex
- Middle Dose (MD): 65/sex
- High Dose (HD): 65/sex

**RAT DOSE LEVELS:**

- Low Dose: 150 mg/kg/day
- Middle Dose: 500 mg/kg/day
- High Dose: 1500 mg/kg/day

BASIS FOR DOSES SELECTED: The high dose was selected based on the MFD.

PRIOR FDA DOSE CONCURRENCE: Yes.

RAT CARCINOGENICITY: Negative

RAT TUMOR FINDINGS: There were no significant treatment-related tumor findings in males or females.

RAT STUDY COMMENTS: The dose selection including the dose volume was per the ECAC recommendations and the high dose was selected based on the MFD. The dose volume was 10 mL/kg as recommended by the ECAC. There were no significant treatment-related non-neoplastic or neoplastic findings in either sex. The study conduct was considered adequate and acceptable.

**CARCINOGENICITY:****Study title:** 104-Week Oral Carcinogenicity Study in Rats**Key study findings:**

- In a 104-week oral (gavage) carcinogenicity study in SD rats, animals were treated with JNJ-27018966-AAA at 0, 150, 500 and 1500 mg/kg/day.
- There were no significant treatment-related effects on mortality in either sex.
- There were no significant treatment-related non-neoplastic findings in males or females.
- There were no significant treatment-related neoplastic findings in males or females.
- The study conduct was considered adequate and acceptable.

**Study number:** 1808-008**Volume #, and page #:** EDR Section dated 4.2.3.4.1.**Conducting laboratory and location:** (b) (4)**Date of study initiation:** May 4, 2011**GLP compliance:** A statement of compliance was included.**QA report:** yes ( X ) no ( )**Drug, lot #, and % purity:** JNJ-27018966-AAA, Batch Nos. ZR497138PFA111 (98.2%), batch ZR497138PFA141 (99.7%), ZR497138PFA151 (100.3%), and batch ZR497138PFA171 (100.2%) of JNJ-27018966-AAA**CAC concurrence:** Yes**Study Type:** 2-year bioassay**Species/strain:** CD® [CrI:CD®(SD)] rats**Number/sex/group; age at start of study; body weight:** 65/sex/group; approximately 6 weeks old; Males: 141-279 g, Females: 162-244 g**Animal housing:** Animals were individually housed in polyboxes in an environmentally controlled room. Fluorescent lighting was provided for approximately 12 hours per day. Temperature and humidity were continuously monitored, recorded, and maintained within the protocol designated ranges of 68 to 79°F and 30 to 70%, respectively.

**Formulation/vehicle:** 0.5% hydroxypropyl methylcellulose (HPMC, high viscosity, hypromellose 2910) in purified water, USP.

**Drug stability/homogeneity:** Dosing formulations were tested for homogeneity and concentration. The following table (from page 14 of the report) shows dosing formulation analysis sample collection schedule.

Dosing Formulation Analysis Sample Collection <sup>a</sup>							
Sample Type	Concentration Sampled (mg/mL)	Stratum	Number of Samples per Concentration			Sample Volume (mL)	Intervals (Week)
			Collected	Analyzed	Backup		
Homogeneity Analyses <sup>b</sup>	15 and 150	Top	3	1	2	1.0	1 and 8
		Middle	3	1	2	1.0	
		Bottom	3	1	2	1.0	
Concentration Analyses <sup>b</sup>	0, 15, 50, and 150	Middle	3	1	2	1.0	1, 2, 3, 4, 8, 12, 14, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 53, 56, 60, 64, 68, 72, 76, 80, 84, 88, 92, and 96
<sup>a</sup> Prior to study initiation, a test batch of JNJ-27018966-AAA was prepared at nominal concentrations of 37.6 and 150 mg/mL. Specific details pertaining to the sampling, analysis results, and disposition of the test batch are not reported but are maintained in the study data. <sup>b</sup> The samples, including backup samples, were stored refrigerated at 2 to 8°C pending analyses or final disposition.							

### **Methods:**

Doses: 0, 150, 500, 1500 mg/kg/day

Dose volume: 10 mL/kg

Basis of dose selection: The doses of 0, 150, 500, and 1500 mg/kg/day for both sexes were selected per the ECAC recommendations including dose volume of 10 mL/kg. The high dose was selected based on the MFD.

Restriction paradigm for dietary restriction studies: N/A

Route of administration: Oral gavage

Frequency of drug administration: Once daily

Dual controls employed: No

Interim sacrifices: None

Study Design: The study design is shown in the table below (from page 16 of the study report).

<b>Group Assignments</b>			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
<b>Main Study Groups</b>			
1	0 <sup>a</sup>	65	65
2	150	65	65
3	500	65	65
4	1500	65	65
<b>Toxicokinetic Groups</b>			
5	0 <sup>a</sup>	12	12
6	150	18	18
7	500	18	18
8	1500	18	18
<b>Sentinel Animals<sup>b</sup></b>			
98	-	35	35

<sup>a</sup> Administered the vehicle formulation only.  
<sup>b</sup> Sentinel animals were not administered the vehicle or JNJ-27018966-AAA formulations.

Satellite group for toxicokinetics: Yes (shown in the above table)

Deviations from original study protocol: Protocol deviations did not adversely affect either the quality or integrity of the study or the interpretation of the results.

Statistical methods:

Survival Data: Survival data from the rat study were analyzed by the Sponsor using the same statistical methodologies that were used to analyze the survival data from the mouse study.

Tumor Data: Tumor data from the rat study were also analyzed by the Sponsor using the same statistical methodologies that were used to analyze the tumor data from the mouse study. The FDA statistical reviewer independently performed survival and tumor data analyses for the rat study. For the rat data, the FDA reviewer used similar methodologies that were used to analyze the data from the mouse study.

**Observations and times:**

Mortality: Mortality was checked twice daily from Weeks 1 through 52 and three times daily starting in Week 53 through study termination.

Clinical Signs: Clinical signs were observed once weekly.

Body weights: Body weights were recorded the day following receipt and prior to randomization (Day -2). Body weights for all surviving main study and TK animals were recorded once weekly for Weeks 1 through 14, bi-weekly from Week 15 through Week 28, and once every 4 weeks thereafter.

Food consumption: Food consumption was recorded for surviving main study animals once weekly during Weeks 1 through 14, bi-weekly from Week 15 through Week 28, and once every 4 weeks thereafter. Food consumption was not measured and recorded for TK animals.

Ophthalmoscopy: Ophthalmoscopy was conducted at pretest and prior to scheduled necropsy.

Clinical Pathology: Clinical pathology evaluations were conducted on all main study animals euthanized *in extremis* and on all surviving main study animals at the scheduled terminal necropsies.

Gross pathology: Gross pathology was conducted at scheduled necropsy.

Histopathology: The following (from page 1045 of the report) tissues were collected for histopathological examination from all main study animals.

The following list constitutes the full complement of organs and tissues:

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- 
- |  |  |
|--|--|
| <ul style="list-style-type: none"> <li>- Adrenal (2)</li> <li>- Aorta</li> <li>- Bone with marrow [femur]</li> <li>- Bone with marrow [sternum]</li> <li>- Bone marrow smear [2 collected]<sup>a</sup></li> <li>- Brain [cerebrum, midbrain, cerebellum, medulla/pons]</li> <li>- Clitoral gland (2)</li> <li>- Epididymis (2)</li> <li>- Eye including optic nerve (2)</li> <li>- GALT [Gut Associated Lymphoid Tissue]</li> <li>- Gastrointestinal tract:             <ul style="list-style-type: none"> <li>esophagus</li> <li>stomach [glandular and nonglandular]</li> <li>duodenum</li> <li>jejunum</li> <li>ileum</li> <li>cecum</li> <li>colon</li> <li>rectum</li> </ul> </li> <li>- Gonads:             <ul style="list-style-type: none"> <li>ovary (2) with oviduct (2)</li> <li>testis (2)</li> </ul> </li> <li>- Gross lesions</li> <li>- Heart</li> <li>- Joint, tibiofemoral</li> <li>- Kidney (2)</li> <li>- Lacrimal gland, exorbital (2)</li> <li>- Larynx</li> <li>- Liver [3 sections collected; 2 examined]</li> </ul> | <ul style="list-style-type: none"> <li>- Lung with bronchi [collected whole; 2 sections examined]</li> <li>- Lymph nodes: mandibular [2 collected; 1 examined], mesenteric, and regional where applicable</li> <li>- Mammary gland [only process females]</li> <li>- Nasal tissue [Levels A, B, C, and D]</li> <li>- Pancreas</li> <li>- Pituitary</li> <li>- Preputial gland (2)</li> <li>- Prostate and seminal vesicle (2)</li> <li>- Salivary gland, mandibular/sublingual [2 collected; 1 examined]</li> <li>- Salivary gland, parotid [2 collected; 1 examined]</li> <li>- Sciatic nerve</li> <li>- Skeletal muscle, biceps femoris</li> <li>- Skin</li> <li>- Spinal cord [cervical, thoracic, and lumbar]</li> <li>- Spleen</li> <li>- Thymus</li> <li>- Thyroid/parathyroid (2)</li> <li>- Tissue masses</li> <li>- Tongue</li> <li>- Trachea</li> <li>- Ureter (2)</li> <li>- Urinary bladder</li> <li>- Uterus [both horns]/Cervix</li> <li>- Vagina</li> <li>- Zymbal's gland (2)</li> </ul> |
|--|--|
- 

<sup>a</sup>Bone marrow smears were collected at necropsies and held.  
(2) Paired organ

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Toxicokinetics: Blood samples were collected from TK animals for determination of the plasma concentrations of JNJ-27018966-AAA. Samples were collected from three TK control animals per sex at 1 hour post-dose on Day 91 and Day 364 and from cohorts of three JNJ-27018966-AAA-treated TK animals per sex at 0.5, 1, 3, 8, 12, and 24 hours post-dose.

**Results:**

Clinical signs: There were no significant treatment-related clinical signs.

Mortality: There were no significant test article-related differences in mortality in either males or females. The survival rates at scheduled sacrifice were 48%, 55%, 49% and 51% in males and 43%, 42%, 42% and 37% in females at 0, 150, 500, or 1500 mg/kg/day, respectively. The following table (from page 1051-1054 of the report) shows the survival data.

(b) (4)  
 Study Number 1808-008  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Rats

**Summary of Survival Estimates - MALE<sup>†</sup>**

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>0 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	0	0	65.0	1.0000	0.0000
	27-39	2	0	65.0	1.0000	0.0000
	40-52	4	0	63.0	0.9692	0.0214
	53-65	3	0	59.0	0.9077	0.0359
	66-78	10	0	56.0	0.8615	0.0428
	79-91	15	0	46.0	0.7077	0.0564
	92-104	11	20 <sup>‡</sup>	21.0	0.4769	0.0620
	105	0	0	0	0	0
<u>150 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	0	0	65.0	1.0000	0.0000
	27-39	2	0	65.0	1.0000	0.0000
	40-52	1	0	63.0	0.9692	0.0214
	53-65	7	0	62.0	0.9538	0.0260
	66-78	14	0	55.0	0.8462	0.0448
	79-91	5	0	41.0	0.6308	0.0599
	92-104	7	29 <sup>‡</sup>	21.5	0.5538	0.0617
	105	0	0	0	0	0
<u>500 mg/kg/day</u>						
	1-13	1	0	65.0	1.0000	0.0000
	14-26	1	0	64.0	0.9846	0.0153
	27-39	1	0	63.0	0.9692	0.0214
	40-52	4	0	62.0	0.9538	0.0260
	53-65	2	0	58.0	0.8923	0.0384
	66-78	9	0	56.0	0.8615	0.0428
	79-91	15	0	47.0	0.7231	0.0555
	92-104	5	27 <sup>‡</sup>	18.5	0.4923	0.0620
	105	0	0	0	0	0
<u>1500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	1	0	65.0	1.0000	0.0000
	27-39	4	0	64.0	0.9846	0.0153
	40-52	2	0	60.0	0.9231	0.0331

<sup>‡</sup> Necropsy count

<sup>†</sup> No statistical significance observed

(b) (4) Study Number 1808-008  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Rats

**Summary of Survival Estimates - MALE<sup>1</sup>**

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
1500 mg/kg/day	53-65	6	0	58.0	0.8923	0.0384
	66-78	9	0	52.0	0.8000	0.0496
	79-91	10	0	43.0	0.6615	0.0587
	92-104	8	25 <sup>2</sup>	20.5	0.5077	0.0620
	105	0	0	0	0	0

<sup>2</sup> Necropsy count

<sup>1</sup> No statistical significance observed

(b) (4) Study Number 1808-008  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Rats

**Summary of Survival Estimates - FEMALE\***

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>0 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	0	0	65.0	1.0000	0.0000
	27-39	0	0	65.0	1.0000	0.0000
	40-52	1	0	65.0	1.0000	0.0000
	53-65	5	0	64.0	0.9846	0.0153
	66-78	13	0	59.0	0.9077	0.0359
	79-91	18	0	46.0	0.7077	0.0564
	92-104	7	21 <sup>&amp;</sup>	17.5	0.4308	0.0614
	105	0	0	0	0	0
<u>150 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	1	0	65.0	1.0000	0.0000
	27-39	3	0	64.0	0.9846	0.0153
	40-52	1	0	61.0	0.9385	0.0298
	53-65	11	0	60.0	0.9231	0.0331
	66-78	13	0	49.0	0.7538	0.0534
	79-91	9	0	36.0	0.5538	0.0617
	92-104	7	20 <sup>&amp;</sup>	17.0	0.4154	0.0611
	105	0	0	0	0	0
<u>500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	0	0	65.0	1.0000	0.0000
	27-39	3	0	65.0	1.0000	0.0000
	40-52	2	0	62.0	0.9538	0.0260
	53-65	9	0	60.0	0.9231	0.0331
	66-78	10	0	51.0	0.7846	0.0510
	79-91	14	0	41.0	0.6308	0.0599
	92-104	12	15 <sup>&amp;</sup>	19.5	0.4154	0.0611
	105	0	0	0	0	0
<u>1500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	1	0	65.0	1.0000	0.0000
	27-39	0	0	64.0	0.9846	0.0153
	40-52	4	0	64.0	0.9846	0.0153

<sup>&</sup> Necropsy count

<sup>\*</sup> No statistical significance observed

(b) (4) Study Number 1808-008  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Rats

**Summary of Survival Estimates - FEMALE\***

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>1500 mg/kg/day</u>						
	53-65	7	0	60.0	0.9231	0.0331
	66-78	15	0	53.0	0.8154	0.0481
	79-91	14	0	38.0	0.5846	0.0611
	92-104	9	15 <sup>&amp;</sup>	16.5	0.3692	0.0599
	105	0	0	0	0	0

<sup>&</sup> Necropsy count

\* No statistical significance observed

(b) (4) Study Number 1808-009  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Mice

