

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**206143Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**NDA:** 206143

**Submission date:** 6/27/2014

**Drug:** ivabradine

**Applicant:** Amgen, Inc.

**Indication:** treatment of stable, symptomatic chronic heart failure in (b) (4) including maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated

**Reviewing Division:** Division of Cardiovascular and Renal Products

**Discussion:** The primary and secondary pharmacology/toxicology reviewers found the information for ivabradine sufficient to support approval for the indication listed above.

An appropriate pharmacologic class for ivabradine would be “hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker”. This would be a new Established Pharmacologic Class.

The primary target of toxicity in animal studies was the heart. This may not be surprising because the primary pharmacologic target is located in the heart. Ivabradine also exhibited some effects on the eye in toxicology studies. This may be related to the activity of ivabradine on an HCN channel located in the retina. It is reasonable to describe these ocular findings in labeling.

The carcinogenicity of ivabradine was assessed in two-year carcinogenicity studies in rats and mice. The executive carcinogenicity assessment committee found these studies to be acceptable and concurred that there were no drug-related neoplasms in either study.

Ivabradine produced heart and other cardiovascular malformations in rat developmental toxicity studies at exposures similar to those achieved in humans at a maximum recommended dose. Use in pregnant women is not recommended.

**Conclusions:** I concur that the nonclinical information is adequate to support approval of ivabradine for the indication listed above.

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PAUL C BROWN  
04/08/2015

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

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION  
Labeling Review**

Application number: 206,143

Supporting document/s: S016 (eCTD0014), S022(eCTD0017)

Applicant's letter date: April 30, 2014, June 27, 2014

CDER stamp date: April 30, 2014, June 27, 2014

Product: Ivabradine

Indication: To reduce the risk of [REDACTED] (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure [REDACTED] (b) (4) [REDACTED] and in sinus rhythm with heart rate  $\geq$  70 beats per minute (bpm), [REDACTED] (b) (4) [REDACTED] maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated [REDACTED] (b) (4)

Applicant: Amgen, Inc.

Review Division: Division of Cardio-Renal Products

Reviewer: Jean Q. Wu

Supervisor/Team Leader: Albert De Felice

Division Director: Norman Stockbridge

Project Manager: Alexis Childers

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summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 206,143.

Note: The drug names ivabradine, S 16257-2 and S 16257, have been used interchangeably in this review. The dose/dosage generally refers to the value in base form unless specified otherwise.

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## Introduction

The goal date of this NDA was extended by 3 months as stated in the Division letter of December 16, 2014. A new timeline for communicating labeling changes was established accordingly. Pregnancy and Lactation Labeling Rule (PLLR) was published on December 4, 2014 and the sponsor intends to submit revised labeling in compliance with the PLLR. This labeling review and recommendations in Section 1.1.3 Labeling is a supplement to the Nonclinical Primary Review of NDA 206,143 dated November 28, 2014. Doses or exposures in animal studies are expressed as multiples of exposures at the maximum recommended human dose (MRHD) of 7.5 mg bid.

As the draft labeling was submitted before the effective date of PLLR, the sponsor agreed to revise the labeling to comply with PLLR in the late cycle meeting of December 11, 2014 (see meeting minutes dated January 9, 2015). The following is the post-meeting comment from preclinical perspective regarding Section 8 conveyed to the sponsor.

“For the animal data, it is noted that teratogenic effects are not explicitly stated in the current draft labeling. In pregnant rats treated during organogenesis, embryofetal toxicity and teratogenic effects, characterized by abnormal heart shape, interventricular septal defects, and complex anomalies of primary arteries, were observed at exposures (AUC<sub>24h</sub>) 1 or 3 times of that at MRHD (maximum recommended human dose). There was increased postnatal mortality associated with the cardiac teratogenic effect in rats. We recommend that study findings, especially teratogenicity, be described clearly in the revised labeling.

In addition, considering lethal cardiac teratogenicity in rats and the potential for ivabradine to transfer into placenta and to be excreted in milk, ivabradine should not be given during pregnancy, particularly at the time of the organogenesis of the heart, or during lactation. This conclusion is stated clearly in both the toxicology-written summary (2.6) and the nonclinical overview (2.4), but not included in the current draft labeling. Please revise the labeling accordingly to reflect such contraindications.”

Division of Pediatric and Maternal Health (DPMH) of CDER was consulted for the compliance of PLLR when an unofficial copy of revised Section 8 was received from the sponsor.

The proposed changes are made in a tracking change format, i.e. cross out means deletion and blue font means insertion. The sentence(s) related to any comments are highlighted.

# 1 Executive Summary

## 1.1 Recommendations

### 1.1.3 Labeling

#### Section 4 Contraindications

Comments: Add "Pregnancy" in the list of contraindications.

*Reviewer Note: Teratogenicity characterized by abnormal shape of the heart, interventricular septal defect and complex anomalies of primary arteries was observed in fetuses of pregnant rats treated during organogenesis at exposures 3 times the human exposure ( $AUC_{0-24hr}$ ) at the maximum recommended human dose (MRHD). An increased postnatal mortality was associated with this cardiovascular teratogenicity. As the fetuses of pregnant women should be considered at potential risk for major cardiovascular anomalies, ivabradine should not be taken during pregnancy. Women should be advised not to become pregnant when taking ivabradine as the clinical benefit of ivabradine is primarily to reduce hospitalization for heart failure rather than to prolong survival. The lethal cardiovascular teratogenicity in rats was discussed in the internal labeling meetings. The final decision for adding "pregnancy" in the contraindication list will be based on Division's risk/benefit assessment.*

#### Section 5.1 Fetal Toxicity

Proposed changes:



#### Section 8.1 Pregnancy/ Risk Summary

Comments: The finding of reduced embryo-fetal survival in pregnant rabbits needs to be clarified (highlighted below).

Proposed changes

Risk Summary

Based on findings in animals, TRADENAME (ivabradine) may cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of TRADENAME in pregnant women to inform any drug-associated risks. In a <sup>(b) (4)</sup> animal reproduction studies, oral administration of ivabradine to pregnant rats during organogenesis at a dosage providing an exposure (AUC<sub>0-24hr</sub>) 1 or 3 times the human exposure at the MRHD resulted in embryo-fetal toxicity and teratogenicity <sup>(b) (4)</sup> as abnormal shape of the heart, interventricular septal defect and complex anomalies of primary arteries <sup>(b) (4)</sup>

<sup>(b) (4)</sup> An increased postnatal mortality was associated with <sup>(b) (4)</sup> teratogenicity in rats. In pregnant rabbits, increased postimplantation loss was noted at an exposure (AUC<sub>0-24hr</sub>) 5 times <sup>(b) (4)</sup> than that of human at the MRHD. <sup>(b) (4)</sup>

### Section 8.1 Pregnancy <sup>(b) (4)</sup>

Comments: The animal data below should be summarized in detail [reviewer note: no line-by-line changes are proposed for this part at current stage]. The sponsor should follow the draft PLLR guidance and provide summaries which include the types of studies completed, species tested, route of administration, doses or exposures expressed as multiples of human exposure at the MRHD, duration and timing of exposure, study findings, presence of absence of maternal toxicity and any study data limitations. As conveyed in the post-meeting comments of the late-cycle meeting, the sponsor should describe the teratogenic findings clearly.

#### Animal Data

<sup>(b) (4)</sup>

### Section 8.2 Lactation

Comments: Women should stop breast-feeding prior to treatment with ivabradine.

*Reviewer note: Animal data indicated that the ivabradine is present in milk. The pre-and postnatal rat study could not exclude the relationship of lactation and the increased postnatal mortality before weaning (birth to Lactation Day 20), though some of the deaths were associated with prenatal cardiac malformations. The data needs to be added to this section (as recommended by DPMH, see below). Women who need treatment with ivabradine should stop breast-feeding.*

Proposed changes: add "Data".

#### Data

Lactating rats received daily oral doses of [<sup>14</sup>C]-ivabradine on post-parturition days 10 to 14; milk and maternal plasma were collected at 0.5 and 2.5 hours post dose on day 14. Ratio of total radioactivity

associated with [<sup>14</sup>C]-ivabradine or its metabolites in milk vs. plasma was 1.5 and 1.8, respectively, indicating that ivabradine is transferred to milk after oral administration.

## Section 12.1 Mechanism of Action

### Comments:

Ivabradine is a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker. The currents carried by HCN channels are generally designated as  $I_f$  in the cardiac pacemaker cells and  $I_h$  in non-pacemaker tissues. It may not (b) (4) lower the heart rate given its potential interaction with HCN channels beyond cardiac pacemaker cells. Suggest modifying the 1<sup>st</sup> sentence in the 1<sup>st</sup> and 2<sup>nd</sup> paragraphs.

### Proposed changes:

#### 12.1 Mechanism of Action

(b) (4)

TRADENAME can also (b) (4) inhibit the retinal current  $I_h$  (b) (4)  $I_h$  (b) (4) curtailing the retinal response to bright light stimuli. Under triggering circumstances (e.g. rapid changes in luminosity), partial inhibition of  $I_h$  by TRADENAME may underlie (b) (4) the luminous phenomena (b) (4) experienced by patients. Luminous phenomena (phosphenes) are described as a transient enhanced brightness in a limited area of the visual field [see (b) (4) *Adverse Reactions (6.1)*].

## Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Comments: Extensive series of in vitro and in vivo genotoxicity tests were conducted with ivabradine and a conclusion from weight of evidence was that ivabradine poses no risk to patients at recommended therapeutic doses. However, for this labeling, each assay and its result, as evidence, should be clearly stated.

*Reviewer note: This request was conveyed to the sponsor in the late-cycle meeting preliminary response dated December 2, 2014.*

### 13.2 Animal Toxicology and/or Pharmacology

Comments: Add specific changes in retinal function and indicate the dose level and exposure as multiples (AUC or  $C_{max}$ ,) of human exposure at MRHD. Specify which "exposures", AUC or  $C_{max}$ , is based and replace "close" with exact value of the multiples.

Proposed changes (see comments for the highlighted part):

**13.2 Animal Toxicology and/or Pharmacology**

Reversible changes in retinal function were observed in dogs administered (b) (4)

These data are consistent with the pharmacological effect of ivabradine related to its interaction with hyperpolarization-activated  $I_h$  currents in the retina which (b) (4) share (b) (4) homology with the cardiac pacemaker  $I_f$  current.

(b) (4)

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JEAN Q WU  
03/04/2015

ALBERT F DEFELICE  
03/09/2015

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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW  
EVALUATION

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Applicant's letter date: April 30, 2014, June 27, 2014  
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Applicant: Amgen, Inc.  
Review Division: Division of Cardio-Renal Products  
Reviewer: Jean Q. Wu  
Supervisor/Team Leader: Albert De Felice  
Division Director: Norman Stockbridge  
Project Manager: Alexis Childers

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# 1 Executive Summary

## 1.1 Recommendations

### 1.1.1 Approvability

There is no approvability issue from pharmacology/toxicology perspective.

### 1.1.2 Additional Non Clinical Recommendations

None.

### 1.1.3 Labeling

See recommendations for labeling in a separate labeling review.

## 1.2 Brief Discussion of Nonclinical Findings

Ivabradine has been developed as a heart rate lowering agent for treatment of chronic heart failure (CHF). The maximum recommended human dose (MRHD) is 7.5 mg bid.

Ivabradine is the first for the class of HCN (hyperpolarization activated cyclic nucleotide-gated) channel blocker. It inhibits  $I_f$  in pacemaker cells of rabbit sinoatrial node (SAN), equivalent  $I_f$  in human atrial cells of patients and  $I_h$  in mouse retina rods with comparable potency,  $IC_{50}$  of  $\sim 3 \mu\text{M}$ . Ivabradine reduced the slope of spontaneous diastolic depolarization without affecting maximal diastolic potential or threshold potential of activation. In vivo, a dose-dependent heart rate reduction was demonstrated in different species with peak effect generally reached within 3-4 hours. The major metabolite S 18982 is similar to ivabradine in pharmacologic effect.

Ivabradine blocked  $I_{kr}$  at an  $IC_{50}$  of  $4.85 \mu\text{M}$ , approximately 200 fold greater than  $C_{max}$  in patients at MRHD (corrected for protein binding) and had no significant effect on QTc in telemetered dogs at an exposure 134-fold of human  $C_{max}$  at MRHD.

The nonclinical safety assessment of ivabradine has been performed in core battery safety pharmacology studies and an extensive toxicology program including toxicity studies from single dose to repeated-dose up to 52 weeks in mice, rats and dogs. The major findings in the heart and the eyes, and other minor findings are discussed below.

The heart is considered the primary organ of toxicity. In rat oral repeated-dose studies up to 52 weeks, myocardial lesions including mucification and metaplasia in the chordae tendinae, ventricular degeneration characterized by cardiomyocyte vacuolation, contraction bands and myoblasts, fibrosis and/or necrosis, and atrial/ventricular

hypertrophy, associated with increased heart weight, were observed at all treated dosages (from 2x3 mg/kg/day in the 52-week study that provided an exposure 3 times that of human  $AUC_{24h}$  at MRHD). The NOAEL could not be established due to the cardiac findings at lowest dosage tested. Although myocardial lesions with similar characteristics were reported as spontaneous observations in rats, especially in males, the extensive lesions, particular in the high dose animals are considered beyond background findings. The result may reflect an exacerbation of a spontaneous myocardial degeneration. It is noted that the findings are similar to the previously reported effects of beta blockers in rodents. Some lesions, such as endocardial necrosis and contraction bands, may also recall those of excess adrenergic stimulation. Similar cardiac lesions were not observed in dogs treated with ivabradine.

Treatment-related ECG findings were observed in dogs during repeated doses up to 52 weeks. These included bradycardia, sinoatrial block or arrest, 1<sup>st</sup> and 2<sup>nd</sup> degree AV block at almost all doses (with  $C_{max}$  at least 15-fold of human  $C_{max}$  at MRHD) and some isolated instances of ventricular escape complexes as well as atrial or ventricular premature complexes at higher dose levels. The ECG was normal 7 days after drug withdrawal. It was reported that SA block or arrest, and 1st and 2nd degree AV block can occur spontaneously in the beagle dog, in association with its inherently high vagal tone.

In 52-week dog study, one female dog at 2x12 mg/kg/day (approximately 40 times of human  $AUC_{24h}$  at MRHD) was found dead on Day 341 without clinical signs preceding the death. ECG evaluation of this dog showed bradycardia with HR of 40-60 bpm without adverse effect on QTc, PR, QRS or rhythm. Gross and histopathologic examination could not reveal the cause of death. A relationship to ivabradine may not be excluded, although bradycardia *per se* would not be expected to be lethal.

Another primary organ of toxicity is the eye. Transient visual symptoms reported in the clinical trials triggered ERG measurement in the 52-week dog study. The dog ERG findings were mainly in the cone system responses, which included decrease in cone b wave amplitude and delay in dark adaptation from lowest dose with exposure 3 times human  $AUC_{24h}$  at MRHD. There were no changes detected from ophthalmoscopic and transmission electron microscopic exams. The ERG was normal after a one week recovery period. In addition, in rats following 3 week s.c. dosing, reduced temporal resolution in response to a sinusoidal light stimulus was observed at a dose of 11 mg/kg with  $C_{max}$  3-5 fold of that at MRHD. The effect was absent after 1 week recovery and there was no effect on cell morphology, HCN distribution and phototransduction. Given that ivabradine inhibits  $I_h$  of the isolated mouse rods with  $IC_{50}$  of 2.7  $\mu M$ , and reduces temporal response of the retina, using light as a stimulus ( $IC_{50}$  of 30  $\mu M$ ), consistent with an effect on  $I_h$ , the adverse effect of ivabradine on visual system might be related to its pharmacologic effect on  $I_h$  at retina which, similar to  $I_f$ , is carried by HCN family.

As for other minor findings, overt signs including decrease activity, abnormal posture and behavior, and tremor generally appeared from exposure level 27 time of human  $C_{max}$  at MRHD in rats and 57 times of human  $C_{max}$  at MRHD in dogs. Convulsions were

observed in dogs at  $C_{max}$  100-fold greater than that at MRHD and were only observed in single dose rat studies ( $\geq 56$  mg/kg iv or po). The transient convulsions were developed within hours post dose. Increased water diuresis or sodium urinary excretion was occasionally observed in rats, but not in dogs. Increased liver weight and/or liver function tests without any associated histopathology were noted across the rat studies. The only histopathology was hepatic congestion found in mice at exposure approximately 40 times of human  $AUC_{24h}$  at MRHD.

Ivabradine was not tumorigenic in dietary 104-week carcinogenicity studies in CD-1 mice and Wistar rats.

Ivabradine was negative in Ames test, mildly induced unscheduled DNA synthesis in primary rat hepatocytes (*ex vivo*) and was weakly positive in mouse lymphoma assay. The genotoxic response were observed at concentrations  $>15000$  times of human  $C_{max}$  at MRHD. The chromosomal aberration test in human lymphocytes was equivocal. Ivabradine was negative in three *in vivo* tests - mouse micronucleus test, rat chromosomal aberration test and rat liver UDS assay. Based on weight of evidence, the genotoxic risk for ivabradine at proposed therapeutic use is considered minimal.

Ivabradine had no effect on fertility in male and female rats. In pregnant rats treated during organogenesis, external abnormal shape of the heart (dysplasia) with or without simple anomalies of the major proximal arteries was observed at exposure close to human  $AUC_{24h}$  at MRHD and above. Teratogenic effects include interventricular septal defect and complex anomalies of the major proximal arteries observed at exposure 3 times human  $AUC_{24h}$  at MRHD. In pregnant rabbits treated during organogenesis, increased postimplantation loss was observed at exposure 5 times of human  $AUC_{24h}$  at MRHD and above. Reduced fetal and placental weights and a small number of fetuses with ectrodactylia were observed at 28 mg/kg (approximately 34 times human  $AUC_{24h}$  at MRHD). In the rat pre-postnatal study, reduced postnatal survival associated with interventricular septum defect and abnormal shape of the heart was observed in the F1 pups delivered by dams treated at 20 mg/kg, a dose that provides approximately 15 times of human  $AUC_{24h}$  at MRHD. Enlargement of the heart was observed in adult F1 rats at dosages  $\geq 7$  mg/kg (approximately 4 times of human  $AUC_{24h}$  at MRHD).

Ivabradine is excreted into milk and transferred to placenta in rats. Taken together, it is recommended that ivabradine be contraindicated in pregnant women, particularly during cardiac organogenesis. In view of enhanced postnatal mortality in rats, it should be avoided by lactating women.

There are no significant nonclinical safety issues for the proposed clinical use when labeling is revised as recommended.

## 2 Drug Information

### 2.1 Drug

### 2.1.1 CAS Registry Number (Optional)

Ivabradine: 155974-00-8

Ivabradine hydrochloride: 148849-67-6

### 2.1.2 Generic Name

ivabradine

### 2.1.3 Code Name

S 16257-2; AMG 998

### 2.1.4 Chemical Name

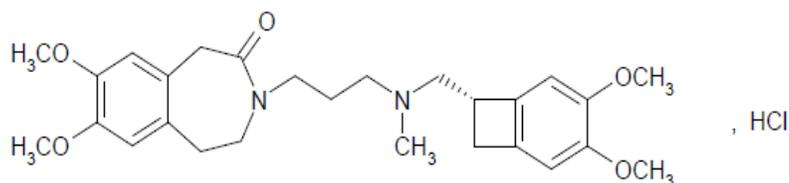
3-(3-(((7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl) methyl amino)propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride

### 2.1.5 Molecular Formula/Molecular Weight

C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>, HCl/505.1 g/mol;

468.593 g/mol (base); Conversion factor from the salt to the base: 0.928

### 2.1.6 Structure



### 2.1.7 Pharmacologic class

hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND (b) (4)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

Ivabradine is provided as a film-coated tablet in two strengths: 5 mg (oval) and 7.5 mg (triangular). The composition of the drug is listed in the table below (excerpted from Module 2.3. P, Table 1, Page 7)

**Table 1. Composition of Ivabradine 5 mg and 7.5 mg Tablets**

Component	5 mg		7.5 mg		Function	Reference to specifications
	Percentage (% w/w)	Quantity (mg/tablet)	Percentage (% w/w)	Quantity (mg/tablet)		
<b>Tablet</b>						
Ivabradine hydrochloride <sup>a</sup> (free base equivalent)	5.39 (5.00)	5.390 (5.000)	8.085 (7.50)	8.085 (7.500)	Drug substance	In-house
Lactose monohydrate	(b) (4)				(b) (4)	USP/NF, PhEur.
Maize starch						USP/NF
Maltodextrin						USP/NF, PhEur
Magnesium stearate						USP/NF, PhEur
Colloidal silicon dioxide						USP/NF, PhEur
(b) (4)						USP
<b>Core Tablet Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>		
<b>Film-Coating</b>						
(b) (4) salmon <sup>c</sup>	(b) (4)				(b) (4)	See 3.2.P.4.1
Polyethylene glycol 6000						USP/NF, PhEur
(b) (4)						USP
<b>Total</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>		

<sup>a</sup> The molecular weights of Ivabradine hydrochloride anhydrous and ivabradine free base are 505.1 g/mol and 468.6 g/mol, respectively. The free base accounts for 92.77% of the salt.

(b) (4)

### 2.3.2 Comments on Novel Excipients

No novel excipients are used in the drug product.

### 2.3.3 Comments on Impurities/Degradants of Concern

The impurities were identified and 36 structures of impurities were assessed via DEREK analysis for mutagenicity potential. Two compounds (b) (4) were reported positive for mutagenicity from DEREK analysis that was communicated with CMC Reviewers, Drs. Wendy Wilson and Pei-I Chu (emails of 7/14/2014). The sponsor established (b) (4) ppm as the TTC for (b) (4) µg/day for each compound based at the maximum daily dose of 15 mg/day (7.5 mg bid). The sponsor is not proposing to include limits for these impurities in the final drug substance as their synthetic process controls presence of each to a level below (b) (4) ppm. Nevertheless, their batch release specifications should specify maximum permissible levels.

For general impurities, there are no control impurities for drug substance and drug product above the ICH limits based on email communication with CMC reviewers, Drs. Wendy Wilson and Pei-I Chu, on 7/14/2014. The discrepancy of the proposed specifications for impurities in drug substance in Toxicology

Tabulated Summary Section 2.6.7 and CMC Section 3.2.S.4.1 was resolved with the sponsor's response dated 7/30/2014 (see below). Therefore, no preclinical qualification studies are required for the impurities.

*"The proposed specifications for impurities in Section 2.6.7 Toxicology Tabulated Summary, Page 22-23 were not the final impurity specifications for commercial drug substance but rather toxicology specifications based on potential impurities that might have been above the ICH limit in the commercial drug substance. Degradation product studies [4-week rat (NP07572) and two genotox studies (NP07357 and NP07671)] were conducted during development to qualify potential impurities. However, as defined in 3.2.S.4.1, the proposed commercial drug substance specifications provided in Module 3 for specific impurities were set below the ICH limit requiring qualification ( $< \frac{(b)(4)}{(4)}5\%$ ), and a limit of  $\leq \frac{(b)(4)}{(4)}\%$  was applied to any impurity that was not specified. Therefore, no additional qualification is required and no control impurities above the ICH qualification limit are proposed, since commercial drug substance specifications were set below the ICH limit."*

## 2.4 Proposed Clinical Population and Dosing Regimen

Ivabradine is indicated to reduce the risk of (b) (4) hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm), (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4).

The proposed starting dose of ivabradine is 5 mg twice daily. After 2 weeks of treatment, if heart rate is 50 - 60 bpm, the dose of 5 mg twice daily should be maintained. The dose should be increased to 7.5 mg twice daily if resting heart rate is persistently above 60 bpm.

As listed in the table below (excerpted from M2/Section 2.4, Page 7), the human plasma exposures of ivabradine and its N-desmethylated metabolite (S 18982) were estimated at steady state in patients receiving the maximum recommended human dose (MRHD) of 7.5 mg bid. These values were derived from a population pharmacokinetic analysis using Phases II and III clinical data [Summary of Clinical Pharmacology Studies, in M2/Section 2.7.2 (2.1.2.2)], and are accepted as references when preclinical doses are expressed as multiples of human exposures for estimation of safety margins in this review, unless otherwise indicated.

**Table 1. Ivabradine and S 18982 (Metabolite) Plasma  $C_{max}$  and Estimated  $AUC_{24}$  at Steady State in Patients at HTD**

	Ivabradine (n=492)	S 18982 (n=541)
Population $C_{max}$ (ng/ml)	31 $\pm$ 9.8	7.9 $\pm$ 2.3
Equivalent $C_{max}$ in $\mu$ M	0.07	0.02 <sup>b</sup>
Population $AUC_{24}$ <sup>a</sup> (ng.h/ml)	346	128

Values are mean  $\pm$  SD;

a: Calculated from AUC over 12 h period from [WS (2.7.2) 3.1/ Table 12; Table 34 and 35]

b: S 18982 MW = (b) (4)

## 2.5 Regulatory Background

There was no IND opened in the US FDA

(b) (4)

(b) (4)

## 3 Studies Submitted

### 3.1 Studies Reviewed

#### Primary Pharmacodynamics

NP07239: Effects of S 16257-2 on rat isolated atria and aorta

NP15295: S16257-2: Time-dependent effects on isolated right rat atria and rabbit sinoatrial node tissue

NP07238: Effects of S 16257-2 on cardiac cellular electrical activity

NP05648: Study of the modes of action and specificity of S 16257.

Assessment of the effects of its metabolite, S 18982, on pacemaker current (I<sub>f</sub>)

NP06823: Effect of S 16257-2 on the I<sub>f</sub> pacemaker current of rabbit isolated sino-atrial node cells

NP08274: S 16257-2: in vitro mechanism of action on pacemaker I<sub>f</sub> channels in rabbit cardiac sinoatrial node cells

NP23227: S 16257-2: Effect of ivabradine on I<sub>f</sub> current in single human atrial myocyte

NP16268: S 16257-2: Mechanism of action on HCN channel isoforms expressed in the cardiac pacemaker tissue

NP07905: In vitro testing of S 16257-2 and its metabolite S 18982-1 on I<sub>f</sub> current expressed by recombinant human HCN2 and HCN4 channels

NP26949: Identification of molecular site of ivabradine binding to HCN4 channels

NP07237: Bradycardic effect of S 16257-2 in conscious normotensive rat after single, intravenous or oral administration

NP06822, Bradycardic activity of S 16257-2 after repeated oral administration in normotensive conscious rat: a comparison with zatebradine

NP05188: Cardiac and regional haemodynamic effects of S 16257 in conscious Long Evans rats.

NP05189: Cardiac and regional haemodynamic effects of chronic treatment with S 16257 in conscious rats

NP05585: Hemodynamic and electrocardiographic effects of S 16257-2 in anesthetized pigs: a comparison with S 16260-2 and zatebradine

NP32698: Improvement of angiogenesis and vascular protection by ivabradine in a rat model of left ventricular dysfunction and chronic heart failure

NP15079: S 16257-2: In vivo Acute and Chronic Effects on Heart Failure in Rats. Investigators: Comparison with Metoprolol

NP23866: S16257-2: Effect of chronic administration of ivabradine on left ventricular contractile properties in a rat model of chronic heart failure.

NP29503: Effect of long-term (3 months) therapy with ivabradine on LV function and remodelling in dogs with chronic heart failure

NP23359: S 16257-2: effect of chronic administration on survival and on the development of heart failure in 132-AR transgenic mice

NP26228: S 16257-2: Effects of sub-chronic administration of ivabradine on pressure overload-induced heart failure in rats

*NP28465: S 16257-2: Long-term effects of the combination ivabradine-perindopril on systemic and cardiac haemodynamics, left ventricular function and remodelling in a rat model of congestive heart failure*

*NP23867: S 16257-2: Effect of chronic administration of ivabradine on survival in a rat model of congestive heart failure*

*NP28523: Effects of long-term ivabradine administration on energetic metabolism and cardiac hypertrophy in a rat model of chronic heart failure*

*NP27414: Effects of long-term ivabradine administration on electrophysiological remodeling in a rat model of chronic heart failure*

*NP28171: Effect of heart-rate reduction (HRR) by ivabradine on endothelial dysfunction and angiogenesis in rats with heart failure (HF).*

*NP29363: S 16257-2: Effects of sub-chronic administration of ivabradine on angiogenesis and function of ischemic myocardium in the rat.*

*NP28361: S 16257-2: Effects of ivabradine on cardiac function, ventricular hyperexcitability and heart rate variability, in a rat model of myocardial infarction with LV dysfunction*

NP08114: Effects of five metabolites of S 16257-2 on isolated right rat atria

NP06262: S 16257-2, S 18982-1, Y 823-1, Y 796-1 AND Y 517-1: Evaluation and comparison of effects on heart rate in the unrestrained conscious rat following single intravenous or oral administration

## **Secondary Pharmacodynamics**

NP06824: Hemodynamic effects of S 16257-2 during exercise in pigs: a comparison with zatebradine and propranolol

NP05163: Comparative effects of S 16257 and propranolol on coronary and systemic hemodynamics and cardiac contractions in conscious dogs at rest and during exercise

NP15062: Comparative effects of ivabradine and atenolol on exercise-induced myocardial stunning in conscious dogs- Determinants of myocardial oxygen balance in control dogs

NP26754: Comparative effects of ivabradine and atenolol on post-systolic wall thickening in normal and stunned myocardium in conscious dog

*NP05587: Effect of S 16257-2 and zatebradine on exercise-induced*

*regional myocardial ischemia in pigs*

NP07580: Comparison of the effects of S 16257-2 and propranolol on exercise-induced regional myocardial ischemia in pigs after oral administration

NP26684: Infarct size reduction by ivabradine/Infarct size reduction by ivabradine given after the onset of ischemia

NP26861: Effects of ivabradine alone or in combination with trimetazidine on the threshold of ischaemic ventricular fibrillation in anaesthetized pigs

NP30119: Dose-effects of ivabradine alone or in combination with trimetazidine on the threshold of ischaemic ventricular fibrillation in anaesthetized pigs

NP07581: Effects of Ivabradine on exercise-Induced myocardial stunning in conscious dogs

NP23528: S 16257-2: Anti-ischaemic effect of ivabradine in isolated rabbit hearts

NP08201: S16257-2 : In Vivo Effect on Contractile Dysfunction during Myocardial Stunning and Cardiogenic Shock. Interactions with Dobutamine

NP29883: Effects of heart rate reduction on the progression of atherosclerosis in hypercholesterolemic rabbits

NP21466: S 16257-2: Effect of a chronic treatment on large artery structure and function in SHR and WKY rats

NP23868: Effect of heart rate reduction by ivabradine on endothelial dysfunction in dyslipidemic mice

NP29505: S 16257-2: Effect of ivabradine on erectile and endothelial dysfunction in Apolipoprotein E-deficient mice model of atherosclerosis

**Safety Pharmacology**

NP07025: Receptor-Binding Studies of S15544-1, and its Enantiomers, S16257-2 and S16260-2, Comparison with Zatebradine (b) (4)

NP07984: S 16257-2: In Vitro testing of S 16257-2 and its metabolite S 18982-1 on recombinant human HERG and KvLQT1 currents and comparison with zatebradine (Non-GLP)

NP07238: Effects of S 16257-2 on cardiac cellular electrical activity

NP07045: S 16257-2. Evaluation of Effect on Cardiac Action Potential in Canine Purkinje Fibers

NP15229: S 16257-2 and S 18982-1: In Vitro Evaluation of S16257-2 and its metabolite S18982-1 on Cardiac Action Potential in Isolated Canine Purkinje Fibers

NP15258: S16257-2 In vivo Evaluation of effects on blood pressure, heart rate and electrocardiogram after repeat oral dosing in conscious dogs

NP15814: S16257-2: In Vivo Effect on Tachycardia induced by dobutamine Infusion and Cardiac Inotropism in halothane Anesthetized Beagle Dog

NP32589: Relationship between the block of If current by IVA and pacemaker activity of isolated sino-atrial node cells

NP15299: In vivo evaluation of positive chronotropic agents on heart rate, electrocardiogram and haemodynamic parameters after single intravenous overdosing of ivabradine in chronically instrumented conscious dogs

NP08442: S 16257-2: In Vitro testing of S 16257-2 and its metabolite S 18982-1 on If current expressed by recombinant mouse HCN1 channel

NP15864: S 16257-2: In vitro effects on electrophysiological responses in mouse retinal cells

NP08129: S 16257-2: Effects on viability and function of retinal rods and cones and pigment epithelial cells

NP08493: S 16257-2: Effect of ivabradine on the immunolocalisation of the proteins involved in the retinal phototransduction cascade (rhodopsin, arrestin, transducin)

NP23620: S 16257-2: In vivo effects of acute and chronic ivabradine administration on pigmented and albino rat ERG, opsin and rhodopsin content

NP25528 S 16257-2: Effect of ivabradine on the progression of the retinal degeneration in a mouse model of retinitis pigmentosa

NP07027: Safety pharmacology evaluation of effects on the central nervous system

NP08101: S 16257-2 and Zatebradine (p.o.) Effects in the primary observation test in the rat

NP07032: S 16257-2: Evaluation of analgesic effect in the hot plate test in the rat following single oral administration

NP07028: S 16257-2: Evaluation of effect on respiration in the unrestrained conscious rat following single oral administration

NP03145: S 16257: Study of the effects of single oral dose on gastrointestinal transit in rats

NP03147: S 16257: Study of the effects of single oral dose on gastric secretion in rats

NP03148: S 16257: Study of the gastric ulcerogenic effects of a single oral dose in rats

NP03160: Fourteen-day continuous intravenous infusion toxicity study with S-16257-2 In the unrestrained rat

NP03146: Study of the effects of a single oral dose on diuresis and urinary electrolytes in rats given a saline overload

## **ADME**

*(Method validation: report number were listed in the review section of ADME)*

NP15185: SUMMARY OF PRECLINICAL BIOANALYTICS FOR DETERMINATION OF S 16257 AND ITS METABOLITE S 18982 - ANA ANALYSIS S 16257, S 18982 ANIMAL PLASMA, URINE

NP05245: PHARMACOKINETICS OF S 16257, ITS N-DEMETHYLATED METABOLITE S 18982 AND OF 14C-LABELLED MATERIAL AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF 14C-S 16257-2 TO MALE WISTAR RATS

NP15152: PHARMACOKINETICS OF S 16257 IN MALE WISTAR RATS AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16257-2

NP05258: Pharmacokinetics and excretion balance of 14C-labelled material after single oral and intravenous administration of 14C-S 16257 to male beagle dogs

NP15001: PHARMACOKINETICS OF S 16257 IN THE MALE BEAGLE DOG AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16257-2

NP16236: Pharmacokinetics of S 16257 and its metabolite S 18982 in male mice after repeat oral administration of ivabradine

NP08033: Pharmacokinetic study of S 16257 after oral administration in rats: building of a population model

NP15186: Pharmacokinetic mixed effects modelling of S 16257 after oral administration in the beagle dog-combined analysis

NP15183: IN VITRO PROTEIN BINDING OF S 16257 AND S 18982, THE N-DESMETHYLATED METABOLITE OF S16257 IN ANIMAL AND HUMAN PLASMA.