**Summary of Survival Estimates - FEMALE\***

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>0 mg/kg/day</u>						
	1-13	1	0	65.0	1.0000	0.0000
	14-26	0	0	64.0	0.9846	0.0153
	27-39	3	0	64.0	0.9846	0.0153
	40-52	8	0	61.0	0.9385	0.0298
	53-65	6	0	53.0	0.8154	0.0481
	66-78	9	0	47.0	0.7231	0.0555
	79-91	12	0	38.0	0.5846	0.0611
	92-104	7	19 <sup>‡</sup>	16.5	0.4000	0.0608
	105	0	0	0	0	0
<u>150 mg/kg/day</u>						
	1-13	1	0	65.0	1.0000	0.0000
	14-26	1	0	64.0	0.9846	0.0153
	27-39	3	0	63.0	0.9692	0.0214
	40-52	3	0	60.0	0.9231	0.0331
	53-65	3	0	57.0	0.8769	0.0407
	66-78	12	0	54.0	0.8308	0.0465
	79-91	13	0	42.0	0.6462	0.0593
	92-104	6	23 <sup>‡</sup>	17.5	0.4462	0.0617
	105	0	0	0	0	0
<u>500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	0	0	65.0	1.0000	0.0000
	27-39	3	0	65.0	1.0000	0.0000
	40-52	1	0	62.0	0.9538	0.0260
	53-65	6	0	61.0	0.9385	0.0298
	66-78	10	0	55.0	0.8462	0.0448
	79-91	13	0	45.0	0.6923	0.0572
	92-104	8	24 <sup>‡</sup>	20.0	0.4923	0.0620
	105	0	0	0	0	0
<u>1500 mg/kg/day</u>						
	1-13	0	0	65.0	1.0000	0.0000
	14-26	1	0	65.0	1.0000	0.0000
	27-39	5	0	64.0	0.9846	0.0153
	40-52	2	0	59.0	0.9077	0.0359

<sup>‡</sup> Necropsy count

<sup>\*</sup> No statistical significance observed

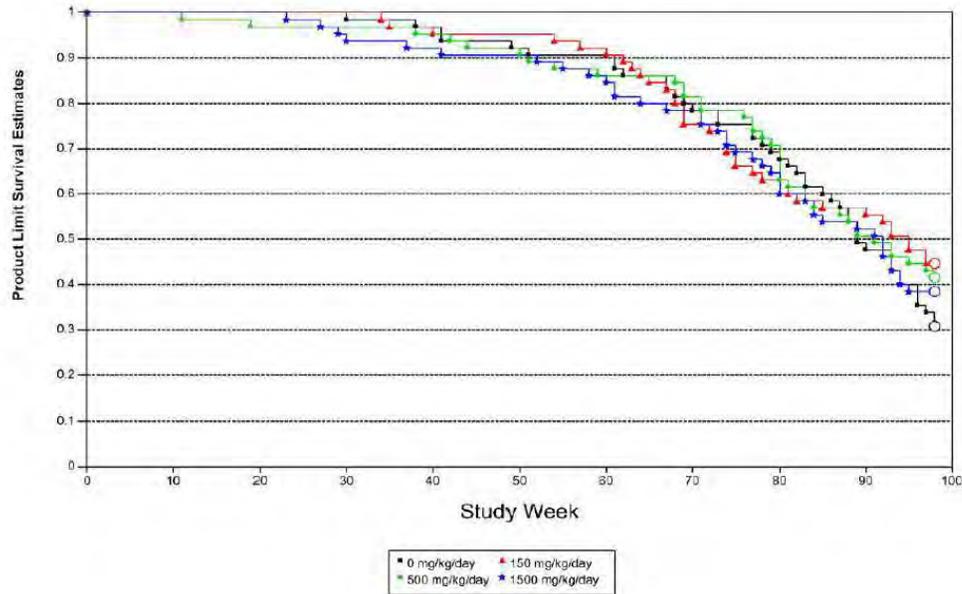
(b) (4)  
 Study Number 1808-009  
 JNJ-27018966-AAA: 104-Week Oral Carcinogenicity Study in Mice

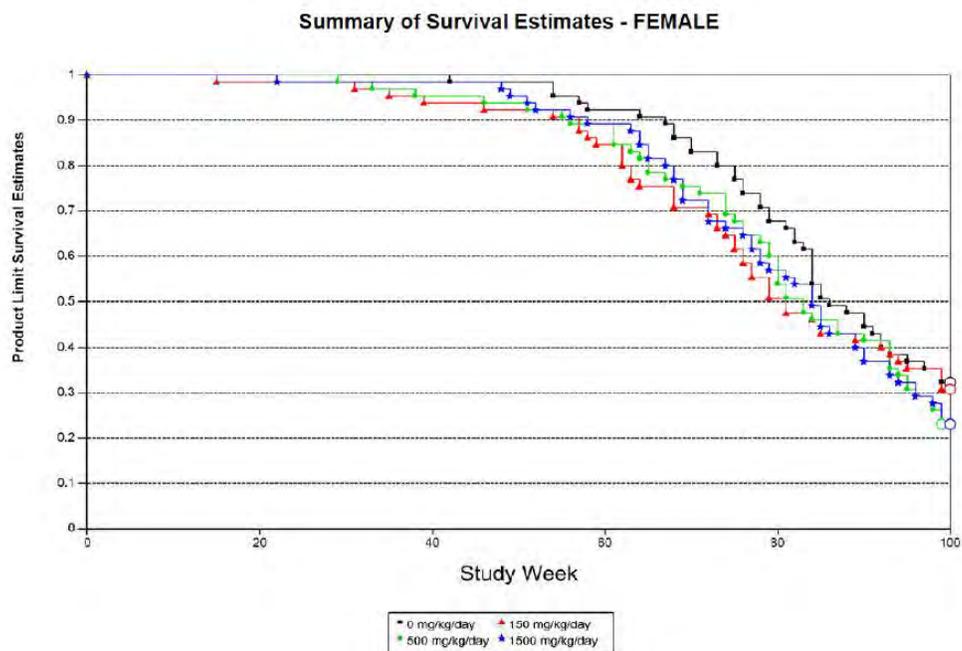
**Summary of Survival Estimates - FEMALE**

Dose Level	Study Interval (Week)	Deaths	Censored	Effective Sample Size	Cumulative Survival	Survival Standard Error
<u>1500 mg/kg/day</u>	53-65	6	0	57.0	0.8769	0.0407
	66-78	10	0	51.0	0.7846	0.0510
	79-91	9	0	41.0	0.6308	0.0599
	92-104	6	26 <sup>A</sup>	19.0	0.4923	0.0620
	105	0	0	0	0	0

Survival curves are shown below (from page 1048 and 1049 of the report).

**Summary of Survival Estimates - MALE**





**Body weights:** The mean initial (Week 1) and final (Week 96) body weights of control males were 309.7 and 872.0 g, respectively. The mean initial (Week 1) and final (Week 96) body weights of control females were 219.7 and 549.8 g, respectively. In males, final body weights were 103%, 104%, and 96% of control at 150, 500 and 1500 mg/kg/day, respectively. In females, final body weights were 94%, 105% and 104% of control at 150, 500 and 1500 mg/kg/day, respectively. There were no significant treatment-related effects on body weights in either sex. The following table (from page 24 of the report) shows the absolute bodyweights (g) for males and females at various time points.

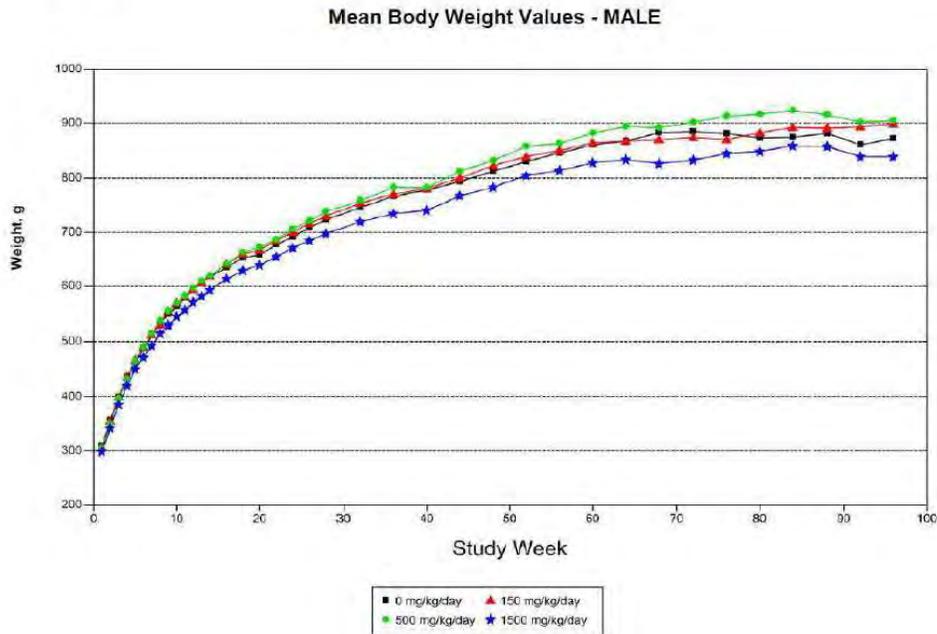
<b>Summary of Group Mean Body Weight (g)</b>								
Dose Level (mg/kg/day)	<b>Main Study Males</b>							
	Week 26	(%)	Week 52	(%)	Week 80	(%)	Week 96	(%)
0	708.9	NA	830.1	NA	872.9	NA	872.0	NA
150	716.7	(+1.1)	839.2	(+1.1)	882.8	(+1.1)	898.6	(+3.1)
500	721.6	(+1.8)	857.9	(+3.4)	916.5	(+5.0)	904.9	(+3.8)
1500	685.0	(-3.4)	804.8	(-3.1)	848.4	(-2.8)	838.9	(-3.8)

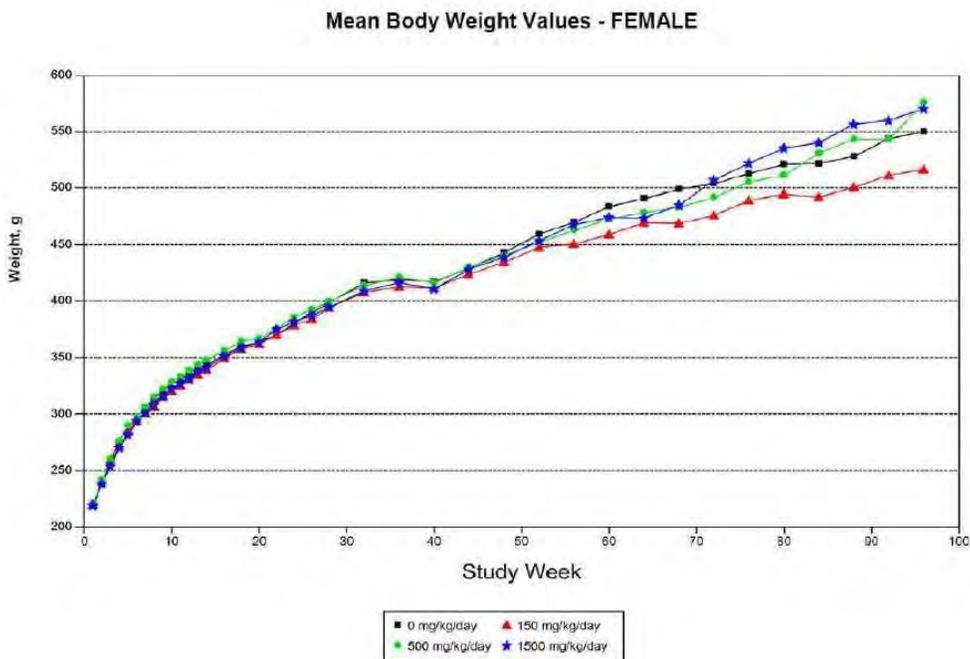
(%) - Percent difference from control  
 NA - Not applicable

Summary of Group Mean Body Weight (g)								
Dose Level (mg/kg/day)	Main Study Females							
	Week 26	(%)	Week 52	(%)	Week 80	(%)	Week 96	(%)
0	389.5	NA	459.5	NA	520.6	NA	549.8	NA
150	383.9	(-1.4)	447.5	(-2.6)	494.6	(-5.0)	516.0	(-6.2)
500	392.7	(+0.8)	452.2	(-1.6)	510.8	(-1.9)	575.7	(+4.7)
1500	387.3	(-0.6)	453.6	(-1.3)	534.9	(+2.8)	570.2	(+3.7)

(%) - Percent difference from control  
 NA - Not applicable

The following figures (from page 83 and 84 of the study report) show the growth curves in males and females.





**Food consumption:** The mean initial (Week 1) and final (Week 96) food consumption in control males was 26.23 and 26.66 g/animal/day, respectively. The mean initial (Week 1) and final (Week 96) food consumption in control females was 19.19 and 20.76 g/animal/day, respectively. There was no significant effect of treatment on food consumption. The following table (from page 25 of the report) shows the food consumption data.

Average Food Consumption; g/animal/day (Week 1 through Week 96)				
Dose Level (mg/kg/day)	Male		Female	
	Mean	(%) Difference from Control	Mean	(%) Difference from Control
0	28.58	NA	20.36	NA
150	29.02	(+1.5)	19.76	(-3.0)
500	29.11	(+1.9)	19.93	(-2.1)
1500	28.39	(-0.7)	20.07	(-1.4)

NA – Not applicable

Ophthalmoscopy: There were no significant treatment-related effects.

Hematology: There were no significant treatment-related effects.

Gross pathology: There were no significant treatment-related effects.

Analysis of Dosing Formulations: The average concentration of the homogeneity samples collected from the formulation prepared for Weeks 1 and 8 were within  $\pm 15\%$  of the nominal concentration; precision was  $\leq 10\%$  relative standard deviation (RSD). The average concentration of samples collected from the formulations prepared for Weeks 1 through 104 and 105 were within  $\pm 15\%$  of the nominal concentration; precision was  $\leq 10\%$  RSD.

Histopathology:

Non-neoplastic: There were no significant treatment-related non-neoplastic findings in either sex.

Neoplastic: There were no significant treatment-related neoplastic findings in males or females.

Toxicokinetics (TK): The  $C_{max}$  of JNJ-27018966 increased more than dose-proportionally from 500 to 1500 mg/kg at 3 months but less than dose-proportionally from 150 to 1500 mg/kg at 12 months. The  $AUC_{0-24h}$  of JNJ-27018966 increased less than dose proportionally from 150 to 500 mg/kg/day after 3 months but more than proportionally from 500 to 1500 mg/kg/day. However, when a direct comparison of  $AUC_{0-24h}$  following 150 and 1500 mg/kg/day was conducted excluding  $AUC_{0-24h}$  at 500 mg/kg/day,  $AUC_{0-24h}$  increased dose proportionally between 150 and 1500 mg/kg/day after 3 months. However,  $AUC_{0-24h}$  increased less than dose proportionally from 150 to 1500 mg/kg/day after 12 months. The  $T_{max}$  varied from 0.5 hour to 1 hour across all dose levels following 3 and 12 months of dosing. Terminal half-lives ranged from 7 to 13 hours. Sex-related difference in exposure to JNJ-27018966 was observed. Males showed higher exposures than females at all dose levels. There was an apparent accumulation of JNJ-27018966 at all doses from 3 months to 12 months. The following table (from page 968 of the report) shows the TK parameters.

**Table 1.1 Toxicokinetic Parameters for JNJ-27018966 in Rats After Daily Oral Administration of 150, 500 and 1500 mg/kg JNJ-27018966-AAA at 3 and 12 Months (Excluding Outliers)**

Month	Dose mg/kg/d	$C_{max}$ (ng/mL)	$T_{max}$ (hr)	$AUC(0-24)$ (hr*ng/mL)	$AUC(0-24)/Dose$ ((hr*ng/mL)/(mg/kg))	$AUC(0-1)$ (hr*ng/mL)	$T_{1/2}$ (hr)	Lamictal-z (1/hr)
3	150	56.35	0.5	83.61	0.56	83.61	-	-
	500	52.07	0.5	160.20	0.32	160.20	-	-
	1500	290.27	0.5	820.00	0.55	820.00	-	-
12	150	150.54	0.5	360.84	2.41	360.84	-	-
	500	102.88	0.5	620.44	1.24	620.44	-	-
	1500	209.34	1.0	872.50	0.58	872.50	-	-

(-) Indicates not applicable or could not be calculated.