NP07530: PRELIMINARY STUDY OF THE IN VITRO PLASMA PROTEIN BINDING AND THE BLOOD TO PLASMA PARTITIONING OF <sup>3</sup>H-S 16257-2 IN RAT, DOG AND MAN

NP05420: QUALITATIVE AND QUANTITATIVE TISSUE DISTRIBUTION OF RADIOACTIVITY IN MALE LONG EVANS RATS FOLLOWING SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF 14C-S 16257-2 AT A DOSE (AS BASE FORM) OF 3 mg/kg

NP08034: S 16257 2 In Vivo ocular distribution and localization after a single administration of 14C-S 16257 in albino and pigmented rats

NP08639: The Secretion of Total Radioactivity in Milk of Lactating Rats Following Repeated Oral Administration of [14C]-S 16257

NP15184: Investigation into the cellular transport of S 16257 and S 18982 using the Caco-2 cell line

NP15182: Investigation into the cellular transport of S 16257 and some metabolites using the blood-brain barrier model

NP06900: In vitro characterization of human P450 isoforms involved in the hepatic metabolism of S 16257 and in vitro interspecies comparison

NP06887: In vitro metabolism of [14C]-S 16257 with rat, dog, monkey and human hepatocytes.

NP15163: INTERSPECIES COMPARISON OF THE IN VIVO METABOLISM OF S 16257

*NP06650: Metabolism of S 16257 in the rat following single oral and intravenous administration of [14C]-S 16257-2*

*NP06651: Metabolism of S 16257 in the dog following single oral and intravenous administration of [14C]-S 16257-2*

NP15187: A study to evaluate the plasma exposure of Wistar rats, CD-1 mice, beagle dogs and healthy volunteers to ten metabolites of S 16257 and S 16257 after repeated oral administration of S 16257-2

NP15001 PHARMACOKINETICS OF S 16257 IN THE MALE BEAGLE DOG AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16257-2.

NP15152: PHARMACOKINETICS OF S 16257 IN MALE WISTAR RATS AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16257-2

NP16270: PHARMACOKINETICS OF S 16260 IN MALE WISTAR RATS AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16260-2

NP05193: EXCRETION BALANCE OF 14C-LABELLED SUBSTANCES AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF 14C-S 16257-2 TO MALE WISTAR RATS

NP05222: BILIARY EXCRETION AND EXCRETION BALANCE OF 14C-LABELLED SUBSTANCES AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF 14C-S 16257-2 TO BILE DUCT CANNULATED RATS

NP06902: Assessment of induction of cytochrome P450 isoenzymes in the wistar rat after oral administration of S16257-2 for 4 weeks

NP15218: Determination of the kinetic parameters (Km, Vmax) of the biotransformation of S 16257 and S 18982 and assessment of drug interaction with therapeutic agents which are substrates or inhibitors of CYP3A4 using human hepatic microsomes

*NP07226: Assessment of any drug-drug interaction by concomitant incubation of human hepatic microsomes with S 16257 and therapeutic agents which are substrates or inhibitors of CYP3A4*

*NP25546: Complementary assessment of drug-drug interactions by concomitant incubation of human hepatic microsomes with S 16257 and therapeutic agents which are substrates or inhibitors of CYP3A4*

NP29916: Investigation of the contribution of cytochrome 3A to the metabolism of [14C]-S 16257

NP16117: Evaluation of the induction of cytochrome P450 3A4 involved in the metabolism of S 16257 by different glucocorticoids using human hepatocytes

NP16173: Activation profile of human nuclear receptor PXR by S 16257 and S 18982

*NP08070: Inhibitory properties of S 16257 on human CYP3A4*

*NP08471: Assessment of the inhibitory properties of S 16257 on human CYP2C18/19 and 2E1*

*NP16016: Complementary assessment of inhibitory properties of S 16257 on human cytochrome P450 using liver microsomes*

*NP31384: Interactions of S 16257 and S18982 with hepatic transporters  
NP32566: In vitro Interaction Studies of S 16257 with P-gp  
(MDR1/ABCB1) and BCRP (ABCG2) in the vesicular transport assay and in  
Bidirectional Transport (Papp) Studies on transfected MDCKII Monolayers and  
with human OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8), OCT1, OCT2,  
OAT1 and OAT3 Uptake Transporters  
NP32565: In vitro Interaction Studies of S 18982 with P-gp  
(MDR1/ABCB1) and BCRP (ABCG2) in the vesicular transport assay and in  
Bidirectional Transport (Papp) Studies on transfected MDCKII Monolayers and  
with human OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8), OCT1 Uptake  
Transporters*

### **General Toxicology**

NP03137: S 16257-02 - Single dose toxicity study by the intravenous route in the mouse.

NP03138: S 16257-02 - Single dose toxicity study by the intravenous route in the rat

NP03143: TOXICOLOGY STUDY OF S 16257-02 FOLLOWING SINGLE INTRAVENOUS ADMINISTRATION IN THE BEAGLE DOG

NP 03163: S 16257-02 - Single dose toxicity study by the oral route (gavage) in the rat.

NP03164: S 16257-02 - Single dose toxicity study by the oral route (gavage) in the mouse

NP03165: S16257-02: SINGLE DOSE ORAL (CAPSULE) TOXICITY STUDY IN THE DOG

NP03169: S 16257-02: 4 week oral (gavage) toxicity in the rat

NP03144: S 16257-02: 4 week oral (gavage) toxicity in the rat

NP05320: S 16257-2: subacute toxicity study four-week oral (gavage) administration in the Wistar rat

NP05319: S 16257-2: subacute toxicity study thirteen-week oral (gavage) administration in the Wistar rat

NP06240: S 16257-2: chronic toxicity study twenty-six week oral (gavage) administration in the Wistar rat

**NP07026: S 16257-2 -52 week oral (gavage) toxicity study in the rat;**

NP05181: S 16257-2: 4 week intravenous toxicity study in the rat

NP03162: Toxicology study of S 16257-2 following repeated administration for 4 weeks by oral route in the beagle dog

NP05574: Toxicity study of S-16257-2 by repeated oral administration for thirteen weeks in the beagle dog

NP06245: Toxicity study of S 16257-2 by repeated oral administration for twenty-six weeks to the beagle dog

**NP15280: S 16257-2 – One year oral (gavage) toxicity study in the beagle dog followed by a 12 week treatment-free period**

NP05108: Toxicity study on S 16257-2 by repeated intravenous administration for four weeks in the beagle dog

**Genetic Toxicology**

See studies listed in the review attached in Appendix 1

**Carcinogenicity**

See studies listed in the review attached in Appendix 2

**Reproductive and Developmental Toxicology**

See studies listed in the review attached in Appendix 3

**Special Toxicology**

NP32768: Acute Eye Irritation/Corrosion study with S 16257-2 (Ivabradine hydrochloride) in the New Zealand White rabbit

NP15279: Determination of the potential immunomodulating effects of S 16257-2 by means of the Plaque-Forming Cell assay and Lymphocyte Subset Analysis after 4 week oral dosing of male and female Wistar rats

NP08592: CYTOTOXICITY ASSAY IN VITRO WITH BALBIC3T3 CELLS:NEUTRAL RED (NR) TEST WITH S 16257-2 AT SIMULTANEOUS IRRADIATION WITH ARTIFICIAL SUNLIGHT

**3.2 Studies Not Reviewed**

*The following studies were not reviewed as they are nonpivotal, less relevant or redundant at the current stage.*

NP16020: S16257-2: Effects on cardiac pacemaker activity: a computer-based numerical simulation in rabbit sinoatrial cells

NP15080: Effects of two metabolites of S 16257-2 on isolated right rat atria (YI016-3 and YI021-3)

NP23256: S 16257-2: Relationship between selective I<sub>f</sub> current inhibition induced by ivabradine and changes in action potential parameters in rabbit sinoatrial node (SAN) cells

NP08000: Four-week Oral Study in Fischer, Sprague-Dawley and Wistar Rats;

NP08002: Four-week Oral Study in Wistar Rats;

NP03166: Two-week IV Study in Sprague-Dawley Rats;

NP15094: Twelve-week Oral study in Beagle Dogs;

NP15093: Two-Week IV Bolus Study in Beagle Dogs;

NP05349: Up to 2 Weeks IV Infusion Study in Beagle Dogs;

NP05514: S 16257-2: 4 week toxicity study by continuous intravenous infusion in beagle dog

NP15300: S 16257-2: In vivo evaluation of dose-effect relationship on blood pressure and heart rate after repeated oral administration in chronically-implanted conscious rats using telemetry

NP08001: EVALUATION OF HEMOLYTIC POTENTIAL OF SALINE SOLUTIONS OF S 16257-2 ON HUMAN BLOOD

NP32767: Assessment of Contact Hypersensitivity to S 16257-2 (Ivabradine Hydrochloride) In the Cba/J Mouse (Local Lymph Node Assay)

NP22686: LOCAL TOLERANCE STUDY BY INTRAVENOUS, PERIVENOUS AND INTRA-ARTERIAL ADMINISTRATION IN MALE NEW-ZEALAND WHITE RABBITS

NP32769: Primary Skin Irritation/Corrosion study with S 16257-2 (ivabradine hydrochloride) in the New Zealand White rabbit (semi-occlusive application)

NP07572: S 16257-2 (Batch FE 349): TOXICITY STUDY BY REPEATED ORAL ADMINISTRATION FOR 4 WEEKS IN WISTAR RATS

NP07357: S 16257-2 (Batch FE 349): DETECTION OF REVERSE MUTATION IN HISTIDINE-REQUIRING SALMONELLA TYPHIMURIUM AND TRYPTOPHAN-REQUIRING ESCHERICHIA COLI (Ames Test)

NP07671: S 16257-2 MICRONUCLEUS CYTOGENETIC ASSAY IN RAT BONE MARROW AFTER ORAL ADMINISTRATION

NP32504: S 16257: In Silico Analysis (Derek Software) For Mutagenicity of Related Structures: Actual and Potential Impurities and Synthesis Intermediates

NP33152: STARTING MATERIALS 2011 DETECTION OF REVERSE MUTATION IN HISTIDINE-REQUIRING SALMONELLA TYPHIMURIUM (AMES II TEST)

### **3.3 Previous Reviews Referenced**

NDA 206,143 Pharmacology/Toxicology Review (Genetic Toxicology) dated November 19, 2014: attached in Appendix 1.

NDA 206,143 Pharmacology/Toxicology Review (Carcinogenicity) dated November 18, 2014: attached in Appendix 2.

NDA 206,143 Pharmacology/Toxicology Review (Reproductive and Developmental Toxicology) dated November 25, 2014: attached in Appendix 3.

## **4 Pharmacology**

### **4.1 Primary Pharmacology**

Primary pharmacodynamics in vitro

**NP07239: Effects of S 16257-2 on rat isolated atria and aorta**

Atria and aorta tissues were prepared from male Wistar rats. Spontaneously beating right atria (n=7) were connected to a beat counter and left atria (n=9) were paced at 3 Hz (180 bpm). Five cumulative concentrations of ivabradine (0.55 - 5.5  $\mu\text{M}$ ) for spontaneously beating right atria and 0.1 - 100  $\mu\text{M}$  for paced left atria) were added at 30 minute intervals. The responses of right atria were recorded in the absence or presence of 0.1  $\mu\text{M}$  atropine. The effect of ivabradine (3  $\mu\text{M}$ ) on the positive chronotropic response to increasing concentrations of isoprenaline (1 - 55 nM) was also evaluated. Aortic rings (n=9) without endothelium were pre-contracted by depolarization with 80 mM external KCl and cumulative concentrations of ivabradine (1 - 100  $\mu\text{M}$ ) were then added to the bath.

In isolated right atria, ivabradine induced a concentration-dependent reduction (over the range 0.55 to 5.5  $\mu\text{M}$ ) of the spontaneous beating rate. After 30 minutes, the concentration that inhibited the initial rate by 30% ( $\text{IC}_{30}$ ) was 2.1  $\mu\text{M}$ . Atropine did not modify the rate-reducing response to ivabradine, demonstrating that the negative chronotropic action of ivabradine was not due to the activation of muscarinic receptors. Moreover, ivabradine did not modify the positive chronotropic response to isoprenaline ( $\text{EC}_{50}$ 's were 1.9 and 1.8  $\mu\text{M}$ , in the absence and presence of 3  $\mu\text{M}$  ivabradine, respectively), ruling out beta-adrenergic receptor antagonism as a potential mechanism.

In isolated paced left atria, ivabradine did not have a negative inotropic effect up to 100  $\mu\text{M}$ . Ivabradine at up to 1  $\mu\text{M}$  did not induce vasorelaxation on pre-contracted aortic rings. A slight vasorelaxant effect was noted at higher concentrations with  $\text{IC}_{50} = 29 \mu\text{M}$ .

**NP15295: S16257-2: Time-dependent effects on isolated right rat atria and rabbit sinoatrial node tissue**

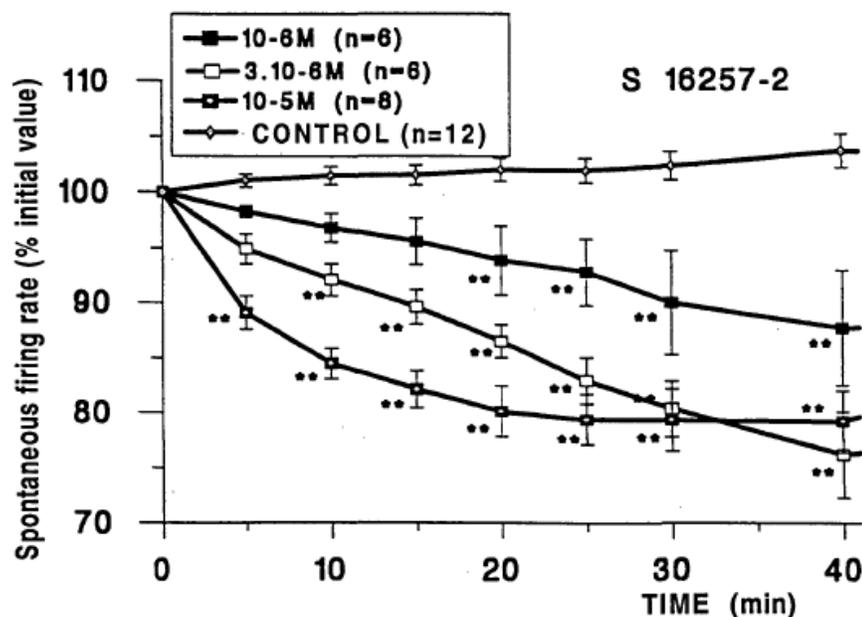
Spontaneous beating rate (beats per minute) was tested in right rat atria incubated with ivabradine at concentrations ranging from 0.056 to 0.56  $\mu\text{M}$  for 3 hours. Spontaneous firing rate (action potentials per min) was evaluated in rabbit SAN preparation for 3, 2 and 1.5 hour at ivabradine concentrations ranging from 0.1 to 1  $\mu\text{M}$  (testing low concentrations in longer durations than other studies).

S16257-2 decreased the atrial beating rate of isolated rat right atria in a time dependent manner, with a maximal effect between 2 and 3 hr. After 3 hr of incubation,  $\text{IC}_{30}$  value was  $\sim 0.2 \mu\text{M}$ . S16257-2 decreased the spontaneous action potential firing rate of rabbit SAN cells in a concentration-dependent manner:  $-10 \pm 1\%$ ,  $-14 \pm 1\%$  and  $-17 \pm 1\%$  reductions of basal rate were observed after S 16257-2 exposure 3 hours at 0.1, 0.3 and 1  $\mu\text{M}$ , respectively. The study indicated that bradycardic effect may result from a reduction in the diastolic depolarization slope, thus prolonging the diastolic depolarization time.

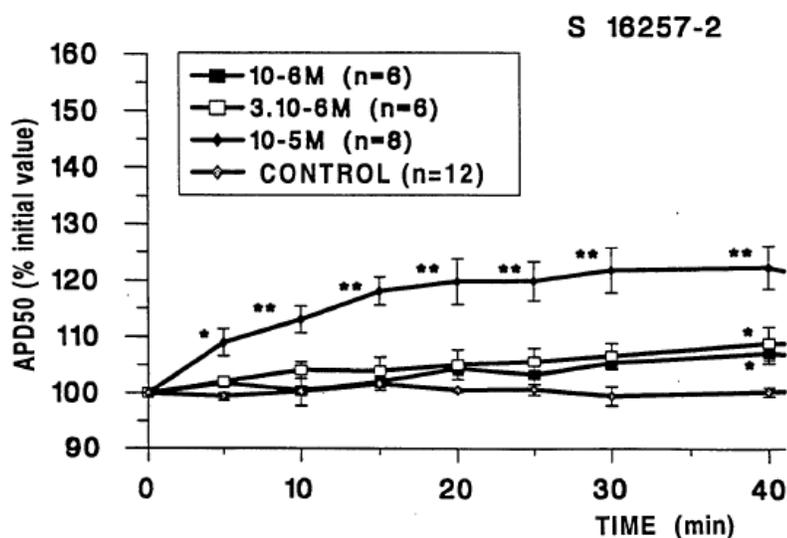
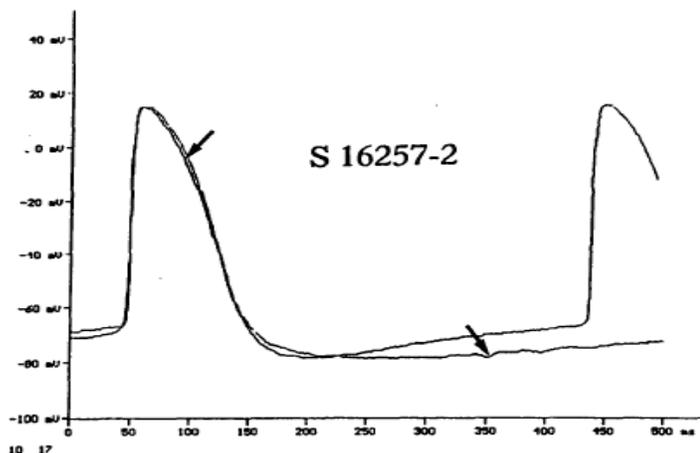
### NP07238: Effects of S 16257-2 on cardiac cellular electrical activity

The effects of S16257-2 on action potential duration in multiple isolated cardiac tissues were reviewed in Section of Safety Pharmacology.

In one part of this study, the electrical activity was evaluated in isolated rabbit sinoatrial node (SAN) preparations (n=6-12) using a conventional microelectrode method. From a concentration of 1  $\mu\text{M}$ , S 16257-2 induced a significant decrease in the spontaneous firing rate of pacemaker cells isolated from rabbit sinoatrial node (SAN). Peak effect, observed after 40 minutes incubation, was  $-12.3 \pm 5.3\%$ ,  $-23.8 \pm 3.9\%$  and  $-20.9 \pm 2.8\%$  at concentrations of 1  $\mu\text{M}$ , 3  $\mu\text{M}$  and 10  $\mu\text{M}$ , respectively (see the figure below, from page 23 of the report). It did not significantly affect maximum diastolic potential (MDP), cycle length or threshold potential (TP) for onset of action potential (AP) recorded from these cells. The representative recording of S 16257-2 at concentration of 3  $\mu\text{M}$  was shown in the figure below (excerpted from page 24 of the report).



At concentration of 3  $\mu\text{M}$ , S16257-2 increased APD50 moderately (maximum prolongation:  $8.9 \pm 2.9\%$ ) and was only significant at time points 30 and 40 minutes (see the figure below for effects of 3 concentrations, from page 26 of the report).



**NP05648: Study of the modes of action and specificity of S 16257. Assessment of the effects of its metabolite, S 18982, on pacemaker current ( $I_f$ )**

The effect of S 16257 on hyperpolarization-activated ( $I_f$ ), delayed-rectifier potassium ( $I_K$ ), T- and L-type calcium ( $I_{Ca,L}$  and  $I_{Ca,T}$ ) currents were evaluated in single pacemaker cells isolated from rabbit SAN using whole cell patch-clamp technique. The functioning of the  $I_f$  channels was carefully observed using the 'cell-attached', macro patch method to record the channel activity of proteins on a whole cell membrane function and 'inside-out patch' method to access the inner surface to study the channels on the cytosolic side.  $I_f$  was elicited by hyperpolarizing steps to -100 mV (1.8 s duration) from a holding potential of -30 mV. Other currents were elicited by depolarizing pulses: from -45 mV to 40 mV for  $I_K$ , from -80 mV to -20 mV for  $I_{Ca,T}$ , from -45 mV to 0 mV for  $I_{Ca,L}$ . For each current, pulse frequency was 1/6 Hz. Increasing concentrations (1 - 100  $\mu$ M) of ivabradine were perfused as indicated.

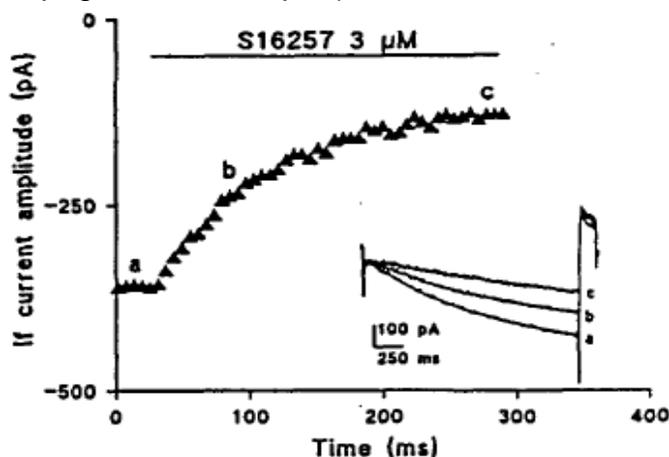
The percentage decrease in  $I_f$  is shown in the table below (excerpted from M2/2.6.2. page 29). Up to 3  $\mu\text{M}$ , ivabradine induced a selective inhibition of  $I_f$  without affecting other tested currents. At 10  $\mu\text{M}$ , it inhibited  $I_f$  by 80% while it had minimal effect on the slow calcium current  $I_{Ca,L}$  and  $I_K$ , and no effect on  $I_{Ca,T}$ .

**Table 6. Effects of ivabradine on  $I_f$ ,  $I_{Ca,L}$ ,  $I_{Ca,T}$  and  $I_K$  in Rabbit SAN Cells**

[ivabradine] ( $\mu\text{M}$ )	% of control current amplitude			
	$I_f$	$I_{Ca,T}$	$I_{Ca,L}$	$I_K$
1	32 $\pm$ 3	-	-	-
3	59 $\pm$ 2	-	0	0
10	80 $\pm$ 2	0	18 $\pm$ 1	16 $\pm$ 1

Values are mean  $\pm$  SEM; n=8 to 12; - not determined

S 16257 rapidly penetrated the cell and then attached itself to the inner surface of the  $I_f$  type channel in the open state. This directly led to a 'use-dependent' phasic blocking of the cytosolic side with a significant concentration-dependent decrease in the pacemaker current  $I_f$ . The reduction in  $I_f$  current caused by S 16257 was not associated with a shift in the activation curve, suggesting a decrease in the overall conductance of  $I_f$ . S 16257 similarly reduced  $I_f$  in both cell-attached and inside-out macro-patch configurations, indicating a direct interaction with f-channels from the inside of the cell. The "use-dependent" blockage over time at 3  $\mu\text{M}$  was representatively shown below (figure excerpted from page 52 of the report).



The graph showed changes in blockage over time of the amplitude of the inward current (evaluated by the difference between the initial current and the final current) before and during perfusion of the cells with S 16257. The figure in the insert show a series of three tracings of the  $I_f$  current recorded sequentially immediately prior to (a) and during perfusion of the cells with S 16257 (b, c).

S 18982, the active metabolite of S 16257, displayed a slower action than S 16257, but demonstrated a similar percentage of blocking of  $I_f$  current.

### **NP06823: Effect of S 16257-2 on the $I_f$ pacemaker current of rabbit isolated sino-atrial node cells**

The effects of S 16257-2 on  $I_f$  in single pacemaker cells isolated from the rabbit SAN were investigated using whole cell patch clamp techniques. Cells were then stimulated with hyperpolarizing voltage pulses (holding potential, HP = -30mV) between -50 and -110 mV during 2.4 s in order to verify the presence of hyperpolarization-activated current  $I_f$ . Only cells exhibiting  $I_f$  current and lacking inwardly rectifying background current  $I_{K1}$  were considered as SAN cells and kept for experiments. Cells were stimulated by a -100 mV hyperpolarizing pulse of 2 s duration, applied from a HP of -30mV and followed by a +10 mV pulse of 150 ms duration. This activation protocol was applied at a rate of 0.33 Hz.

S 16257-2 concentration-dependently reduced the pacemaker current  $I_f$ . It blocked the  $I_f$  current in a use-dependent manner without causing a shift of its activation curve. Under steady state conditions, the  $IC_{50}$  for inhibition of  $I_f$  amplitude was determined at 3  $\mu$ M. The concentration-dependent response curve was shown below (excerpted from page 13 of the report).

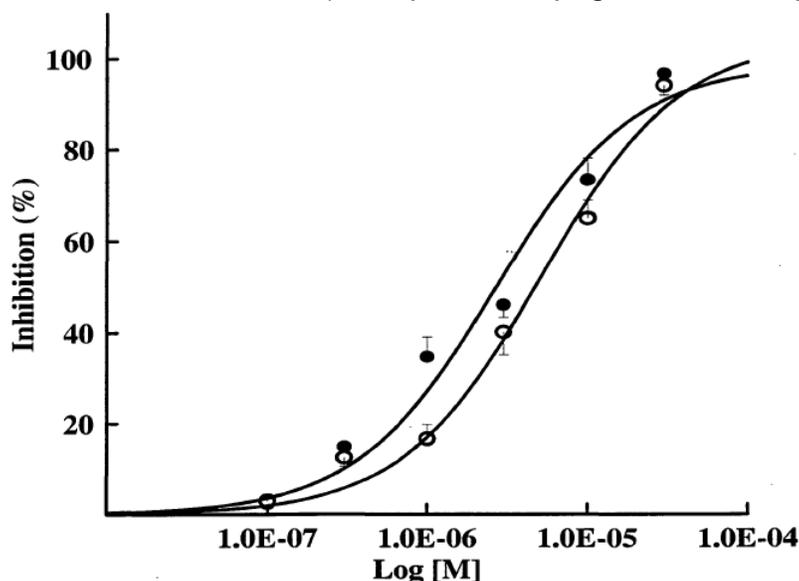


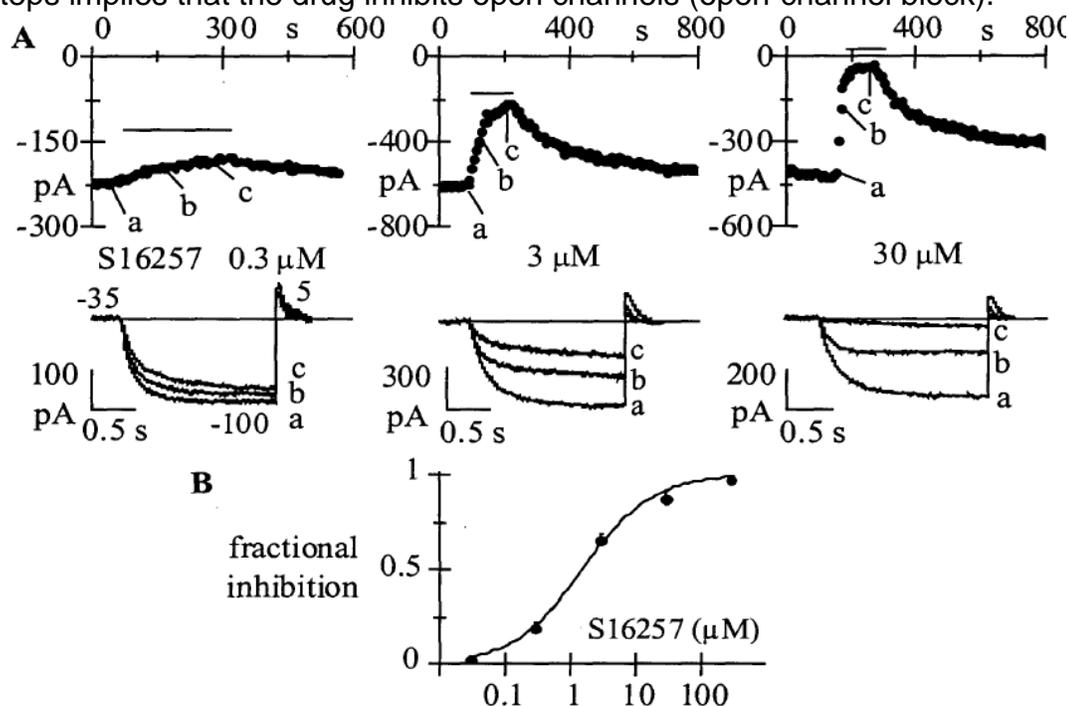
Figure 4 : Concentration - dependent response curve use - dependent block of  $I_f$  during application of S 16257-2 (●) or Zatebradine (○).

#### NP08274: S 16257-2: in vitro mechanism of action on pacemaker $I_f$ channels in rabbit cardiac sinoatrial node cells

In this study, the mechanism involved in the blockade of  $I_f$  channel was further characterized in isolated rabbit SAN using the patch-clamp technique in whole-cell configuration. The  $I_f$  current amplitude was measured at 32°C using a standard repetitive activation/deactivation protocol (-100 mV for 1.8 s/+5 mV for 0.45 s at 1/6 Hz), from a holding potential of -35 mV. On each cell, a given concentration of ivabradine (0.03 – 300  $\mu$ M) was perfused to construct the concentration-response curve. The use-dependent inhibition was further characterized using 3  $\mu$ M ivabradine and applying specific pulse protocols to establish the current-block relationships in normal and low- $Na^+$  media.

The parameters in the evaluation included: Use-dependence (block amplitude obtained with different voltage protocols);  $I_f$  block reversibility (time courses of  $I_f$  increase after S 16257-2 removal); Open channel block (evaluation of fractional block amplitude); Close-channel block (evaluation of fractional block amplitude); Voltage-dependence (fully activated  $I/V$  relation of  $I_f$  in control conditions and after correcting for steady-state block); Current-dependence (evaluation of block in open but caesium(Cs)-blocked channels, evaluation of block at the same voltage with different driving force voltage or current dependency, interaction with open channels and reversibility of the interaction).

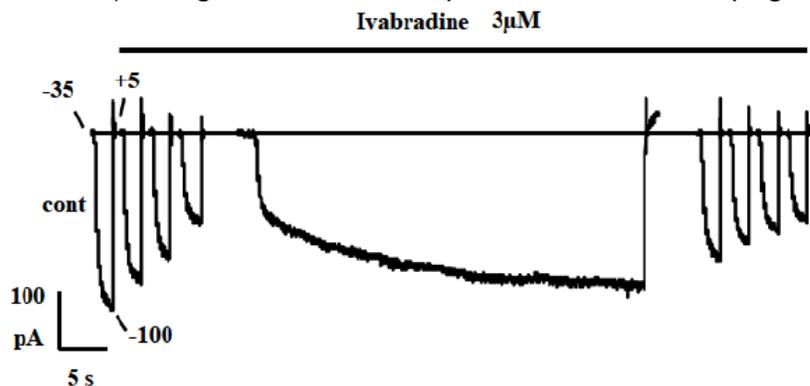
Using a standard activation/deactivation protocol, ivabradine showed a concentration-dependent block of  $I_f$  with  $IC_{50}$  of  $1.5 \mu\text{M}$ . At  $3 \mu\text{M}$ , ivabradine induced  $I_f$  blockage was use-dependent (as shown in the figure below, excerpted from page 20 of the report). The lack of current-block in the absence of activating steps implies that the drug inhibits open channels (open-channel block).



**Fig. 1. S16257 blocks  $I_f$ .** Activating/ deactivating steps ( $-100 \text{ mV} \cdot 1.8 \text{ s} / +5 \text{ mV} \cdot 0.45 \text{ s}$ ) were applied every 6 s from a holding potential of  $-35 \text{ mV}$ , and a given concentration of S16257 perfused until full block developed. A: time-course of  $I_f$  amplitude at  $-100 \text{ mV}$  during block onset and removal for three cells challenged with  $0.3$  (left),  $3$  (middle) and  $30 \mu\text{M}$  drug concentration (right). Lower panels show current traces recorded just before and during block development (a to c). B: dose-response relationship of  $I_f$  block by S16257 from a total of  $n=32$  cells (mean  $\pm$  SEM). Each cell was exposed to one drug dose only. The Hill equation fitted to data points resulted in a half-block concentration of  $1.5 \mu\text{M}$  and a Hill factor of  $0.8$ .

Ivabradine ( $3 \mu\text{M}$ ) achieved  $65.9 \pm 2.4\%$   $I_f$  inhibition ( $n=19$ ) with the standard protocol. However, when applied during a long lasting inward current elicited by a 40 s-long hyperpolarizing step at  $-100 \text{ mV}$ , it only slightly blocked steady-state  $I_f$  with  $6.4 \pm 1.6\%$  inhibition ( $n=5$ ). The results suggested that the block preferentially occurred when the current deactivated on depolarization

(deactivation step +5 mV), and was relieved when it activated on hyperpolarization (see figure below, excerpted from M2/2.6.2, page 35).



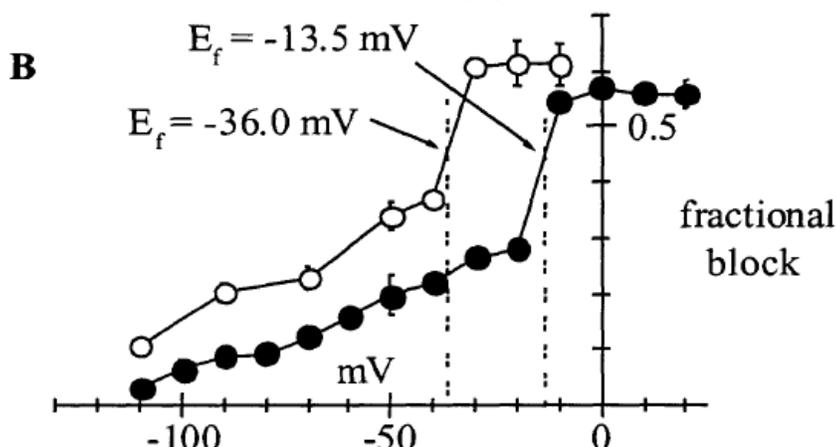
A 40 s-long hyperpolarizing step to -100 mV was preceded and followed by standard activation/deactivation protocols (-100/+5 mV; 1/6 Hz) during perfusion of ivabradine (3  $\mu$ M). Notice that  $I_f$  slowly increased during the long -100 mV pulse and the subsequent  $I_f$  amplitude using activation/deactivation protocol was higher than before the long hyperpolarization pulse.

The steady-state blockade in Tyrodes solution and low sodium solution was summarized in the table below (excerpted from page 6 of the report) and the block-curve was shown in the figure below (excerpted from page 26 of the report). The data suggested that voltage-dependence of S 16257 block may not be an intrinsic property of the binding, but was secondary to the current flow. And the block depended on the current direction.

**$I_f$  steady-state blockade by S 16257-2 3  $\mu$ M :**

mV	Tyr solution		Low Na solution	
	n	%block Mean $\pm$ sem	n	%block Mean $\pm$ sem
+20	3	55.8 $\pm$ 2.7		
+10	3	56.0 $\pm$ 1.3		
0	3	56.9 $\pm$ 1.3		
-10	3	54.5 $\pm$ 1.0	4	61.1 $\pm$ 3.8
-20	3	28.0 $\pm$ 1.2	5	61.5 $\pm$ 4.0
-30	3	26.6 $\pm$ 1.4	3	60.7 $\pm$ 1.6
-40	5	22.0 $\pm$ 1.2	5	37.1 $\pm$ 1.1
-50	3	19.6 $\pm$ 3.6	5	33.8 $\pm$ 2.6
-60	4	15.9 $\pm$ 1.6		
-70	4	12.4 $\pm$ 1.9	5	22.8 $\pm$ 2.1
-80	4	9.3 $\pm$ 1.3		
-90	5	8.7 $\pm$ 0.8	4	20.4 $\pm$ 1.2
-100	5	6.4 $\pm$ 3.0		
-110	4	3.0 $\pm$ 0.6	6	10.8 $\pm$ 0.9

**Mean Fractional Block-curve in Normal Tyrode Solution (Filled Circles) and in Low Na<sup>+</sup> Solution (Open Circles)**



Values are mean  $\pm$  SEM;  $n=3-7$  cells. Vertical dotted lines correspond to the  $I_f$  reversal potentials ( $E_f$ ). Arrows show the intercepts of the block curves with corresponding  $E_f$  values.

Overall, ivabradine is an open-channel blocker of  $I_f$  with an  $IC_{50}$  of  $1.5 \mu\text{M}$ . The use-dependent inhibition appears to be dependent on driving-force (current-dependent) than on voltage.

#### **NP23227: S 16257-2: Effect of ivabradine on $I_f$ current in single human atrial myocyte**

Right atrial appendages were obtained from 20 patients undergoing cardiac surgery (aorta-coronary bypasses, aortic valve replacements). Effect of ivabradine on human atrial  $I_f$  was investigated on the isolated human atrial myocytes using the patch-clamp technique. The HCN isoforms in human atria were characterized by multiplex RT-PCR. To examine the time course of the  $I_f$  block induced by ivabradine ( $0.1, 0.3, 1, 3, 10$  and  $30 \mu\text{M}$ ), a train of pulses from  $-30$  to  $-120 \text{ mV}$  was applied at the frequency of  $1/6 \text{ Hz}$  in whole-cell conditions at  $32^\circ\text{C}$  on these cells ( $n = 3 - 8$ ).

Ivabradine induced a marked concentration and use-dependent block of  $I_f$  with an  $IC_{50}$  at steady state of  $2.79 \pm 0.01 \mu\text{M}$ . A time constant of block development ( $\tau_{on}$ ) of  $98.8 \pm 4.8 \text{ s}$  ( $n = 8$ ),  $79.1 \pm 6.8 \text{ s}$  ( $n = 10$ ),  $37.3 \pm 4.4 \text{ s}$  ( $n = 6$ ) and  $16.3 \pm 4.0 \text{ s}$  ( $n = 3$ ) was observed for  $1, 3, 10$  and  $30 \mu\text{M}$  ivabradine, respectively. The concentration-dependence of  $I_f$  reduction in human atrial myocytes was comparable to that reported previously in rabbit cardiac pacemaker cells.

Recovery of  $I_f$  occurred preferentially upon hyperpolarization. Use-dependent block induced by ivabradine ( $3 \mu\text{M}$ ) was not modified in the presence of cAMP ( $10 \mu\text{M}$ ) in human atrial myocytes.

HCN gene subtypes, HCN 1, 2 and 4 in absence of HCN3, were detected using Multiplex RT-PCR on different biopsies extracted from human atrial tissues.

Major HCN gene subtype detected using Multiplex single cell RT-PCR on atrial single cell was HCN2 whereas HCN4 expression was weak and HCN1 expression was non-significant.

Overall, ivabradine blocked  $I_f$  in human atria from patients undergoing cardiac bypass surgery with characteristics similar to those described previously in rabbit sinus node cells. HCN2 was predominantly expressed in these human tissues.

### **NP16268: S 16257-2: Mechanism of action on HCN channel isoforms expressed in the cardiac pacemaker tissue**

$I_f$  is carried by hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels. Four different members of the HCN family (HCN1-4) have been identified and cloned in mammalian cells.

In this study, the effect of ivabradine on HCN channels were investigated in HEK293 cells transiently transfected with HCN clones (mouse HCN1 and human HCN4). HCN current were recorded using the patch clamp technique in a whole-cell configuration. Parameters to determine the mechanism of action on ivabradine on HCN1 and HCN4 channels included voltage or current-dependency, reversibility of the interaction, interaction with open and/or closed channel. Tested ivabradine concentrations in solution ranged between 0.03 and 300  $\mu$ M.

The results were summarized below (page 8 of the report):

<b>Effect of ivabradine on hHCN4 and mHCN1 channels</b>		
	<b>HCN4</b>	<b>HCN1</b>
<b>Activating/deactivating protocol: -100 mV*1.80 s; +5 mV*0.45 s, 1/6 Hz</b>		
<b>Ivabradine (3<math>\mu</math>M)</b>		
. Block (%)	58.2 $\pm$ 2.0 (n=5)	86.9 $\pm$ 1.6 (n=3)
. $\tau_{on}$ (s)	53.1 $\pm$ 5.5 (n=3)	63.1 $\pm$ 3.3 (n=3)
. Block reversibility	Limited	Limited
<b>Ivabradine concentration-response (0.03, 0.3, 3, 30, 300 <math>\mu</math>M)</b>		
. IC <sub>50</sub>	2.0 (n=22)	0.93 (n=25)
. Hill coefficient	0.8 (n=22)	1.3 (n=25)
. Open-channel block	Yes	No
. Close-channel block	No	Yes
. Use-dependent block	Yes	Yes
. Hyperpolarization block relieve	Yes	No
. Current-dependent block	Yes	No

Ivabradine blocked mHCN1 and hHCN4 in a concentration-dependent manner with similar affinity as that reported for native  $I_f$ -channel in rabbit SAN cells (1.5  $\mu$ M). However, the block of mHCN1 and hHCN4 was not identical in the following aspects:

- HCN4 block occurred only when hHCN4 channels were open whereas for mHCN1, ivabradine reaches its site of action also when channels were closed, although less easily.
- HCN4 block depends on the direction of current flow, although much less evident than for native  $I_f$ -channels.
- HCN1 block differs from that of hHCN4 and native rabbit  $I_f$ -channels. Ivabradine has no effect when HCN1 channels are kept open and moreover, the hyperpolarization is unable to induce the removal of the block. The binding/unbinding reactions are not allowed when channels are open, and the current flow does not affect the drug-channel interaction.

The study indicated a specific mechanism of action of ivabradine according to the type of HCN isoform.

#### **NP07905: In vitro testing of S 16257-2 and its metabolite S 18982-1 on $I_f$ current expressed by recombinant human HCN2 and HCN4 channels**

HEK293 cells were transiently transfected with hHCN2 and hHCN4 cDNA. HCN activities were recorded using the patch clamp technique in a whole-cell configuration. Currents were measured at room temperature, 2 to 3 days after transfection. Inward currents were elicited by hyperpolarizing pulses at -140 mV for 3 s (HCN2) or 7.5 s (HCN4) from -40 mV holding potential. This pulse-protocol was repeated every 20 s. After determination of the initial current amplitude, cells were perfused for 5 minutes with ivabradine (1 - 100  $\mu$ M) and the corresponding percent inhibition was calculated.

Both S16257-2 and S18982-1 inhibited  $I_f$  current induced by hHCN2 and hHCN4. Both of them showed greater affinity for hHCN4 than hHCN2 by 2-3 folds (IC<sub>50</sub> of S16257-2:  $3.6 \pm 0.4 \mu$ M and  $10.2 \pm 1.1 \mu$ M, respectively; IC<sub>50</sub> of S18982-1:  $2.6 \pm 0.4 \mu$ M and  $6.9 \pm 1.6 \mu$ M, respectively).

In both channels, the onset of block by S16257-2 and S18982-1 was slow (time constants in the range of minutes) and not reversible after washout. S 16257-2 and S 18982-1 did not alter the activation kinetics and voltage dependence of hHCN2 and hHCN4.

#### **NP26949: Identification of molecular site of ivabradine binding to HCN4 channels**

In this study, a molecular mapping of the binding site of hHCN4 was performed with a focus on residues of interest (A503, Y506, M508 and I510) based on experience of other compounds. WT and mutated (Mut) channels were expressed in HEK293 cells and the voltage dependence of kinetic parameters and affinity to ivabradine binding were measured by patch clamp technique in whole-cell configuration (32°C). The ivabradine affinity of Mut channels was measured by applying different concentrations of the drug during repetitive activation/deactivation steps (-140/+5 mV, 0.5 Hz; holding potential = -35 mV).

The results showed:

Residues Y506 and I510 are relevant for ivabradine affinity for the channel;  
Residues C478 and M5086 do not appear to determine the affinity but are relevant for the use-dependent properties;  
Residue A503 is important in controlling current recovery upon drug removal.

Overall, the study identified a series of residues that appear to control several aspects of ivabradine-induced block and removal of HCN4 channel.

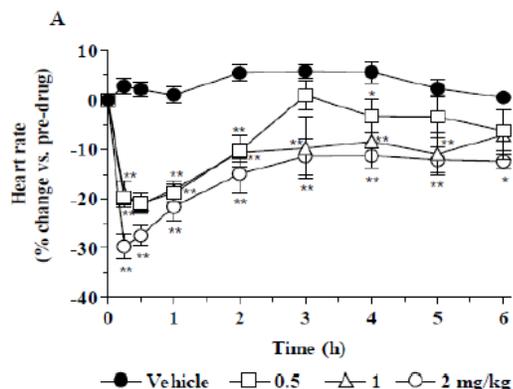
### Primary pharmacodynamics *in vivo*

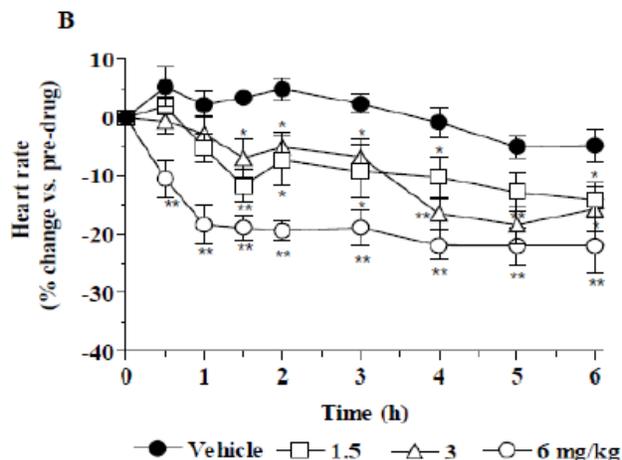
#### **NP07237: Bradycardic effect of S 16257-2 in conscious normotensive rat after single, intravenous or oral administration**

Male Wistar rats (n=6/dose) received ivabradine as a single intravenous (iv) bolus (jugular vein) at 0 (water), 0.5, 1, or 2 mg/kg or as a single oral dose (gavage) at 0 (water), 1.5, 3 or 6 mg/kg. MBP was recorded via femoral artery in unrestrained animals at regular intervals during the first 6 hours post-dose and after 24 hours. HR was integrated from the arterial pressure signal. Results were compared to those in vehicle-treated group.

A single iv dose of ivabradine induced a dose-dependent HR reduction (HRR) reaching ~20 and 30% at 0.5 and 2 mg/kg, respectively. As shown in the figures below (excerpted from M2/2.6.2. page 44), this effect occurred within 15 to 30 minutes and remained significant for 2 hours at 0.5 mg/kg and up to 6 hours at the highest dose. After single oral dosing, a dose-related HRR was also observed, reaching reduction of 14 to 22% within 6 hours post-dose. HR returned to baseline after 24 hours. MBP remained unchanged.

#### **Effect of ivabradine on HR After Single iv (A) or Oral (B) Administration in Conscious Rats**



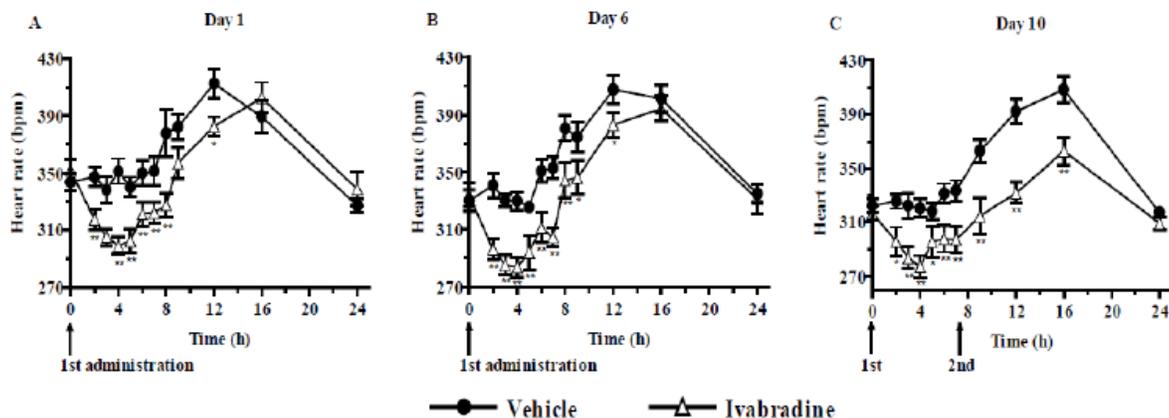


**NP06822, Bradycardic activity of S 16257-2 after repeated oral administration in normotensive conscious rat: a comparison with zatebradine**

Male Wistar rats (n=12) chronically instrumented with telemetric pressure transmitters were continuously monitored for HR and MBP after oral gavage of 3 mg/kg/d ivabradine for 10 days. On Day 10, a second administration was performed 7.5 hours after the first dosing.

During the 10-day treatment period of 3 mg/kg/d ivabradine, daily HR changes were similar from Day 1 with a significant HRR observed 2 hours after dosing, reaching maximal effect (~15% HRR) after 4 hours, and lasting up to 12 hours. The HR changes were shown in the figure below (excerpted from M2/2.6.2 page 45). There was no residual HRR 24 hours post-dosing. Rather, the marked rebound increase in heart rate would be noteworthy were it not also observed in the control cohort. On Day 10, a second oral gavage prolonged the HR reducing activity. MBP remained unchanged throughout the treatment period.

Overall, after oral administration of ivabradine at 3 mg/kg/d, a similar negative chronotropic effect is observed from Day 1 through Day 10, without any effect on mean blood pressure and no residual HRR 24 hours after the last administration.



Values are mean ± SEM; n=12  
(2<sup>nd</sup>) oral gavage

\*: p≤0.05, \*\*: p≤0.01 vs. vehicle

On Day 10, rats received a second

## Hemodynamic effects

### **NP05188: Cardiac and regional haemodynamic effects of S 16257 in conscious Long Evans rats.**

(note: the table summary sheet in this report should belong to Study NP05189)

Male Long Evans rats were chronically instrumented for the measurement of cardiac or regional haemodynamics (n=9 in each group). On separate experimental days, rats were randomized to receive iv. bolus of vehicle (5% dextrose) or S16257 at doses of 1 or 10 mg/kg. The two doses of S16257 were separated by 48 h washout period.

The result of hemodynamic changes (the analysis of integrated responses 1 h following injection) was summarized in the table below (excerpted from M2/2.6.2. page 46, same data from page 20 of the report). Ivabradine caused dose-dependent reductions in HR, MBP, cardiac index, and total peripheral conductance, together with dose-dependent increases in stroke index, peak aortic flow, dF/dtmax and central venous pressure.

% change vs. pre-drug over 1 h post-dose	Vehicle	ivabradine	
		1 mg/kg iv	10 mg/kg iv
Heart rate	-3±1	-33±2*	-57±9*
MBP	-3±1	-8±1*	-19±3*
Cardiac Index	-3±1	-18±1*	-41±2*
Stroke Index	-2±1	+21±2*	+32±3*
Peak aortic flow	-2±1	+4±1*	+8±1*
dF/dt <sub>max</sub>	-3±1	+2±2*	+6±2*
Total peripheral conductance	+4±1	-10±1*	-28±2*
Central venous pressure	-9±3	+9±2*	+49±8*

Values are mean ± SEM; n=9      \*: p<0.05 vs. vehicle

For regional hemodynamics, the bradycardic and depressor effects of S16257 were accompanied dose-dependent reductions in renal, mesenteric and hindquarters blood flows and vascular conductances over the first 1 h when compared to the control group.

At a single iv dose of 1 mg/kg, ivabradine produced a marked HRR reaching 33% within the 1st hour and remaining significant up to 6 hours. There was no sign of negative inotropic effect while slight increases in peak aortic flow and dF/dt<sub>max</sub> were observed.

At dose of 10 mg/kg, the extensive HRR of 57% was associated with more marked cardiac and regional hemodynamic changes, including a reduction in cardiac index that could not be prevented despite the increase in stroke index. Secondary to bradycardia, further reduction in MBP, total peripheral and regional conductance were also observed, i.e. increases in systemic and regional vascular resistance.

Overall, these results indicate that the bradycardic action of S16257 is not associated with any signs of negative inotropic action. The fall in cardiac index appears to be accounted for entirely by the profound reduction in heart rate, in spite of a substantial increase in stroke index. However, reduction in MBP, total peripheral and regional conductance (renal, mesenteric and hindquarters) are noteworthy.

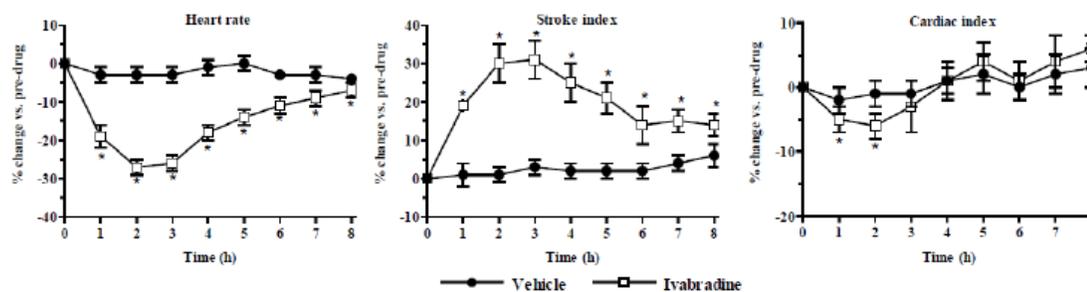
#### **NP05189: Cardiac and regional haemodynamic effects of chronic treatment with S 16257 in conscious rats**

*(note: the table summary sheet in this report should belong to Study NP05188)*

This study model was same as the above study (NP05188) except that vehicle (5% dextrose) or ivabradine (1 mg/kg/d) were administered subcutaneously for 4 days (n=9/group). Cardiac and regional hemodynamics parameters same as the study NP05188 were monitored each day for 8 h post-dosing.

For cardiac haemodynamics (figure below, excerpted from M2/2.6.2, page 47), S 16257 caused a marked HRR (20 - 25%), maximal 2-3 hours after injection and HRR lasted for ~8 hours. The extent of HRR was similar from Day 2 to Day 4, and was significantly greater than that seen on Day 1. A substantial increase in stroke index was observed, which may prevent a significant fall in cardiac index (peak effects on cardiac index were -6 and -14% from Day 1 and to Day 4, respectively). The MBP was only slightly reduced by 5% at the time of peak effect on HR on Day 1. The peak aortic flow and dF/dtmax slightly increased, suggesting the lack of direct negative inotropic effect.

**Figure 16. Hemodynamic Effects of ivabradine (1 mg/kg/d sc) in Rats on Day 1**



Values are mean  $\pm$  SEM; n=9      \*: p<0.05 vs. vehicle

For regional haemodynamics, no reduction in renal, mesenteric and hindquarters flows nor vascular conductances were observed, when compared to the controls.

Overall, treatment with S16257 at 1 mg/kg/day sc for 4 days resulted in substantial long duration bradycardia (no evidence of desensitization) without significant negative inotropic effects or impairment of regional perfusion.

#### **NP05585: Hemodynamic and electrocardiographic effects of S 16257-2 in anesthetized pigs: a comparison with S 16260-2 and zatebradine**

Anesthetized open-chest Large-White pigs received, at 30 minute intervals, four bolus iv doses of either vehicle (water, n=10) or ivabradine (n=10) at doses of 0.03, 0.1, 0.3 and 1 mg/kg. Animals were instrumented for monitoring of aortic and coronary blood flows (CBF), ventricular and arterial pressures and for recording of Lead II-ECG at 1, 3, 5, 10, 20 and 30 minutes after dosing. Myocardial oxygen consumption (MVO<sub>2</sub>) was calculated based on CBF and the difference between the oxygen content in aortic and coronary sinus blood samples.

The results were summarized in the table below (excerpted from M2/2.6.2, page 48). In control group, over the 2 hour period, heart rate and electrocardiogram intervals (PR and QTc) were stable along the experiment, but a progressive rise in total peripheral resistance and mean coronary vascular resistance, as well as a reduction of cardiac output, stroke volume and myocardial contractility (measured as LV dP/dt) and a slight increase in MBP were observed. These observations reflected the hemodynamic changes in the context of anesthesia.

	Mean % changes (vs. pre-drug)							
	Vehicle				ivabradine (mg/kg, iv)			
	1 <sup>st</sup> iv	2 <sup>nd</sup> iv	3 <sup>rd</sup> iv	4 <sup>th</sup> iv	0.03	0.1	0.3	1
Heart rate	-0.1	+1.0	+4.4	+3.5	-6.2	-14.1**	-23.5**	-29.3**
Mean blood pressure	+4.8	+4.6	+8.2	+9.2	+4.0	+4.1	-3.5	-5.5
LVdP/dt	-3.0	-9.3	-13.8	-20.2	-10.1	-17.4	-23.7	-28.5
Cardiac output	-2.0	-7.5	-9.3	-14.3	-8.4	-17.7	-23.8*	-28.3*
Stroke volume	-1.8	-8.1	-11.9	-16.8	-3.4	-3.4	+0.4	+2.7
Total peripheral resistance	+7.6	+13.8	+20.6	+28.7	+11.1	+22.8	+24.1	+28.9
Mean coronary vascular resistance	+10.6	+20.0	+28.2	+44.3	+9.4	+23.6	+33.8	+54.8
Myocardial oxygen consumption	+3.4	+6.4	-0.9	-4.5	-1.5	-11.7*	-21.4*	-31.6*
O <sub>2</sub> delivery / MVO <sub>2</sub> ratio	-1.8	-4.2	-4.6	-7.6	-2.6	-3.4	-5.2	-7.2

n=7-10 \*: p≤0.05, \*\*: p≤0.01 vs. vehicle

In this setting ivabradine induced a significant and dose-related HRR. This effect occurred within 20 minutes after dosing (1 minute at the highest dose), reaching 16, 27 and 37% vs. vehicle at 0.1, 0.3 and 1 mg/kg, respectively. PR-duration, QTc (Bazett's correction formula), MBP, LVdp/dt and total peripheral resistance were unchanged with treatment relative to vehicle. Cardiac output decreased significantly from 0.3 mg/kg with a maintained stroke volume, indicating the effect on stroke volume was not sufficient to prevent a reduction in CO greater than that observed with vehicle. In parallel with HRR, treatment of ivabradine resulted in a dose-related reduction MVO<sub>2</sub>, but did not modify the ratio of myocardial oxygen delivery/ MVO<sub>2</sub>.

#### Efficacy in LV dysfunction and heart failure models

##### **NP32698: Improvement of angiogenesis and vascular protection by ivabradine in a rat model of left ventricular dysfunction and chronic heart failure**

Myocardial infarction was induced by left coronary artery ligation in 10 week old male Wistar rats (CHF animals). In order to assess the respective contribution of acute and long-term HRR, CHF rats were randomly allocated to the following groups: untreated CHF rats (n=15 to 19), or CHF rats administered with ivabradine (10 mg/kg/day in diet) as a long-term treatment (90 days, starting 7 days after ligation; n = 11 to 18); or a delayed short-term treatment (4 days, starting 93 days after ligation; n = 6 to 9). A sham group was included.

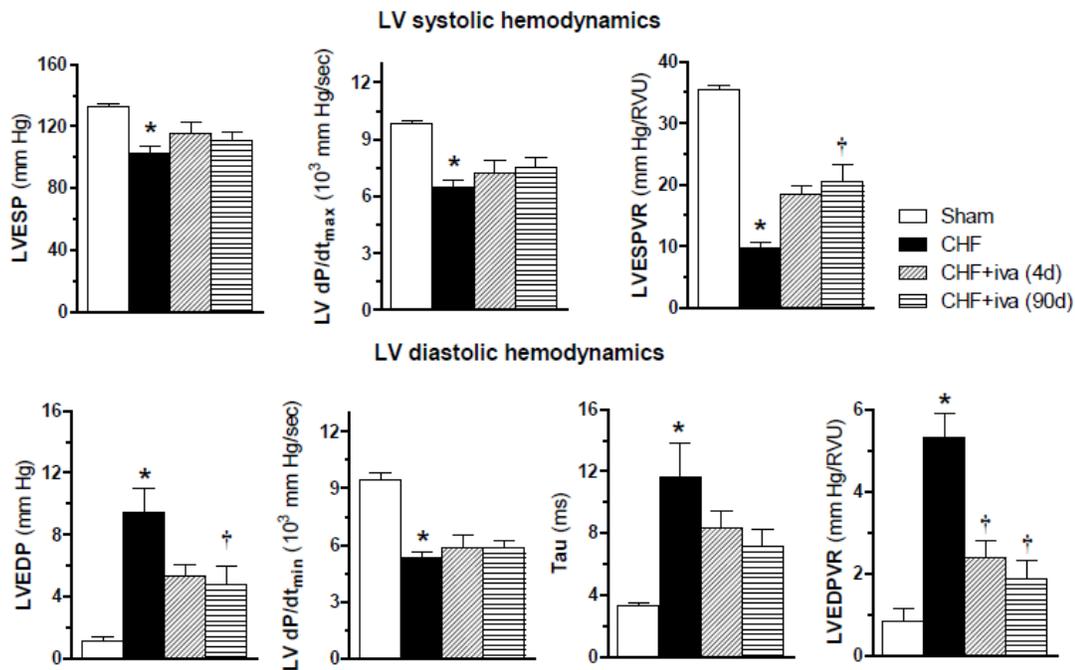
Parameters measured included cardiac function (echocardiography) assessed prior to (D7 or D93 post-ligation) and at the end of study period (D97 post-

ligation), systemic and LV hemodynamics (including pressure-volume curves) and LV myocardial interstitial collagen density assessed at the end of treatment period (D97 post-ligation). Arteriolar growth and endothelial function were also evaluated.