The following animals (times) were outliers at 12 months and not included in the primary analysis: Animal 1619 (0.5 hour, 500 mg/kg/day) and Animal 1639 (1 hour, 1500 mg/kg/day).

**Summary of individual study findings:**

Adequacy of the carcinogenicity study and appropriateness of the test model: The doses including the dose volume (10 mL/kg) were selected per the ECAC recommendations; high dose being selected based on the MFD. The test species is appropriate and acceptable. Overall, the study conduct was adequate and acceptable.

Evaluation of tumor findings: There were no significant treatment-related neoplastic findings in males or females.

**Carcinogenicity Summary:** In a 104-week oral (gavage) carcinogenicity study in SD rats, animals were treated with JNJ-27018966-AAA at 0, 150, 500 and 1500 mg/kg/day (10 mL/kg). The doses were selected per the ECAC recommendations (ECAC meeting minutes dated March 16, 2011, Attachment-1); high dose being selected based on the MFD. There were no significant treatment-related effects on mortality in either sex. There were no significant treatment-related non-neoplastic or neoplastic findings in males or females.

**Carcinogenicity conclusions:**

1. The study was adequate and acceptable.
2. There were no significant drug-related neoplastic findings in male or female rats.

**Integrated Summary and Safety Evaluation**

JNJ-27018966 is a locally active, mixed mu-opioid receptor agonist and delta-opioid receptor antagonist. This drug is being developed for the treatment of IBS-d. The sponsor submitted the reports of 2-year oral (gavage) carcinogenicity study with JNJ-27018966 in CD1 mice (Study No. 1808-009) and SD rats (Study No. 1808-008).

In a 104-week oral (gavage) carcinogenicity study in CD1 mice, animals were treated with JNJ-27018966-AAA at 0, 150, 500 and 1500 mg/kg/day. The doses were selected per the ECAC recommendations (ECAC meeting minutes dated March 16, 2011, Attachment-1); high dose being selected based on the maximum feasible dose (MFD). There were no significant treatment-related effects on mortality in either sex. There were no significant treatment-related non-neoplastic findings in males or females. There were no significant tumor findings in either sex. Overall, this study appears to be negative for tumor findings in both sexes. The study conduct was considered adequate and acceptable.

In a 104-week oral (gavage) carcinogenicity study in SD rats, animals were treated with JNJ-27018966-AAA at 0, 150, 500 and 1500 mg/kg/day. The doses were selected per the ECAC recommendations (ECAC meeting minutes dated March 16, 2011, Attachment-1); high dose being selected based on the MFD. There were no significant

treatment-related effects on mortality in either sex. There were no significant treatment-related non-neoplastic or neoplastic findings in males or females. The study conduct was considered adequate and acceptable.

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

#### Study title: Fertility and Early Embryonic Development to Implantation in Rats

Study no.:	1808-003
Study report location:	EDR 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 3, 2010
Date of study completion:	December 22, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	JNJ-27018966-AAA, ZR497138PFA011, 95.5%

#### Key Study Findings:

- In an oral fertility and early embryonic development study in rats, JNJ-27018966-AAA was administered at 100, 300 and 1000 mg/kg/day.
- Estrous cyclicity (mean cycle length and number of cycles) was unaffected by the treatment.
- There were no significant treatment related effects on mating, fertility, and fecundity indices.
- The pregnancy index and uterine parameters (mean number of corpora lutea, number of implantation sites, number of viable embryos, number of resorptions, and pre- and post-implantation loss) in all groups were unaffected by the treatment.
- JNJ-27018966-AAA did not cause any significant adverse effects on fertility and early embryonic development in this study.

**Methods:**

Doses: 100, 300 and 1000 mg/kg/day  
 Frequency of dosing: Daily. Treatment began 28 days prior to pairing for the males, and 14 days prior to pairing for the females. Dosing of the males continued through the mating and postmating periods to euthanasia, while dosing of the females continued through the mating period to gestation day 7 (GD7).  
 Dose volume: 10 mL/kg  
 Route of administration: Oral (gavage)  
 Formulation/Vehicle: 0.5% Hydroxypropyl methylcellulose (HPMC, high viscosity) in purified water, USP.  
 Species/Strain: SD rats  
 Number/Sex/Group: 25/sex/dose  
 Satellite groups: None  
 Study design: Shown below  
 Deviation from study protocol: Protocol deviations did not affect the quality or integrity of the study.

The following table (from page 14 of the report) shows the study design.

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1	0	25	25
2	100	25	25
3	300	25	25
4	1000	25	25

Basis of dose selection: Dose levels were selected based on the results of the 13-week toxicology study (TOX8677) in rats. The NOAEL was 1000 mg/kg/day. The low and mid dose levels were selected to examine potential dose-response effects. It appears that higher doses should have been tested as no significant toxicity was observed at 1000 mg/kg/day in the above 13-week study in rats.

**Observations:**

Mortality: Mortality was observed twice daily.

Clinical Signs: Clinical signs were examined daily.

Body Weight: Body weights were recorded on GD 0, 4, 7, 10, and 13.

**Food Consumption:** Food consumption for animals was recorded weekly prior to pairing for mating.

**Toxicokinetics:** Not conducted

**Dosing Solution Analysis:** Dosing formulations were analyzed for homogeneity and concentration. The following table (from page 13 of the report) shows the dosing formulation analysis sample collection schedule.

<b>Dosing Formulation Analysis Sample Collection</b>							
Sample Type	Dose Level Sampled (mg/kg/day)	Stratum	Number of Samples per Concentration			Sample Volume (mL)	Intervals (Week)
			Collected	Analyzed	Backup		
Homogeneity Analyses <sup>a</sup>	100 and 1000	Top	3	1	2	1	1
		Middle	3	1	2	1	
		Bottom	3	1	2	1	
Concentration Analyses <sup>a</sup>	0, 100, 300, and 1000	Middle	3	1	2	1	1, 2 <sup>b</sup> , 4, 8
<sup>a</sup> The samples, including backup samples, were stored refrigerated at 2 to 8°C pending shipment.							
<sup>b</sup> Samples at 0 mg/kg/day were collected in error and discarded without analysis.							

**Necropsy:** Necropsy examinations were performed on all animals at the scheduled necropsy. The abdominal, thoracic, and cranial cavities were examined for abnormalities, and the organs were removed and examined.

**Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.):** On GD 13, each female was euthanized and the uterus and ovaries were collected. The number of viable embryos, resorptions, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded. Uteri from females that appeared non-gravid were collected for detection of implantation sites.

## **Results:**

**Mortality:** All animals survived to terminal euthanasia.

**Clinical Signs:** There were no significant treatment related clinical signs.

**Body Weight:** The mean initial (GD0) and final (GD13) gestational body weights of control animals were 242 and 313 g, respectively. The final body weights of treated animals were 97%, 98% and 99% of control at 100, 300 and 1000 mg/kg/day,

respectively. There were no significant treatment related effects on gestational body weights.

Food Consumption: The mean initial (GD0-4) and final (GD10-13) gestational food consumption of control animals were 26.1 and 29.6 g/animal/day, respectively. There were no significant treatment related effects on gestational food consumption.

Dosing Solution Analysis: The formulations were homogenous. For the low dose, percent expected concentration values were 100%, 101%, and 99%, respectively, for the top, middle and bottom samples. For the high dose, all values were 92%. Percent expected concentration values ranged from 95% to 103% for samples collected throughout the course of the study.

Necropsy/Microscopy: There were no significant test article related macroscopic or microscopic findings.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.): Mean estrous cycle length and number of cycles in the treated groups were comparable to controls. The male and female mating, fertility, and fecundity indices in the treated groups ranged from 92% to 100% and were comparable to controls. Copulatory interval for the treated groups ranged from 2.9 days to 3.3 days and was comparable to controls (3.7 days). Pregnancy index and uterine parameters (mean number of corpora lutea, number of implantation sites, number of viable embryos, number of resorptions, and pre- and post-implantation loss) in the treated groups were comparable to controls and were unaffected by the treatment. Sperm motility at 100 and 1000 mg/kg/day was comparable to controls and were unaffected by the treatment. The following tables (from page 85, 86 and 87 of the report) show the uterine data.

(b) (4) Study Number 1808-003  
JNJ-27018966-AAA: Study of Fertility and Early Embryonic Development to Implantation in Rats

Summary of Maternal and Developmental Observations at Uterine Examination				
Endpoint	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Females on Study	25	25	25	25
No. Not Pregnant	2	1	0	0
No. Pregnant	23	24	25	25
Pregnancy Index Percent	92.0	96.0	100.0	100.0
No. Died Pregnant	0	0	0	0
No. Abortions	0	0	0	0
No. Early Deliveries	0	0	0	0
No. Females with All Resorptions	0	0	0	0
No. Females with Viable Embryos Day 13 Gestation	22	22	23	22
No. Pregnant Females with No Confirmed Mating Date	1	2	2	2

(b) (4) Study Number 1808-003  
 JNJ-27018966-AAA: Study of Fertility and Early Embryonic Development to Implantation in Rats

**Summary of Maternal and Developmental Observations at Uterine Examination**

Endpoint		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Corpora Lutea No. per Animal	Mean	16.5	15.8	15.7	16.3
	SD	2.72	3.55	2.05	2.75
	N	22	22	23	22
Implantation Sites No. per Animal	Mean	15.0	13.6	14.7	14.6
	SD	2.82	3.20	1.50	2.67
	N	22	22	23	22
Preimplantation Loss % per animal	Mean	8.75	13.10	6.19	9.17
	SD	14.284	15.234	8.807	16.515
	N	22	22	23	22
Viable Embryos No. per Animal	Mean	14.1	12.9	14.0	13.6
	SD	2.78	3.69	1.52	2.94
	N	22	22	23	22
Postimplantation Loss % Implants per Animal	Mean	5.77	7.14	4.01	7.15
	SD	7.507	17.700	6.211	8.720
	N	22	22	23	22

N - Number of measures used to calculate mean  
 SD - Standard Deviation  
 No. - Number

(b) (4) Study Number 1808-003  
 JNJ-27018966-AAA: Study of Fertility and Early Embryonic Development to Implantation in Rats

		<b>Summary of Maternal and Developmental Observations at Uterine Examination</b>			
Endpoint		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Nonviable Embryos No. per Animal	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
	N	22	22	23	22
Litter Size No. per Animal	Mean	14.1	12.9	14.0	13.6
	SD	2.78	3.69	1.52	2.94
	N	22	22	23	22
Resorptions: Early + Late No. per Animal	Mean	0.9	0.7	0.6	1.0
	SD	1.23	1.20	0.94	1.31
	N	22	22	23	22

N - Number of measures used to calculate mean  
 SD - Standard Deviation  
 No. - Number

## 9.2 Embryonic Fetal Development

### Study title: Oral/Subcutaneous Embryofetal Development Study of JNJ-27018966-AAA in the Rat

Study no.: TOX8398  
 Study report location: EDR 4.2.3.5.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: July 31, 2007  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAA, batch No. 30205959, 98%

### Key Study Findings:

- In an oral/SC embryofetal development study in rats, animals were treated at 100, 300 or 1000/5 mg/kg/day PO/SC.

- There were no significant treatment related effects on uterine parameters e.g., the numbers of corpora lutea, implantations, early and late resorptions, live fetuses, pre- or post-implantation loss, or sex ratio.
- There were no significant treatment related fetal external or visceral changes.
- Some of the skeletal changes (incomplete ossifications and wavy ribs) did not appear to be treatment related in the absence of clear dose response.
- A slightly higher incidence of incompletely descended thymus was observed at 1000/5 mg/kg/day PO/SC. This was not dose related and was seen in control animals and was not considered treatment related.
- JNJ-27018966-AAA did not appear to cause significant adverse effect on embryofetal development at any of the tested doses in this study.

**Methods:** The study was conducted to examine the potential effects of JNJ 27018966-AAA on embryofetal development, when administered orally by gavage and orally in combination with subcutaneously to the pregnant females once daily during the period of organogenesis (Days GD6 to GD17). Three groups of twenty-four, timed-mated, female rats were dosed by oral gavage with an aqueous solution of JNJ- 27018966-AAA at 100, 300 or 1000 mg/kg/day. In addition each female at 1000 mg/kg/day received a further administration of a 5 mg/kg/day dose by the subcutaneous (SC) route. The combination of the oral and subcutaneous route was chosen to obtain a higher systemic exposure.

Doses: Oral: 100, 300 and 1000 mg/kg/day; SC: 5 mg/kg/day  
 Frequency of dosing: Daily (GD6 to GD17)  
 Dose volume: 10 mL/kg (Oral), SC (1 mL/kg)  
 Route of administration: Oral gavage + SC  
 Formulation/Vehicle: Oral: 0.5% w/v Methocel (hydroxypropyl methylcellulose) in water  
 SC: 10% w/v hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD)  
 Species/Strain: Sprague-Dawley (SD) rats  
 Number/Sex/Group: 23/group  
 Satellite groups: 5/group  
 Study design: Shown below  
 Deviation from study protocol: The Applicant stated that protocol deviations did not have any impact on the validity or integrity of the study. However, the Applicant did not submit the protocol in the study report.

The following table (from page 18 of the report) shows the study design.

Code	Group	Cage card	Female numbers		Dose level (mg/kg body weight/day)
			Main	TK Satellite (Toxicokinetics)	
V	Vehicle	Blue	1 – 24	TK 25 – TK 27	00
L	Low	Red	31 – 54	TK 55 – TK 60	100
M	Medium	Yellow	51 – 74	TK 85 – TK 90	300
H	High	Green	91 – 114	TK 115 – TK 120	1000/5

**Basis of dose selection:** The dose selection for the present study was based upon the results of a pilot oral embryofetal development study in rats (TOX8260). In this study pregnant SD rats were orally (gavage) administered from GD 6 through 17 with JNJ-2701866-AAA (as an aqueous suspension of 0.5% Methocel) at 200 or 1000 mg/kg/day. Two additional groups of six females received the same dose levels via oral gavage but the doses were given in combination with a daily subcutaneous dose of JNJ-28630368-AAA in hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) at 5 mg/kg/day. All females were sacrificed on GD 21 of pregnancy. At necropsy, dams were examined for macroscopic abnormalities. The uterus was examined for numbers of corpora lutea of pregnancy, implantations, early and late resorptions and live and dead fetuses. Fetuses in all groups were weighed and examined externally. There were no treatment related mortalities. Uterine parameters (numbers of corpora lutea, implantations, live fetuses and pre- and post-implantation losses) were unaffected by the treatment. Based upon the above findings, the high dose was selected at 1000/5 mg/kg/day PO/SC. The low and mid doses were set at 100 and 300 mg/kg/day PO, respectively.

### **Observations:**

**Mortality:** All animals were observed once a day for mortality.

**Clinical Signs:** All animals were observed once daily for clinical signs.

**Body Weight:** Body weights were recorded on Days 0, 4, 6, 10, 14, 18 and 21 of pregnancy.

**Food Consumption:** Food consumption was recorded on gestation days 0, 6, 10, 14, 18 and 21.

**Toxicokinetics:** On GD6 and GD16, blood samples were collected from all vehicle treated rats at 1, 2, 4, 7, and 24 hours post-dose. Samples were collected from the first three treated rats at 1, 4, and 24 hours postdose and the second three treated rats at 2 and 7 hours postdose.