As compared to Sham, 90 days after ligation, untreated animals showed overt CHF with marked LV hemodynamic impairment, dilatation, and increase LV weight.

Ivabradine reduced HR after both short-term (-18%) and long-term (-14%) treatments, increased fractional shortening (short-term: +30%; long-term: +197%), and preserved cardiac output (CO) through increase in stroke volume (SV) (short-term and long-term +28%). After short-term ivabradine, this increase in SV was attributable in part to the Frank-Starling mechanism, as shown by the increase in LV diastolic diameter (CHF: +4%), associated with unchanged LV systolic diameter. Inversely, after long-term ivabradine, the improvement of SV was related to a slight decrease in LV diastolic diameter (-5%), associated with a marked decrease in LV systolic diameter (-20%).

Both short-term and long-term ivabradine treatment increased LV end-systolic pressure-volume relation (+92% and +114%), improved diastolic function as shown by improved LV filling (reduced LV end-diastolic pressure: -43% and -50%), isovolumic relaxation (Tau -29% and -39%), and compliance (LV end-diastolic pressure-volume relation: -55% and -65%). The maximal rates of increase/decrease of LV pressure (LV dP/dt<sub>max/min</sub>) and LV end-systolic pressure were not modified by both treatment durations.



\* p<0.05 vs Sham, † p<0.05 vs CHF; Iva: ivabradine

Systolic parameters: LVESP: left ventricular end-systolic pressure; LV dP/dt<sub>max</sub>: maximal rate of rise of left ventricular pressure; LVESPVR: left ventricular end-systolic pressure-volume relation.

Diastolic parameters: LVEDP: left ventricular end-diastolic pressure; LV dP/dt<sub>min</sub>: maximal rate of decrease of left ventricular pressure; LVEDPVR: left ventricular end-diastolic pressure-volume relation.

Overall, in a rat model of CHF, both short-term and long-term HRR by ivabradine improves LV systolic and diastolic dysfunction, demonstrating the contribution of acute beneficial effect of ivabradine, in addition to the long-term indirect effects such as decrease in LV dilatation and preservation of myocardial vascularization. The shift in pressure-volume relations suggests directly improved contractility, perhaps due in part to some reversal of structural LV defects shown in the rat CHF model reviewed below (NP23866)

#### **NP15079: S16257-2: In vivo Acute and Chronic Effects on Heart Failure in Rats. Investigators: Comparison with Metoprolol**

#### **NP23866: S16257-2: Effect of chronic administration of ivabradine on left ventricular contractile properties in a rat model of chronic heart failure.**

The same experimental model of chronic heart failure induced by post-myocardial infarction in rats was also used in these two studies to evaluate the beneficial effects of 3 months ivabradine treatments on LV remodeling *in vivo* [Report NP15079], as well as *ex vivo* [Report NP23866] LV function. Most results were similar to the study NP32698 above, which demonstrated beneficial effects of long-term treatment with ivabradine on LV function with an increase in stroke volume (SV) and a preserved cardiac output despite HRR.

The two original measurements from study NP15079 included plasma noradrenaline assessed at the end of treatment, and cardiac function (via echocardiography) assessed 3 days after treatment cessation. Long-term ivabradine treatment decreased the sympathetic nervous activity (D90: plasma noradrenaline -16% at 10 mg/kg/d) stimulated in chronic heart failure and partially normalized LV capillary and LV collagen densities (-12%) without change in heart weight. Three days after interruption of long-term treatment, the beneficial effects of ivabradine on LV geometry, LV shortening, and SV persisted despite normalization of HR, indicated improvement of myocardial LV structure as a basis for the improved pressure-volume indices of inherent contractility.

*In addition to the above three studies in rat ischemic heart failure model, the sponsor conducted other studies in rat heart failure model to explore different aspects related to the efficacy of ivabradine (Study Nos. NP28465, NP23867, NP28523, NP27414, NP29363, NP28171, NP28361). The main findings are highlighted in the table below (excerpted from M2/2.6.2, page 19).*

Rat (post-myocardial infarction) / po: 0.3 – 10; ip: 10.5	<ul style="list-style-type: none"> <li>- Increased stroke volume and maintained cardiac output.</li> <li>- Improvement of systolic and diastolic LV function.</li> <li>- Improved maximal myocardial perfusion and coronary reserve.</li> <li>- Increased cardiac angiogenesis.</li> <li>- Improved coronary and peripheral endothelial function.</li> <li>- No effect on survival.</li> <li>- Improved cardiac reverse remodeling:             <ul style="list-style-type: none"> <li>- reduced fibrosis.</li> <li>- down-regulation of local and central RAAS.</li> <li>- maintenance of cardiac energetic charge by optimizing energy consumption.</li> <li>- prevention of cardiac electrophysiological remodeling.</li> </ul> </li> <li>- Additive improvement of diastolic function in combination with an ACE-inhibitor</li> </ul>
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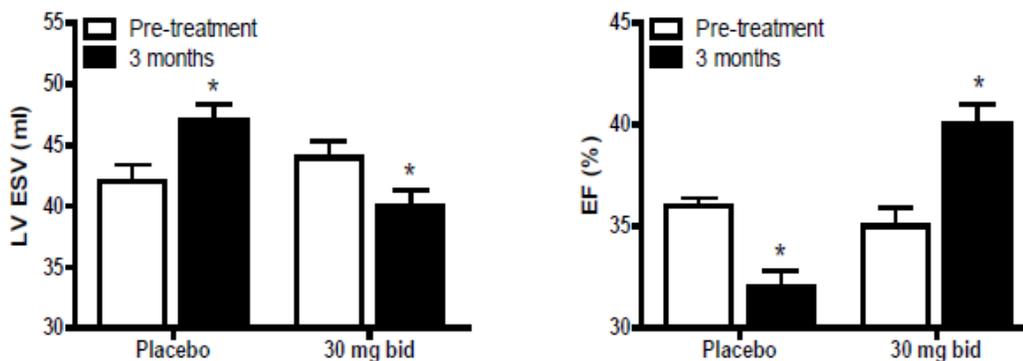
### **NP29503: Effect of long-term (3 months) therapy with ivabradine on LV function and remodelling in dogs with chronic heart failure**

CHF was produced by intracoronary microembolizations into the circumflex and anterior descending coronary arteries once weekly until LV ejection fraction (EF), reached 30 to 40%. Dogs were randomized to 3 months oral monotherapy with a low dose of ivabradine (15 mg twice daily, n=8) or a higher dose of ivabradine (30 mg twice daily, n=8) or no therapy at all (placebo, n=8).

Hemodynamic, ventriculographic, echocardiographic/Doppler measurements and 24 hour ambulatory ECG Holter monitoring studies were performed in all dogs at baseline, at pre-treatment and 3 months after initiating therapy. Multiple endpoints or markers for potential biochemical events/pathways were assessed.

Basically, HR was reduced by 13.8% (from  $91 \pm 11$  at pre-treatment to  $79 \pm 9$  bpm post-treatment) by ivabradine after 3 months of treatment. LV systolic function was significantly improved as evidenced by a decrease in LV end systolic volume (LV ESV) leading to an increased LV EF (see figure below, excerpted from M2/2.6.2, page 70).

**Figure 29. Effect of ivabradine on LV ESV and LV EF in Dog Model of HF**



Overall, the beneficial effect of long-term (3 months) monotherapy with ivabradine was demonstrated in this moderate heart failure model, shown by improving LV systolic and diastolic function and preventing progressive LV remodeling. The study also indicated that slowing down of the HR in experimental ischemic heart failure might initiate cellular and biochemical events that improve LV systolic and diastolic function in heart failure.

**NP23359: S 16257-2: effect of chronic administration on survival and on the development of heart failure in 132-AR transgenic mice**

**NP26228: S 16257-2: Effects of sub-chronic administration of ivabradine on pressure overload-induced heart failure in rats**

Chronic ivabradine treatment (delivered in drinking water or in food diet) was assessed in two models of CHF of non-ischemic origin. The murine model uses transgenic cardiac over-expression of  $\beta_2$ -adrenergic receptors (study NP23359). The rat model uses aortic banding to induce pressure overload (study NP26228). The reduction of heart rate with ivabradine neither reversed the progression of this cardiomyopathy and subsequent left ventricular dysfunction and cardiac death in the transgenic model; nor improved LV dysfunction in a rat model of pressure overload-induced heart failure. Although the sponsor dismisses these two specific models as either not predictive of clinical efficacy (transgenic mouse model), or not pertinent in regard to the targeted HF population for ivabradine (rat aortic banding), the lack of effect is noteworthy.

Pharmacodynamics of metabolites

**NP08114: EFFECTS OF FIVE METABOLITES OF S 16257-2 ON ISOLATED RIGHT RAT ATRIA**

A series of cleaved and uncleaved metabolites of ivabradine for their potential to reduce HR was tested in the right atria dissected from male Wistar rats (n=5-7/test compound). Tested compounds included ivabradine, 5 cleaved derivatives (Y 596 (M9), Y 609 (M3), Y 492, Y 831 (M10), Y 1044 (M30) and 2 uncleaved metabolites (Y 1016 (M28) and Y 1021 (M32)). Testing concentrations ranged 0.1-10  $\mu$ M. The two uncleaved metabolites, Y 1016 and Y 1021, each reduced atrial beating rate with a similar potency as ivabradine, with an IC<sub>30</sub> of 3.6  $\mu$ M and 4.9  $\mu$ M, respectively, vs. 2.1  $\mu$ M for ivabradine. In contrast, the five cleaved metabolites did not affect atrial spontaneous beating rate.

**NP06262: S 16257-2, S 18982-1, Y 823-1, Y 796-1 AND Y 517-1: Evaluation and comparison of effects on heart rate in the unrestrained conscious rat following single intravenous or oral administration**

Male Wistar rats (n=5-6) were instrumented for continuous Lead II-ECG recording. Each animal received on different days (at least 2-days apart) a single oral or i.v. dose of vehicle (saline) or S 18982 (M29), Y 823 (M22b), Y 796 (M26b) or Y 517 (M31) (6 mg/kg po or 2 mg/kg iv). ECG recordings were performed over 24 hours post dose.

Following i.v. administration, ivabradine and all administered metabolites induced a significant HRR over ~5 hours, with a maximal effect reached within 15 minutes to 2 hours. S 18982 was the most effective of the articles tested. After oral administration, only ivabradine, S 18982, and Y 517 significantly reduced HR over ~10 hours, with ivabradine and S 18982 similar in effect. The maximal effect was reached from 2.5 h to ~3.5 hours after oral administration.

**4.2 Secondary Pharmacology**Hemodynamic effects during exercise**NP06824: Hemodynamic effects of S 16257-2 during exercise in pigs: a comparison with zatebradine and propranolol**

Yucatan micro pigs (n=5) chronically instrumented for LV pressure, arterial pressure, and aortic blood flow monitoring, received on different days and in a randomized order, either a single oral dose of vehicle (water), ivabradine (5 mg/kg), or propranolol (5 mg/kg). Each experiment included four similar standardized treadmill-exercises (3.5 km/h for 5 minutes), i.e. before treatment and 1, 3 and 5 hours after dosing.

The effects of ivabradine on HR, LVdP/dt, cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR) were presented below (excerpted from M2/2.6.2. page 78). The results indicated that ivabradine or propranolol at the

same oral dose of 5 mg/kg produced comparable HRR, either at rest or during exercise. Ivabradine preserved the exercise-induced rise in myocardial contractility, supporting the absence of negative inotropic effect and compliments the increase in cardiac output induced by exercise, through an increase in stroke volume. Propranolol markedly differs from ivabradine with respect to the adaptation of myocardial contractility and cardiac output during exercise.

**Table 19. Hemodynamic Effects of ivabradine and Propranolol in Conscious Pigs During Exercise 3 hours Post-Treatment**

	HR	LVdP/dt	CO	SV	TPR
	Mean $\Delta\%$ change vs. control exercise				
Vehicle	+3.6 $\pm$ 3.4	+1.9 $\pm$ 6.1	+1.7 $\pm$ 6.3	-2.5 $\pm$ 2.1	-3.9 $\pm$ 5.0
ivabradine	-22 $\pm$ 3.9**	-6.5 $\pm$ 2.3	-12.4 $\pm$ 4.6*	+14.5 $\pm$ 7.8	+9.1 $\pm$ 8.5
Propranolol	-17.7 $\pm$ 1.2**	-25.0 $\pm$ 5.7**	-21.6 $\pm$ 3.1**	-4.6 $\pm$ 3.9	+33.6 $\pm$ 11.5**

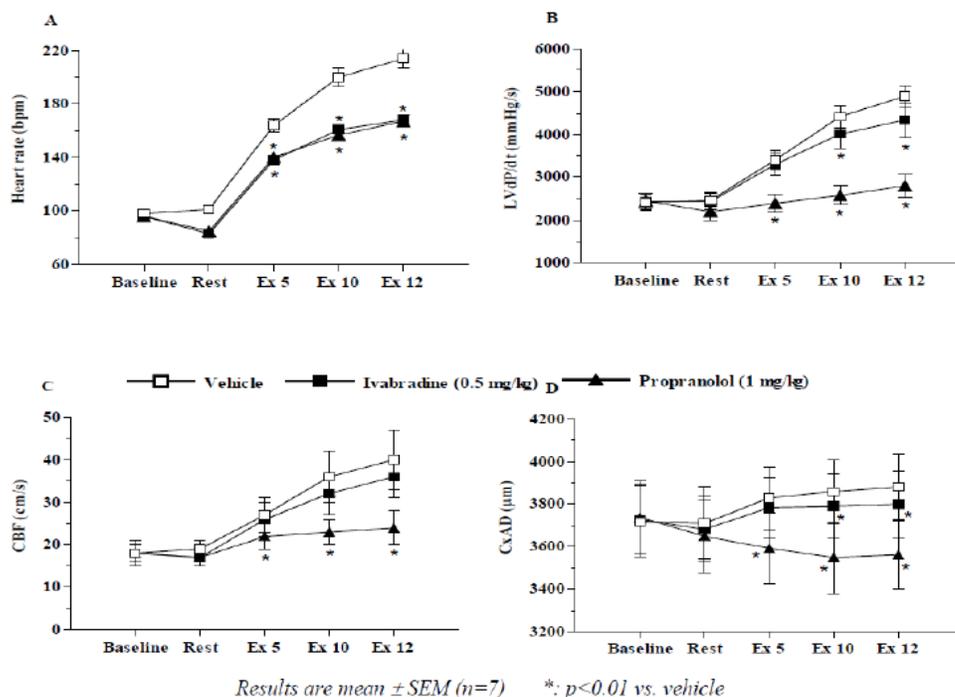
Values are mean $\pm$ SEM; n=5 (n=4 for CO and TPR); . \*:p<0.05, \*\*:p<<0.01 vs. vehicle.

### **NP05163: Comparative effects of S 16257 and propranolol on coronary and systemic hemodynamics and cardiac contractions in conscious dogs at rest and during exercise**

Male mongrel dogs were chronically instrumented for continuous monitoring of HR, hemodynamic (n=5) and coronary dynamic (n=7) parameters. Each dog received, on separate days and in a randomized order, a single i.v. dose of vehicle (saline), ivabradine (0.1, 0.3, 0.5 or 1 mg/kg) or propranolol (1 mg/kg). Each experimental sequence included measurements at baseline, at rest, and during graded treadmill exercise (5, 10 and 12 km/h, 5% slope- 3 min/grade). In 3 animals, the measurements were conducted under atrial pacing (~200 bpm). Ivabradine induced a dose-related HRR, significant at rest from 0.5 mg/kg i.v. (-16 and -23% at 0.5 and 1 mg/kg, respectively) and during exercise from 0.3 mg/kg (~ -10 and -30% at 0.3 and 1 mg/kg, respectively), as shown in the figure below (excerpted from M2/2.6.2. page 80).

In contrast to propranolol, ivabradine reduced HR without inotropic effects at rest and during exercise, and did not exert vasoconstriction of large coronary arteries.

**Figure 31. Effects of ivabradine and Propranolol on HR, LVdP/dt<sub>max</sub>, Coronary Blood Flow (CBF) and Coronary Artery Diameter (CxAD) in Conscious Dogs**



**NP15062: Comparative effects of ivabradine and atenolol on exercise-induced myocardial stunning in conscious dogs- Determinants of myocardial oxygen balance in control dogs**

Male mongrel dogs (n=8) were chronically instrumented for continuous monitoring of HR, hemodynamic parameters, arterial and venous blood O<sub>2</sub> content at rest or during exercise. Treatment with single iv dose of ivabradine (1 mg/kg) or atenolol (1 mg/kg) reduced HR similarly (~ -30% from 222  $\pm$  5 bpm). LV mean ejection wall stress was not altered. LVdP/dt<sub>max</sub> was reduced by atenolol but not ivabradine. MVO<sub>2</sub> was significantly lower under ivabradine and atenolol when compared to the control (6.7  $\pm$  0.6 and 4.7  $\pm$  0.4 vs. 8.1  $\pm$  0.6 ml/min, respectively). Under atrial pacing, MVO<sub>2</sub> was similar between ivabradine and saline, but significantly reduced with atenolol. Overall, ivabradine reduced myocardial stunning while atenolol worsened it due to its negative inotropic effect. The HRR and negative inotropic may independently contribute to the MVO<sub>2</sub> during exercise.

This study also demonstrated ivabradine preserved the exercise-induced acceleration of the rate of LV isovolumic relaxation, while atenolol, at similar HRR, markedly decreased the rate and extent of LV relaxation process, both at rest and during exercise (negative lusitropic effect).

**NP26754: Comparative effects of ivabradine and atenolol on post-systolic wall thickening in normal and stunned myocardium in conscious dog**

Male Mongrel dogs (n=6) were chronically instrumented for continuous monitoring of HR and hemodynamic parameters, in particular post-systolic wall thickness (PSWT), in a setting similar to the above dog studies. Treatment with a single iv dose of ivabradine or atenolol at 1 mg/kg caused similar HRR. However, ivabradine, in contrast to atenolol, did not alter PSWT and preserved a segment of thickening devoted to ejection in healthy dogs.

*More secondary pharmacodynamics studies (see the study numbers listed below) were conducted to further evaluate cardiovascular effects of ivabradine in disease models.*

*For Anti-ischemic activities:*

*Pig studies: NP05587, NP07580, NP26684, NP26861, NP30119;  
rabbit ex vivo: NP23528; Dog studies: NP07581, NP15062\*, NP26754\*  
\*these studies were reviewed separately above for hemodynamic effects.*

*For Other effects:*

*NP08201, NP29883 (rabbit), NP21466, NP23868, NP29505*

*The main outcomes of the studies are summarized in the table below (excerpted from M2/2.6.2, page 20).*

Test system / Dose range tested (mg/kg or as specified)	Main outcomes
<b>Anti-ischemic activity</b>	
Pig (fixed partial stenosis of LAD) / po: 5; iv: 0.5 Dog (partial stenosis of the LCX) / iv: 1	<ul style="list-style-type: none"> <li>- Significant limitation of ST-segment shift and myocardial contractility dysfunction in ischemic zone.</li> <li>- Rapid recovery of post-ischemic myocardial stunning.</li> <li>- Decreased myocardial O<sub>2</sub> consumption and increased diastolic perfusion time.</li> <li>- No effect on post-systolic wall thickening (PSWT) (contractility index, sign of ventricular performance) at rest and during exercise in normal dog whereas atenolol significantly prolongs this parameter.</li> <li>- Improvement of systolic function during stunning by conversion of ineffective PSWT to effective systolic fraction.</li> </ul>
Pig (total occlusion of LAD) / iv: 0.25 - 0.625	<ul style="list-style-type: none"> <li>- Protection against ventricular fibrillation and myocardial mitochondrial ultrastructural damage during acute ischemia.</li> </ul>
Pig (LAD hypoperfusion/reperfusion) / iv: 0.6	<ul style="list-style-type: none"> <li>- Reduction in infarct size with ivabradine given prior or after the onset of ischemia, increase in ischemic regional blood flow and contractile function.</li> </ul>
Isolated rabbit heart (ischemia/reperfusion) / 0.3 - 3 μM	<ul style="list-style-type: none"> <li>- Improvement of LV mechanical function; decrease in noradrenaline and creatine kinase releases; improved cardiac mitochondrial function; preservation of ATP content.</li> </ul>
<b>Acute effect of ivabradine in combination with dobutamine in contractile dysfunction models</b>	
Rat (stunning; aortic banding) / iv: 2	<ul style="list-style-type: none"> <li>- Prevention of dobutamine-induced tachycardia.</li> <li>- Preservation of the positive inotropic effect of dobutamine.</li> <li>- Augmentation of systolic ejection volume.</li> </ul>
<b>Effects on diastolic function</b>	
Hypercholesterolemic rabbit / po: 17	<ul style="list-style-type: none"> <li>- Prevention of diastolic dysfunction and cardiac fibrosis.</li> </ul>
<b>Effects on vascular structure and function</b>	
Rat (spontaneously hypertensive and normotensive) / sc: 8.4	<ul style="list-style-type: none"> <li>- Improvement in the mechanical properties of the carotid artery wall.</li> <li>- Anti-hypertrophic effects on the media of the thoracic aorta.</li> </ul>
Dyslipidemic mouse / po: 10 - 15	<ul style="list-style-type: none"> <li>- Prevention of endothelial dysfunction in aorta, corpus cavernosum, cerebral and renal arteries.</li> <li>- Prevention of aortic atherosclerotic lesions.</li> </ul>

LAD: Left anterior descending coronary artery; LCX: left circumflex coronary artery; LV: left ventricular; MPTP: Mitochondrial permeability transition pore; PSWT: post-systolic wall thickening

### 4.3 Safety Pharmacology

#### Receptor binding profile

#### NP07025: Receptor-Binding Studies of S15544-1, and its Enantiomers, S16257-2 and S16260-2, Comparison with Zatebradine <sup>(b) (4)</sup>

The receptor binding study was performed on 18 receptors and binding sites and the result for S16257-2 is shown in the table below (excerpted from page 8 of the report).

Receptor	Ligand	Reference	K <sub>0.5</sub> (M)				
			S 15544-1	S 16257-2	S 16260-2		
5HT <sub>1A</sub>	[ <sup>3</sup> H] 80 HDPAT	80HDPAT	2.0x10 <sup>-9</sup>	> 10 <sup>-4</sup>	2.9x10 <sup>-5</sup>	2.1x10 <sup>-5</sup>	
5HT <sub>1B</sub>	[ <sup>125</sup> I] Cyano-pindolol	Serotonin	4.1x10 <sup>-8</sup>	> 10 <sup>-4</sup>	1.2x10 <sup>-5</sup>	6.4x10 <sup>-5</sup>	
5HT <sub>2</sub>	[ <sup>3</sup> H] Ketanserin	Ketanserin	8.7x10 <sup>-9</sup>	1.4x10 <sup>-5</sup>	> 10 <sup>-4</sup>	2.6x10 <sup>-5</sup>	
5HT <sub>3</sub>	[ <sup>3</sup> H] BRL 43694	GR38032F	0.5x10 <sup>-9</sup>	» 10 <sup>-4</sup>	7.2x10 <sup>-5</sup>	5.6x10 <sup>-5</sup>	
Alpha 1 (-)	[ <sup>3</sup> H] Prazosin	Prazosin	1.2x10 <sup>-9</sup>	> 10 <sup>-4</sup>	5.8x10 <sup>-5</sup>	4.4x10 <sup>-5</sup>	
Alpha 2 (-)	[ <sup>3</sup> H] RX821002	(-) Adrenaline	4.9x10 <sup>-8</sup>	> 10 <sup>-4</sup>	> 10 <sup>-4</sup>	> 10 <sup>-4</sup>	
Beta	[ <sup>3</sup> H] Dihydro-alprenolol	Alprenolol	2.2x10 <sup>-9</sup>	> 10 <sup>-4</sup>	» 10 <sup>-4</sup>	» 10 <sup>-4</sup>	
D1	[ <sup>3</sup> H] SCH23390	SCH23390	0.8x10 <sup>-9</sup>	» 10 <sup>-4</sup>	> 10 <sup>-4</sup>	> 10 <sup>-4</sup>	
D2	[ <sup>3</sup> H] Raclopride	(+)Butaclamol	6.5x10 <sup>-9</sup>	» 10 <sup>-4</sup>	4.0x10 <sup>-5</sup>	5.9x10 <sup>-5</sup>	
Ach-M	[ <sup>3</sup> H] QNB	Atropine	4.0x10 <sup>-9</sup>	1.5x10 <sup>-5</sup>	1.3x10 <sup>-5</sup>	1.9x10 <sup>-5</sup>	
GABA <sub>A</sub>	[ <sup>3</sup> H] SR95531	SR95531	4.3x10 <sup>-8</sup>	> 10 <sup>-4</sup>	> 10 <sup>-4</sup>	> 10 <sup>-4</sup>	
H <sub>1</sub>	[ <sup>3</sup> H]Mepyramine	Promethazine	5.1x10 <sup>-9</sup>	> 10 <sup>-4</sup>	2.1x10 <sup>-5</sup>	1.0x10 <sup>-5</sup>	
Central benzodiazepine	[ <sup>3</sup> H] Ro15-1788	Ro15-1788	1.7x10 <sup>-9</sup>	» 10 <sup>-4</sup>	» 10 <sup>-4</sup>	» 10 <sup>-4</sup>	
A1	[ <sup>3</sup> H] R(-) Pia	R(-)Pia	5.5x10 <sup>-8</sup>	» 10 <sup>-4</sup>	» 10 <sup>-4</sup>	» 10 <sup>-4</sup>	
Mu opioid	[ <sup>3</sup> H] DAGO	DAGO	1.9x10 <sup>-8</sup>	6.4x10 <sup>-5</sup>	5.6x10 <sup>-5</sup>	7.5x10 <sup>-5</sup>	
Na <sup>+</sup> channel	[ <sup>3</sup> H] BTX	Veratridine	K <sub>0.5</sub>	2.2±0.3x10 <sup>-6</sup>	5.3±0.2x10 <sup>-6</sup>	7.0±0.2x10 <sup>-6</sup>	7.1±1.1x10 <sup>-6</sup>
			K <sub>1</sub>	1.1±0.1x10 <sup>-6</sup>	36±0.1x10 <sup>-6</sup>	4.0±0.3x10 <sup>-6</sup>	4.3±0.3x10 <sup>-6</sup>
Ca <sup>2+</sup> channel	[ <sup>3</sup> H] PN 200-110	Nifedipine	K <sub>0.5</sub>	2.2±0.5x10 <sup>-8</sup>	> 10 <sup>-4</sup>	> 10 <sup>-4</sup>	» 10 <sup>-4</sup>
			K <sub>1</sub>	1.5±0.3x10 <sup>-8</sup>			
Ca <sup>2+</sup> channel	[ <sup>3</sup> H] D888	D888	K <sub>0.5</sub>	2.5±0.5x10 <sup>-9</sup>	1.9±0.1x10 <sup>-6</sup>	1.9±0.1x10 <sup>-6</sup>	4.7±0.9x10 <sup>-6</sup>
			K <sub>1</sub>	2.0±0.4x10 <sup>-9</sup>	1.8±0.4x10 <sup>-6</sup>	9.2±0.9x10 <sup>-7</sup>	1.9±0.1x10 <sup>-6</sup>

The result showed lack of affinity of S 16257-2 for adrenergic (α<sub>1</sub>, α<sub>2</sub> and β), serotonin (5HT<sub>1A</sub>, 5HT<sub>1B</sub>, 5HT<sub>2</sub> and 5HT<sub>3</sub>), central benzodiazepine, dopamine (D1 and D2), adenosine (A1), histamine (H1), GABA<sub>A</sub>, mu-opioid, muscarinic cholinergic receptors and dihydropyridine binding site of the Ca<sup>2+</sup> channel. There was low affinity for the

phenyl-alkyl-amine binding site of the L-type  $\text{Ca}^{2+}$  channel ( $K_i = 0.9 \mu\text{M}$ ) and for the voltage-dependent  $\text{Na}^+$  channel ( $K_i=4.0 \mu\text{M}$ ) in the testing system. However, low affinity of binding may not predict a functional activity. At 2 and 3  $\mu\text{M}$  (concentrations close to the  $\text{IC}_{50}$  for If inhibition), ivabradine had no effect on  $I_{\text{Ca,L}}$ ,  $I_{\text{Ca,T}}$  and  $I_{\text{Kr}}$  in rabbit SAN. Only at high concentration (10  $\mu\text{M}$ ), when more than 80% If is blocked, is 18% reduction of  $I_{\text{Ca,L}}$  observed.

### Cardiovascular System

#### **NP07984: S 16257-2: In Vitro testing of S 16257-2 and its metabolite S 18982-1 on recombinant human HERG and KvLQT1 currents and comparison with zatebradine (NonGLP)**

The whole-cell patch-clamp technique was used to record HERG and KvLQT1 currents in transiently transfected COS-7 cells maintained at 35°C. The concentrations tested for HERG current were 0.3 to 10  $\mu\text{M}$ .

The results showed that S16257-2 induced a concentration-dependent block of HERG current, reaching significance for concentrations  $\geq 3 \mu\text{M}$ . The  $\text{IC}_{50}$  of S 16257-2 was  $4.85 \pm 0.88 \mu\text{M}$  and the current was blocked by  $69 \pm 5\%$  at a concentration of 10  $\mu\text{M}$ . S18982-1 was less active: the  $\text{IC}_{50}$  for S 18982-1 was  $15.88 \pm 5.2 \mu\text{M}$  and the current was blocked by only  $43 \pm 6\%$  at 10  $\mu\text{M}$ . The reference substance, zatebradine, blocked HERG current with an  $\text{IC}_{50}$  of  $3.02 \pm 0.55 \mu\text{M}$  and the positive control substance, E 4031, blocked the HERG current by  $78 \pm 5\%$  at a concentration of 0.1  $\mu\text{M}$ . S 16257-2 was approximately 100 fold less potent than E 4031 under the testing condition.

At concentration of 3  $\mu\text{M}$ , S 16257-2, S 18982-1 and zatebradine did not block recombinant KvLQT1-minK current (Iks).

#### **NP07238: Effects of S 16257-2 on cardiac cellular electrical activity**

Rabbit right atrium and purkinje fibers, and guinea pig papillary muscles were prepared. Action potential (AP) was recorded using conventional intracellular microelectrodes filled with 3M KCl (giving tip resistances of 15-20  $\text{M}\Omega$ ). In papillary muscle, cumulative concentrations of test articles were applied every 30 min over the range 0.1 - 10  $\mu\text{M}$ , and recordings were performed every 10 min. Effects on rabbit Purkinje fibers were assessed at 3 and 10  $\mu\text{M}$  over 40-min experiments, and reversibility of the effects was assessed after a 30-min washout period. For sinoatrial node (SAN), spontaneous firing rate was studied and the following parameters measured: AP threshold, maximum diastolic potential and cycle length (time between two APs). In all the preparations studied, action potential durations at 50%, 75% and 90% repolarization (APD50, APD75 and APD90) were analyzed. For papillary muscle, AP amplitude, resting potential and fast-phase depolarization rate (dV/dt) were also measured.

*The result from rabbit SAN was discussed in the primary pharmacodynamics section.*

AP data in guinea pig papillary muscles and rabbit purkinje fibers were summarized in the table below (excerpted from M2, section 2.6.2, page 106).

In guinea pig papillary muscles paced at 1Hz (60bpm), S 16257-2 produced slight AP prolongation  $+2.8 \pm 0.7\%$  (APD50 and APD90) and  $9.0 \pm 1.0\%$  (APD90) at concentrations of  $0.3 \mu\text{M}$  and  $3 \mu\text{M}$ , respectively and had no effect on the other papillary muscle AP parameters (amplitude, resting potential,  $dV/dt$ ).

In rabbit Purkinje fibers paced at  $0.25\text{Hz}$  (15bpm), at  $3 \mu\text{M}$ , S 16257-2 resulted in a slight and reversible AP prolongation (increased APD90 by  $+14.8 \pm 3.3\%$ , APD50 by  $+14.1 \pm 5.0\%$ ) when compared to zetabradine which prolonged APD90 by  $+86.0 \pm 15.4\%$  at the same concentration.