Dosing Solution Analysis: For oral dosing, the concentration and stability of JNJ-27018966-AAA in the firstly prepared formulations were checked at 4 and 24 days after preparation. The homogeneity (top, middle and bottom samples) of the samples was determined. For SC dosing, the concentration and stability of JNJ-27018966-AAA in the formulations were checked on the day of preparation and 14 and 21 days after preparation.

Necropsy: On Day 21 of pregnancy, animals were sacrificed for necropsy.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): Animals were sacrificed on GD21 and the females were examined for macroscopic abnormalities, pregnancy status, the numbers of corpora lutea of pregnancy, implantations, early and late resorptions and live and dead fetuses.

Offspring (Malformations, Variations, etc.): The fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

## **Results**:

Mortality: There were no deaths or premature sacrifices.

Clinical Signs: Skin irritation was observed in 2 animals at 1000/5 mg/kg/day PO/SC. This was considered to be related to the subcutaneous dose administration.

Body Weight: The Applicant did not provide body weight data and provided summary of weight gain. Initially, there was a non-dose related reduction in body weight gain in all treated groups. However, body weight gain during the remainder of the study was comparable with that of the vehicle control group. The following table (from page 40 and 43 of the report) shows the body weight gain data.

Treatment unit: mg/kg		Vehicle 0	Low 100	Medium 300	High 1000/5
<b>ADULT DATA</b>					
Number of dosed females		24	24	24	24
Number of pregnant females/terminally sacrificed	(2)	23/24	24/24	23/24	23/24
Number of dead or sacrificed females/dosed females	(1)	0/24	0/24	0/24	0/24
Body weight gain (d0-d3)	(3)	21	20	21	16
Body weight gain (d4-d5)	(3)	8	9	8	8
Body weight gain (d6-d9)	(3)	22	18 *	16 ***	16 ***
Body weight gain (d10-d13)	(3)	24	23	27	25
Body weight gain (d14-d17)	(3)	46	48	43	48
Body weight gain (d18-d20)	(3)	44	51	45	49
Corrected mean maternal weight gain	(3)	43.7	37.8	31.6 *	36.8 *
Food consumption (d0-d5)	(3)	147	147	148	143
Food consumption (d6-d9)	(3)	105	98	91 ***	83 ***
Food consumption (d10-d13)	(3)	115	112	108 *	103 ***
Food consumption (d14-d17)	(3)	120	113	113	112 **
Food consumption (d18-d20)	(3)	88	90	85	93

All weights are in gram  
 Corrected mean maternal weight gain = BW last day - (BW day 6 + uterus weight)  
 Significances computed by:  
 (1) Fisher Exact Test: Right Tail probability (Mid P Value) (2) Fisher Exact Test: Left Tail probability (Mid P Value) (3) Mann-Whitney U Test: Two Tailed

Reference group: Vehicle  
 \*: p<0.05 \*\*: p<0.01 \*\*\*: p<0.001

Treatment unit: mg/kg		Vehicle 0	Low 100	Medium 300	High 1000/5
<b>Body Weight Gain</b>	<b>Day 0-3</b>	21 (1)	20 (1)	21 (2)	16 (3)
		-	0.5414	0.8428	0.0740
<b>Body Weight Gain</b>	<b>Day 4-5</b>	8 (1)	9 (1)	8 (1)	8 (2)
		-	0.1118	0.9823	0.1560
<b>Body Weight Gain</b>	<b>Day 6-9</b>	22 (1)	18 (1)	16 (1)	16 (1)
		-	0.0271*	0.0004***	0.0004***
<b>Body Weight Gain</b>	<b>Day 10-13</b>	24 (1)	23 (2)	27 (1)	25 (1)
		-	0.8394	0.0921	0.6047
<b>Body Weight Gain</b>	<b>Day 14-17</b>	46 (4)	48 (5)	43 (5)	48 (2)
		-	0.4368	0.7415	0.6129
<b>Body Weight Gain</b>	<b>Day 18-20</b>	44 (5)	51 (3)	45 (6)	49 (2)
		-	0.3428	0.3789	0.9037

All weights are in gram  
 Corrected mean maternal weight gain = BW last day - (BW day 6 + uterus weight)  
 Significances computed versus Vehicle by Mann-Whitney U Test: Two Tailed

Mean (S.E.) / p-value Significance / Number  
 \*: p<0.05 \*\*: p<0.01 \*\*\*: p<0.001

**Food Consumption:** The Applicant did not provide food consumption data and provided summary of food consumption. There was no effect of treatment on food consumption at 100 mg/kg/day. However, food consumption was decreased at 300 and 1000/5 mg/kg/day from D6-13 and from D6-17, respectively. The above table shows the food consumption values.

**Toxicokinetics:** The  $t_{max}$  values ranged from 1.00 to 4.00 hours, and elimination was moderate to slow based on half-life values ranging from 2.65 to 5.61 hours. Systemic exposures (AUC and  $C_{max}$ ) to JNJ-27018966-AAA increased in a dose related manner. Following 11 days of dosing in the PO/SC dose group, there appeared to be a slight increase in exposure, while the oral only groups showed a decrease in exposure from Day 6 to Day 16. The following table (from page 257 of the report) shows the TK parameters.

Table SD3: JNJ-27018966 Plasma Toxicokinetic Parameters in Female Rats Following a Single or 11 Daily Oral or Oral plus Subcutaneous Doses of JNJ-27018966-AAA [100, 300, or 1000 (p.o.) plus 5 (s.c.) mg/kg/day] (TOX8398)

Route	Day	Dose (mg/kg)	$C_{max}$ (ng/mL)	$t_{max}$ (h)	AUC (0-∞) <sup>a</sup> (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h·kg)	AUC/ Dose	$C_{max}$ / Dose
PO	6	100	6.17	1.00	58.3	5.61	1710000	0.583	0.0617
		300	21.1	1.00	113	5.31	2650000	0.377	0.0703
	16	100	4.89	4.00	43.9	2.65	2990000	0.439	0.0489
		300	9.45	1.00	71.8	4.74	4030000	0.239	0.0315
PO/S C	6	1000/5	394	1.00	1070	5.46	943000	1.06	0.392
	16	1000/5	489	1.00	1230	4.21	807000	1.22	0.487

<sup>a</sup> AUC (0-24) hours on Day 16.

PO = oral

SC = subcutaneous

**Dosing Solution Analysis:** The concentration, homogeneity and stability of the test article JNJ-27018966-AAA in the formulations fell within the predefined acceptance criteria. Formulations were stable for up to 24 days after preparation when stored refrigerated (2-8°C).

**Necropsy:** There were no significant treatment related changes.

**Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):** At necropsy 23, 24, 23 and 23 females were pregnant (out of 24 /group) at 0, 100, 300 and 1000/5 mg/kg/day, respectively. There were no significant treatment related effects on pregnancy parameters i.e., the numbers of corpora lutea, implantations, early and late resorptions, live and dead fetuses, pre- and post-implantation losses, fetal weight or sex ratio. The following tables (from page 47 and 48 of the report) show the cesarean section data.

Treatment unit: mg/kg	Vehicle 0	Low 100	Medium 300	High 1000/5
<b>Corpora Lutea</b>	16.4 (0.3)	16.6 (0.4)	16.1 (0.3)	15.7 (0.6)
	-	0.5568	0.7540	0.4466
	22	23	23	23
<b>Implantations</b>	13.7 (0.8)	14.2 (0.9)	14.6 (0.6)	14.4 (0.8)
	-	0.4396	0.4364	0.5860
	23	24	23	23
<b>Pre-implantation loss</b>	13.36 (3.61)	12.11 (3.38)	9.52 (3.22)	10.48 (4.11)
	-	0.9354	0.4739	0.4286
	22	23	23	23
<b>Early Resorptions</b>	1.22 (0.38)	0.75 (0.22)	1.26 (0.39)	0.70 (0.25)
	-	0.4047	0.5601	0.2035
	23	24	23	23
<b>Late Resorptions</b>	0.65 (0.61)	0.04 (0.04)	0.87 (0.65)	0.00 (0.00)
	-	0.5138	0.9821	0.1528
	23	24	23	23
<b>Total Resorptions</b>	1.87 (0.67)	0.79 (0.23)	2.13 (0.86)	0.70 (0.25)
	-	0.2190	0.8181	0.0954
	23	24	23	23
<b>Post-implantation loss</b>	18.56 (6.31)	10.31 (4.34)	14.19 (5.76)	4.58 (1.73)
	-	0.2922	0.7874	0.0415*
	23	24	23	23
<b>Live fetuses</b>	11.7 (1.1)	13.3 (0.9)	12.5 (1.0)	13.7 (0.8)
	-	0.2308	0.7314	0.3231
	23	24	23	23
<b>Dead fetuses</b>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	-	-	-	-
	23	24	23	23
<b>Litter size</b>	11.7 (1.1)	13.3 (0.9)	12.5 (1.0)	13.7 (0.8)
	-	0.2308	0.7314	0.3231
	23	24	23	23

Mean (S.E.) / p-value Significance / Number  
Significances computed versus Vehicle by Mann-Whitney U Test: Two Tailed

\*: p< 0.05 \*\*: p<0.01 \*\*\*: p<0.001

Treatment unit: mg/kg	Vehicle 0	Low 100	Medium 300	High 1000/5
<b>Placental weight (gram)</b>	- (-)	- (-)	- (-)	- (-)
	0	0	0	0
<b>Male fetal weight (gram)</b>	5.9 (0.1)	5.8 (0.1)	5.8 (0.1)	5.7 (0.1)
	-	0.7337	0.6966	0.1143
	21	22	21	22
<b>Female fetal weight (gram)</b>	5.6 (0.1)	5.5 (0.1)	5.4 (0.2)	5.3 (0.1)
	-	0.4217	0.9584	0.0642
	20	23	21	23
<b>Overall fetal weight (gram)</b>	5.8 (0.1)	5.6 (0.1)	5.6 (0.2)	5.5 (0.1)
	-	0.3782	0.6966	0.0761
	21	23	21	23
<b>Sex ratio (%M)</b>	51.8 (3.9)	49.3 (3.6)	50.8 (2.9)	49.9 (3.7)
	-	0.8598	1.0000	0.9343
	21	23	21	23

Mean (S.E.) / p-value Significance / Number  
Significances computed versus Vehicle by Mann-Whitney U Test: Two Tailed

\*: p< 0.05 \*\*: p<0.01 \*\*\*: p<0.001

**Offspring (Malformations, Variations, etc.):** There were no significant treatment related effects on fetal external or visceral observations. There were some skeletal aberrations. There was an increase in the incidence of wavy ribs in all treated groups when compared to the control group. However, these changes are not considered as malformation. In the absence of any clear dose response, these changes were not considered treatment related. There was a slightly higher incidence of incomplete ossification of vertebrae and ribs in treated animals compared to control. However,

there was no dose response. These skeletal changes are not considered malformation and were not considered treatment related. A slightly higher incidence of incompletely descended thymus was observed at 1000/5 mg/kg/day. However, this was not dose related and was seen in control animals and was not considered related to the treatment. In addition, one fetus (No. 7) from one dam (Dam No. 111) had marked dilatation of the lateral ventricles with compensatory pressure atrophy of nervous tissue. This was an isolated incidence and did not appear to be treatment related. The following tables (from page 53 and 57 of the report) show the above changes.

Group	Fetuses				Litters			
	Vehicle	Low	Medium	High	Vehicle	Low	Medium	High
Treatment unit: mg/kg	0	100	300	1000/5	0	100	300	1000/5
Number examined	135	158	142	157	21	23	21	22
	#M:71 #F:64	#M:89 #F:69	#M:72 #F:70	#M:79 #F:78				
sacral vertebra(e), arch(es): incomplete ossification	0	1 (0.4)	0	0	0	1 (4.3)	0	0
Spine caudal vertebra(e)								
caudal vertebra(e): incomplete ossification	0	0	0	3 (3.4)	0	0	0	1 (4.5)
caudal vertebra(e): less than 4 ossified	0	0	1 (2.4)	0	0	0	1 (4.8)	0
Rib(s)								
rib(s): incomplete ossification bil	5 (3.3)	7 (3.7)	8 (5.5)	5 (2.9)	2 (9.5)	5 (21.7)	4 (19.0)	3 (13.6)
rib(s): incomplete ossification uni	3 (3.7)	10 (5.9) *	6 (4.0)	5 (2.8)	3 (14.3)	9 (39.1) *	5 (23.8)	4 (18.2)
rib(s): short bil	1 (0.6)	0	0	0	1 (4.8)	0	0	0
rib(s): wavy	15 (11.8)	42 (27.9) ***	40 (28.2) ***	37 (22.3) **	7 (33.3)	14 (60.9) *	14 (66.7) *	14 (63.6) *
Sternum								
sternum bone(s) - extra ossification site(s)/bone(s): fused	1 (0.6)	6 (3.9)	2 (1.2)	5 (3.2)	1 (4.8)	5 (21.7)	2 (9.5)	4 (18.2)
sternum bone(s): asymmetrical	2 (1.2)	2 (1.3)	0	2 (1.1)	2 (9.5)	2 (8.7)	0	2 (9.1)
sternum bone(s): bifurcated	0	1 (0.7)	1 (0.8)	0	0	1 (4.3)	1 (4.8)	0
sternum bone(s): bipartite	1 (0.7)	1 (0.7)	0	1 (0.5)	1 (4.8)	1 (4.3)	0	1 (4.5)
sternum bone(s): cleft	0	1 (0.7)	0	0	0	1 (4.3)	0	0

@: smaller number of examined fetuses is used as denominator  
 No of fetuses affected (mean % fetuses affected per litter) and No of litters affected (% litters affected) \*: p< 0.05 \*\*: p<0.01 \*\*\*: p<0.001  
 Comparison of the number of fetuses / litters with an abnormality between the dosed group and the reference group (Vehicle) computed by Fisher Exact Test: Right tail probability (Mid P Value)

(b) (4)

Group	Fetuses				Litters			
	Vehicle	Low	Medium	High	Vehicle	Low	Medium	High
Treatment unit: mg/kg	0	100	300	1000/5	0	100	300	1000/5
Number examined	135	161	145	158	21	23	21	23
	#M:69 #F:66	#M:73 #F:88	#M:74 #F:71	#M:83 #F:75				
Head / Neck								
thyroid gland(s): enlarged	1 (0.7)	0	0	0	1 (4.8)	0	0	0
Head / neck								
brains, ventricle(s): dilated lateral	0	0	0	1 (0.9)	0	0	0	1 (4.3)
Thorax								
lung lobe(s): abnormal lobulation	1 (0.7)	0	0	0	1 (4.8)	0	0	0
thymus:incompletely descended	9 (7.0)	12 (7.2)	12 (8.2)	18 (14.3)	8 (38.1)	9 (39.1)	9 (42.9)	12 (52.2)
Thorax heart / bloodvessel(s)								
aorta, arch: right sided	1 (0.7)	0	0	0	1 (4.8)	0	0	0
aorta: right descending	1 (0.7)	0	0	0	1 (4.8)	0	0	0
azygos vein: double	1 (0.7)	0	0	0	1 (4.8)	0	0	0
ductus arteriosus: connected with right sided aortic arch	1 (0.7)	0	0	0	1 (4.8)	0	0	0
heart, ventricular septum defect	1 (0.7)	0	0	0	1 (4.8)	0	0	0
heart: malpositioned	1 (0.7)	0	0	0	1 (4.8)	0	0	0

@: smaller number of examined fetuses is used as denominator  
 No of fetuses affected (mean % fetuses affected per litter) and No of litters affected (% litters affected) \*: p< 0.05 \*\*: p<0.01 \*\*\*: p<0.001  
 Comparison of the number of fetuses / litters with an abnormality between the dosed group and the reference group (Vehicle) computed by Fisher Exact Test: Right tail probability (Mid P Value)

**Study title: Oral/Subcutaneous Embryofetal Development Study of JNJ-27018966-AAA in the Rabbit**

Study no.: TOX8376  
 Study report location: EDR 4.2.3.5.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: June 13, 2007  
 Date of study completion: April 15, 2008  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: JNJ-27018966-AAA, batch No. 30205959, 98%

**Key Study Findings:**

- In an oral/SC embryofetal development study in rats, animals were treated at 100, 300 or 1000/5 mg/kg/day PO/SC.
- There were no significant treatment related effects on uterine parameters e.g., numbers of corpora lutea, implantations, early and late resorptions, live fetuses, pre- or post-implantation loss, or sex ratio.
- There were no significant treatment related fetal external, visceral or skeletal changes.
- JNJ-27018966-AAA did not appear to cause significant adverse effects on embryofetal development at any of the tested doses.