**Table 22. Effects of ivabradine on APD in Guinea-pig Papillary Muscle Paced at 1 Hz and Rabbit Purkinje Fibers Paced at 0.25 Hz (% Change vs. Pre-drug)**

[ivabradine] $\mu\text{M}$	Guinea-pig papillary muscle (n = 11)		Rabbit Purkinje fibers (n = 5)			
	ivabradine		ivabradine		Washout	
	$\Delta\%$ APD50	$\Delta\%$ APD90	$\Delta\%$ APD50	$\Delta\%$ APD90	$\Delta\%$ APD50	$\Delta\%$ APD90
0.1	$+1.7 \pm 0.6$	$+1.5 \pm 0.5$	-	-	-	-
0.3	$+2.7 \pm 0.8^*$	$+2.8 \pm 0.7^*$	-	-	-	-
1	$+6.1 \pm 0.6^{**}$	$+6.7 \pm 0.6^{**}$	-	-	-	-
3	$+7.3 \pm 1.1^{**}$	$+9.0 \pm 0.9^{**}$	$+14 \pm 5$	$+15 \pm 3$	$+0.6 \pm 3.8$	$+3.4 \pm 2.2$
10	$+4.4 \pm 1.2^{**}$	$+7.8 \pm 1.2^{**}$	$+46 \pm 7$	$+40 \pm 7$	$+15 \pm 4$	$+18 \pm 4$

APD = action potential duration at 50% or 90% repolarization; SEM = standard error of the mean. Values are mean  $\pm$  SEM. \*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  vs. vehicle. Purkinje fibers: no statistics. -: not determined

### NP07045: S 16257-2. Evaluation of Effect on Cardiac Action Potential in Canine Purkinje Fibers

AP was recorded using a conventional microelectrode method in isolated dog Purkinje fibers (n=6 preparations). Concentrations tested were  $0.1$ ,  $1$  and  $10 \mu\text{M}$ . Vehicle control and the method-control, astemizole at  $3 \mu\text{M}$ , were also included. Measured parameters included resting potential amplitude, maximal rate of depolarization, and duration of the action potential (APD30, APD50, APD70 and APD90).

S 16257-2 at concentrations of  $0.1$  and  $10 \mu\text{M}$  under normal stimulation frequency ( $60 \text{ ppm}$ ) induced no statistically significant changes on action potential parameters (resting potential (RP), amplitude of the action potential (APA), maximal rate of depolarization ( $V_{\text{max}}$ ) and duration of the action potential (APD30, APD50, APD70, APD90)) in isolated canine Purkinje fibers. At a concentration of  $1 \mu\text{M}$ , a statistically significant increase in APD70 and APD90 were observed. However, changes in APD70 and APD90 were very slight and were not confirmed at the concentration of  $10 \mu\text{M}$ . Therefore, these changes could be due to variability of parameters rather than to a specific effect attributable to S 16257-2.

### NP15229: S 16257-2 and S 18982-1: In Vitro Evaluation of S16257-2 and its metabolite S18982-1 on Cardiac Action Potential in Isolated Canine Purkinje Fibers

AP were recorded using a conventional microelectrode method in isolated beagle dog Purkinje fibers (n = 6/test compound). S16257-2, S18982-1 and the method-control, cisapride, were tested as separated groups each included 6 preparations. Measured parameters included APD50, APD70, and APD90. Five cumulative concentrations of test substance were tested over the range 0.1 - 10  $\mu\text{M}$  (every 30 min), each at 4 successive stimulation rates of 60, 40, 20 and 12 ppm. The method-control substance, cisapride (0.3  $\mu\text{M}$ ), was perfused at the end of the experiments with vehicle-Tyrode, and induced a significant increase in APD90 at all rates of stimulation.

S16257-2 and its metabolite, S18982-1, shared common electrophysiological characteristics regarding their effects on cardiac action potential. At all stimulation rates (from 60 to 12 ppm), S16257-2 and S18982-1 (0.1 - 10  $\mu\text{M}$ ) had no effect on AP resting potential, amplitude, and maximal rate of depolarization. S 16257-2 had no effect on APD50 at all stimulation rates at all doses whereas S18982-1 showed a slight but significant reduction of APD50 (-64 ms) at 10  $\mu\text{M}$  under rate of 60 ppm and an increase in APD50 (+65 ms) at 3  $\mu\text{M}$  under rate of 12 ppm. Both compounds at all doses at 60 and 40 ppm did not affect APD70 and APD90 but increased APD70 and APD90 at very low stimulation rates from 3  $\mu\text{M}$  at 20 ppm and from 0.3-1  $\mu\text{M}$  at 12 ppm. No early after-depolarization was observed. The effects on APD are illustrated in the table show below (excerpted from M2, section 2.6.2, page 108).

**Table 23. Effect of ivabradine on APD in Dog Purkinje Fibers (Change vs. Control of Mean APD Values)**

[ivabradine] ( $\mu\text{M}$ )	Stimulation frequency (ppm)			
	60	40	20	12
<b><math>\Delta</math> APD70 (ms)</b>				
1	-	-	-	+58*
3	-	-	+53*	+94***
10	-	-	+58*	+109***
<b><math>\Delta</math> APD90 (ms)</b>				
1	-	-	-	+70*
3	-	-	+69*	+119***
10	-	-	+95**	+145***

-: no effect. APD = action potential duration at 70% or 90% repolarization. \*: p<0.05, \*\*: p<0.01, \*\*\*: p<0.001 vs. vehicle

### NP15258: S16257-2 In vivo Evaluation of effects on blood pressure, heart rate and electrocardiogram after repeat oral dosing in conscious dogs

Six beagle dogs (n = 3/gender) were chronically instrumented with telemetric transmitters for continuous recording of BP and Lead II-ECG. Each dog received by gavage vehicle (saline) and ivabradine (0.5, 1.5, 5 and 15 mg/kg twice daily) for 5 days; each treatment was separated by a 48 h drug-free period. Telemetric measurements of

arterial blood pressure, heart rate and electrocardiogram started at least 24 hours before the first administration and were continued during the 5 days of each treatment period. Data were analyzed on Day 1 and 4 of treatment. Electrocardiogram by 6 leads was carried out and clinical observations were recorded on day 5 of each treatment period, before dosing and 3 hours post-dosing. Both qualitative and quantitative analyses of ECG parameters were performed on recordings from D1, D4 and D5. QT-interval was analyzed using both the Fridericia's correction for HR (QTcF) and the Sarma's analysis that compares the QT/RR relationship in treated animals with that observed in vehicle-treated animals. Blood samples were collected on day 5 of treatment for the assay in plasma of ivabradine and its metabolite S 18982.

On Day 1, the pharmacological activity of ivabradine on heart rate (HR) was not clearly established. On Day 4, a significant heart rate reduction (HRR) was observed from the dose of 1.5 mg/kg twice daily as shown in the table below (excerpted from page 12 of the report). The lowest heart rate was observed within 2 to 4 hours after dosing and was similar in the animals treated at 5 and 15 mg/kg twice daily. The larger magnitude of heart rate reduction was not achieved at highest dose when compared to that in the mid-dose, indicating a plateau effect. A dose-related increased incidence of occasional bradycardia (HR  $\leq$  50 bpm) was noted in animals treated at 1.5 (2 dogs), 5 (2 dogs) and 15 (4 dogs) mg/kg twice daily, respectively.

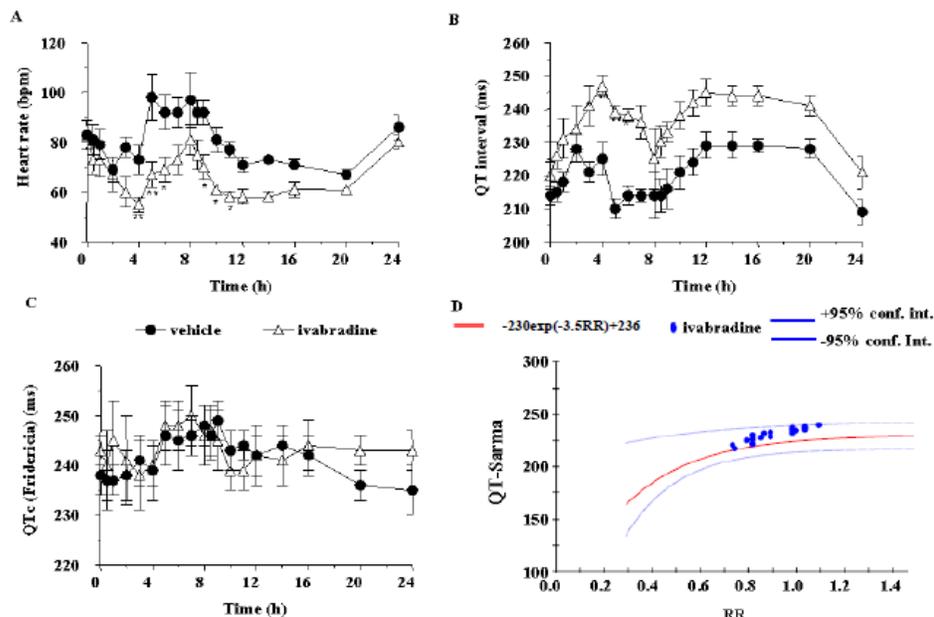
Table 1: Effect on heart rate on Day 4

Effect on heart rate on Day 4 (mean $\pm$ s.e.m)	Ivabradine (mg/kg twice daily)				
	Vehicle	0.5	1.5	5	15
Time of lowest HR (h)	4 $\pm$ 1	2 $\pm$ 0.4	2 $\pm$ 0.4	4 $\pm$ 0.2	4 $\pm$ 1
Lowest HR (bpm)	64 $\pm$ 3	63 $\pm$ 3	60 $\pm$ 4	54 $\pm$ 3	56 $\pm$ 4
(% change vs vehicle)	/	-1 $\pm$ 2	-7 $\pm$ 3	-15 $\pm$ 7	-12 $\pm$ 17
Mean daily HR (bpm)	78 $\pm$ 2	76 $\pm$ 3	73 $\pm$ 4	65 $\pm$ 3*	65 $\pm$ 3*
(% change vs vehicle)	/	-2 $\pm$ 2	-7 $\pm$ 4	-17 $\pm$ 2	-16 $\pm$ 2

Ivabradine did not affect diastolic or mean arterial pressure. Systolic pressure was slightly increased at the 2 highest doses (e.g. Emax: +24% and +20% at 5 mg/kg and 15 mg/kg, respectively).

PR interval, QRS complex duration, as well as QT corrected for heart rate, both using Fridericia's formula and Sarma's equation were not affected by ivabradine. The representative data at 2x5 mg/kg/d is shown in the figure below (excerpted from M2, section 2.6.7, page 112).

**Figure 46. Effects of ivabradine (5 mg/kg b.i.d. after 4 days on HR (A), QT-interval (B) QTcF (C), and QT-Sarma (D)**



Values are mean  $\pm$  standard error of the mean; n = 6. \*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  vs. vehicle

T-wave morphology was normal for all dogs. The main dose-related ECG changes included occasional bradycardia ( $\geq 2 \times 1.5$  mg/kg/d), sinoatrial block and sinoatrial arrest (from  $2 \times 5$  mg/kg/d). There was one animal with pre-existing ventricular escape complexes, and two with preexisting sinoatrial block which were not considered to affect the overall quality of the study.

The PK parameters and the exposure ratios based on human exposures are summarized in the table below (excerpted from M2, section 2.6.2. page 111).

**Table 25. Mean Plasma C<sub>max</sub> and AUC<sub>24</sub> for S 16257 and S 18982 on Day 5**

Dose (mg/kg/d)	C <sub>max</sub> (ng/ml)				AUC <sub>24</sub> (ng.h/ml)			
	Males		Females		Males		Females	
	Day 5	Multiple of hC <sub>max</sub>	Day 5	Multiple of hC <sub>max</sub>	Day 5	Multiple of hAUC <sub>24</sub>	Day 5	Multiple of hAUC <sub>24</sub>
<b>S 16257</b>								
2 x 0.5	62	2	24	0.8	183	0.5	75	0.2
2 x 1.5	391	13	198	6	1166	3	604	1.5
2 x 5	1572	51	935	30	5577	16	3769	11
2 x 15	4141	134	2911	94	23551	68	1506 2	44
<b>S 18982</b>								
2 x 0.5	2.3	0.7	-	-	-	-	-	-
2 x 1.5	13	1.5	6.1	0.8	-	-	-	-
2 x 5	158	20	56	7	406	3	316	2
2 x 15	446	56	252	32	2855	22	1830	14

-: not determined (insufficient data points). AUC<sub>24</sub> = Area under the concentration curve over 24 hours; C<sub>max</sub> = maximum concentration; hC<sub>max</sub> = Mean plasma maximum concentration at steady state in patients at the highest oral therapeutic dose.

Overall, treatment with ivabradine at dosages up to 2x15 mg/kg/day for 5 days (equivalent to C<sub>max</sub> up to 134 times of that in patients at MRHD) resulted in a specific HR reducing effect, without affecting MBP, DBP, and ECG parameters (PR interval, QRS complex duration and corrected QT interval). It is possible that sinoatrial arrest and/or sinoatrial block maybe the result of an exaggerated pharmacological effect in beagle dogs that present with an inherently high degree of vagal tone.

#### **NP15814: S16257-2: In Vivo Effect on Tachycardia induced by dobutamine Infusion and Cardiac Inotropism in halothane Anesthetized Beagle Dog**

Ten beagle dogs were anesthetized with thiopental (20 mg/kg, iv) followed by halothane (1.5%)/oxygen. Animals were instrumented with catheters and Doppler probe for monitoring of cardiac hemodynamics and contractility parameters and for ECG recording. Dogs were randomized in two groups and received dobutamine iv infusion with doses progressively increased to obtain a major tachycardia (+40 ± 10% of heart rate) that was maintained for at least one hour. Each group (n = 5) was then administered an iv bolus of vehicle or ivabradine (0.5 mg/kg) followed by a 1-hour infusion (0.5 mg/kg/h) with vehicle or ivabradine. All parameters studied were recorded at baseline, at predose (immediately prior to ivabradine or vehicle dosing), and at 2, 5, 10, 15, 30, 45, and 60 minutes after the start of ivabradine or vehicle infusion.

Dobutamine (mean cumulative dose of  $871 \pm 174 \mu\text{g/kg}$ ) induced tachycardia (heart rate: +49%,  $p < 0.05$ ), a slight increase in diastolic (+17%, NS), systolic (+17%,  $p < 0.05$ ), and mean blood pressures (+19% vs. baseline,  $p < 0.05$ ). Mean coronary flow was also increased by 115% ( $p < 0.05$ ), associated with a 40% ( $p < 0.05$ ) decrease in coronary resistance. Dobutamine infusion did not affect cardiac output but decreased stroke volume by -32% (vs. baseline,  $p < 0.05$ ). Ivabradine rapidly reduced heart rate and mainly reversed the dobutamine-induced tachycardia. Blood pressures progressively returned to baseline values during ivabradine administration. Dobutamine-related effects on mean coronary flow and coronary resistance were maintained throughout the one-hour infusion of vehicle or ivabradine. Ivabradine restored stroke volume to baseline values, thus maintaining cardiac output.

Dobutamine infusion markedly increased LVdP/dt(+), LV work and systolic LVP by 135%, 41% and 39%, respectively (vs. baseline,  $p < 0.05$ ), consistent with the marked positive inotropic effect of dobutamine. Ivabradine did not modify the positive inotropic effects of dobutamine on LVdP/dt(+), LV work and systolic LVP, although slight but non-significant variations occurred after 30 min of infusion.

Dobutamine decreased QT interval by  $34 \pm 8 \text{ ms}$  (vs. baseline,  $p < 0.01$ ) but did not affect QTcF interval. PR-interval was also decreased with dobutamine ( $-24 \pm 3 \text{ ms}$ ,  $p < 0.05$ ). QRS-complex duration remained unchanged. Ivabradine rapidly (within 5 min) restored QT and PR intervals to near baseline values, which remained stable up to the end of administration.

### **NP32589: Relationship between the block of $I_f$ current by IVA and pacemaker activity of isolated sino-atrial node cells**

$I_f$  and the Cav1.3-mediated L-type  $\text{Ca}^{2+}$  currents are important determinants of the diastolic depolarization and pacemaker activity. In humans, genetic mutations affecting  $I_f$ - and Cav1.3 channels underlie sino-atrial node (SAN) dysfunction. In the mouse, genetic inactivation of Cav1.3 channels induces a phenotype that closely mimics the characteristics of human "sick sinus syndrome" (SND). The current study was to evaluate the effect of ivabradine on ECG parameters measured in WT C57BL6/J mice and in Cav1.3<sup>-/-</sup> mice in which cardiac pacemaker activity has been affected by the deletion of Cav1.3 gene expression coding for a subunit of the L-type calcium channel (Cav1.3<sup>-/-</sup>).

Mice [WT ( $n = 6 - 7$ ) and Cav1.3<sup>-/-</sup> ( $n = 10 - 11$ )] received saline or ivabradine once i.p. (bolus) in a cross over design at doses of 0.1, 1, 3 and 6 mg/kg. Telemetric ECGs were recorded continuously over 8 hours. Quantitative (RR, PQ, QRS and QT intervals) and qualitative (AVB, VPC, APC) ECG analyses were performed. Each ECG tracing was qualitatively examined for any abnormality over a total duration of 2 minutes at -1, 0, 0.5, 1, 2, 4, 6 and 8 hours. After administration of each dose, some animals were given 4 days for washing-out of the drug.

In WT and Cav1.3<sup>-/-</sup> mice, Ivabradine dose-dependently reduced HR with a plateau-effect of -21% and -31%, respectively. Plateau on HR reduction occurred from 3 mg/kg (p<0.01 vs. saline) which corresponded to plasma concentrations of Ivabradine ranging from 0.6 µM (at 3 mg/kg) to 3 µM (at 6 mg/kg) in both strains.

In WT mice, ivabradine did not significantly affect ECG parameters other than HR. In Cav1.3<sup>-/-</sup> mice, most of ECGs presented typical abnormalities (2°AVB, 3°AVB, VPC, APC) of this engineered strain. Ivabradine up to 6 mg/kg abolished 2°AVB, in the absence of other adverse ECG effects.

In vitro, whole-cell patch-clamp technique on mouse SAN cells was used to evaluate the dose response of pacemaker activity and the quantity of I<sub>f</sub> blocked at the same Ivabradine plasma concentrations observed in mice. In isolated SAN cells from WT mice, pacemaker activity was concentration-dependently reduced by Ivabradine from 0.3 µM (-12%) to 1 µM (-38%), with a plateau-effect starting from 1 µM Ivabradine. Furthermore, Ivabradine concentration dependently inhibited I<sub>f</sub> activating during the whole diastolic depolarization and reached a plateau-effect at 1 µM.

**NP15299: In vivo evaluation of positive chronotropic agents on heart rate, electrocardiogram and haemodynamic parameters after single intravenous overdosing of ivabradine in chronically instrumented conscious dogs**

This study was to assess whether positive chronotropic agents used in the clinic, such as isoprenaline, atropine or dobutamine, would reverse the exaggerated cardiac effects that may occur in case of an accidental overdose with ivabradine.

Male mongrel dogs (n = 6) were chronically instrumented to continuously monitor cardiac function. Each dog participated to 10 successive treatment sessions, each including ivabradine iv infusion for 5 min (0.5 or 5 mg/kg) and 15 min later, an iv infusion of a positive chronotropic agent (isoprenaline, atropine or dobutamine) or vehicle. Since the dose of 5 mg/kg was well tolerated, the effects of ivabradine at maximal tolerated dose (MTD) (10 mg/kg iv) followed by either saline or isoprenaline were also assessed. Each treatment was separated by at least 48 h washout period. Plasma levels of ivabradine and its metabolite S 18982 were determined after dosing at 0.5 and 5 mg/kg.

The iv dose of 5 mg/kg was associated, after 20 min, with a mean plasma level of ivabradine 178-fold greater than the mean plasma C<sub>max</sub> in patients at MRHD and resulted in HR reduction by 25% without other adverse effect on ECG recordings. Slight and transient hemodynamic changes (slight increases in SBP, LVSP, LVEDP and LVdP/dtmin) were observed during and after the 5-min infusion. Isoprenaline and dobutamine (mean cumulated doses of 0.50 and 200 µg/kg, respectively) efficiently reversed HR reduction. In addition to the effect on HR, dobutamine increased SBP, MBP and LV contractility parameters, and decreased PR-interval; isoprenaline also increased SBP and LV contractility, although to a lower extent than dobutamine, and decreased DBP, MBP and PR-interval. Atropine (up to 82 µg/kg) was less consistent in

reversing HRR, but had a weak effect, if any, on all other ECG and hemodynamic parameters.

The subsequent dose of 10 mg/kg ivabradine induced marked overt signs, including subdued behavior, abnormal posture and tremor, and HR was reduced by 22% in only 3 out of 6 dogs. Transient cardiac hemodynamic changes were of greater magnitude than those observed at 5 mg/kg, including increases in SBP, MBP and LV contractility. Isoprenaline (0.46 µg/kg) increased HR back to basal values in the 3 dogs presenting HRR in response to ivabradine.

### Ophthalmology/Visual System

#### **NP08442: S 16257-2: In Vitro testing of S 16257-2 and its metabolite S 18982-1 on $I_f$ current expressed by recombinant mouse HCN1 channel**

The present study was to evaluate the effects of ivabradine (S 16257-2) and of its main metabolite S 18982-1 on  $I_f$  current as expressed by the mouse HCN1 (mHCN1) channel isoform in a heterologous expression system. Six cumulative concentrations of S16257-2 or S18982-1 (1, 3, 10, 30, 100, and 300 µM) were tested in mammalian HEK 293 cells transiently transfected with mHCN1 cDNA using whole cell patch-clamp technique.

The results showed that extracellular S16257-2 and S18982-1 exerted a dose-dependent inhibition of currents induced by mHCN1 with  $IC_{50}$  values of  $16.1 \pm 0.5$  µM (n=7) and  $26.6 \pm 3.5$  µM (n=6), respectively. S18982-1 also shifts the voltage-dependence of channels activation to more hyperpolarizing voltages and decreases the slope of the activation curves. The onset of block was slow and, under the experimental conditions, not reversible after washout.

#### **NP15864: S 16257-2: In vitro effects on electrophysiological responses in mouse retinal cells**

Rods and bipolar neurons and retina were isolated from adult male mice (C57BL/ 6J) for testing effects of ivabradine on 1) retinal ionic currents, including  $I_h$ , using patch-clamp techniques in isolated photoreceptors; and 2) Signal transmission from photoreceptors to single second order neurons, using microelectrode recordings from horizontal cells in intact isolated retina.

Effect of ivabradine on retinal currents in rods:

- Mouse rod photoreceptors express several types of voltage-dependent currents, including  $I_h$  and  $I_{Kx}$ , which gate in response to membrane hyperpolarization ranging from -35 to -70 mV, the same range of the rod response to light stimuli.
- Analysis of the activation kinetics is consistent with rod h-channels being HCN1 homomeric. This is in agreement with immunocytochemistry data, showing that **HCN1, but not HCN2, are expressed in the inner segment of mouse rods.**
- Ivabradine dose-dependently suppresses  $I_h$  in mouse rods, with a full block at 30 µM and  **$IC_{50}$  of 2.7 µM.** Full block requires repetitive stepping from -80 to -30

mV from a holding potential of -35 mV, suggesting that the block is use-dependent.

- $I_h$  block does not promptly reverse upon drug washout. Recovery has been observed only in a few experiments during 20 s-long membrane hyperpolarization to -120 mV.
- Analysis of the I/V relationship before and during full  $I_h$  block at 30  $\mu\text{M}$  ivabradine indicates that the drug affects neither the voltage dependence nor the maximal conductance of  $I_{Kx}$ .

Effect on signal transmission from photoreceptors to single second-order neurons:

- The response of horizontal cells is affected dose-dependently by ivabradine. However, even at 30  $\mu\text{M}$  ivabradine, the temporal resolution of horizontal cells does not approach that of the transductive cascade, consistent with ivabradine blocking  $I_h$  but not  $I_{Kx}$ .
- h-currents were identified in some neurons, post-synaptic to photoreceptors, such as bipolar cells, but not in horizontal cells. In general agreement with electrophysiological data, **immunocytochemistry confirmed the expression of HCN2 isoforms by a subset of bipolar cells**, but not by horizontal cells.

#### **NP08129: S 16257-2: Effects on viability and function of retinal rods and cones and pigment epithelial cells**

In Vitro assay

RPE cells form a monolayer between the choroid circulation and the neuroretina, and serve as a selective permeability barrier ensuring appropriate ionic balance critical to neuroretinal cell functioning. Potential effects of ivabradine on the function and the structure of the retinal pigment epithelium (RPE) were assessed in vitro by examining transepithelial resistance (TER) and cells tight junctions, for evaluation of RPE permeability function and structural integrity, respectively. TER was measured on primary cultured rat RPE cell monolayers, after 24, 48 or 72 h incubation in the presence of 0, 0.1, 1 or 10  $\mu\text{M}$  ivabradine ( $n = 12 - 20/\text{dose}$ ). Immunohistochemistry of tight junction proteins, zonula occludens (ZO-1) and F-actin, was performed after 72 h incubation.

The results showed that ivabradine concentrations up to 10  $\mu\text{M}$  (i.e. more than 100-fold the plasma  $C_{\text{max}}$  in patients at MRHD) for 72-hours, did not alter TER and the integrity of RPE tight junction proteins, ZO-1 and F-actin, indicating no effect on the permeability barrier function of RPE cell monolayers and the integrity of RPE tight junctions under testing conditions.

In vivo part:

Male Wistar rats ( $n = 5/\text{group}$ ) received oral (gavage) administration of 0 (vehicle-water), 6 or 60 mg/kg/d of ivabradine for 4 weeks. At the end of the treatment period, ocular globes were dissected and fixed. Sections were prepared for in situ DNA strand breaks detection by the TUNEL method and for transmission electron microscopy (TEM) examination.

Examination of DNA strand breaks evidenced the absence of apoptosis in retinal and glial cells in all groups. Examination by light microscopy of ocular globes semi-thin sections showed that rods, cones and RPE were similar in all groups. Electron microscopy confirmed a strictly normal ultrastructure of retinal layers in all ivabradine-treated groups, including a regular chromatin distribution in RPE and photoreceptors nuclei, the presence of phagosomes in the RPE cytoplasm, and normal size and numbers of photoreceptors.

Overall, there is no evidence of apoptosis or cell damage, and normal ultrastructural morphology is observed in all retinal cell layers after treatment with ivabradine at 6 or 60 mg/kg/d for 4 weeks.

**NP08493: S 16257-2 : Effect of ivabradine on the immunolocalisation of the proteins involved in the retinal phototransduction cascade (rhodopsin, arrestin, transducin)**

Male Wistar rats (n = 5/group) received oral (gavage) administration of 0 (vehicle-water) or 5.6 mg/kg/d of ivabradine for 1 to 7 days. The effects of drug administration on phototransduction were assessed in an indirect manner by immunolocalization and semi-quantitation of rhodopsin, arrestin and alphotransducin in the four photoreceptor compartment levels (outer segment, inner segment, outer granule and outer plexus). The parameters were studied under standard lighting conditions (after treatment for 1 to 7 days), during a light-adaptation period (after treatment for 5 days) and during a dark-adaptation period (after treatment for 5 days). Lastly, the potential toxicity of ivabradine on the retina was assessed by histological control of cell integrity on sections from control and treated rats.

Under the various conditions tested, either standard illumination or during adaptation to light or to dark, the expression of rhodopsin, arrestin and transducin was similar in photoreceptors from control and treated animals. After 5 days administration, the physiological redistribution of arrestin and transducin into distinct compartments of the photoreceptors in response to dark or to light adaptation was unaffected. Finally, post-mortem examination of ocular sections by light microscopy showed normal ocular structures, including choroid, retina, ciliary body and iris.

Overall, treatment with ivabradine at 5.6 mg/kg/d for up to 5 days did not result in any adverse effect on the expression of the main effectors of the phototransduction cascade (i.e. rhodopsin, arrestin and transducin) and their regulation during adaptation to light or to dark.

**NP23620: S 16257-2: In vivo effects of acute and chronic ivabradine administration on pigmented and albino rat ERG, opsin and rhodopsin content**

The purpose of this study was to assess the impact of acutely and chronically administered ivabradine on the electroretinographic response (ERG), on retinal

morphology, on distribution of HCN channels and on 11-cis chromophore binding to opsin. The role of melanin binding on these effects was also evaluated.

		Long Evans rats	Wistar rats
Number of animals		at least n=6/group	
Gender		male	
<i>Acute study</i>			
Administration		intravenous	
Vehicle (saline)		x	x
Ivabradine	(mg/kg)		
	3	x	
	6	x	
	12	x	x
Evaluated criteria			
-	ERG	x	x
-	ECG	x	x
<i>Chronic study</i>			
Administration		3-week oral (subcutaneous, minipump)	
-	Vehicle (saline)	x	x
-	Ivabradine*	x	x
-	Ivabradine* + 1-week off-dose	x	x
Evaluated criteria			
-	ERG effect and reversibility	x	x
-	Rhodopsin/opsin content	x	x
-	HCN1 and HCN2 protein	x	x
-	ECG	x	x

\*: Dose selected according to acute ERG results (~11 mg/kg/d)

As shown in study method table above (excerpted from page 11 of the report), both Long Evans rats (pigmented rats) and Wistar rats were treated with vehicle or ivabradine in acute and chronic (3-week) conditions. Before the experimental session, rats were dark-adapted for at least one hour and then anesthetized with urethane (i.p. 120 mg/100g). In both conditions, ERG were recorded in intact anesthetized animals in response to flashes or to sinusoidal light stimuli and ECG (i.e. HR) was checked. Retinal integrity and HCN distribution were assessed by immunocytochemistry, confocal microscopy and western blot analyses only after chronic administration of the drug.

#### Results:

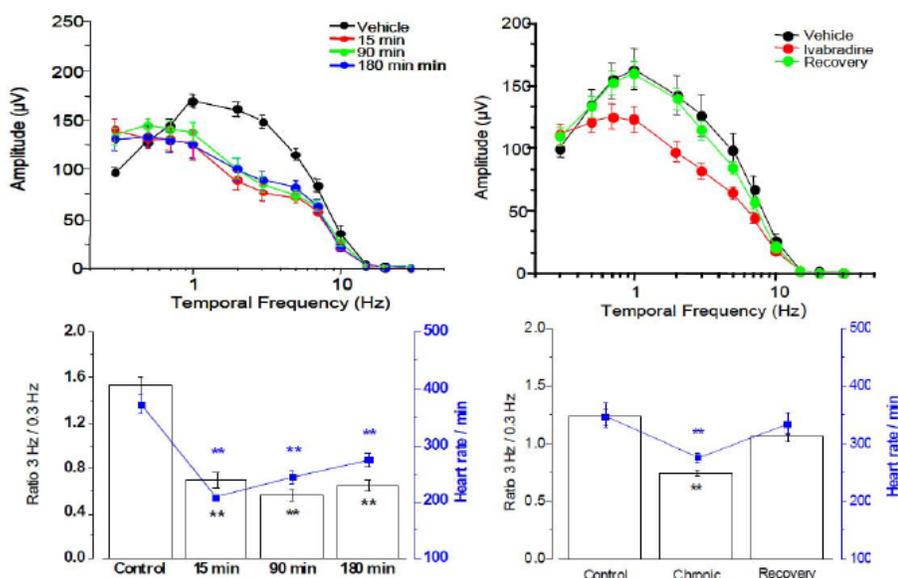
In pigmented rats, the amplitude of ERG response to flashes of light is not significantly modified and neither a- nor b-waves are significantly affected by acute or chronic ivabradine administration. A small rebound in the decaying phase of the b-wave may be observed under ivabradine treatment in acute condition. This phenomenon is consistent with the suppression of the negative feed-back action consequent to  $I_h$  inhibition.

Retinal morphology, isoform distribution of HCN channels in retina and rhodopsin/opsin content are preserved in eyes of pigmented rats chronically treated with ivabradine.