**Methods:** The study was conducted to examine the potential effects of JNJ 27018966-AAA on embryofetal development, when administered orally by gavage and orally plus subcutaneously to pregnant females once daily during the period of organogenesis (Days GD6 to GD19). Three groups of twenty, timed-mated, female New Zealand white rabbits were treated by oral gavage with an aqueous solution of JNJ- 27018966-AAA at 100, 300 or 1000/5 mg/kg/day PO/SC. Each female at 1000 mg/kg/day received a further administration of 5 mg/kg/day by the SC route in combination with the oral dose. The combination of the oral and subcutaneous route was chosen to obtain a higher systemic exposure.

Doses: Oral: 100, 300 and 1000 mg/kg/day; SC: 5 mg/kg/day  
 Frequency of dosing: Daily (GD6 to GD17)  
 Dose volume: 10 mL/kg (oral); 1 mL/kg (SC)  
 Route of administration: Oral gavage + SC  
 Formulation/Vehicle: Oral: 0.5% w/v Methocel (hydroxypropyl methylcellulose) in water;  
 SC: Aqueous solution containing 10% w/v hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD)  
 Species/Strain: New Zealand white rabbits

Number/Sex/Group: 20/group  
 Satellite groups: 3/group  
 Study design: Shown below  
 Deviation from study protocol: The Applicant stated that protocol deviations did not have any impact on the validity or integrity of the study. However, the Applicant did not submit the protocol in the study report.

The following table (from page 16 of the report) shows the study design.

Code	Group	Cage card	Female numbers		Dose level (mg/kg/day)
			Main	Toxicokinetics	
V	Vehicle	Blue	1 – 20	11 – 13	00
L	Low	Red	31-50	41 – 43	100
M	Medium	Yellow	61-80	71 – 73	300
H	High	Green	91-110	101 – 103	1000/5

**Basis of dose selection:** Dose selection for the present study was based upon the results of a pilot embryofetal development study in pregnant rabbits (TOX8261). In this study, four groups of five, timed-mated New Zealand white rabbits were treated by oral gavage with an aqueous solution of JNJ-27018966-AAA at 200, 1000, 200/5 or 1000/5 mg/kg/day PO/SC. The oral formulation was an aqueous solution containing 0.5% w/v hydroxypropyl methylcellulose (Methocel) and the dose volume used was 10 mL/kg. The subcutaneous formulation was an aqueous solution containing 10% w/v HP- $\beta$ -CD and was administered at a dose volume of 1 mL/kg. A control dosed group was also included and received 0.5% w/v Methocel together with a 'sham' subcutaneous administration. All females were sacrificed on GD 28 and a necropsy was performed. At necropsy, dams were examined for macroscopic abnormalities. The uterus was examined for numbers of corpora lutea of pregnancy, implantations, early and late resorptions and live and dead fetuses. Fetuses in all groups were weighed and examined externally.

There were no treatment related mortalities. All females at 1000/5 mg/kg dose exhibited reduced general activity during the dosing period. There was also an increased incidence of reduced fecal output at 200/0, 1000/0 or 1000/5 mg/kg/day PO/SC. There were no other treatment related findings. Uterine parameters (numbers of corpora lutea, implantations, live fetuses and pre- and post-implantation losses) were unaffected by

the treatment. There was no significant treatment related effect on fetal weight. Based upon the above findings, the high dose for the current study was set at 1000/5 mg/kg/day PO/SC. The low and mid doses were set at 100 and 300 mg/kg/day PO, respectively.

### **Observations:**

Mortality: All animals were observed for mortality on a daily basis.

Clinical Signs: All animals were observed once daily for clinical signs.

Body Weight: Body weights were recorded on Days 2, 4, 6, 9, 13, 16, 20, 22, 25 and 28 of pregnancy.

Food Consumption: Food consumption was recorded on gestation days 2, 6, 9, 13, 20, 25 and 28.

Toxicokinetics: On GD6 and GD19, blood samples were collected from all rabbits at 0.5, 1, 2, 4, 7, and 24 hours postdose.

Dosing Solution Analysis: The concentration and stability of JNJ-27018966-AAA in the firstly prepared formulations were checked at 5, 14 and 21 days after preparation. The homogeneity (top, middle and bottom samples) of the oral and SC dose formulations was also examined.

Necropsy: On GD28, animals were sacrificed for necropsy.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): Animals were sacrificed on GD28 and a necropsy was performed during which the females were examined for macroscopic abnormalities, pregnancy status, the numbers of corpora lutea of pregnancy, implantations, early and late resorptions and live and dead fetuses.

Offspring (Malformations, Variations, etc.): The fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

### **Results:**

Mortality: There were no deaths or premature sacrifices.

Clinical Signs: There was a dose related increase in the incidence of reduced fecal output in all treated groups compared to the vehicle control group. These findings were considered to be consistent with the observed reduction in food consumption and with the pharmacologic effect of reduced gastrointestinal motility.

**Body Weight:** The Applicant did not provide body weight data and provided summary of weight gain. No treatment related effects were observed. The following table (from page 36 and 43 of the report) shows the body weight gain data.

Observation Treatment unit: mg/kg	Vehicle 0	Low 100	Medium 300	High 1000/5
<b>ADULT DATA</b>				
Number of dosed females	20	20	20	20
Number of pregnant females/terminally sacrificed (2)	18/20	17/20	17/20	17/20
Number of dead or sacrificed females/dosed females (1)	0/20	0/20	0/20	0/20
Body weight gain (d2-d3) (3)	4	35	51	0
Body weight gain (d4-d5) (3)	43	43	43	46
Body weight gain (d6-d8) (3)	14	4	20	13
Body weight gain (d9-d12) (3)	79	71	76	74
Body weight gain (d13-d15) (3)	78	78	93	78
Body weight gain (d16-d19) (3)	91	90	77	111
Body weight gain (d20-d21) (3)	62	-25 ***	-34 ***	-78 ***
Body weight gain (d22-d24) (3)	26	79 **	49	61
Body weight gain (d25-d27) (3)	30	57 *	79 **	64
Corrected mean maternal weight gain (3)	-79.2	-30.0	-74.6	-109.1
Food consumption (d2-d5) (3)	706	732	723	692
Food consumption (d6-d8) (3)	554	463	408 **	342 ***
Food consumption (d9-d12) (3)	749	748	743	699
Food consumption (d13-d19) (3)	1290	1263	1264	1260
Food consumption (d20-d24) (3)	750	671	588 **	623 *
Food consumption (d25-d27) (3)	349	407 *	376	369

All weights are in gram

Reference group: Vehicle

Significances computed by:

\*: p&lt;0.05 \*\*: p&lt;0.01 \*\*\*: p&lt;0.001

(1) Fisher Exact Test: Right Tail probability (Mid P Value) (2) Fisher Exact Test: Left Tail probability (Mid P Value) (3) Mann-Whitney U Test: Two Tailed

**Food Consumption:** The Applicant did not provide food consumption data and provided summary of food consumption. Food consumption in all treated groups was reduced in a dose related manner between GD6 and GD8 compared to controls. Throughout the remainder of the treatment period, food consumption was generally comparable with the control at 100 or 300 mg/kg/day but was slightly reduced at 1000/5 mg/kg/day. The above table shows the food consumption values.

**Toxicokinetics:** Following oral dosing, mean  $t_{max}$  values ranged from 0.667 to 3.33 hours, and elimination appeared to be slow based on mean half-life values ranging from 12.1 to 24.9 hours. Systemic exposure to JNJ-27018966-AAA (mean  $C_{max}$  or AUC) was dose related. Following 14 daily oral doses of JNJ-27018966, increases in mean  $C_{max}$  values were observed, indicating possible accumulation. Following a single 1000/5 mg/kg/day PO/SC dose, mean  $t_{max}$  was 0.667 hours, and mean half-life was 5.54 hours. There was no apparent accumulation of the drug following repeated exposure. The following table (from page 209 of the report) shows the TK parameters.

Table SD4: Mean (SD) JNJ-27018966 Plasma Toxicokinetic Parameters in Pregnant Female Rabbits Following a Single or 14 Daily Oral or Oral Plus Subcutaneous Doses of JNJ-27018966-AAA (100/0, 300/0, and 1000/5 mg/kg/day) (TOX8376)

Day	Dose <sup>a</sup> (mg/kg)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC (0-∞) (ng·h/mL)	AUC (0-24 h) (ng·h/mL)	t <sub>1/2</sub> (h)	AUC/ Dose	C <sub>max</sub> / Dose
6	100/0	9.92 (2.98)	3.33 (3.21)	143 <sup>b</sup> NA	126 (6.23)	13.9 (5.02)	1.43 NA	0.0992 (0.0298)
	300/0	11.9 (4.41)	2.33 (1.53)	ND NA	168 (62.0)	24.9 <sup>c</sup> NA	NA NA	0.0397 (0.0147)
	1000/5	1140 (55.1)	0.667 (0.289)	2990 (189)	2880 (251)	5.54 (1.88)	2.98 (0.190)	1.14 (0.0548)
19	100/0	31.3 (19.0)	0.667 (0.289)	NR NA	124 (46.0)	15.5 (1.86)	1.24 (0.461)	0.313 (0.190)
	300/0	109 (95.8)	0.667 (0.289)	NR NA	369 (123)	12.1 (3.03)	1.23 (0.412)	0.364 (0.319)
	1000/5	1020 (301)	0.667 (0.289)	NR NA	2750 (961)	5.03 (0.964)	2.73 (0.961)	1.02 (0.299)

<sup>a</sup> Dose administered Oral/Subcutaneous

<sup>b</sup> N=1. Two AUC values were excluded from the mean due to extrapolation >25%.

<sup>c</sup> N=2. Unable to calculate for one animal.

NA: Not Applicable

ND: Not Determined because the AUC was excluded from the Mean calculation.

NR: Not Reported for multiple dose days.

**Dosing Solution Analysis:** Concentration, homogeneity and stability of JNJ-27018966-AAA in the formulations were within the predefined acceptance criteria. Formulations were stable for up to 21 days after preparation when stored at 2-8°C.

**Necropsy:** There were no significant treatment related changes.

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

There were 18 of 20, 17 of 20, 17 of 20 and 17 of 20 pregnant females at 0, 100, 300 and 1000/5 mg/kg/day, respectively. There were no significant treatment related effects on the numbers of corpora lutea, implantations, early and late resorptions, live and dead fetuses, pre- and post-implantation losses, fetal weight or sex ratio. Higher post-implantation losses were observed at 100 or 300 mg/kg/day, however, in the absence of any increase at 1000/5 mg/kg/day this was not considered treatment related. The following tables (from page 44 and 45 of the report) show the cesarean section data.

Treatment unit: mg/kg	Vehicle 0	Low 100	Medium 300	High 1000/5
<b>Corpora Lutea</b>	11.9 (0.4)	11.8 (0.5)	12.2 (0.4)	12.6 (0.6)
	-	0.8280	0.8271	0.6041
	18	17	17	17
<b>Implantations</b>	10.2 (0.4)	8.9 (0.7)	10.2 (0.5)	10.2 (0.6)
	-	0.1773	0.7892	0.7769
	18	17	17	17
<b>Pre-implantation loss</b>	13.96 (3.28)	25.66 (4.38)	16.27 (3.71)	18.46 (3.77)
	-	0.0605	0.5613	0.3509
	18	17	17	17
<b>Early Resorptions</b>	0.56 (0.27)	0.82 (0.30)	1.06 (0.26)	0.59 (0.17)
	-	0.4001	0.0804	0.3456
	18	17	17	17
<b>Late Resorptions</b>	0.00 (0.00)	0.41 (0.12)	0.12 (0.08)	0.18 (0.10)
	-	0.0027**	0.1396	0.0662
	18	17	17	17
<b>Total Resorptions</b>	0.56 (0.27)	1.24 (0.33)	1.18 (0.27)	0.76 (0.24)
	-	0.0238*	0.0421*	0.2838
	18	17	17	17
<b>Post-implantation loss</b>	5.63 (2.79)	15.30 (4.18)	12.19 (2.78)	7.62 (2.38)
	-	0.0210*	0.0456*	0.2864
	5.63	15.30	12.19	7.62
<b>Live fetuses</b>	9.7 (0.5)	7.7 (0.7)	9.1 (0.6)	9.5 (0.6)
	-	0.0580	0.4626	0.7519
	18	17	17	17
<b>Dead fetuses</b>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	-	-	-	-
	18	17	17	17
<b>Litter size</b>	9.7 (0.5)	7.7 (0.7)	9.1 (0.6)	9.5 (0.6)
	-	0.0580	0.4626	0.7519
	18	17	17	17

Mean (S.E.) / p-value Significance / Number  
Significances computed versus Vehicle by Mann-Whitney U Test: Two Tailed

\*: p< 0.05 \*\*: p<0.01 \*\*\*: p<0.001

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Treatment unit: mg/kg	Vehicle 0	Low 100	Medium 300	High 1000/5
<b>Placental weight (gram)</b>	- (-)	- (-)	- (-)	- (-)
	-	-	-	-
	0	0	0	0
<b>Male fetal weight (gram)</b>	32.1 (0.8)	34.0 (0.9)	32.6 (1.0)	30.4 (0.7)
	-	0.1375	0.7166	0.1657
	18	17	17	17
<b>Female fetal weight (gram)</b>	31.6 (0.6)	33.1 (1.3)	31.5 (0.9)	29.9 (0.8)
	-	0.6048	0.7414	0.0747
	18	16	17	17
<b>Overall fetal weight (gram)</b>	31.8 (0.6)	33.7 (1.0)	32.2 (0.9)	30.2 (0.7)
	-	0.2098	0.8173	0.0747
	18	17	17	17
<b>Sex ratio (%M)</b>	44.9 (4.3)	58.7 (4.9)	56.1 (4.1)	56.5 (3.8)
	-	0.0389*	0.0575	0.0455*
	18	17	17	17

Mean (S.E.) / p-value Significance / Number  
Significances computed versus Vehicle by Mann-Whitney U Test: Two Tailed

\*: p< 0.05 \*\*: p<0.01 \*\*\*: p<0.001

**Offspring (Malformations, Variations, etc.):** There were no significant treatment related fetal external, visceral or skeletal observations. There were some skeletal aberrations. There was an increase in the incidence of additional ribs at 300 and 1000/5 mg/kg/day. However, these changes are not considered as malformation. It has been reported that extra ribs in the rabbit are frequently observed when maternal toxicity has been produced (Khera, K. S., 1985, Maternal Toxicity: A Possible Etiological Factor in

Embryo-Fetal Deaths and Fetal Malformations of Rodent-Rabbit Species, Teratology, 31:129-153). These changes could be due to the observed maternal toxicity (reduced food consumption, reduced fecal output) and may not be a direct effect of the test article on the developing fetus. The following tables (from page 49 and 50 of the report) show the above changes.