Acute and chronic ivabradine administration induces similar significant changes in the ERG response to periodic stimuli consisting in a sinusoidal modulation of luminance at various temporal frequencies. In pigmented (Long Evans) rats, the frequency-response curves (FRC), reporting the response amplitude as a function of the temporal frequency

of the sinusoidal stimuli, are enhanced at the lowest frequencies and reduced in the 1-7 Hz range with ivabradine. The representative figure is shown below (excerpted from M2, section 2.6.2. page 122). This effect may be described as a transition from a band-pass mode of retinal filtering to a low-pass mode. A similar behavior was consistent with the suppression of a negative feedback mechanism as that exerted by  $I_h$  on both rods and bipolar cells. This effect, conveniently expressed as the ratio between response amplitude at 3 and 0.3 Hz ( $R_{3\text{Hz}} / R_{0.3\text{Hz}}$ ) or by the 2Hz amplitude response is dose-dependent and is completely reversed one week after discontinuation of ivabradine dosing.

**Figure 49. Effect of Acute (left) and Chronic Doses of ivabradine (11-12 mg/kg/d) (right) on the FRC Over Time in Pigmented Rats**

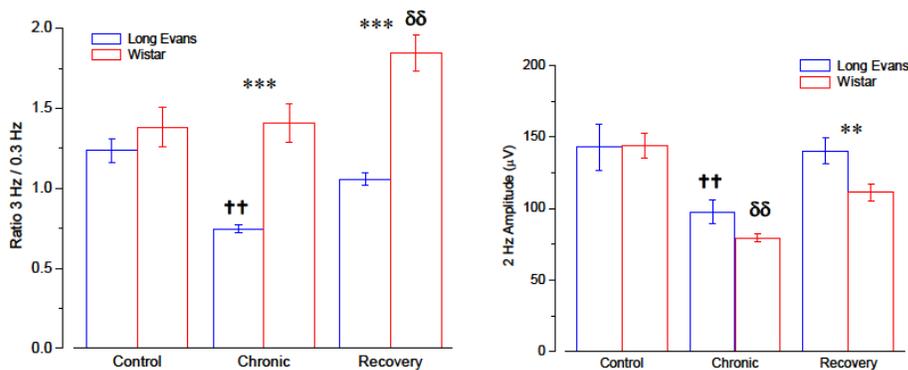


\*\* Both  $R_{3\text{Hz}} / R_{0.3\text{Hz}}$  and HR changes are statistically significant ( $p < 0.01$ ).

In albino (Wistar) rats lacking melanin pigment, isoform distribution of HCN channels in retina and rhodopsin/opsin content were not changed in eyes of ivabradine chronically treated rats when compared to pigmented rats. After acute and chronic administration, ivabradine decreased the temporal resolution of the ERG response and the effect is also reversible (strain comparison was shown in the figure below, excerpted from page 49 of the report).

Overall, the effect of acute and chronic administration of ivabradine on the ERG response is small and reversible and restricted to very few numbers of parameters in pigmented and albino rats. The decrease in the ERG response is revealed by the harmonic analysis of responses to periodic stimuli whose mean luminance is modulated sinusoidally with no marked differences between the two strains. In pigmented rats, melanin binding was not associated with a deleterious visual effect.

Fig. 34. Strain comparison (L.E. and W.A.) of the ratio  $R_{3\text{Hz}} / R_{0.3\text{Hz}}$  and on the response amplitude to stimuli of 2 Hz frequency in control, after chronic administration of ivabradine and recovery



Strain effect at fixed treatment: statistically significant \*with  $P < 0.05$ ; \*\* with  $P < 0.01$

Treatment effect at fixed strain: †† $p < 0.01$  treatment effect vs. control in Long Evans (Tukey test); ††† $p < 0.01$  treatment effect vs. control in Wistar rats (Tukey test).

### NP25528 S 16257-2: Effect of ivabradine on the progression of the retinal degeneration in a mouse model of retinitis pigmentosa

The effect of S 16257-2 on the rate of degeneration and remodeling (morphological changes and function) was also evaluated in the Rd10 mouse, a convenient model of human retinitis pigmentosa (RP), and also in WT mice. Retinal degeneration in Rd10 mouse begins around the age of 20 days (P20) with the thickness of the outer nuclear layer halved by 25 days old (P25).

WT and Rd10 mice (12 days-old) were treated with ivabradine 12 mg/kg/d (sc) for 10 days as shown in the table below (excerpted from page 12 of the report). ECG was recorded for HR check. At the end of treatment, the mouse retinas were analyzed using immunocytochemistry (ICCH) for different retinal cell markers (PKC, HCN1 and HCN2, GFAP), TUNEL staining for apoptosis determination and western blotting (WB) for opsin, Glial Fibrillary Acid Protein (GFAP) and actin (for normalization of opsin and GFAP).

Strain	Treatment	Start	End	Number of animals		
				HR	ICCH	WB
WT	Vehicle	P12	P21	8	4	4
WT	Iva 12 mg/Kg/day	P12	P21	10	4	5
Rd10	Vehicle	P12	P21	17	4	7
Rd10	Iva 12 mg/Kg/day	P12	P21	17	4	9

HR: heart rate; ICCH: immunocytochemistry; WB: western blot

In Rd10 mice, ivabradine treatment had no significant effect on the survival of photoreceptors, or on the progression of retinal degeneration. Consistent with the characteristics of this mutant, TUNEL analysis revealed a high incidence of apoptotic

figures with a different distribution in different retinal regions in control rd10 mice vs. WT mice. These characteristics were not influenced by ivabradine.

At P21, the rhodopsin content of Rd10 mouse retinas was low and the expression of GFAP was high as a consequence of the on-going degeneration. This pattern was similar in both control and ivabradine-treated mice.

The immunolabeling studies showed that chronic ivabradine treatment did not alter HCN1 and HCN2 isoforms composition or distribution in WT or Rd10 mouse retina.

Overall, ten days of treatment with 12 mg/kg/day of ivabradine resulted in a marked reduction of HR but did not affect morphology or retinal specific proteins expression in either WT or Rd10 mutant mice, indicating that ivabradine has no deleterious influence on the retinal degenerative processes.

## CNS

### **NP07027 (GLP): Safety pharmacology evaluation of effects on the central nervous system**

Part I: Evaluation of effect on spontaneous locomotor activity in the rat following single oral administration.

Male Wistar rats (n = 8/group) received a single oral dose of ivabradine at 0 (vehicle-water), 5, 20 or 80 mg/kg. A positive-control, chlorpromazine, 20 mg/kg (p.o.) was included. At approximately one hour post dose, animals were placed onto an open-field arena in a quiet, dark room, and locomotor activity was assessed by counting the number of passages through the open-field over a 10-min period. In addition, numbers of rearing (exploratory behavior) and grooming (stereotypic) events were recorded.

Ivabradine up to 80 mg/kg had no effect on spontaneous locomotor activity, exploratory behavior or stereotypes whereas the positive control, chlorpromazine at 20 mg/kg induced, as expected, a decrease in spontaneous locomotor activity, exploratory behavior (peripheral rearing) and grooming.

Part II: Evaluation of interaction with hexobarbital-induced sleep in the rat following single oral administration.

Male Wistar rats (n = 8/group) received a single oral dose of ivabradine at 0 (vehicle-water), 5, 20 or 80 mg/kg. A positive-control, clonidine, 1 mg/kg (p.o.) was included. At approximately one hour post dose, each animal was injected intraperitoneally with 125 mg/kg of hexobarbital. The time taken to fall asleep and the duration of sleep were measured. Sleeping was assessed by gently pinching the hind paw at 1-min intervals over the 2-hour examination period.

Ivabradine up to 80 mg/kg had no effect on the time taken to fall asleep or on the duration of hexobarbital-induced sleep whereas the positive control (clonidine, 1 mg/kg, p.o.) induced, as expected, its typical sedative effect characterized by a statistically significant increase in the duration of sleep.

Part III: Evaluation of proconvulsant effect in the rat following single oral administration.

Male Wistar rats (n = 8/group) received a single oral dose of ivabradine at 0 (vehicle-water), 5, 20 or 80 mg/kg. A positive-control, caffeine, 150 mg/kg (po) was included. At approximately one hour post dose, each animal was injected intraperitoneally with pentylenetetrazole at an expected subconvulsant dose of 40 mg/kg. Individuals were then observed for 10 min, and the numbers of full clonic and tonic seizures were counted.

Ivabradine up to 80 mg/kg did not promote convulsions when compared with the control. Under the same conditions, the positive control (caffeine, 150 mg/kg, p.o.) induced, as expected, its typical proconvulsant effect characterised by a statistically significant increase in the proportion of animals with full clonic/tonic seizures.

#### **NP08101: S 16257-2 and Zatebradine (p.o.) Effects in the primary observation test in the rat**

Male Wistar rats (n = 3/group) received a single oral dose of ivabradine at 0 (vehicle-water), 15, 30, 59, 119 or 238 mg/kg. Behavioral and physiological changes were recorded on a standardized observation grid, at 15, 30, 60, 120, and 180 min and 24 hr after dosing.

Up to 59 mg/kg, ivabradine had no effect on measured parameters. At 119 and 238 mg/kg, a moderate sedation and a slight hypothermia (-1.1°C at 119 mg/kg and -1.5°C at 238 mg/kg) were observed 15 and 30 min after dosing.

#### **NP07032: S 16257-2: Evaluation of analgesic effect in the hot plate test in the rat following single oral administration (GLP\*)**

Male Wistar rats (n = 8/group) received a single oral dose of ivabradine at 0 (vehicle-water), 5, 20 or 80 mg/kg. The positive-control, buprenorphine, 3 mg/kg (po) was included. At approximately one hour post dosing, each animal was placed on a hot plate maintained at a constant temperature of 52.2°C. The latencies for forepaw and hind paw licking were recorded.

Ivabradine did not affect the latencies for reaction to pain whereas the positive control (buprenorphine, 3 mg/kg, p.o.) induced, as expected, its typical analgesic effect characterized by a statistically significant increase in the latency of reaction to pain of forepaws and hindpaws.

#### Respiratory System

**NP07028: S 16257-2: Evaluation of effect on respiration in the unrestrained conscious rat following single oral administration (GLP)**

Male Wistar rats (n = 8/group) received a single oral dose of ivabradine at 0 (vehicle, water), 5, 20, or 80 mg/kg. The positive-control, clonidine, 30 mg/kg po was included. Immediately after dosing, each animal was placed in a plethysmograph for 4 h. During the experimental period, respiration was recorded for 10 s every 5 min using a Gould DASA 4600 acquisition system. Each respiratory cycle was analyzed and the following parameters were determined: respiratory rate, peak inspiratory flow, peak expiratory flow, inspiration time, expiration time, airway resistance, and tidal volume.

At 5 mg/kg, ivabradine had no effect on any measured respiratory parameters. At 20 and 80 mg/kg, there was a transient increase in respiratory rate, which peaked 30 min after dosing (Emax = +17% ( $p \leq 0.05$ ) and +18% ( $p \leq 0.01$ ), respectively). No other respiratory parameters were affected.

Under the same condition, the positive control, clonidine, 30 mg/kg, po increased airway resistance (Emax: +91%, Tmax: 30 min), respiratory rate (Emax: +99%, Tmax: 60 min), peak inspiratory (Emax: +65%, Tmax: 30 min) and expiratory (Emax: +114%, Tmax: 30 min) flows; and decreased inspiration (Emax: -39%, Tmax: 30 min), expiration (Emax: -57%, Tmax: 90 min) times, and tidal volume (Emax: -31%, Tmax: 90 min).

Gastrointestinal system**NP03145: S 16257: Study of the effects of single oral dose on gastrointestinal transit in rats (GLP)**

Male Wistar rats (n = 8/ group) received a single oral (gavage) dose of ivabradine at 0 (vehicle, water), 5, 15, or 30 mg/kg. The positive-control was atropine, 10 mg/kg po. The rats were not given any fluid for 24 hr before treatment. At approximately 45 minutes post dosing, 2 ml of an aqueous solution of 10% fuchsin (a colored marker) and 2.5% carboxymethylcellulose were orally administered; 15 minutes later, animals were sacrificed, and a section of the intestine from the pylorus to the end of the cecum was removed. The distance travelled by the progression of the alimentary bolus stained with fuchsin was immediately measured and expressed as a percent of the total length of the intestine.

GI transit (measured as the distance travelled by an alimentary bolus) was slightly but significantly retarded in animals treated with ivabradine, with a dose-related reduction of 7%, 17% and 20% at the doses of 5, 15, and 30 mg/kg, respectively. The positive control, atropine at 10 mg/kg, reduced the GI transit by 37% when compared to control.

**NP03147: S 16257: Study of the effects of single oral dose on gastric secretion in rats (GLP)**

Male Wistar rats (n = 8/ group) received a single oral (gavage) dose of ivabradine at 0 (vehicle, water), 5, 15 or 30 mg/kg; the positive control was atropine, 10 mg/kg po. At

approximately 30 minutes after dosing, the pylorus was ligated under anesthesia and gastric secretions were collected over 4 hours. The volume, pH, buffer strength, free and total acidity of the gastric secretions were measured.

Ivabradine did not affect volume, pH and buffer strength of the gastric secretions at any dose. A significant reduction of free (-60%) and total (-47%) acidities ( $p < 0.05$ ) was observed in the 5 mg/kg dose group. However, this effect was not observed in the animals at the higher doses of 15 and 30 mg/kg.

**NP03148: S 16257: Study of the gastric ulcerogenic effects of a single oral dose in rats (GLP)**

Male Wistar rats ( $n = 8/\text{group}$ ) received a single oral (gavage) dose of ivabradine at 0 (vehicle, water), 5, 15, or 30 mg/kg. The positive control was indomethacin, 10 mg/kg po. The animals were not given any fluid starting 24 hr prior to dosing. Animals were sacrificed 24 hr after dosing, and the stomachs were dissected and examined by stereomicroscopy. Ulcers were scored on a scale from 0 (no lesions) to 8 (more than 10 linear ulcerations with a length  $> 3\text{mm}$ ).

Ivabradine at 5 or 15 mg/kg was not ulcerogenic. The highest dose of 30 mg/kg was slightly ulcerogenic (median rating level of 3,  $p < 0.01$ ). In rats receiving indomethacin, a mean rating level of 6 was observed ( $p < 0.01$ ).

**NP03160: Fourteen-day continuous intravenous infusion toxicity study with S-16257-2 In the unrestrained rat (GLP)**

Male Sprague-Dawley rats ( $n = 13/\text{group}$ ) received vehicle (saline) or ivabradine by i.v. infusion (3 hr/day) at a rate of 4 mL/kg/hr for 14 days, which was intended to deliver a dose of 28 mg/kg/day. A satellite group ( $n = 6$ ) was designated for toxicokinetic evaluation. At necropsy of the main-study animals, blood samples were collected for hematology and clinical chemistry, and the gastrointestinal tract was examined histologically for evidence of ulcerogenic effects.

Ivabradine did not elicit any hematological or clinical chemical changes, and there was no histopathological change in the gastric mucosa. The i.v. administration of 28 mg/kg/d was associated with a mean plasma exposure ( $\text{AUC}_{24\text{h}}$  on Day 14) to ivabradine of 7414 ng.ml/h, which is 21-fold of that measured in patient at MRHD.

A single iv dose of 28 mg/kg did not result in an ulcerogenic effect in rats, suggesting the slight ulcerogenic effect observed after single oral dose of 30 mg/kg in the above study might result from a local effect related to the oral administration.

Renal system

**NP03146: Study of the effects of a single oral dose on diuresis and urinary electrolytes in rats given a saline overload (GLP)**

Male Wistar rats (n = 10/group) received a single oral (gavage) dose of ivabradine at 0 (vehicle, water), 5, 15, or 30 mg/kg. The positive control was frusemide, 10 mg/kg po. Immediately after dosing, the animals received 50 mL/kg of isotonic saline solution to provoke a diuresis. Urine was collected over 4 h for measurement of volume, pH, sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) levels.

A dose-related increase in diuresis was observed with urine volume increasing by 35%, 40% and 58% at doses of 5, 15 and 30 mg/kg vs. 40% at frusemide dose of 10 mg/kg, all when compared to control.

The effect on ion secretion and urine ion concentration is shown in the table below (excerpted from page 18 of the report). At the two higher doses of 15 and 30 mg/kg, sodium excretion was significantly increased (+37 % and +34 %, respectively) and urine concentration of K<sup>+</sup> were reduced. Urine sodium concentration was also reduced (-12%) at 30 mg/kg. The Na<sup>+</sup>/K<sup>+</sup> ratio was not significantly increased in any ivabradine-treated group whereas it was significantly increased in the positive control group (Frusemide 10 mg/kg) which was associated with a significant reduction in urine potassium level. A slight but significant increase in urinary pH was observed in the groups treated with 15 and 30 mg/kg.

PRODUCTS		SODIUM (mmol/hour)	POTASSIUM (mmol/hour)	SODIUM (mmol/hour)	POTASSIUM (mmol/L)	Na/K
VEHICLE Sterile water for irrigation	Med	0.144	0.037	145	43.0	3.30
	N	10	10	10	10	10
FRUSEMIDE 10 mg/kg	Med	0.174	0.039	143	30.0	4.53
	N	10	10	10	10	10
	%	+ 20.8	+ 4.0	-1.4	- 30.3	+37.1
	P	NS	NS	NS	**	*
S16257 5 mg/kg	Med	0.162	0.046	129	35.5	3.41
	N	10	10	10	10	10
	%	+ 12.6	+ 24.6	- 11.4	- 17.6	+ 3.2
	P	NS	NS	NS	NS	NS
S16257 15 mg/kg	Med	0.197	0.050	138	35.6	4.05
	N	10	10	10	10	10
	%	+ 37.3	+ 35.8	- 5.2	- 17.3	+22.6
	P	*	NS	NS	*	NS
S16257 30 mg/kg	Med	0.192	0.045	127	31.4	3.94
	N	10	10	10	10	10
	%	+ 33.6	+ 21.2	- 12.8	- 27.1	+19.3
	P	*	NS	*	*	NS

Reviewer's note: in the table above, the unit (mmol/hour) for Sodium in the head of 5<sup>th</sup> column should be read as mmol/L; the original one might be a typo.

Overall, single oral doses of ivabradine in the range of 5 to 30 mg/kg increased urine volume in saline-overloaded Wistar rats. At two higher doses, sodium excretion was increased, urine potassium was reduced and urine pH was slightly increased. The interpretation of this result is unclear since furosemide, as tested, was neither natriuretic or kaliuretic.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

#### Methods of analysis

The analytical method validation reports were listed in the table below (excerpted from M2/2.6.5, page 7). All toxicokinetic (TK) studies, and the majority of PK studies, used a specific liquid chromatography (LC) method with native fluorescence detection (LC-Fluorescence=LC-Fluo) for analysis of ivabradine (S 16257) and M29 (S 18982) in animal matrices: ivabradine in human, rat and dog plasma (Report NP06841); ivabradine in human, rat, rabbit, and dog plasma (Report NP03157); ivabradine and S 18982 in human, rat and dog plasma, and in human urine (Report NP06903). A sensitive LC-MS-MS (MS=mass spectrometry) method was developed for the analysis of ivabradine and ten metabolites in animal plasma (Report NP15187) and validated for the analysis of ivabradine and S 18982 in mouse, rat, rabbit, dog, and pig plasma (Report NP16131). A chiral LC method using either tandem mass spectrometry (rat) or native fluorescence (dog) detection was also applied in the enantio-selective determination of ivabradine (S 16257, S-enantiomer) and S 16260 (R-enantiomer) in plasma. The overall analytical approach employed in the nonclinical program was compiled in a summary report, together with all validation parameters (Report NP15185).

Type of study	Test system	Analytical method	Testing facility	Report number
<b>Analytical methods and validation reports</b>				
Summary of nonclinical bio-analytics	Mouse, rat, rabbit and dog plasma	LC / Chiral LC	(b) (4)	NP15185
Validation for the measurement of S 16257 in plasma	Rat, dog and human plasma	LC-Fluo		NP06841
Validation for the measurement of S 16257 in plasma	Rat, rabbit, dog and human plasma	LC-Fluo		NP03157
Validation for the measurement and short-term stability of S 16257 and S 18982 in plasma	Rat, dog and human plasma	LC-Fluo		NP06903
Determination of S 16257 and S 18982 in mouse plasma	Mouse plasma	LC-Fluo		NP05454
Validation for the measurement of S 16257 and S 18982 in plasma	Mouse, rat, dog and human plasma	LC-Fluo		NP05116
Additional validation for the measurement of S 16257 and S 18982 in plasma (additional tests)	Mouse, rat, rabbit and dog plasma	LC-Fluo		NP15283
Determination of S 16257, S 18982 and 9 additional metabolites in plasma	Mouse, rat, dog and human plasma	LC-MS-MS		NP15187
Validation for the measurement of S 16257 and S 18982 in plasma	Mouse, rat, rabbit, dog and pig plasma	LC-MS-MS		NP16131
Enantioselective determination of S 16257 and S 16260 in plasma	Rat plasma	Chiral LC-MS-MS		NP15152
Enantioselective determination of S 16257 and S 16260 in plasma	Dog and human plasma	Chiral LC-Fluo		NP15192
Long-term stability of S 16257 in plasma up to 12 months	Rat, dog and human plasma	LC-Fluo		NP06819
Long-term stability of S 18982 in plasma up to 12 months	Rat, dog and human plasma	LC-Fluo		NP06852
Long-term stability of S 16257 and S 18982 in plasma up to 6 months	Mouse and rabbit plasma	LC-Fluo		NP15123

#### Additional information:

All above mentioned reports contain a GLP Compliance Statement.

Two [<sup>14</sup>C]-radiolabelled isotopomers of ivabradine were synthesized: [benzazepinone-<sup>14</sup>C]-ivabradine and [dexbicyclamin-<sup>14</sup>C]-ivabradine for use in various nonclinical studies. [<sup>3</sup>H]-ivabradine was synthesized and used for the in vitro determination of blood to plasma ratio and assessment of auto-induction. The radiochemical purity was at least

98%. Radioactivity analysis was applied to both in vitro and in vivo nonclinical studies. Biological matrices, supernatants and digested tissues were analyzed by liquid scintillation counting (LSC) or LC coupled with radioactivity detection. Tissue sections were analyzed using autoradiography.

#### Absorption

#### **NP05245: PHARMACOKINETICS OF S 16257, ITS N-DEMETHYLATED METABOLITE S 18982 AND OF 14C-LABELLED MATERIAL AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF 14C-S 16257-2 TO MALE WISTAR RATS**

The plasma concentration versus time course of S 16257 and its metabolite, S 18982, were characterized after single administration of <sup>14</sup>C-S 16257-2 to male Wistar rats at doses of 3 mg/kg (oral and iv administration) and 200 mg/kg (oral dosing only). The concentrations of S 16257 and its N-demethylated metabolite S 18982 in plasma were determined according to the analytical method that uses liquid-solid extraction followed by liquid chromatography with native fluorescence detection. The limit of quantitation of S 16257 and S 18982 was 0.50 ng/ml using 1.0 ml of plasma. All the concentrations are expressed as S 16257 and S 18982 base form. Radioactivity was then measured in a Packard Tri-Carb model 2200 CA liquid scintillation spectrometer.

The resulting PK parameters are summarized below (excerpted from page 34 of the report).

Plasma pharmacokinetic parameters of the total radioactivity (Rad) and unchanged S 16257 after single oral and intravenous administration of <sup>14</sup>C-S 16257-2 to male Wistar rats

Parameter	Dose of 3 mg/kg S 16257 base form				Dose of 200 mg/kg S 16257 base form	
	Intravenous administration		Oral administration		Oral administration	
	Rad	S 16257	Rad	S 16257	Rad	S 16257
C <sub>max</sub> (ng eq/ml or ng/ml)	1234	997	235	54	6389	3224
t <sub>max</sub> (h)	0.083*	0.083*	0.5	0.17*	0.17*	0.17*
t <sub>1/2,z</sub> (h)	53 (123)	0.89	45 (68)	4.8	47	4.8**
AUC (µg eq.h/ml or µg.h/ml)	2.3	0.69	1.7	0.18	123	13
AUMC (ng.h <sup>2</sup> /ml)		537	-	-	-	-
CL (ml/min/kg)	-	72	-	-	-	-
V <sub>ss</sub> (l/kg)	-	3.4	-	-	-	-
F	-	-	-	0.26	-	-

\* : first sampling time.

\*\* : estimated value (cf. page 23)

Note 1 : numbers in brackets give the terminal half-life in whole blood.

Note 2 : AUC (µg.h/ml) for S 18982 were 0.017 (IV ; 3 mg/kg), 0.0065 (PO ; 3 mg/kg) and 0.73 (PO ; 200 mg/kg)

#### NP15152: PHARMACOKINETICS OF S 16257 IN MALE WISTAR RATS AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16257-2

Nine male non-fasted rats per route of administration received a single oral or iv administration of S 16257-2 (expressed as free compound) at doses of 3 mg/kg (oral and iv administration) and 200 mg/kg (oral dosing only). Urine samples were collected over a 24 or 30 h period. The measurement of S 16257 and S 18982 in plasma and urine samples was performed according to an achiral analytical method using solid-phase extraction (for plasma samples) followed by LC-MS/MS detection (LOQ = 0.2 ng/ml) on a C18 column. The result was summarized in the table below (excerpted from page 6 of the report).

Plasma parameters :	S 16257		S 18982	
	IV 3 mg/kg	PO 3 mg/kg	IV 3 mg/kg	PO 3 mg/kg
C <sub>max</sub> (ng/ml) :	-	151	13.6	9.35
t <sub>max</sub> (h) :	-	0.25	0.083	0.25
AUC <sub>t</sub> (ng.h/ml) :	741	206	8.42	8.51
AUC (ng.h/ml) :	742	206	8.86	11.5
CL(ml/min/kg) :	67.4	-	-	-
t <sub>1/2,z</sub> (h) :	2.44	1.50	0.955	3.69
V <sub>ss</sub> (l/kg)	2.98	-	-	-
Absolute bioavailability (%) (CV %):	-	27.8 (39.1)	-	-
Met <sub>ratio</sub> (%)	-	-	1.23	5.75
R <sub>240 day1</sub> (AUC/ AUC <sub>24</sub> ) :	1.00	1.00	1.05	1.35
<b>Excretion (Urine) :</b>				
% dose in urine (0-24 h)	5.05	2.42	0.191	0.249
CL <sub>R</sub> (ml/min/kg) :	3.41	5.87	11.0	10.0
Met <sub>ratio</sub> (%)	-	-	3.90	10.6

The drug is eliminated with a high total plasma clearance (CL : 67.4 ml/min/kg) and a bioavailability of 28 % (inter-individual variability close to 39%) was determined at 3 mg/kg. This value was slightly higher than predicted (<20 %) when comparing the hepatic blood clearance of 70 ml/min/kg (CL - CLR, corrected for the Blood/Plasma ratio) with the hepatic blood flow. These results confirmed a possible saturation of the first pass effect at this dose level, as demonstrated in the rat with a population approach combining the results of several studies.

#### **NP05258: Pharmacokinetics and excretion balance of 14C-labelled material after single oral and intravenous administration of 14C-S 16257 to male beagle dogs**

Two male beagle dogs received a single administration of 1 mg/kg 14C-S 16257-2 in a cross-over fashion either the oral dose or the 10 min iv infusion and a single oral dose of 10 mg/kg 14C-S 16257-2. The absorption of the drug and the disposition of drug and drug-related material, i.e. radioactivity, from plasma were determined using LC-FLuo method. The limit of quantitation of S 16257 and S 18982 was 0.50 ng/ml using 1.0 ml of plasma.

The study result is summarized in the table below for study NP05258 (excerpted from M2/2.6.3, page 15). In all animals, the maximum plasma concentrations of total radioactivity, unchanged drug and the N-demethylated metabolite S 18982 were observed at 2 h post-dose (the earliest sampling time). At this time, the plasma levels of S 16257 represented at most 50 % of the radioactivity concentrations. After that time, the concentrations of S 16257 decreased rapidly (terminal half-life of about 1 to 2 h); plasma levels of radiolabelled material decreased much slower with a terminal half-life ranging from 103 to 139 h.

#### **NP15001: PHARMACOKINETICS OF S 16257 IN THE MALE BEAGLE DOG AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16257-2**

Two male dogs received in a cross-over fashion either the oral or the intravenous dose, in study sequences 1 and 2, with a wash-out period of 7 days between periods. Urine and plasma were collected up to 24 h after dosing. S 16257 was quantified in plasma

and urine using an analytical procedure (achiral method) involving liquid-liquid extraction, followed by liquid chromatography with native fluorescence detection (the limit of quantitation was 1 ng/mL using 500  $\mu$ L of plasma or urine).

The PK parameters are summarized in the table below for study NP15001 (excerpted from M2/2.6.3, page 15).

Test article: Ivabradine hydrochloride						
Species	Rat	Rat	Dog	Dog	Dog	Human <sup>b</sup>
Strain	Wistar	Wistar	beagle	beagle	beagle	Caucasian
Number of animals / Gender (M/F)	15M	15M	2M	2M	2M	44M
Feeding condition	Fed	Fed	Fasted	Fasted	Fasted	-
Formulation / Vehicle	Solution / saline	Powder / capsule				
Method of administration	Oral (gavage)	Oral				
Dose (mg/kg)*	3	200	1	3	10	20 mg
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	S 16257					
Assay	LC-Fluo	LC-Fluo	LC-Fluo	LC-Fluo	LC-Fluo	LC-Fluo
PK parameters:						
t <sub>max</sub> (h)	0.17 <sup>a</sup>	0.17 <sup>a</sup>	2.0 <sup>a</sup>	0.67	2.0 <sup>a</sup>	1.8
C <sub>max</sub> (ng/mL)	54	3224	64	759	2208	57
AUC (ng.h/mL)	180	13000	nd	1653	nd	529
t <sub>1/2,1</sub> (h)	-	-	-	-	-	2.3
t <sub>1/2,z</sub> (h)	4.8	nd	1.0	1.0	2.4	37
F (%)	26	nd	nd	40	nd	44 <sup>c</sup>
Location in CTD	4.2.2.5	4.2.2.5	4.2.2.5	4.2.2.5	4.2.2.5	5.3.3.2
Report Number	NP05245	NP05245	NP05258	NP 15001	NP05258	NP15388

**Additional information:**

Extent of oral absorption of the radioactive dose:

- between 64 and 83% - male rats (at 3 or 200 mg/kg, 2.8 MBq/kg) - fed conditions [Report NP05193, Section 4.2.2.5];
- between 80 and 87% - male bile duct-cannulated rats (at 3 or 200 mg/kg, 2.8 MBq/kg) - fed conditions [Report NP05222, Section 4.2.2.5];
- at least 31% to 38% - male dogs (at 1 or 10 mg/kg, 0.37 MBq/kg) - fasting conditions [Report NP05258, Section 4.2.2.5].

a: First sampling time

b: Patients

c: Data obtained after single oral administration of 5 mg (Report NP15305)

\*: Unless otherwise indicated

### NP16236: Pharmacokinetics of S 16257 and its metabolite S 18982 in male mice after repeat oral administration of ivabradine (non-GLP)

Twenty-eight C57BJ6 male mice received twice daily oral administration of 5 mg/kg of ivabradine for 3 weeks. Blood samples were collected on Day 22 prior to dosing and 15 min, 30 min, 1 hr, 4 hr and 8 hr post dosing. Plasma concentration of S 16257 and its metabolite S 18982 were determined using solid-phase extraction followed by liquid chromatography with tandem mass spectrometric detection. The limit of quantitation was 0.250 ng/mL using a plasma sample volume of 50  $\mu$ L over a range of concentrations of 0.250 to 250 ng/mL for each compound.

As shown in the table below (excerpted from page 7 of the report). The peak plasma concentration of S 16257 was reached within 0.5 hr post dosing, suggesting a rapid absorption. S 16257 was rapidly metabolized into its N-desmethylated S 18982, according to the T<sub>max</sub> observed within 0.5 hr. The plasma exposure of S 18982 represents 20% of the overall plasma exposure of the unchanged drug, S 16257. Elimination was characterized by a short terminal half-life (T<sub>1/2</sub> < 2 hr) although it was possibly underestimated due to the last sampling time used for calculations (8 hr).