Group	Fetuses				Litters			
	Vehicle	Low	Medium	High	Vehicle	Low	Medium	High
Treatment unit: mg/kg	0	100	300	1000/5	0	100	300	1000/5
<b>Number examined</b>	<b>174</b> <sup>84</sup>	<b>131</b> <sup>67</sup>	<b>154</b> <sup>76</sup>	<b>161</b> <sup>83</sup>	<b>18</b>	<b>17</b>	<b>17</b>	<b>17</b>
	<small>#M:77 #F:97</small>	<small>#M:74 #F:57</small>	<small>#M:86 #F:68</small>	<small>#M:88 #F:73</small>				
cervical vertebra(e), centrum: incomplete ossification	0	1 (0.7)	0	0	0	1 (5.9)	0	0
cervical vertebra, rib(s): full rib uni	0	1 (0.5)	0	0	0	1 (5.9)	0	0
<b>Spine thoracic vertebra(e)</b>								
thoracic vertebra(e), arch(es): fused	1 (0.7)	1 (1.5)	0	0	1 (5.6)	1 (5.9)	0	0
thoracic vertebra(e), arch(es): incomplete ossification	2 (1.4)	0	0	0	2 (11.1)	0	0	0
thoracic vertebra(e), centrum: bipartite	0	1 (1.5)	0	0	0	1 (5.9)	0	0
thoracic vertebra(e), centrum: dumbbell shaped	1 (0.7)	0	0	0	1 (5.6)	0	0	0
thoracic vertebra(e), centrum: hemicentrum	1 (0.7)	2 (2.1)	0	0	1 (5.6)	2 (11.8)	0	0
thoracic vertebra(e), centrum: incomplete ossification	1 (0.7)	0	0	0	1 (5.6)	0	0	0
thoracic vertebra(e): displaced	1 (0.7)	1 (1.5)	0	0	1 (5.6)	1 (5.9)	0	0
<b>Spine lumbar vertebra(e)</b>								
lumbar vertebra L1: extra rib(s) bil full	101 (57.4)	76 (62.1)	111 (68.9) **	111 (64.6) *	18 (100.0)	17 (100.0)	17 (100.0)	16 (94.1)
lumbar vertebra L1: extra rib(s) bil ru	4 (2.1)	2 (1.3)	1 (0.7)	1 (0.6)	3 (16.7)	2 (11.8)	1 (5.9)	1 (5.9)
lumbar vertebra L1: extra rib(s) uni full	16 (9.6)	12 (9.1)	5 (3.4)	11 (8.3)	11 (61.1)	9 (52.9)	4 (23.5)	7 (41.2)

@: smaller number of examined fetuses is used as denominator  
 No of fetuses affected (mean % fetuses affected per litter) and No of litters affected (% litters affected) \*: p<0.05 \*\*: p<0.01 \*\*\*: p<0.001  
 Comparison of the number of fetuses / litters with an abnormality between the dosed group and the reference group (Vehicle) computed by Fisher Exact Test: Right tail probability (Mid P Value)

Group	Fetuses				Litters			
	Vehicle	Low	Medium	High	Vehicle	Low	Medium	High
Treatment unit: mg/kg	0	100	300	1000/5	0	100	300	1000/5
<b>Number examined</b>	<b>174</b> <sup>84</sup>	<b>131</b> <sup>67</sup>	<b>154</b> <sup>76</sup>	<b>161</b> <sup>83</sup>	<b>18</b>	<b>17</b>	<b>17</b>	<b>17</b>
	<small>#M:77 #F:97</small>	<small>#M:74 #F:57</small>	<small>#M:86 #F:68</small>	<small>#M:88 #F:73</small>				
lumbar vertebra L1: extra rib(s) uni full-uni ru	7 (3.6)	5 (4.5)	13 (9.7)	4 (2.7)	5 (27.8)	5 (29.4)	10 (58.8) *	3 (17.6)
lumbar vertebra L1: extra rib(s) uni ru	6 (3.2)	8 (6.5)	5 (3.4)	7 (4.6)	6 (33.3)	7 (41.2)	3 (17.6)	7 (41.2)
lumbar vertebra(e): displaced	0	1 (1.5)	0	0	0	1 (5.9)	0	0
lumbar vertebra(e): supernumerary	4 (2.5)	6 (4.0)	25 (14.4) ***	14 (6.9) **	3 (16.7)	3 (17.6)	8 (47.1) *	6 (35.3)
<b>Rib(s)</b>								
rib(s), cartilage: abnormal association	0	0	0	2 (1.4)	0	0	0	2 (11.8)
rib(s), first rib(s): rudimentary bil	0	0	0	1 (0.5)	0	0	0	1 (5.9)
rib(s): discontinuous bil	1 (0.4)	0	0	0	1 (5.6)	0	0	0
rib(s): discontinuous uni	2 (1.6)	0	0	1 (0.8)	1 (5.6)	0	0	1 (5.9)
rib(s): fused uni	0	3 (2.5) *	0	0	0	2 (11.8)	0	0
rib(s): short bil	0	0	0	1 (0.6)	0	0	0	1 (5.9)
<b>Sternum</b>								
sternum bone(s): fused	11 (6.8)	4 (4.0)	1 (0.7)	2 (1.5)	6 (33.3)	4 (23.5)	1 (5.9)	2 (11.8)
sternum bone(s): asymmetrical	8 (4.7)	7 (6.6)	4 (2.5)	3 (1.9)	6 (33.3)	6 (35.3)	2 (11.8)	3 (17.6)
sternum bone(s): bifurcated	1 (0.5)	0	0	1 (0.5)	1 (5.6)	0	0	1 (5.9)
sternum bone(s): bipartite	4 (2.5)	2 (2.6)	1 (0.7)	2 (1.1)	3 (16.7)	2 (11.8)	1 (5.9)	2 (11.8)

@: smaller number of examined fetuses is used as denominator  
 No of fetuses affected (mean % fetuses affected per litter) and No of litters affected (% litters affected) \*: p<0.05 \*\*: p<0.01 \*\*\*: p<0.001  
 Comparison of the number of fetuses / litters with an abnormality between the dosed group and the reference group (Vehicle) computed by Fisher Exact Test: Right tail probability (Mid P Value)

### 9.3 Prenatal and Postnatal Development

#### Study title: Oral Pre and Postnatal Development Study including Maternal Function in Rats

Study no.:	1808-019
Study report location:	EDR 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 15, 2012
Date of study completion:	August 23, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	JNJ-27018966-AAA, ZR497138PFA141, 99.7%

#### Key Study Findings:

- In an oral pre and postnatal development study in rats, animals were treated with JNJ-27018966-AAA from GD 6 to LD 20 at 100, 300 and 1000 mg/kg/day.
- In maternal animals, there was no significant treatment related effect on survival. There were no significant treatment related clinical signs, body weight changes, or changes in food consumption in maternal females.
- In F1 pups, no significant effect of treatment was seen during preweaning (sex ratios, survival to weaning, body weights, clinical findings, or behavior, developmental and sensory evaluations) and postweaning [survival, growth, sexual maturation, behavioral evaluations (motor activity and learning and memory), reproductive performance, and fertility] evaluations.
- JNJ-27018966-AAA was secreted into the milk of lactating rats at all tested doses.
- JNJ-27018966-AAA did not appear to cause significant adverse effect on pre and postnatal development of rats in this study.

**Methods:**

Doses: 100, 300 and 1000 mg/kg/day  
 Frequency of dosing: The vehicle or the test article was administered once daily via oral gavage beginning on Gestation Day (GD) 6 and continuing through lactation up to and inclusive of lactation day 20 (LD20).  
 Dose volume: 10 mL/kg  
 Route of administration: Oral gavage  
 Formulation/Vehicle: 0.5% Hydroxypropyl methylcellulose (HPMC) (high viscosity) in purified water, USP  
 Species/Strain: SD rats  
 Number/Sex/Group: 25/group  
 Satellite groups: None  
 Study design: Shown in the table below  
 Deviation from study protocol: None of the protocol deviations affected the quality or integrity of the study.

The following table (from page 15 of the report) shows the study design.

<b>Group Assignments</b>		
<b>Group Number</b>	<b>Dose Level (mg/kg/day)</b>	<b>Number of Time-mated Females</b>
1	0	25
2	100	25
3	300	25
4	1000	25

**Basis of dose selection:** The dose levels were selected based on the results of the fertility and early embryonic development study (1808-003) in rats (reviewed above). In this study, JNJ-27018966-AAA was administered at 100, 300 and 1000 mg/kg/day. There were no significant treatment related effects on mating, fertility, and fecundity indices. The pregnancy index and uterine parameters (mean number of corpora lutea, number of implantation sites, number of viable embryos, number of resorptions, and pre- and post-implantation loss) in all groups were unaffected by the treatment. JNJ-27018966-AAA did not cause any significant adverse effects on fertility and early embryonic development in this study. Based on these results, the low, mid and high doses were selected as 100, 300 and 1000 mg/kg/day for the current study.

**Observations and Results:****F0 Dams:**

Mortality: Mortality was observed twice daily. Two females at 1000 mg/kg/day were found dead during the lactation period. Animal number 284 was found dead on LD9. Macroscopic findings consisted of white foreign material in the thoracic cavity and a severe laceration of the esophagus. This death was attributed to dosing error. Animal number 294 was found dead on LD2. Macroscopic findings consisted of a blood clot and red fluid within the pericardial cavity. No other macroscopic findings were observed in other animals at 1000 mg/kg/day and this death was not considered test article related.

Clinical Signs: Clinical signs were observed on a daily basis. There were no significant treatment related clinical signs.

Body Weight: Body weights were recorded on GD 0, 6, 10, 14, 17, and 20, and on LD 0, 4, 7, 10, 14, 17, and 21. The mean initial (GD 0) and final (GD 20) body weights of control animals were 207 and 364 g, respectively. There were no significant treatment related effects.

Food Consumption: Food consumption was recorded on GD 0, 6, 10, 14, 17, and 20, and on LD 0, 4, 7, 10, 14, 17, and 21. There were no significant treatment related changes.

Uterine Data: Females were examined twice daily for signs of parturition. Duration of gestation was calculated. Litter size, number of stillborn and live born pups, number of males and females, individual body weights, and gross abnormalities of the pups were recorded for each litter. There were no significant treatment related effects on parturition. Pregnancy rates were 100% in all groups. Gestation length and all litter parameters in the treated groups were comparable to controls. The following tables (from page 97-100 of the report) show the delivery and litter data.

(b) (4) Study Number 1808-019  
 JNJ-27018966-AAA: An Oral Study of Toxic Effects on Pre-and Postnatal Development, Including Maternal Function in Rats

Summary of P Natural Delivery and Litter Data					
Endpoint		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Females on Study	N	25	25	25	25
No. Females Pregnant	N	25	25	25	25
Females Delivering Litters <sup>1</sup>	N	25	25	25	25
	%	100.0	100.0	100.0	100.0
With Stillborn Pups <sup>1</sup>	N	4	2	1	4
	%	16.0	8.0	4.0	16.0
With All Stillborn <sup>1</sup>	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Gestation Length (Days)	Mean	21.8	22.0	21.9	22.0
	SD	0.47	0.29	0.28	0.20
	N	25	25	25	25
No. of Pups at Day 0 (Total Pups Born/Litter)	Mean	11.1	11.5	11.8	11.3
	SD	2.33	2.12	1.89	1.84
	N	25	25	25	25

N- Number of measures used to calculate mean  
 SD- Standard Deviation  
<sup>1</sup>Not statistically analyzed  
 No.- Number

(b) (4) Study Number 1808-019  
 JNJ-27018966-AAA: An Oral Study of Toxic Effects on Pre-and Postnatal Development, Including Maternal Function in Rats

**Summary of P Natural Delivery and Litter Data**

Endpoint		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. of Pups at Day 0 cont.					
Liveborn/Litter	Mean	10.9	11.3	11.8	10.9
	SD	2.60	2.50	1.85	2.31
	N	25	25	25	25
Stillborn/Litter	Mean	0.2	0.2	0.0	0.4
	SD	0.50	0.62	0.20	1.08
	N	25	25	25	25
Gestation Index	%	100.0	100.0	100.0	100.0
	N	25	25	25	25
Stillborn Index	Mean %/Litter	3.64	2.16	0.29	3.56
	SD	11.034	8.765	1.429	10.837
	N	25	25	25	25
Total Implantation Scars/Litter	Mean	11.7	12.2	12.1	12.0
	SD	2.44	1.91	1.83	1.80
	N	25	25	25	25

N- Number of measures used to calculate mean  
 SD- Standard Deviation  
 No.- Number

(b) (4)  
 Study Number 1808-019  
 JNJ-27018966-AAA: An Oral Study of Toxic Effects on Pre-and Postnatal Development, Including Maternal Function in Rats

**Summary of P Natural Delivery and Litter Data**

Endpoint		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
<b>No. Live Pups/Litter</b>					
Day 4 (Preculling)	Mean	10.6	10.9	11.6	10.9
	SD	2.62	2.42	1.68	2.36
	N	24	23	25	24
Day 4 (Postculling)	Mean	7.6	7.8	8.0	7.8
	SD	1.53	0.83	0.00	0.83
	N	24	23	25	24
Day 7	Mean	7.9	7.8	7.9	7.8
	SD	0.63	0.83	0.28	0.83
	N	23	23	25	24
Day 14	Mean	7.8	7.8	7.9	7.8
	SD	0.83	0.83	0.28	0.85
	N	23	23	25	23
Day 21	Mean	7.8	7.8	7.8	7.5
	SD	1.09	0.89	0.44	1.10
	N	21	20	21	22
Day 28	Mean	7.8	7.8	7.7	7.5
	SD	1.09	0.89	0.56	1.10
	N	21	20	21	22

N- Number of measures used to calculate mean  
 SD- Standard Deviation  
 No.- Number

(b) (4)  
Study Number 1808-019  
JNJ-27018966-AAA: An Oral Study of Toxic Effects on Pre-and Postnatal Development, Including Maternal Function in Rats

		Summary of P Natural Delivery and Litter Data			
Endpoint		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Sex Ratio (% Males per Animal)					
Pups Day 0	Mean %/Litter	55.96	51.39	55.79	47.80
	SD	18.817	18.506	13.282	14.745
	N	25	25	25	25
Pups Day 4 (Preculling)	Mean %/Litter	57.56	51.73	55.24	47.87
	SD	18.178	18.758	13.485	14.327
	N	24	23	25	24
Pups Day 4 (Postculling)	Mean %/Litter	55.31	51.63	52.50	49.33
	SD	14.583	15.218	5.103	9.707
	N	24	23	25	24
Pups Day 21	Mean %/Litter	53.37	53.75	51.45	47.48
	SD	12.263	13.512	5.831	9.904
	N	21	20	21	22
Pup Survival Indices					
Viability Index	Mean %/Litter	93.52	89.02	98.84	94.20
	SD	20.600	27.166	2.728	20.599
	N	25	25	25	25
Lactation Index	Mean %/Litter	85.83	86.96	81.50	88.91
	SD	34.125	34.435	36.643	28.474
	N	24	23	25	24

N- Number of measures used to calculate mean  
SD- Standard Deviation  
No.- Number

**Maternal Milk:** On LD12, milk was collected from the first six surviving lactating females per group. Milk samples were analyzed for JNJ-27018966. JNJ-27018966 was secreted in milk of lactating rats at all three dose levels. Mean concentrations of the drug in the milk were approximately dose related. The following table (from page 28 of the report) shows the mean concentrations of JNJ-27018966-AAA in the milk samples.

Mean Concentration (ng/mL) of JNJ-27018966 in LD 12 Rat Milk Samples			
	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
<b>Mean</b>	2.78	5.49	44.02
<b>N</b>	5	6	5

It is to be mentioned here that quantifiable levels of the test article were also found in two control samples. No cause could be identified after an investigation at the testing facility (b) (4) and an anomaly review at (b) (4). The Applicant stated that there were adequate data from the other samples analyzed to provide for a thorough and accurate interpretation of the test article exposure in milk samples. Per the Applicant, there was no impact on the integrity or quality of the study.

Necropsy: There were no significant treatment related changes.

Toxicokinetics: Not conducted

Dosing Formulation Analysis: Dosing solutions were analyzed from the dose formulations prepared for Weeks 1, 3, and 5 of the study. All study samples analyzed had mean concentrations ranging from 96% to 100% of the theoretical concentrations. No test article was found in the control samples.

### **F1 Generation**:

Survival: Survival was observed on a daily basis. There was no significant treatment related effect on F1 pup survival. Viability index in the treated groups ranged from 89% to 99% and was comparable to controls (94%).

Clinical Sign: Clinical signs were observed daily. There were no significant treatment related clinical signs. Several pups in litters for which the dams (first six surviving dams per group) were used for milk collection showed clinical signs, which included decreased activity, thin appearance, and/or skin cold to touch. Many of these pups either died or were euthanized *in extremis*. It was determined that many of the F0 dams, following the milk collection procedures on LD12, were not producing enough milk to sustain their pups. Based on these, morbidity/mortality observed in all groups including the controls was attributed to injury to the milk ducts during the milk collection procedure and were not considered treatment related.

Body Weight: Body weight was recorded on LD 0, 4, 7, 14, and 21. Mean initial (Day 0) and final (Day 28) weights of control animals (males and females combined) were 6.76 and 85.06 g, respectively. No significant treatment related effects were observed.

Food Consumption: Not recorded

Physical development: Physical development including pinna detachment and cliff aversion were examined on LD2 and eye opening on LD13. F1 females were examined for vaginal opening beginning on postnatal day 28 (PND28) and for preputial separation on PND35. Slightly earlier onset of pinna detachment was seen at 2.3 days at 1000 mg/kg/day relative to controls at 2.9 days. However, the onset for pinna detachment in the high dose group was within the historical control ranges (2.2 to 2.9 days) and was not considered treatment related. There were no other significant treatment related changes.

Neurological Assessment: On LD21, neuropharmacological evaluations were conducted on each pup using Irwin test. Auditory (Preyer's) response was evaluated for each pup at PND22. Locomotor activity and passive avoidance test (learning and memory) were conducted on PND35 and PND70-85, respectively. No significant effect of treatment was observed on sensory and reflex evaluations. Slightly earlier onset of static righting reflex was observed at 2.3 days at 1000 mg/kg/day relative to controls (2.5 days).

However, the onset for static righting reflex at the high dose was within the historical control ranges (2.3 to 2.6 days) and was not considered treatment related. In males at 1000 mg/kg/day, basic and fine movement and total distance were statistically significantly lower at the 10-15 minute interval in comparison to controls. These changes were predominantly within the range of the historical control data, apparent in only one sex and were not considered toxicologically meaningful or test article related. In addition, no significant treatment related effects on learning and memory was observed in passive avoidance test.

Reproduction: On Postnatal Day (PND) 28, 25 male and 25 female F1 pups were randomly selected from each group to continue on study for evaluation of growth, sexual maturation (vaginal opening, preputial separation), behavior (motor activity and learning and memory [step-through passive avoidance]), reproductive performance, and fertility. The latter was evaluated by mating animals within treatment group (1 male:1 female). F1 pups not selected to continue on study were euthanized and subjected to a complete necropsy on PND 28. Mated F1 females were euthanized on GD13. Uterus was excised and the total number of normally developing embryos, resorptions, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded. Uteri from females that appeared nongravid were opened for detection of implantation sites.

No significant treatment related effects were observed on mating performance and fertility of the F1 animals. Mating, fertility, and fecundity indices in the treated groups (males and females) were comparable to controls and within the range of the historical control data for the laboratory. Mean copulatory interval (i.e., number of days to mating) in the F1 treated groups ranged from 2.9 to 3.8 days and was comparable to controls (3.2 days). The following tables (from pages 161-162 of the report) show the F1 reproductive and fertility data.

(b) (4)  
 Study Number 1808-019  
 JNJ-27018966-AAA: An Oral Study of Toxic Effects on Pre- and Postnatal Development, Including Maternal Function in Rats

Summary of F <sub>1</sub> Reproductive and Fertility Parameters				
Endpoint	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Females on Study	25	25	25	25
No. Females Paired	25	25	25	25
No. Females Mated	23	24	23	24
No. Pregnant	23	21	21	24
Female Mating Index (%)	92.0	96.0	92.0	96.0
Female Fertility Index (%)	92.0	84.0	84.0	96.0
Female Fecundity Index (%)	100.0	87.5	91.3	100.0
No. Males on Study	25	25	25	25
No. Males Paired	25	25	25	25
No. Males Mated	23	24	23	24
No. Males Impregnating a Female	23	21	21	24
Male Mating Index (%)	92.0	96.0	92.0	96.0
Male Fertility Index (%)	92.0	84.0	84.0	96.0

No. - Number

(b) (4)  
 Study Number 1808-019  
 JNJ-27018966-AAA: An Oral Study of Toxic Effects on Pre- and Postnatal Development, Including Maternal Function in Rats

Summary of F <sub>1</sub> Reproductive and Fertility Parameters					
Endpoint		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Male Fecundity Index (%)		100.0	87.5	91.3	100.0
Females with Confirmed Mating Day		23	23	22	23
Copulatory Interval (Days)	Mean	3.2	3.7	3.8	2.9
	SD	2.15	2.89	4.62	1.93
	N	23	23	22	23

N - Number of measures used to calculate mean  
 SD - Standard Deviation

No significant effect of treatment was seen on uterine implantation data. At 1000 mg/kg/day, mean pre-implantation loss was higher (10.60%) compared to controls (5.35%), however, the value was not statistically significant and within the historical control range (5.9 to 11.9%) and was not considered test article related. For all other uterine parameters, values in the treated groups were comparable to controls. The following tables (from pages 165 and 166 of the report) show the uterine data.

(b) (4) Study Number 1808-019  
JNJ-27018966-AAA: An Oral Study of Toxic Effects on Pre- and Postnatal Development, Including Maternal Function in Rats

Endpoint		Summary of F <sub>1</sub> Maternal and Developmental Observations at Uterine Examination			
		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Corpora Lutea No. per Animal	Mean	17.8	16.9	16.9	16.6
	SD	2.33	2.75	1.73	3.12
	N	23	20	20	23
Implantation Sites No. per Animal	Mean	16.7	15.8	15.4	15.1
	SD	1.58	2.94	2.64	4.00
	N	23	20	20	23
Preimplantation Loss % per Animal	Mean	5.35	6.88	7.93	10.60
	SD	8.303	10.211	15.509	16.926
	N	23	20	20	23
Viable Embryos No. per Animal	Mean	15.9	14.8	14.7	14.7
	SD	1.66	2.86	2.90	4.00
	N	23	20	20	23
Postimplantation Loss % per Animal	Mean	4.90	6.18	5.00	4.43
	SD	4.987	6.251	7.016	10.924
	N	23	20	20	23

N - Number of measures used to calculate mean  
SD - Standard Deviation  
No. - Number

(b) (4)  
 Study Number 1808-019  
 JNJ-27018966-AAA: An Oral Study of Toxic Effects on Pre- and Postnatal Development, Including Maternal Function in Rats

		Summary of F <sub>1</sub> Maternal and Developmental Observations at Uterine Examination			
Endpoint		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Resorptions: Early + Late No. per Animal	Mean	0.8	1.0	0.7	0.4
	SD	0.83	0.97	0.98	0.79
	N	23	20	20	23

N - Number of measures used to calculate mean  
 SD - Standard Deviation  
 No. - Number

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## 10 Special Toxicology Studies

### Neutral Red Uptake Phototoxicity Assay of JNJ-27018966 in Balb/c 3T3 Mouse Fibroblasts (Study No. 20046999)

**Methods:** The objective of this study was to evaluate the phototoxicity potential of JNJ-27018966. In this study, viability of Balb/c 3T3 mouse fibroblasts exposed to JNJ-27018966 and ultraviolet radiation (+UVR) was assessed and then compared with the viability of fibroblasts exposed to JNJ-27018966 in the absence of ultraviolet radiation (-UVR). The vehicle was 1% DMSO/DPBS (Dulbecco's Phosphate Buffered Saline). Promethazine hydrochloride was used as the positive control. The following table (from page 10 of the report) shows the concentrations tested. The cells were exposed to 5 J/cm<sup>2</sup> of UVA and 22 mJ/cm<sup>2</sup> of UVB from a xenon arc solar simulator.

Text Table 1  
Assay Concentrations in 1% DMSO/DPBS (mg/L)

Test Material	Range-Finding Assay							
JNJ-27018966	0.030	0.100	0.300	1.00	3.20	10.2	32.2	102

Test Material	Definitive Assays 1 and 2							
Promethazine	0.032	0.100	0.316	1.00	3.16	10.0	31.6	100
JNJ-27018966	1.78	3.16	5.62	10.0	17.8	31.6	56.2	100

**Results:** JNJ-27018966 did not show any cytotoxicity or phototoxicity in this assay. The following table (from page 10 of the report) shows the assay results.

Range-Finding Assay					
Test Material	IC <sub>50</sub> (mg/L) -UVR (cytotoxicity)	IC <sub>50</sub> (mg/L) +UVR (phototoxicity)	Photoirritancy Factor (PIF)	Mean Photo Effect (MPE)	Phototoxic Potential
JNJ-27018966	-	-	*1	-0.006	Non-Phototoxic
Definitive Assays					
Promethazine	64.09	0.9797	65.436	0.473	Phototoxic
JNJ-27018966 (Assay 1)	-	-	*1	-0.012	Non-Phototoxic
JNJ-27018966 (Assay 2)	-	-	*1	0.008	Non-Phototoxic

IC<sub>50</sub>: 50% Inhibitory Concentration.

+UVR: with exposure to 5 J/cm<sup>2</sup>UVA and 22 mJ/cm<sup>2</sup> of UVB in the range-finding and definitive assays

- UVR: without UVR exposure

\* 1: Both IC<sub>50</sub>(-UVR) and IC<sub>50</sub>(+UVR) could not be calculated, this indicated a lack of phototoxic potential.

### **Murine Local Lymph Node Assay with JNJ-27018966 (Study No. TOX8122)**

**Methods:** The objective of this study was to evaluate skin sensitization potential of JNJ-27018966 using lymphocytes from the auricular lymph nodes of topically treated CBA/J mice. JNJ-27018966 was administered to animals at a maximum concentration of 50% (w/w) in N,N-dimethylformamide (DMF). This study was divided into toxicity and irritation screening phase and a local lymph node assay (LLNA) phase as shown in the following (from page 7 of the report) table.

**Toxicity and Irritation Screening Phase**

Group	No. of Females	Treatment <sup>a</sup>	Dose Level (% w/w)
1 (Control)	2	<i>N,N</i> -dimethylformamide	0
2	2	JNJ-27018966 in <i>N,N</i> -dimethylformamide	10
3	2	JNJ-27018966 in <i>N,N</i> -dimethylformamide	25
4	2	JNJ-27018966 in <i>N,N</i> -dimethylformamide	50

a The dose volume was 25 microliters/ear.

**Local Lymph Node Assay Phase**

Group	No. of Females	Treatment <sup>a</sup>	Dose Level (% w/w)
1 (Naïve Control) <sup>b</sup>	5	Naïve	0
2 (Negative Control)	5	Acetone/olive oil (4:1)	0
3 (Negative Control)	5	<i>N,N</i> -dimethylformamide	0
4	5	JNJ-27018966 in <i>N,N</i> -dimethylformamide	10
5	5	JNJ-27018966 in <i>N,N</i> -dimethylformamide	25
6	5	JNJ-27018966 in <i>N,N</i> -dimethylformamide	50
7 (Positive Control)	5	Hexylcinnamaldehyde in acetone/olive oil (4:1)	25% (v/v)

a The dose volume was 25 microliters/ear.

b Animals were not treated.

The toxicity and irritation screening phase was conducted to determine the maximum concentration of JNJ-27018966 to be tested in the LLNA phase. Animals were treated once daily for 4 days and subsequently observed for mortality, any abnormalities, and signs of pain or distress. The ears of each animal were observed at predose, approximately 4 hours and 22 to 26 hours postdose. Any reactions observed were scored for redness and swelling according to a 4-point scale.

**Results:** Criteria for contact sensitizer in the LLNA assay included a stimulation index (SI) of 3.0 or greater at any dose level, statistically significant differences from control values, and evidence of a dose response (National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Public Health Service, Department of Health and Human Services, *The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds*, NIH Publication No. 99-4494, 1999). Mean Si scores were 1.0, 1.3, and 1.2 at 10, 25, and 50% (w/w), respectively. The absence of statistically significant SI values > 3.0 at all tested dose levels indicated that JNJ-27018966 was not a contact sensitizer under the conditions of this study. The following table (from page 29 of the report) shows the results.

**Table 7**  
**Incorporation of <sup>3</sup>H-Thymidine into Auricular Lymph Nodes**

Group	Vehicle	Animal Number	dpm	Mean dpm	SD dpm	% RSD	SI	Mean SI	SD SI	% RSD
1	Naive Control <sup>a</sup>	A48507	325	507	345	68	0.5	0.8	0.6	68
		A48508	1099				1.8			
		A48509	219				0.4			
		A48510	468				0.8			
		A48511	422				0.7			
2	AOO negative control	A48512	315	577	178	31	0.5	1.0	0.3	31
		A48513	636				1.1			
		A48514	NA <sup>b</sup>				NA			
		A48515	647				1.1			
		A48516	710				1.2			
3	DMF negative control	A48517	517	612	368	60	0.8	1.0	0.6	60
		A48518	397				0.6			
		A48519	186				0.3			
		A48520	843				1.4			
		A48521	1115				1.8			
4	JNJ-27018966 in DMF 10% (w/w)	A48522	927	640	282	44	1.5	1.0	0.5	44
		A48523	384				0.6			
		A48524	740				1.2			
		A48525	300				0.5			
		A48526	850				1.4			
5	JNJ-27018966 in DMF 25% (w/w)	A48527	1393	784	368	47	2.3	1.3	0.6	47
		A48528	808				1.3			
		A48529	655				1.1			
		A48530	646				1.1			
		A48531	418				0.7			
6	JNJ-27018966 in DMF 50% (w/w)	A48532	809	717	239	33	1.3	1.2	0.4	33
		A48533	470				0.8			
		A48534	592				1.0			
		A48535	628				1.0			
		A48536	1086				1.8			
7	HCA in AOO positive control 25% (v/v)	A20909	3349	3577	363	10	5.8	6.2	0.6	10
		A20910	3471				6.0			
		A20911	4075				7.1			
		A20912	3813				6.6			
		A20913	3175				5.5			

AOO Acetone/ olive oil (4:1).