Pharmacokinetic parameters	S 16257	S 18982
$C_{min}$ (ng/mL)	6.20	1.53
$C_{max}$ (ng/mL)	450	74.6
$t_{max}$ (ng/mL)	0.500	0.500
AUC <sub>8</sub> (ng.h/mL)	645	119
AUC (ng.h/mL)	683	129
$\lambda_z$ (h <sup>-1</sup> )	0.357	0.360
$t_{1/2,z}$ (h)	1.94	1.93
number of points *	3	3
MET ratio (%) **	-	19.5

\* Number of points used in calculation of  $\lambda_z$

\*\* MET ratio: molar AUC ratio of S 18982 to S 16257

**NP08033: Pharmacokinetic study of S 16257 after oral administration in rats: building of a population model (GLP\*)**

**NP15186: Pharmacokinetic mixed effects modelling of S 16257 after oral administration in the beagle dog-combined analysis (GLP\*)**

In these two PK modeling studies, rat and dog PK population models for ivabradine were developed by combining data from nonclinical PK and TK studies. For the rat, data used for the building process were taken from twelve pharmacokinetic and toxicokinetic studies, including single oral administration in male Wistar and/or Sprague-Dawley rats, and repeated oral administration with different treatment duration in male and female Wistar, Sprague-Dawley and/or Fischer rats. For the dog, data used were taken from all pharmacokinetic and toxicokinetic studies including single and repeated oral administration with various treatment durations in the beagle dog (1 day to 52 weeks). The table below (excerpted from M2/2.6.4. page 19) showed model-dependent parameters in rats and dogs. In both species, the PK modeling was performed using the NONMEM (NONlinear Mixed Effects Modeling) software. Models were defined in terms of apparent clearance (CL/F), apparent volume of the central compartment (Vc/F), apparent inter-compartmental clearance (Q/F), apparent volume of the peripheral compartment (Vp/F) and absorption rate constant (ka).

**Table 3-1. Model-dependent Absorption Parameters for Ivabradine in Rat and Dog Following Single or Repeated Oral Administration**

Species	Strain	Dose range (mg/kg/d)*	Gender	Daily dosing	Range <sup>a</sup> of t <sub>max</sub> (h)	ka (1/h)	F <sub>(%)</sub> (%)	Range of AUC <sub>24</sub> (ng.h/mL)	Report number
Rat	Wistar, SD, Fischer	3 to 223	M	Once	0.24 to 0.28	10 fixed	40 <sup>b</sup>	310 to 45000 <sup>c</sup> 965 to 71700 <sup>c</sup>	NP08033
			F	Twice	0.29 to 0.35		60		
Dog	Beagle	0.23 to 42 0.5 to 15 BID	M/F	Once	0.53 to 1.1	3.4	15 / 40 <sup>d</sup>	136 to 24700 <sup>c</sup> 129 to 19306	NP15186 NP15258
			M/F	First Second	0.47 to 0.72 0.97 to 1.5	3.4 0.77	10 to 51		

SD: Sprague-Dawley \* : Unless otherwise indicated

a: Regardless of the dose. b: For a dose of 3 mg/kg. c: AUC<sub>pop</sub> (ng.h/mL), calculated from population primary pharmacokinetic parameters. d: For doses of ≤1 and >1 mg/kg, respectively.

The results were summarized in the table below (excerpted from M2/2.6.5, page 16).

Species	Rat	Dog
Strain	Wistar, Sprague-Dawley, Fischer	beagle
Number of animals / Gender (M/F)	234M+232F	64M+63F
Feeding condition	Fasting; fed	Fasting; fed <sup>e</sup>
Formulation / Vehicle	Solution / saline, purified or demineralized water	Solution / saline, purified or demineralized water
Method of administration	Oral (gavage)	Oral (gavage)
Duration of treatment	Up to 52 weeks	Up to 52 weeks
Dose (mg/kg/d)*	2.8 to 279 (M), 2.3 to 223 (F)	0.23 to 42
Sample	Plasma	Plasma
Analyte	S 16257	S 16257
Assay	LC-Fluo	LC-Fluo
Occasion	-	-
PK parameters:		
AUC <sub>24</sub> (ng.h/mL)	310 to 45000 (M), 965 to 71700 (F) <sup>a</sup>	136 to 24700 <sup>a</sup>
t <sub>1/2,1</sub> (h)	0.61 to 0.73 <sup>c</sup> (M), 0.82 (F)	0.76 to 2.0
% AUC <sub>1</sub> (%) <sup>b</sup>	16 to 43	≥80
t <sub>1/2,z</sub> (h)	5.8 to 8.6 <sup>c</sup> (M), 14 (F)	8.9 to 22
% AUC <sub>z</sub> (%) <sup>b</sup>	57 to 84	<20
V <sub>d</sub> /F (l/kg)	18	2.4 <sup>f</sup>
CL/F (mL/min/kg)	162 to 91 <sup>c</sup> (M), 52 (F)	27
F <sub>(%)</sub>	40 (M), 60 (F) <sup>d</sup>	15 / 40 <sup>g</sup>
Location in CTD	4.2.2.2	4.2.2.2
Report Number	NP08033	NP15186

In rats, significant relationships were found between CL/F and gender, CL/F and dose. The rat strain, the body weight and the duration of the treatment did not influence CL/F and none of the covariates tested was found to influence significantly any other parameter.

In male and female rats, the absolute bioavailability (F<sub>(%)</sub>) was approximately 40% and 60%, respectively. As noted above (NP15001), it is also approx. 40% in dog and human. In male rats, when the dose increased, the S 16257 apparent clearance decreased. Depending on the dose administered in males, the apparent clearance in female rats was from 43 to 68 % lower than in male rats. For a given dose of S 16257, the exposure of females is higher than the exposure of males. Moreover, the exposure of males increases more than proportionally to the dose certainly because of a saturation of the hepatic first-pass effect. In the rat, the terminal half-life was up to 17.6 h in males and 18.2 h in females at 90 mg/kg/day over 52 weeks.

In dogs, the covariates gender and bodyweight do not significantly influence the parameters CL/F, Q/F, Vc/F, Vp/F and ka. The following covariates were found to significantly influence the pharmacokinetics of S 16257. When the dose increases between 1.60 mg and 376 mg, the volume of the central compartment increases by 2.5-fold. This could be attributed to the increase of the unbound plasma fraction with the concentration (and therefore with the dose). F is estimated to 40 % for doses higher than 1 mg/kg and to 15% for doses lower or equal to 1 mg/kg. This could be attributed to a saturation of an appreciable first-pass effect that is also evident in rat and human. In the 2 studies where S 16257 was administered twice-a-day, the absorption after the second administration was slower than after the first administration (the absorption rate decreased by about 5-fold and Cmax by about 2-fold) while the exposure after the second administration was about 15 % lower than after the first administration. This could be attributed to a food effect, the food being given between the 2 administrations. In the dog, the first exponential phase half-life ( $t_{1/2,\alpha}$ ) was up to 1.39h and the second elimination phase half-life ( $t_{1/2,\beta}$ ) was up to 22 h, estimated for a dose regimen of 2x20 mg/kg/d calculated from 26-week and 52-week studies.

### Distribution

#### **NP15183: IN VITRO PROTEIN BINDING OF S 16257 AND S 18982, THE N-DESMETHYLATED METABOLITE OF S16257 IN ANIMAL AND HUMAN PLASMA.**

The in vitro protein binding of S 16257 using [<sup>14</sup>C]-S16257 as tracer compound and the in vitro protein binding of S 18982, were assessed in heparinized plasma of CD1 mouse, Wistar rat, New Zealand rabbit, beagle dog and Human by ultrafiltration (S 16257 and S 18982) and equilibrium dialysis (S 16257 only). Concentrations of S 16257 (expressed as base form) were 10, 150 and 2500 ng/mL in human plasma, 10, 250, 5000 ng/mL in mouse and rabbit plasma, 10, 400, 15000 ng/mL in rat and dog plasma. Concentrations of S 18982 (expressed as base form) were 10, 70 and 500 ng/mL in animal and human plasma. For S 16257, analysis of the total radioactivity was performed by liquid scintillation spectrometry. For S 18982, analysis was performed by high performance liquid chromatography with tandem mass spectrometric detection.

The ultrafiltration results are shown in the table below (excerpted from page 7 of report). S 16257 is weakly to moderately bound in plasma and the unbound fraction of S 16257 can be ranked as follows (at the intermediate concentration) : rabbit > mouse > rat > human > dog. Except for mouse, this grading is comparable to that obtained for the unbound fraction of S 18982: rat > human > dog > mouse. Moreover, the unbound fraction of S 16257 has been determined by equilibrium dialysis. There was no significant difference between ultrafiltration and equilibrium dialysis for determination of the unbound fraction of S 16257.

**Results : Unbound fraction (fu) of S 16257 and S 18982 in animal and human plasma (ultrafiltration results)**

Plasma	S 16257 Concentration (ng/ml)	fu (%)	S 18982 Concentration (ng/ml)	fu (%)
Mouse	10	53.0	10	12.0
	250	50.5	70	18.7
	5000	53.5	500	13.4
Rat	10	41.0	10	37.1
	400	43.5	70	36.0
	15000	57.5	500	44.4
Rabbit	10	48.4		
	250	53.8		
	5000	61.1		
Dog	10	27.4	10	21.4
	400	28.6	70	25.0
	15000	62.2	500	29.1
Human	10	24.0	10	25.8
	150	30.6	70	29.9
	2500	32.0	500	24.5
Human	150	50.5		
	150	37.4		
	150	31.1		
	150	27.1		
	150	29.0		

**NP07530: PRELIMINARY STUDY OF THE IN VITRO PLASMA PROTEIN BINDING AND THE BLOOD TO PLASMA PARTITIONING OF <sup>3</sup>H-S 16257-2 IN RAT, DOG AND MAN**

The in vitro protein binding and the blood to plasma partitioning using <sup>3</sup>H-S 16257-2 as tracing compound were assessed in human, dog and rat by equilibrium dialysis method.

The median binding percentage of S 16257 was 69.7 % and 68.9 % in human plasma (at concentrations of 10 and 2500 ng/mL, respectively), 87.3 % and 72.7 % in dog plasma (at concentrations of 10 and 5000 ng/mL, respectively), and 58.0 % and 50.8 % in rat plasma (at concentrations of 10 and 5000 ng.m<sup>-1</sup>, respectively).

The blood to plasma ratio of ivabradine ranged from 0.92 to 1.0 in rat (10-5000 ng/mL), 0.76 to 0.95 in dog (10-5000 ng/mL), and 0.65 to 0.69 in human (10-2500 ng/mL). These results indicated that ivabradine distributed evenly between plasma and red blood cells with partial exclusion from the latter in humans.

**NP05420: QUALITATIVE AND QUANTITATIVE TISSUE DISTRIBUTION OF RADIOACTIVITY IN MALE LONG EVANS RATS FOLLOWING SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF <sup>14</sup>C-S 16257-2 AT A DOSE (AS BASE FORM) OF 3 mg/kg (GLP\*)**

Male Long Evans (pigmented) rats received a single oral or iv administration at dose (base form) of 3 mg/kg <sup>14</sup>C-S 16257-2. One Long Evans rat was sacrificed at each of

the following time-points, i.e. 10 min, 1 hr, 6 hr, 24 hr and 168 hr post dosing. Whole-body autoradiography was done and autoradiograms were analyzed using image analysis for quantitation of the radioactivity.

The distribution results for male Long Evans rats were summarized in the tables below (excerpted from M2/2.6.5, pages 17-18). For iv administration, the highest levels of radioactivity were mostly observed at 10 min. Very high concentrations, ranging from about 10000 to 25000 ng eq/g, were observed in tissues as the pituitary gland, adrenals, kidneys, liver, lacrymal gland, spleen, submaxillary glands, thyroid gland and pancreas. The concentration in heart (4085 ng eq/g) was less than 10000 ng eq/g but well above the blood level. The elimination of the radioactivity was somewhat delayed in the adrenals, meninx, pigmented skin and the uveal tract which were still labelled at 168 hr.

For oral administration, the highest levels of radioactivity were observed at about 1 hr. The tissues which concentrations were above the blood levels were the heart, the brown fat and the liver. The maximal tissue concentrations after oral dosing were lower than those observed with the same iv dose. The radioactivity levels in brain, spinal cord and bone mineral were always below the limit of quantitation for the oral route whereas the peak levels of radioactivity in the brain were 0.023% of dose/g brain tissue after iv dosing. At 168 hr after oral dosing, meninx and the uveal tract were still labelled.

There was no accumulation over time in cardiac tissues following either iv or oral dosing.

**Concentration of radioactivity in tissues and Tissue/Blood concentration ratio of male Long Evans rats following single intravenous administration of 14C-S 16257-2 at a target dose of 3 mg/kg (expressed as base form) and 2.78 MBq/kg (specific activity=0.93 MBq/mg).**

Tissues / Organs <sup>o</sup>	Concentration (ng eq/g tissue)					Tissue/blood concentration ratio			
	Time (h)	0.17	1	6	24	168	0.17	1	6
Blood		1942	781	66	blq	blq			
Kidney (medulla)		24668	5071	231	blq	blq	13	6.5	3.5
Submaxillary glands		17855	12008	2396	427	blq	9.2	15	36
Adrenal medulla		14857	5158	442	379	blq	7.6	6.6	6.7
Liver		14304	7060	1542	220	blq	7.4	9.0	23
Kidney (cortex)		13092	4525	212	blq	blq	6.7	5.8	3.2
Adrenal cortex		11707	5012	691	401	96	6.0	6.4	10
Lachrymal glands		11076	5286	231	44	blq	5.7	6.8	3.5
Kidney (corticomedulla)		10656	6151	538	79	blq	5.5	7.9	8.2
Uveal tract		7367	7040	3074	2616	2404	3.8	9.0	47
Heart		4085	1309	82	blq	blq	2.1	1.7	1.2
Urinary bladder wall		2987	4716	478	blq	blq	1.5	6.0	7.2
Skin (pigmented)		2628	2443	1094	639	185	1.4	3.1	17
Skin (non-pigmented)		2628	1271	70	blq	blq	1.4	1.6	1.1
Meninges		2297	3054	1170	1011	1031	1.2	3.9	18
Seminal vesicles		2158	20795	185	blq	blq	1.1	27	2.8
Testes		179	212	231	blq	blq	0.1	0.3	3.5
Brain		152	192	blq	blq	blq	0.1	0.2	-

Additional information: Limit of quantitation = 26 ng eq/g tissue; blq: Below the limit of quantitation

Tissues and organs such as bone surfaces, bone marrow, brown fat, colon, intestine wall, lung, pancreas, pineal gland, pituitary gland, prostate, skeletal muscle, spinal cord, spleen, stomach wall, thymus and thyroid gland were examined but are not reported here; except for the intestine wall (blq at 168 h), the concentrations in these tissues were blq at 24 h.

a: Mixture of the two [<sup>14</sup>C]-ivabradine chemical entities b: Tissues and organs are ranked based upon their tissue/blood concentration ratio at 0.17 h

**Concentration of radioactivity in tissues and Tissue/Blood concentration ratio of male Long Evans rats following single oral administration of 14C-S 16257-2 at a target dose of 3 mg/kg (expressed as base form) and 2.78 MBq/kg (specific activity=0.93 MBq/mg).**

Tissues / Organs <sup>b</sup>	Concentration (ng eq/g tissue)					Tissue/blood concentration ratio		
	Time (h)	0.17	1	6	24	168	1	6
Blood		blq	134	77	blq	blq		
Liver	1166	3604	1809	213	blq	blq	27	23
Kidney (corticomedulla)	139	1764	875	69	blq	blq	13	11
Uveal tract	blq	1734	1630	2683	1530	blq	13	21
Adrenal medulla	195	1604	465	209	blq	blq	12	6.0
Submaxillary glands	83	1544	1799	104	blq	blq	12	23
Adrenal cortex	214	1479	923	293	blq	blq	11	12
Kidney (medulla)	86	1291	499	blq	blq	blq	9.6	6.5
Kidney (cortex)	100	1045	339	blq	blq	blq	7.8	4.4
Urinary bladder wall	blq	883	208	285	blq	blq	6.6	2.7
Lachrymal glands	blq	561	337	blq	blq	blq	4.2	4.4
Skin (pigmented)	blq	423	527	294	blq	blq	3.2	6.8
Seminal vesicles	blq	266	140	blq	blq	blq	2.0	1.8
Heart	blq	260	103	blq	blq	blq	1.9	1.3
Skin (non-pigmented)	blq	225	92	blq	blq	blq	1.7	1.2
Meninges	blq	blq	953	703	654	-	-	12
Testes	blq	blq	108	blq	blq	-	-	1.4
Brain	blq	blq	blq	blq	blq	-	-	-

Additional information: Limit of quantitation = 26 ng eq/g tissue; blq: Below the limit of quantitation

Tissues and organs such as bone surfaces, bone marrow, brown fat, colon, intestine wall, lung, pancreas, pineal gland, pituitary gland, prostate, skeletal muscle, spinal cord, spleen, stomach wall, thymus and thyroid gland were examined but are not reported here; the concentrations in these tissues were blq at 24 h.

**NP08034: S 16257 2 In Vivo ocular distribution and localization after a single administration of 14C-S 16257 in albino and pigmented rats (GLP)**

Fifteen male Long Evans rats and fifteen male Wistar rats received a single iv bolus at dose (base form) of 3.9 mg/kg 14C-S 16257-2. Three rats of each strain were sacrificed at each of the following time-points, i.e. 10 min, 1 hr, 6 hr, 24 hr and 168 hr post dosing. Eyeballs (autoradiography) or eyelids, cornea, aqueous humor, iris-ciliary body, lens, vitreous, choroid-retina, sclera, optic nerve, lacrimal gland, heart, brain, skin, whole blood and plasma (liquid scintillation) were sampled at designated time points and analyzed using radioactivity counting by liquid scintillation and autoradiography method.

The result was summarized in the table below (excerpted from M2/2.6.5, page 19), left panel for Wistar rats and right panel for Long Evans rats. It showed that S16257 distributed to various extents in heart, skin and brain, and in all ocular structures, mainly in pigmented and/or vascularized structures, including iris ciliary process, chorioretina, sclera, eyelids and lacrimal gland (from the highest to the lowest exposure). The distribution was larger in pigmented than in albino eyes, indicating affinity for ocular pigments and slower elimination from pigmented structures. Micro-autoradiography data confirmed the binding of 14C-labelled substances to the pigment in iris, ciliary process and choroid of pigmented animals and the absence of binding to the retina. No detectable labelling was observed in albino rats.

Tissues / Organs	Time (h)	Concentration (ng eq/g tissue)					AUC <sub>168</sub> (ng eq.h/g tissue)	Concentration (ng eq/g tissue)					AUC <sub>168</sub> (ng eq.h/g tissue)	Pigmente d/albino AUC ratio	
		0.17	1	6	24	168		0.17	1	6	24	168			t <sub>1/2,z</sub> (h)
Plasma	5908	3027	595	36	7.6	22	22100	7674	2369	408	33	7.2	22	18660	0.84
Aqueous humor	58	45	12	blq	blq	2.6	298	29	30	blq	blq	blq	nd	102	0.35
Chorio-retina	830	382	92	17	blq	4.8	3967	6827	11845	11408	11441	8047	>168	1675214	422
Cornea	266	228	27	blq	blq	1.7	1105	212	1280	309	321	414	>168	63222	57
Iris-ciliary body	74	185	6.6	blq	blq	1.4	652	2810	4594	8062	10408	2205	136	1109351	1702
Eyelids	1761	1097	129	47	20	36	10835	2382	1676	886	840	287	70	104970	9.8
Lachrymal gland	4812	5075	314	35	2.7	18	23824	5230	3870	135	58	8.3	23	20728	0.87
Lens	28	28	15	5.5	blq	10	710	28	38	17	13	blq	19	1351	1.9
Optic nerve	509	418	62	blq	blq	1.9	2193	260	109	19	7.0	blq	5.4	1227	0.56
Sclera	573	506	77	8.1	blq	4.0	3303	3483	6210	2303	3043	3120	>168	517478	157
Vitreous humor	139	95	12	blq	blq	1.7	484	772	2932	439	630	128	56	74213	153

blq: Below the limit of quantitation

Additional information: Limit of quantitation depending on tissue, i.e. ranging from 1.5 (lachrymal gland) to 27 ng eq/g tissue (iris-ciliary body)

*NP15280: this is a 52-week dog toxicity study which was reviewed in section of general toxicology below. Limited tissue distributions of S 16257 and its metabolite, S18989 in the heart and eyes were determined as part of toxicity study evaluation.*

*NP05035: this study was reviewed in section of reproductive toxicology. Concentrations of S 16257 and its metabolite, S18989 were measured in amniotic fluid. See review of reproductive toxicology in Appendix 3.*

**NP08639: The Secretion of Total Radioactivity in Milk of Lactating Rats Following Repeated Oral Administration of [<sup>14</sup>C]-S 16257 ( (b) (4) )**

Six lactating female rats received oral administration of [<sup>14</sup>C]-S 16257 at target dose level of 7 mg base/kg/day on Days 10, 11, 12 13 and 14 after parturition. Samples of milk were collected at 0.5 and 2.5 h post dose. Animals were sacrificed after milk collection and a sample of whole was collected at the termination.

Mean concentration of total radioactivity in milk and plasma following multiple doses is shown in the table below (excerpted from page 20 of the report). The results demonstrated that the radioactivity associated with [<sup>14</sup>C]-S 16257, or its metabolites, were easily transferred into milk after oral administration, and that ivabradine concentrated somewhat in milk.

Sample	Time			
	0.5h		2.5h	
	Mean	SD	Mean	SD
Milk	1801	294	1139	242
Plasma	1233	288	627	142
Ratio	1.5	0.1	1.8	0.1

Ratio = ratio of total radioactivity in milk to plasma

**NP15184: Investigation into the cellular transport of S 16257 and S 18982 using the Caco-2 cell line**

Directional transport of ivabradine or S 18982 was measured across Caco-2 cell monolayers, with kinetics determined across a range of substrate concentrations (1, 5, 25, 125, and 250  $\mu$ M). The transport of ivabradine and S 18982 across Caco-2 cells was also measured in the absence or presence of verapamil (100  $\mu$ M), a known inhibitor of Pgp.

Caco-2 cell monolayers in vitro net apparent permeability ( $P_{app}$ ) was  $1.37 \times 10^{-6}$  cm/s for ivabradine and  $7.39 \times 10^{-6}$  cm/s for verapamil. The kinetic parameters of the active efflux (e.g. P-gp) of ivabradine (10  $\mu$ M) and S 18982 (10  $\mu$ M) were determined in Caco-2 cells. Ivabradine had a  $V_{max}$  of 13.2 pmol/min/cm<sup>2</sup>,  $K_m$  of 310  $\mu$ M, and intrinsic clearance ( $CL_{int}$ ) of  $4.25 \times 10^{-5}$  mL/min/cm<sup>2</sup>. S 18982 had a  $V_{max}$  of 3.85 pmol/min/cm<sup>2</sup>, a  $K_m$  of 15  $\mu$ M, and  $CL_{int}$  of  $25.3 \times 10^{-5}$  mL/min/cm<sup>2</sup>. Correlation of passive permeability

with absorption in human of reference drugs predicted an in vivo absorbed fraction in humans of 91% for ivabradine, and 32% for S 18982.

### **NP15182: Investigation into the cellular transport of S 16257 and some metabolites using the blood-brain barrier model**

Test compounds (10  $\mu$ M) including ivabradine and seven of its primary metabolites: S 18982 (M29), M3, M10, M22, M26, M28 and M31, were added separately to a confluent monolayer of bovine brain capillary endothelial cells (passage 5) previously cultured with astrocytes. The rate of apical to basolateral transport was then measured by sampling the basolateral medium at 10, 20, 30, 40, and 60 minutes, and analyzing for test compound using LCMS. Results indicated that permeability was low for M3 and S 18982, intermediate for M10, M22 and M26, and high for ivabradine, M28 and M31.

### Metabolism

### **NP06900: In vitro characterization of human P450 isoforms involved in the hepatic metabolism of S 16257 and in vitro interspecies comparison**

The metabolism of S 16257 was studied in rat, dog, monkey, mouse, rabbit and human liver microsomes using new incubation conditions allowing the measurement of the main primary S 16257 metabolites: various hydroxy-S 16257, various demethylated-S 16257 (e.g. M29 - S 18982) and various cleavage compound (e.g. Y 492).

Results of the percentage S 16257 remaining (percentages of total radioactivity in the metabolic profiles) in the course of incubations are shown in the table below (excerpted from page 44 of the report).

**Table 3B: Metabolism of S 16257 (1 and 100  $\mu$ M) following incubation (15 to 90 min with hepatic microsomes (clearance study)).**

S 16257	S 16257 area %				
	15 min	30 min	45 min	60 min	90 min
1 $\mu$ M					
Rat	66	58	56	49	44
Dog	86	78	72	64	54
Human	88	72	66	61	45
100 $\mu$ M					
Rat	90	76	70	67	69
Dog	96	90	88	84	79
Human	89	84	75	67	60

Values expressed as S 16257 percentage area (percentages of total metabolic profile)

Liver microsomes converted S 16257 to about 13 metabolites. The metabolic profile in microsomes at 60 min of incubation was shown in the tables below (excerpted from page 9 of the report).

**Metabolic profiling in hepatic microsomes (60 min). results are expressed as % of the total radioactivity**

Species	Parent	Cleavage compound		
		M3 (Y609)	M5	Y 492 *
Mouse	52.8	5.6	5.5	1.8
Rat	49.2	1.0	4.6	3.9
Rabbit	9.5	0.5	-	0.8
Dog	46.6	3.6	4.4	1.2
Monkey	-	21.7	8.7	8.5
Human	37.1	7.2	3.8	3.2

Species	Hydroxylated / O- or N- desmethylated derivatives														Other
	M16 M17 (a)	M18	M21	M21 (b)	M20 (b)	M22 (c)	M25 (c)	M26 (c)	M27 (d)	M26 (d)	M22 (e)	M29 (e)	M28 (f)	M25 (f)	
Mouse	2.1		2.6	4.4		-			4.6		14.4		3.8		-
Rat	3.3		3.6	7.5		4.6			3.3		12.1		3.6		-
Rabbit	-	18.6	8.3	-		8.2			-		53.1		-		-
Dog	-		2.4	3.5		-			16.9		10.3		7.4		-
Monkey	20.8	8.8	9.3	-		-			7.1		-		-		-
Human	7		2.3	5.1		-			5.6		14		8.2		2.31

- : not detected; S: metabolite only observed in *in vitro* samples; (a) : M16 or M17 ; (b) : M21 coeluted with M20; (c) : M22 coeluted with M25 and M26 ; (d) : M27 coeluted with M26; (e) : M22 coeluted with M29 ; (f) : M28 coeluted with M25

For (a), (b), (c), (d), (e) and (f), the percentages of radioactivity indicated in the table are those of the sum of the identified metabolites.

S 16257 was slowly metabolized by rat, dog, mouse and human microsomes, and almost completely or completely metabolized by rabbit and monkey microsomes, respectively. The metabolic profiles observed in rat, dog and man were similar; the profile in mouse was closer to that in man than to that in rabbit and monkey. Predicted values for the hepatic clearance represented between 24 and 41 % of the hepatic blood flow. For human, apparent  $K_m$  and  $V_m$  were 146 pM and 2.2 pmole/min/g protein, respectively, and the extrapolated hepatic clearance represented 36% of the hepatic blood flow. The extrapolated *in vivo* hepatic clearances were ranked as follows: rat < dog < human, which was in good agreement with the actual *in vivo* clearances, and partially accounts for the first pass effect (i.e.,  $F < 100\%$ ) noted in all species.

The total S 16257 metabolism inhibition observed *in vitro* in presence of clotrimazole, a global cytochrome P450 inhibitor, showed the involvement of the cytochrome P450 enzyme system in the metabolic pathways of S 16257 in human. The effects of selective inhibitors of various cytochrome P450 isoforms (CYP) on S 16257 metabolic pathways were investigated. The result is shown in the table below (excerpted from page 10 of the report). It showed that CYP3A4 was the main enzyme involved in S 16257 metabolism (partial inhibition by troleandomycin and complete inhibition by ketoconazole). These results were confirmed using human CYP expressed in human lymphoblastoid cell lines because S 16257 was only metabolized by microsomes of CYP3A4 transfected cells and lead to all the metabolites formed by human microsomes.

Overall, CYP3A4 appeared to be the main human cytochrome P450 isozyme involved in all primary routes of metabolism of S 16257 (hydroxylation, demethylation and cleavage), indicating a potential risk of drug-drug interaction with CYP3A4 inhibitors.

CYP isoform	Constitutive expression in liver (%)	Gentest	Inhibitors	Correlation with CYP activities	Inhibitor effect
1A1	13%	-	$\alpha$ -naphthoflavone	nd	phenacetin +
1A2		-	furafylline	-	
2A6	4%	-	pilocarpine	nd	coumarine nd
2B6	1%	-	orphenadrine	nd	coumarine nd
2C8/9	18%	-	sulfaphenazole	-	tolbutamide -
2C18/19		-	S-mephenytoin	nd	S-mephenytoin nd
2D6	2%	-	quinidine	-	dextromethorphan -
2E1	7%	-	disulfiram	nd	chlorzoxazone nd
3A4	28%	+	TAO ketoconazole	+++ +++	testosterone -
4A9		nd		-	lauric acid nd

#### NP06887: In vitro metabolism of [<sup>14</sup>C]-S 16257 with rat, dog, monkey and human hepatocytes. (GLP\*)

Cryopreserved hepatocytes from Wistar rat, Beagle dog, Macaca fascicularis monkey and man were incubated with 10 NM of [<sup>14</sup>C]-S 16257 for 3 and 24 hours. Measurement of radioactivity at the end of the incubation with hepatocytes lead to total recoveries ranging from 89 to 101 %.

The rate of metabolism ranked as follows: monkey > rat > dog>human. The levels of unchanged drug remaining after a 3 hour or a 24 hour hepatocyte culture in dog and human were not significantly different from those measured in the control indicating that there was almost no metabolism of [<sup>14</sup>C]-S 16257 by hepatocytes from these species. In conclusion, the results observed with hepatocytes were similar to those observed with hepatic microsomes and indicated a slow in vitro metabolism of S 16257 in dog and human while the most rapid metabolism was observed with monkey hepatocytes.

#### NP15163: INTERSPECIES COMPARISON OF THE IN VIVO METABOLISM OF S 16257

Results from three metabolism studies (study Nos. NP06650, NP06651 and NP06963) were compiled in this report, which enables interspecies comparison. These data were obtained following single oral administration of [<sup>14</sup>C]-S 16257-2 to rats, dogs and human at 3 mg/kg, 1 mg/kg and 20 mg, respectively, where S 16257 metabolism was investigated in plasma, urine and feces samples.

The plasma exposure to S 16257 and its main primary metabolites was then assessed following repeated oral administration of unlabelled S 16257-2 to mice and rats at the highest doses of the carcinogenicity studies (i.e. 405 mg/kg/d and 120 mg/kg/d, respectively), to dogs at an intermediate dose used in toxicology studies (10 mg/kg/d) and to human at a therapeutic dose (20 mg/d) for 42, 28, 5 and 5 days, respectively.

As shown in the summary table below (excerpted from page 6 of the report), S 16257 was extensively metabolized, with more than twenty metabolites recovered in plasma and urine and between ten and twenty in feces regardless of the species. The major S 16257 metabolic pathways observed in plasma, urine and faeces were similar for the three species. They were identified as N-dealkylations, hydroxylations (mono or dihydroxylations) and N- or O-desmethylations.