DMF N,N-dimethylformamide

dpm Disintegrations per minute.

HCA Hexylcinnamaldehyde.

NA Not applicable.

SD Standard deviation.

SI Stimulation index. Calculation: (dpm for individual animal)/(Mean dpm of solvent or vehicle control group)

%RSD SD/mean x 100.

Note: The SI calculations were as follows: Groups 1 and 4 through 6 were compared to the Group 3 negative control, and Group 7 (positive control) was compared to Group 2.

a Animals were not treated.

b Not applicable due to a misdose of <sup>3</sup>H-thymidine.

### **In Vitro Bovine Corneal Opacity-Permeability Eye Irritation Test (Study No. TOX8226)**

**Methods:** The aim of this study was to assess in vitro eye irritating potential of JNJ-27018966-AAA for occupational safety. This study was conducted using bovine corneas to examine opacity and permeability. The values obtained from both the parameters were combined, and the resulting in vitro score was compared to a previously established scale of ocular irritancy, which consisted of five broad categories: no irritation ( $\leq 3.0$ ), mild (3.1 to 25.0), moderate (25.1 to 55.0), severe (55.1 to 80.0) and very severe ( $\geq 80.1$ ) irritation. This assay is an in vitro alternative to the in vivo Draize

eye irritation test. The bovine corneal opacity-permeability (BCOP) assay using isolated bovine corneas has been (Gautheron P *et al.*, 1992, Bovine Cornea Opacity and Permeability Test: An In Vitro Assay for Ocular Irritancy. *Fundam Appl Toxicol*, 18:442-449) demonstrated to produce consistent results with in vivo results from the Draize eye test (Gautheron *et al.*, 1992, Gautheron, P. *et al.*, 1994, Interlaboratory Assessment of the Bovine Corneal Opacity and Permeability (BCO-P) Assay, *Toxicol in Vitro*, 8:381-392; Vanparys, Ph. *Et al.*, 1993, Evaluation of the Bovine Cornea Opacity-Permeability Assay as an In Vitro Alternative to the Draize eye irritation test, *Toxicol in Vitro*, 7:471-476). Three corneas were treated per dose group. The following three dose groups were used:

- Vehicle control [0.9 % (w/v) NaCl in water]
- Positive control [20 % (w/w) imidazole in the vehicle]
- JNJ-27018966-AAA [20 % (w/w) in the vehicle]

Corneas were incubated for 4 hours at 32°C in a water-bath, solutions were removed and the corneal epithelium was washed at least 3 times with MEM (Minimal Essential Medium) solution. Then opacity was measured using the opacitometer. The permeability of the corneas was evaluated immediately after measuring the opacity. The medium was removed from the anterior compartment and replaced by 0.5% sodium-fluorescein solution. Corneas were incubated for 90 minutes at 32°C in a water-bath. After incubation, medium from the posterior chamber was removed, and its optical density (OD) was determined spectrophotometrically at 490 nm.

**Results:** JNJ-27018966-AAA did not increase corneal opacity (0.3) or permeability (0.111). The corneas treated with the positive control imidazole showed a marked increase in opacity and permeability. Imidazole was classified as a very severe eye irritant. Based on an in vitro score of 2.0, JNJ-27018966 was classified as a non-eye irritant under the conditions of the experiment. The following table (from page 21 of the report) shows the results.

Table 4: Individual and mean values of opacity, permeability and in vitro scores

Cornea	Treatment with	Opacity at			Permeability	In vitro score
		t0	t240	t240 - t0		
1	Vehicle control	0	0	0	0.025	0.4
2	NaCl	0	0	0	0.026	0.4
3	0.9 % (w/v)	1	1	0	0.015	0.2
	Mean ± S.D.	0.0 ± 0.0			0.022 ± 0.006	0.3 ± 0.1
				Corrected value	Corrected value	
4	Positive control	1	164	163	163.0	236.5
5	Imidazole	0	151	151	151.0	197.7
6	20% (w/w)	0	152	152	152.0	215.0
	Mean ± S.D.	155.3 ± 6.7			4.070 ± 0.899	216.4 ± 19.4
				Corrected value	Corrected value	
7	JNJ-27018966-AAA	0	0	0	0.013	-0.1
8	20% (w/w)	0	1	1	1.0	3.7
9		0	0	0	0.0	2.4
	Mean ± S.D.	0.3 ± 0.6			0.111 ± 0.104	2.0 ± 1.9

## 11 Integrated Summary and Safety Evaluation

Eluxadoline is a locally active, mu opioid receptor ( $\mu$ OR) agonist and delta opioid receptor ( $\delta$ OR) antagonist. The Applicant is seeking approval for Eluxadoline for the treatment of diarrhea-predominant irritable bowel syndrome (IBS-d) in adult men and women. The recommended oral dose of eluxadoline is 100 mg twice daily. The mechanism of action of eluxadoline is based on the hypothesis that the agonist activity at the  $\mu$ OR inhibits GI transit while antagonism at the  $\delta$ OR prevents excessive inhibition of GI motility. Several studies have demonstrated that the side effects of  $\mu$ OR agonists can be suppressed by  $\delta$ OR antagonists. Thus, eluxadoline is expected to decrease adverse events (e.g., constipation) associated with pure  $\mu$ OR agonists. In addition, low oral bioavailability of eluxadoline is expected to limit the central nervous system (CNS) effects.

Eluxadoline has been evaluated in a comprehensive program of nonclinical studies which included pharmacology, ADME (absorption, distribution, metabolism and excretion), acute toxicology (mouse and rat), 2-week intravenous (IV) studies in rats and

Cynomolgus monkeys and repeated dose toxicology (up to 13-week study in mice, up to 26-week study in rats, and up to 9-month study in Cynomolgus monkeys), juvenile toxicology (4-week study in juvenile rats), genotoxicity [Ames test, chromosome aberration assay, lymphoma cell (L5178Y/TK<sup>+/+</sup>) forward mutation test, and rat bone marrow micronucleus test], carcinogenicity (2-year carcinogenicity studies in mice and rats), reproductive toxicity (fertility and early embryonic development in rats, embryofetal development in rats and rabbits and pre and post-natal development in rats) and special toxicology.

Several *in vitro* and *in vivo* pharmacology studies have been conducted with eluxadoline. *In vitro* receptor binding and function profiling studies demonstrated that eluxadoline is a  $\mu$ OR agonist ( $K_i = 1.0$ - $1.8$  nM) and  $\delta$ OR antagonist ( $K_i = 4.4$  nM), with moderate kappa opioid receptor (OR) agonist ( $K_i = 55$  nM) activity. In animal efficacy studies, eluxadoline has shown efficacy in normalizing GI transit and defecation in several animal models of altered GI function induced by stress, castor-oil or GI inflammation. Eluxadoline also reversed hyperalgesic responses in a rat model of acute colitis-induced visceral pain. Eluxadoline reduced GI transit and fecal output in stressed and non-stressed mice over a wide dose-range without fully inhibiting gastrointestinal transit. In secondary pharmacology studies, eluxadoline (10  $\mu$ M) inhibited the binding of control ligands ( $\geq 30\%$  inhibition) to four non-opioid receptors: histamine H2 (32%), muscarinic M1 (62%), serotonin 5-HT6 (31%) and somatostatin (31%); and the calcium-dependent potassium channel, SK<sup>+</sup>Ca<sup>2+</sup> (48%).

Eluxadoline did not cause significant inhibition of  $I_{Kr}$  in *in vitro* hERG assay up to 3  $\mu$ M concentration and had no significant effect on the rate and the force of contraction up to 10  $\mu$ M concentration in isolated guinea pig atrium. Eluxadoline did not show significant electrophysiological effects on isolated rabbit Purkinje fibers up to 10  $\mu$ M ( $> 1000$  times the  $C_{max}$  of 3 ng/mL after 100 mg oral dose in humans). In anesthetized dogs, eluxadoline did not show any significant cardiovascular adverse effects up to an IV cumulative dose of 1.443 mg/kg [approximately 124 times the  $C_{max}$  of 2-3 ng/mL in humans at the 100 mg dose]. In conscious telemetered monkeys, QT and QTc intervals were slightly prolonged (106% to 112% of control?) at 5, 15 and 30 mg/kg. In a respiratory safety pharmacology study in rats at IV doses of 5, 10 and 20 mg/kg, depressive changes in breathing, consistent with  $\mu$ OR agonists, were observed. No significant safety signals were identified in the CNS safety pharmacology studies in rats at oral doses of up to 300 mg/kg.

Pharmacokinetics (PK) of eluxadoline has been studied in rodent and nonrodent species using different routes of administration. Oral bioavailability of eluxadoline was low ( $\leq 0.83\%$ ) in mice, rats, and Cynomolgus monkeys, which is consistent with humans (oral bioavailability of 1.3%). In tissue distribution studies in rats, highest exposure to eluxadoline was observed in the gastrointestinal tract and the radioactivity was below the lower limit of quantification in the brain. *In vivo* metabolism studies in animals revealed that unchanged drug was the major drug-related component in rats and Cynomolgus monkeys, consistent with humans. One metabolite (acyl glucuronide M11, urine) identified in humans was also detected in the plasma of monkeys and in the urine

of rats and monkeys. In rats, after oral administration, eluxadoline was excreted primarily through the feces (97%) and the urinary excretion was low (0.5%).

In acute oral studies, the maximum non-lethal dose was 500 mg/kg in mice and  $\geq 2000$  mg/kg in rats. In acute intraperitoneal (IP) studies, the maximum non-lethal dose was  $\geq 500$  mg/kg in mice and 62.5 mg/kg in male rats and 125 mg/kg in female rats.

Chronic oral toxicology studies were conducted in rats (6-month) and monkeys (9-month) to support chronic use of eluxadoline. The no-observed-adverse-effect-levels (NOAELs) in rats and monkeys were 2000 and 200 mg/kg/day, respectively (about 11 and 14 times, respectively, the human AUC of 24 ng.h/mL after a single oral dose of 100 mg). In a 4-week oral toxicology study in juvenile rats, the NOAEL was 1500 mg/kg/day.

Eluxadoline was negative in the Ames test, chromosome aberration assay in human lymphocytes, the mouse lymphoma cell (L5178Y/TK<sup>+/-</sup>) forward mutation test and the in vivo rat bone marrow micronucleus test. Oral administration of eluxadoline for 104 weeks did not produce tumors in mice and rats at up to 14 and 36 times, respectively, the human AUC of 24 ng.h/mL after a single oral dose of 100 mg.

Eluxadoline at oral doses up to 1000 mg/kg/day [about 10 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg] was found to have no adverse effect on fertility and reproductive performance of male and female rats. Embryofetal development studies in rats and rabbits at oral/SC doses up to 1000/5 mg/kg/day (about 51 and 115 times, respectively, the human AUC after a single oral dose of 100 mg) did not cause any adverse effects on embryofetal development. A pre and postnatal development study in rats showed no evidence of any adverse effect on pre and postnatal development at oral doses of eluxadoline up to 1000 mg/kg/day (about 10 times the human AUC after a single oral dose of 100 mg). Eluxadoline was secreted in the milk of lactating rats at oral doses of 100, 300 and 1000 mg/kg/day (about 1.8, 3 and 10 times the human AUC of 24 ng.h/mL after a single oral dose of 100 mg). Mean concentrations of eluxadoline in the milk of lactating rats on lactation day 12 were 2.78, 5.49 and 44.02 ng/mL at 100, 300 and 1000 mg/kg/day, respectively.

Impurities, heavy metals and residual solvents in the drug substance (DS) were evaluated. Organic impurities were either qualified in toxicology studies or were detected at a level at which qualification was not required. However, one of the impurities, (b) (4), was present at higher than the ICH reportable levels in the latest batches of the DS. The current specification of NMT (not more than) (b) (4) % w/w will result in the maximum exposure of (b) (4) mg per day in humans at the recommended dose of 100 mg BID. The (b) (4) impurity was considered toxicologically qualified and the above limit of NMT (b) (4) % was found to be acceptable. A genotoxic impurity assessment on the process related impurities was also performed using computational toxicology assessment using Derek software. No potential genotoxic impurities were identified. (b) (4) level in the DS was (b) (4) ppm. The exposure to (b) (4) would be about (b) (4)  $\mu$ g per day at the recommended dose of

100 mg BID. The <sup>(b) (4)</sup> levels were well below the proposed USP <231> limit of <sup>(b) (4)</sup> ppm for oral drug products with a maximum daily dose ≤ 10 g per day or the maximum exposure to <sup>(b) (4)</sup> is less than the Permitted Daily Exposure (PDE) of <sup>(b) (4)</sup> µg per day per the ICH Q3D guidelines (Step 2b, July 2013) for elemental impurities. Residual solvent levels were also below the ICH Q3C limits.

Overall, the NOAELs in rats and monkeys were 2000 and 200 mg/kg/day, respectively (about 11 and 14 times, respectively, the human AUC of 24 ng.h/mL after a single oral dose of 100 mg). Eluxadoline was not genotoxic and did not produce tumors in mice and rats up to 1500 mg/kg/day. Eluxadoline at oral doses up to 1000 mg/kg/day did not have any adverse effect on fertility and reproductive performance of male and female rats. In embryofetal development studies in rats and rabbits at oral/SC doses up to 1000/5 mg/kg/day, eluxadoline did not cause any adverse effects on embryofetal development. A pre and postnatal development study in rats showed no evidence of any adverse effect on pre and postnatal development at oral doses of eluxadoline up to 1000 mg/kg/day.

In conclusion, this NDA contains adequate nonclinical studies and satisfies the criteria for marketing authorization of eluxadoline. From a nonclinical perspective, this NDA is recommended for approval for its proposed use as indicated in the label.

## 12 Appendix/Attachments

None

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/s/  
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TAMAL K CHAKRABORTI  
01/23/2015

SUSHANTA K CHAKDER  
01/23/2015

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

**NDA Number: 206940**      **Applicant: Furiex Pharmaceuticals, Inc.**      **Stamp Date: 6/27/2014**

**Drug Name: Eluxadoline**      **NDA Type: New NDA**      **Submit Date: 6/26/2014**

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?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
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)?	√		
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?	√		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement  
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA/BLA or Supplement**

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	√		The proposed labeling sections relevant to nonclinical studies may need to be revised during the labeling review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		
11	Has the applicant addressed any abuse potential issues in the submission?	√		
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 is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant. **N/A**

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter. **None**

Tamal Chakraborti, Ph.D. August 12, 2014  
 \_\_\_\_\_  
 Reviewing Pharmacologist Date

Sushanta Chakder, Ph.D. August 12, 2014  
 \_\_\_\_\_  
 Supervisor Date

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/s/  
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TAMAL K CHAKRABORTI  
08/12/2014

SUSHANTA K CHAKDER  
08/12/2014