**Metabolic pattern : Plasma (results are expressed as % of radioactivity)**

Short name	S 16257	Cleavage compounds									
		M1	M2	M3 (Y609)	M4	M5	M6	M7	M8	M9 (Y596)	M10 (Y831)
Standardised Rt (min)	82	11	13	23	29	31	40	45	53	60	
Rat PO 3 ma/ka (1+6+8 h)	15	-	5.3	32	MS	5.8	7.5	2.8	-	MS	
Doa PO 1 ma/ka (1 h)	22	-	MS	23	MS	19	MS	2.0	1.8	MS	3.7
Human PO 20 ma (1 h)	27	-	-	29	-	7.9	6.1	-	-	-	5.6
Human PO 20 ma (5 h)	15	-	-	14	-	13	11	-	MS	14	

Short name	Conjugated derivatives				
	M11	M12	M13	M14	M15
Standardised Rt (min)	61	66	69	72	72
Rat PO 3 ma/ka (1+6+8 h)	-	14	7.0	-	-
Doa PO 1 ma/ka (1 h)	-	2.1	1.6	-	-
Human PO 20 ma (1 h)	-	-	-	-	-
Human PO 20 ma (5 h)	-	-	-	-	-

Short name	Hydroxylated / O- or N- desmethylated derivatives														
	M16	M17	M18	M19	M20	M21	M22 *	M23 (Y659)	M24	M25	M26 **	M27	M28 (Y1016)	M29 (S 18982)	M32 (Y1021)
Standardised Rt (min)	72	72	74	76	76	76	76	76	76	78	78	78	79	80	86
Rat PO 3 mg/kg (1+6+8 h)	MS	-	-	-	-	MS	-	-	-	MS	-	-	MS	MS	na
Dog PO 1 mg/kg (1 h)	3.3	-	MS	-	MS	3.5	-	-	MS	3.4	MS	2.4	MS	MS	na
Human PO 20 mg (1 h)	3.9	-	-	-	-	2.0 <sup>a</sup>	-	-	-	2.0 <sup>a</sup>	-	6.1	-	-	-
Human PO 20 mg (5 h)	6.9	-	MS	MS	-	MS	4.4 <sup>a</sup>	MS	MS	MS	4.4 <sup>a</sup>	-	13	MS	na

Short name	Others metabolites	
	M30 (Y1044)	M31 (S 33170)
Standardised Rt (min)	81	84
Rat PO 3 ma/ka (1+6+8 h)	MS	-
Doa PO 1 ma/ka (1 h)	MS	MS
Human PO 20 ma (1 h)	4.9	-
Human PO 20 ma (5 h)	6.8	MS

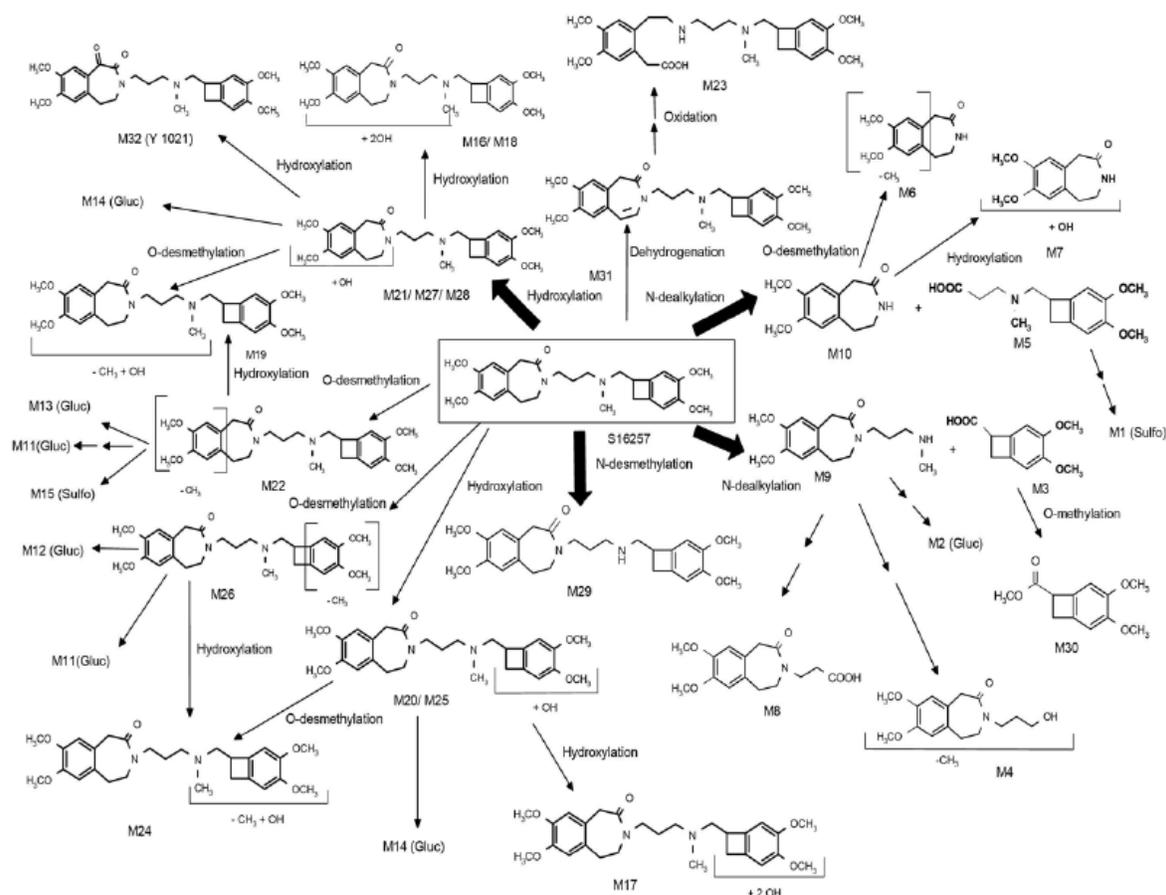
**Additional information :** "MS" : metabolite only detected by mass spectrometry. For human plasma, mass spectrometry analysis was performed on the 5 h samples only.

"-" : not detected ; "na" : not analysed; \* : M22 (S 33173 or S 33174), \*\* : M26 (S 33171 or S 33172).

a : in human plasma M22 coeluted with M26 (representing together 2.0 % of the dose at 1 h and 4.4 % of the dose at 5 h).

Overall, S 16257 was extensively but not completely metabolized. Qualitatively, all the metabolites observed in human were also detected in the animal species, and quantitatively, dog is the closest species to human. Moreover, the plasma exposure to S 16257 and its main primary metabolites following repeated oral administration of relevant doses to animals and human, shows at least a 2 to > 3500-fold higher exposure in animals than in human, validating therefore the mouse, the rat and the dog as relevant species for safety assessment.

A postulated interspecies metabolic pathway of S 16257 is shown in the figure below (excerpted from page 53 of the report).



**NP15187: A study to evaluate the plasma exposure of Wistar rats, CD-1 mice, beagle dogs and healthy volunteers to ten metabolites of S 16257 and S 16257 after repeated oral administration of S 16257-2**

The repeat dose interspecies metabolism was evaluated in this study based on multiple oral studies in mice, rats, dogs and humans. The dosing regimens in these studies are listed in the summary table below (excerpted from M2/2.6.5, page 32). S 16257 and 10 of its metabolites were quantified in plasma using an analytical procedure involving solid phase extraction on OASIS extraction 96-well plates, followed by liquid chromatography (2 methods: isocratic mobile phase for quantification of S 16257, S 18982, S 33170, Y 1016, Y 1021, Y 609 and Y 831 and using a gradient for S 33171, S 33172, S 33173 and S 33174) with tandem mass spectrometry detection (ESI+). The limit of quantitation (LOQ) was 0.2 ng/ml for S 16257 and 9 metabolites and 2 ng/ml for Y 609 using a sample size of 250  $\mu$ l.

As shown in the summary table below, all the circulating metabolites present in human were present in animal plasma. Animals were always more exposed to S 16257 and all the metabolites than humans. Despite a slow rate of formation in rats and dogs of S 18982 (active metabolite) in comparison to the other species, the animals were more exposed than humans, with the animal/human AUC<sub>24</sub> ratio ranged from 2.4 to 3.5 in

rats and dogs and > 45 in mice. The O-desmethylated derivatives of S 16257 (S 33171, S 33172, S 33173, S 33174) were only detected in animal species. Some gender differences were observed in mice and rats while no gender difference was noticed in dogs and humans.

*Plasma exposure to ivabradine and ten metabolites after repeated oral doses*

Species		Mouse	Rat	Dog	Human							
Strain		CD-1	Wistar	beagle	Caucasian							
Number of animals / Gender (M/F)		24M+24F	6M+6F	4M+4F	4M+4F							
Feeding condition		Fed	Fed	Fed	Fasted							
Formulation / Vehicle		Diet	Diet	Solution / purified water	Solution / drinking solution							
Method of administration		Oral	Oral	Oral (gavage)	Oral							
Duration of treatment		4 weeks	6 weeks	5 days	5 days							
Dose (mg/kg/d)*		405	120	2 x 5	2 x 10 mg							
Assay		LC-MS-MS	LC-MS-MS	LC-MS-MS	LC-MS-MS							
Occasion		D28	D42	D5	D5							
Species	Gender	Metabolite/ivabradine AUC <sub>24</sub> molar ratio <sup>a</sup>										
		M3	M10	M22a <sup>a</sup>	M22b <sup>a</sup>	M26a <sup>b</sup>	M26b <sup>b</sup>	M28	M29 (S 18982)	M31	M32	
Mouse	Male	36	0.46	0.0010	0.0021	0.0019	0.0020	0.12	0.48	0.0045	0.026	
	Female	5.8	0.21	0.00085	0.0017	0.0019	0.0014	0.097	0.27	0.0068	0.022	
Rat	Male	5.2	0.51	0.0091	0.0065	0.0053	0.014	0.14	0.057	0.0099	0.042	
	Female	1.2	0.079	0.0043	0.0038	0.0030	0.0058	0.10	0.023	0.011	0.021	
Dog	Male	11	0.16	0.060	0.0064	0.0015	0.052	0.096	0.070	0.0069	0.016	
	Female	13	0.17	0.047	0.0077	0.0016	0.064	0.11	0.10	0.0072	0.019	
Human	Male	0.45	0.61	-	-	-	-	0.16	0.38	0.049	0.089	
	Female	0.79	0.66	-	-	-	-	0.16	0.41	0.045	0.084	
Species	Gender	Animal/human AUC <sub>24</sub> ratio										
		Ivabradine	M3	M10	M22a <sup>a</sup>	M22b <sup>a</sup>	M26a <sup>b</sup>	M26b <sup>b</sup>	M28	M29 (S 18982)	M31	M32
Mouse	Male	47	3685	35	-	-	-	-	37	58	4.3	14
	Female	71	520	22	-	-	-	-	44	46	11	19
Rat	Male	23	267	20	-	-	-	-	21	3.5	4.7	11
	Female	59	94	7.1	-	-	-	-	38	3.3	14	15
Dog	Male	18	443	4.6	-	-	-	-	11	3.2	2.5	3.1
	Female	9.8	163	2.6	-	-	-	-	6.8	2.4	1.6	2.2

Dog and human plasma samples were obtained from 2 different studies (Reports NP15258 and NP15383, respectively).

a: Two O-desmethylated isomers of M22 (M22a = S 33173 and M22b = S 33174)

b: Two O-desmethylated isomers of M26 (M26a = S 33171 and M26b = S 33172)

\*: Unless otherwise indicated -: AUC not calculable in humans

## Bioconversion (Chiral conversion)

### NP15001 PHARMACOKINETICS OF S 16257 IN THE MALE BEAGLE DOG AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16257-2.

### NP15152: PHARMACOKINETICS OF S 16257 IN MALE WISTAR RATS AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16257-2

### NP16270: PHARMACOKINETICS OF S 16260 IN MALE WISTAR RATS AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF S 16260-2

S 16260 and S 16257 are the R and S enantiomers of S 15544, respectively. The bioconversion between S16260 and S16257 was assessed in the above three studies. The measurement of S 16260 and S 16257 in plasma specimens was performed according to a chiral analytical method using solid-phase extraction followed by LC-MS/MS detection (LOQ = 0.2 ng/mL) on a Chiralpak AD-RH column. In rats and dogs, no in vitro inversion at the stereocenter of ivabradine was observed in plasma samples from PK studies, i.e. there was neither *in vivo* interconversion of the S-enantiomer,

ivabradine (S 16257) into the R-enantiomer S 16260 (rat Study NP15152; dog Study NP15001), nor *in vivo* inter-conversion of S 16260 to ivabradine (rat Study NP16270).

### Excretion

Excretion after a single iv dose summarized from several PK studies was presented below (M2/2.6.5, page 49). The relevant information was also reviewed in the PK studies in absorption section, which included NP05245 (rat, single dose), NP15152 (rat, single dose, 67.4), NP15001 (dog), NP16236 (mouse, single dose), rat population PK model: Report NP08033; dog population PK model: Report NP15186.

#### *Excretion after a single intravenous dose*

Species	Rat	Rat	Dog	Dog	Human <sup>c</sup>
Strain	Wistar	Wistar	beagle	beagle	Caucasian
Number of animals / Gender (M/F)	12M	4F	3M	3F	8M
Feeding condition	Fed	Fed	Fasted	Fasted	Fed
Formulation / Vehicle	Solution / saline				
Method of administration	iv (bolus)				
Dose (mg/kg)*	3	2.3	3.7	3.7	24 mg
Sample	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	S 16257				
Assay	LC-Fluo	LC-Fluo	LC-Fluo	LC-Fluo	LC-Fluo
PK parameters:					
t <sub>max</sub> (h)	0.083 <sup>a</sup>				
C <sub>max</sub> (ng/mL)	997	1132	4130	5660	1279
AUC (ng.h/mL)	690	1009	4197	5265	920
V <sub>ss</sub> (l/kg)	3.4	3.3	0.97 <sup>b</sup>	0.90 <sup>b</sup>	0.92 <sup>b</sup>
CL (mL/min/kg)	72	38	15	14	6.6 <sup>b</sup>
t <sub>1/2,z</sub> (h)	0.89	1.4	0.76	0.75	6.4
Location in CTD	4.2.2.5	4.2.3.2	4.2.3.2	4.2.3.2	5.3.4.1
Report Number	NP05245	NP05181	NP05108	NP05108	NP06965

The major route of excretion of unchanged ivabradine and/or metabolites was in the feces of rats and dogs (intact rats: NP05193, intact dog: NP05258). In bile duct-cannulated rats, most of the radioactivity was excreted into the bile.

### **NP05193: EXCRETION BALANCE OF <sup>14</sup>C-LABELLED SUBSTANCES AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF <sup>14</sup>C-S 16257-2 TO MALE WISTAR RATS**

Male Wistar rats received a single oral or intravenous administration of <sup>14</sup>C-S 16257-2 at doses of 3 mg/kg (PO and IV dosing) and 200 mg/kg (PO administration only). Animals were kept in metabolism cages allowing the separate collection of urine and feces at 168 hour post dosing. The results were summarized in the table on the next page (excerpted from M2/2.6.4, page 28, Table 6-1). In all cases, the radioactivity was mainly eliminated via the feces (likely due to biliary excretion) and represented on the average 76 to 81 % of the dose. The mean total recovery after 168 h varied between 98 and 102 % of the dose, indicating that there was no retention of radioactivity.

### **NP05222: BILIARY EXCRETION AND EXCRETION BALANCE OF <sup>14</sup>C-LABELLED SUBSTANCES AFTER SINGLE ORAL AND INTRAVENOUS ADMINISTRATION OF <sup>14</sup>C-S 16257-2 TO BILE DUCT CANNULATED RATS**

Bile duct-cannulated male Wistar rats (at least 3 rats for each dose) received a single oral or intravenous administration of <sup>14</sup>C-S 16257-2 at doses of 3 mg/kg (PO and IV

dosing) and 200 mg/kg (PO administration only). Animals were kept in metabolism cages allowing the separate collection of bile, urine, feces and cage washes for 72 h (after the low dose) or 144 h (after the high dose). The excretion of radioactivity in samples were quantified. The results were summarized in the table below (excerpted from M2/2.6.4, page 28, Table 6-1). In bile duct-cannulated rats, most of the radioactivity was excreted into the bile. Recovery of radioactivity was high (90% to 100%) after single oral administration (3 or 200 mg/kg) or intravenous administration (3 mg/kg) of [<sup>14</sup>C]-ivabradine to bile duct-cannulated male Wistar rats.

In the Study NP05258 (reviewed in the Absorption section, 2 dogs following oral or iv dosing), elimination of the radioactivity was initially rapid and at 72 h after dosing about 89 to 94 % was already recovered. After that time, the elimination proceeded less rapidly and the excretion balance after 168 h varied between 92 and 97 % in individual animals. The excretion data was summarized in the table below (excerpted from M2/2.6.4, page 28, Table 6-1).

**Table 6-1. Excretion Balance in Rat, and Dog Following Single Oral and Intravenous Administration of [<sup>14</sup>C]-Ivabradine**

Species (Strain)	Gender	N	Route	Dose		Duration (h)	Bile % of radioactive	Urine	Feces	Total	Report number
				(mg/kg)	(MBq/kg)*						
Rat (Wistar)	male	4	oral	3	2.8	168	-	16	81	98 <sup>a</sup>	NP05193
		4	oral	200	2.8	168	-	21	79	100 <sup>a</sup>	
		4	iv	3	2.8	168	-	26	76	102 <sup>a</sup>	
	male <sup>c</sup>	4	oral	3	2.8	72	59	28	12	99 <sup>a</sup>	NP05222
		3	oral	200	2.8	144	44	36	9.2	90 <sup>a</sup>	
		4	iv	3	2.8	72	63	31	4.8	99 <sup>a</sup>	
Dog (beagle)	male	2	oral	1	0.37	168	-	31	62	94 <sup>b</sup>	NP05258
		2	oral	10	0.37	168	-	38	52	93 <sup>b</sup>	
		2	iv	1	0.37	168	-	27	68	95 <sup>b</sup>	

a: Total includes the recovery in the cage washes. b: Total includes the recovery in the cage washes and cage debris.

c: Bile duct-cannulated rats.

### Induction/Inhibition of Drug-Metabolizing Enzymes

#### **NP06902: Assessment of induction of cytochrome P450 isoenzymes in the wistar rat after oral administration of S16257-2 for 4 weeks**

Five male wistar rats per dose group received oral administration of S16257-2 at 2x60, 2x90, and 2x125 mg/kg/day for 4 weeks. Hepatic microsomes were prepared from liver biopsies of animals following 4-week dosing. Liver to body weight ratios, microsomal protein concentrations, total CYP levels and related CYP activities (ethoxyresorufin and phenacetin O-dealkylations, dextromethorphan O-demethylation, chlorzoxazone 6-hydroxylation and testosterone hydroxylations) were determined. CYP2C1, 2B1/2, 1A1, 1A2, 3A1 and 3A2 apoprotein content were measured by Western immunoblotting analysis. The effect of repeated dosing of S 16257 on its own *in vitro* metabolism was also investigated.

No effect was observed in the liver to body weight ratio, the microsomal protein

concentrations, the total CYP levels as well as for activities related to the CYP2A, 2B, 2C, 2D, 2E and 3A after dosing of S 16257 at the highest toxicological dose D4 (2 x 125 mg/kg/d) as compared with the control dose (0 mg/kg/d). No modification of S 16257 own metabolism (i.e. auto-induction) was observed.

For CYP1A subfamily, the enzyme -activities (ethoxyresorufin and phenacetin dealkylations) and the related (CYP1A2) protein expression were slightly increased (factor 1.8 to 2.4) at the highest dose. A significant increase in the paracetamol formation was also observed at low and mid-dose levels but with an induction factor less than 2. However, in comparison with the effects observed with well-known inducers like 3-methylcholanthrene (factor 35), the effect observed with S 16257 could not be attributed to an induction process.

For CYP3A, only a slight decrease in CYP3A2 protein concentration was observed by Western blotting but with no significant implication on CYP3A activity (2R testosterone hydroxylase).

**NP15218: Determination of the kinetic parameters (Km, Vmax) of the biotransformation of S 16257 and S 18982 and assessment of drug interaction with therapeutic agents which are substrates or inhibitors of CYP3A4 using human hepatic microsomes**

In human liver microsomes, biotransformation of S16257 (10  $\mu$ M and 300  $\mu$ M ) or S 18982 (variously 5  $\mu$ M, 10  $\mu$ M, and 300  $\mu$ M) was measured. Chemical inhibition were assessed using human liver microsomes and S16257 or S 18982 (1, 10, 100  $\mu$ M) with and without ketoconazole (1  $\mu$ M). The effect of 53 known CYP3A4 substrates and/or inhibitors (at 3 concentrations) on the metabolism of S16257 (10  $\mu$ M) was determined. Inhibition constants (Ki) were determined for 30 drugs using S16257 or S 18982 at 10  $\mu$ M and 300  $\mu$ M, and various concentrations of inhibitors.

Results showed that S16257 was a potent CYP3A4 inhibitor. In human liver microsomes, ketoconazole was a potent inhibitor of the metabolism of S16257 (~85% remaining) and of S 18982 (~100% remaining). S16257 was metabolized with a Km of  $209 \pm 68 \mu$ M; S 18982 was metabolized with a Km of  $178 \pm 68 \mu$ M. The most potent inhibitors of S16257 in this study ( $IC_{50} < 30 \mu$ M) were cisapride, cyclosporin A, dihydroergotamine, josamycin, ketoconazole, lacidipine, lercanidipine, lovastatin, midazolam, nefazodone, nicardipine, sildenafil and simvastatin.

**NP07226: Assessment of any drug-drug interaction by concomitant incubation of human hepatic microsomes with S 16257 and therapeutic agents which are substrates or inhibitors of CYP3A4**

**NP25546: Complementary assessment of drug-drug interactions by concomitant incubation of human hepatic microsomes with S 16257 and therapeutic agents which are substrates or inhibitors of CYP3A4**

Similar to studies NP06900 and NP15218, above two studies assessed the effect of known diverse CYP3A4 substrates and/or inhibitors (at various concentrations) on the metabolism of S16257 (10  $\mu\text{M}$ ) in human liver microsomes. Inhibition constants ( $K_i$ ) were determined using S16257 at 10  $\mu\text{M}$  and 300  $\mu\text{M}$ . The results showed:

Among the 21 drugs tested (Study NP07226), the followings inhibited metabolism of S16257: cyclosporine A, erythromycin, josamycin, quinidine, verapamil, midazolam, nifedipine, diltiazem, and diazepam. The inhibition constants ( $K_i$ ) determined for the 5 inhibitors were: 3.5  $\mu\text{M}$  for Cyclosporin, 13  $\mu\text{M}$  for Josamycin, 16  $\mu\text{M}$  for Midazolam, 20  $\mu\text{M}$  for Verapamil, and 118  $\mu\text{M}$  for Quinidine.

Among the drugs tested (Study NP25546), ritonavir was the most potent inhibitor with  $\text{IC}_{50} = 0.23 \mu\text{M}$ . Ritonavir was not a time-dependent inhibitor of ivabradine metabolism. The most potent time-dependent inhibitor was erythromycin with a  $K_i = 0.498 \mu\text{M}$  and  $K_{\text{inact}} = 0.00397 \text{ min}^{-1}$ . Compounds with  $\text{IC}_{50} < 30 \mu\text{M}$  were ritonavir, vardenafil, pristinamycin, telithromycin and dalfopristin. Compounds with  $30 \mu\text{M} < \text{IC}_{50} < 100 \mu\text{M}$  were tadalafil and quinupristin.

#### **NP29916: Investigation of the contribution of cytochrome 3A to the metabolism of [14C]-S 16257**

S16257 at (10  $\mu\text{M}$ ) was incubated with recombinant enzymes expressing individual human (rhCYP3A4, 3A5, 3A7), human liver microsomes (HLM) and in HLM genotyped for allelic variants of CYP3A5 (\*1\*1, \*1\*3 and \*3\*3). Inhibition by S16257 (0.1  $\mu\text{M}$  to 100  $\mu\text{M}$ ) of the metabolism of midazolam (1  $\mu\text{M}$ ) was also evaluated in HLM and hrCYP3A4 and 3A5.

S16257 was metabolized predominantly by CYP3A4, with no significant metabolism by CYP3A5 or CYP3A7. There was no significant correlation between clearance of S16257 and the CYP3A5 genotype in HLM donors. Ivabradine inhibited midazolam metabolism with  $\text{IC}_{50} > 100 \mu\text{M}$  for HLM,  $\text{IC}_{50}$  of 46  $\mu\text{M}$  for CYP3A4 and  $\text{IC}_{50}$  of 17  $\mu\text{M}$  for CYP3A5.

#### **NP16117: Evaluation of the induction of cytochrome P450 3A4 involved in the metabolism of S 16257 by different glucocorticoids using human hepatocytes**

Human hepatocytes were treated for at least 72 h with glucocorticoids (beclometasone dipropionate, budesonide, triamcinolone acetonide, flunisolide, fluticasone propionate) at concentrations corresponding to their reported  $C_{\text{max}}$  after a therapeutic dose and the estimated concentration in the hepatic portal vein ( $C_{\text{in}}$ ). Rifampicin (50  $\mu\text{M}$ ) and dexamethasone (10  $\mu\text{M}$ , 100  $\mu\text{M}$ ) were tested as positive and negative controls, respectively. CYP3A4 activity in the hepatocytes was confirmed at 72 h, with optimal conditions for S16257 shown to be 3  $\mu\text{M}$  at 8 h, and nifedipine at 200  $\mu\text{M}$  and 2 h.

Potent CYP3A4 induction can increase the metabolism of S16257 in human hepatocytes. Glucocorticoids (beclometasone dipropionate, budesonide, triamcinolone acetonide, flunisolide and fluticasone propionate) did not induce CYP3A4 in human

hepatocytes and had no effect on the metabolism of S16257 or nifedipine. Rifampicin induced the metabolism of S16257 by 3.1-fold and nifedipine by 7.5-fold.

#### **NP16173: Activation profile of human nuclear receptor PXR by S 16257 and S 18982**

Human retinal pigment epithelial cells (ARPE-19) transfected with human nuclear pregnane X receptor (PXR) were incubated with incubated for 24 h with vehicle, S16257 or S 18982 (1, 3, or 10  $\mu$ M), or 10  $\mu$ M rifampicin as a positive control. Aliquots of cell extracts (n=3/well) were then assayed for luciferase and  $\beta$ -galactosidase activities. There was no consistent activation of human PXR by S16257 and S 18982.

#### **NP08070: Inhibitory properties of S 16257 on human CYP3A4**

S 16257 (0, 1, 10, 100, 250  $\mu$ M) was incubated with midazolam (5  $\mu$ M), testosterone (100  $\mu$ M), or nifedipine (10  $\mu$ M) in human liver microsomes. S 16257 did not significantly inhibit CYP3A4 in human liver microsomes. S 16257 at >100  $\mu$ M weakly inhibited the three CYP3A-substrates assessed, with a  $K_i$  of 140  $\mu$ M for midazolam 1'-hydroxylation. S 18982 weakly inhibited testosterone 6 $\beta$ -hydroxylation.

#### **NP08471: Assessment of the inhibitory properties of S 16257 on human CYP2C18/19 and 2E1**

Ivabradine (0, 1, 10, 100  $\mu$ M) did not inhibit CYP2C18/19 or 2E1 in human liver microsomes.

#### **NP16016: Complementary assessment of inhibitory properties of S 16257 on human cytochrome P450 using liver microsomes**

Ivabradine (1, 10, 50, 100  $\mu$ M) did not inhibit CYP2A6, 2B6, or 2C8 in human liver microsomes.

#### Other: In Vitro Transport and Inhibition

#### **NP31384: Interactions of S 16257 and S18982 with hepatic transporters**

There was lower accumulation of ivabradine and S 18982 in P-gp overexpressing cells (K562R/7 and MCF7R) compared with their parental cell lines (K562 and MCF7). The established P-gp inhibitors, verapamil, cyclosporine, rifampicin, and RU486 inhibited the active transport of ivabradine and S 18982 in MCF7R cells.

#### **NP32566: In vitro Interaction Studies of S 16257 with P-gp (MDR1/ABCB1) and BCRP (ABCG2) in the vesicular transport assay and in Bidirectional Transport (Papp) Studies on transfected MDCKII Monolayers and with human OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8), OCT1, OCT2, OAT1 and OAT3 Uptake Transporters**

Ivabradine was not subject to BCRP-mediated efflux (MDCKII-BCRP cells), or OATP1B1, OATP1B3 and OCT1 active uptake (in individual transporter over-expressing cell systems).

**NP32565: In vitro Interaction Studies of S 18982 with P-gp (MDR1/ABCB1) and BCRP (ABCG2) in the vesicular transport assay and in Bidirectional Transport (Papp) Studies on transfected MDCKII Monolayers and with human OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8), OCT1 Uptake Transporters**

Similar to ivabradine, S 18982 was not subject to BCRP-mediated efflux (MDCKII-BCRP cells), or OATP1B1, OATP1B3 and OCT1 active uptake.

## 5.2 Toxicokinetics

TK data were reviewed with the correspondent toxicity studies.

# 6 General Toxicology

## 6.1 Single-Dose Toxicity

Single-dose studies included an observation period of 2 weeks post-dose were performed in OF-1 mice, SD rats (NP03163, NP03164, NP03137, NP03138) and beagle dogs (NP03165, NP03143) via oral gavage and iv administration. The study design, observed maximum non-lethal or minimum lethal doses and main findings for single oral dosing are presented in tables below (excerpted from M2/2.6.6. pages 18-19). In rodents, overt signs of acute toxicity consisted of behavioral changes and death. Effects occurred earlier and at lower doses following iv injection at dosage of 56-74 mg/kg. In dogs, maximal nonlethal oral dose was about 11 mg/kg due to premature kill and behavioral changes at higher doses. Behavioral changes were also observed at iv doses up to 9.3 mg/kg.

Species (strain) (doses, mg/kg) Report number	Observed maximum non-lethal dose (mg/kg)		Observed minimum lethal dose (mg/kg)		Noteworthy findings
	6M	6F	6M	6F	
<b>Oral route</b>					
<b>Mice (OF1)</b> (0, 371, 557, 742, 928, 1114) <a href="#">NP03164</a>	557	742	742	928	Deaths often preceded by clonic convulsions, Subdued behavior, blepharoptosis and/or tremor at $\geq 371$ (dose-related), Reduced bodyweight gain over the observation period for female mice at $\geq 928$ and rats at $\geq 371$ , No target organ pathology identified.
<b>Rats (Sprague-Dawley)</b> (0, 371, 557, 742, 928, 1114) <a href="#">NP03163</a>	557	371	742	557	

Table 5. Single or Dose-escalation Toxicity Studies in Beagle Dogs

Study type (doses, mg/kg) <sup>a</sup> Report number	Maximum non-lethal dose (mg/kg)	Maximum tolerated dose (mg/kg)	Main findings
<b>Oral route (1M+1F per dose)</b>			
Single Dose (37, 70)  NP03165	nd	nd	Premature kill of both dogs given 37 and male given 70 (due to excessive toxicity including stiffness, hunched posture, tremor and/or convulsions)  Similar changes in surviving female given 70 with sinus arrhythmia and decreased heart rate  Discolouration of the gastrointestinal tract at necropsy  No target organ pathology identified
<b>Oral route (1M+1F)</b>			
Dose escalation (2.8 mg/kg/d for 2 d, 5.6 mg/kg/d for 3 d, 11 mg/kg/d for 2 d, 22 mg/kg/d for 2 d)  NP03165	11	>11 and <22	Premature kill of both animals after second dosing at 22 (due to excessive toxicity including ataxia, stiffness, hunched posture and/or loss of balance)  Marginal bodyweight loss (female) associated with reduced food consumption (at ≥11)  Sinus arrhythmia (at ≥11) with sinus arrest at 22  Discolouration of the gastrointestinal tract at necropsy  No target organ pathology identified

The TK parameters were analyzed following single oral or iv dose in dogs, as shown below (excerpted from M2/2.6.7, page 27).

TK after single oral dosing in dogs					TK after single iv dosing in dogs				
Dose (mg/kg/ d)	C <sub>max</sub> (ng/ml)		AUC <sub>24</sub> (ng.h/ml)		Dose (mg/kg)	C <sub>Smin</sub> (ng/ml) <sup>a</sup>		AUC <sub>24</sub> (ng.h/ml)	
	M	F	M	F		M	F	M	F
2.8	212	281	nd	nd	4.6	4780	2770	7034	4124
5.6	1360	1330	2710	2440	9.3	5170	4710	11389	6306
11	3080 <sup>a</sup>	2210 <sup>a</sup>	3740 <sup>a</sup>	5300 <sup>a</sup>					
22	6980 <sup>a</sup>	6270 <sup>a</sup>	nd	nd					

## 6.2 Repeat-Dose Toxicity

### Rat

Repeat-dose toxicity studies in rats were conducted in durations of 4-week, 13-week, 26-week and 52-week with oral gavage route of administration and in durations of 4-week with iv route of administration. For the 4- and 13-week studies, daily oral doses up to 175 mg/kg/day were applied. For the chronic studies (26- and 52-week), twice daily dose up to 2x90 mg/kg/day were applied in order to mimic clinical dosing regimen.

Parameters generally measured in these studies [*standard parameters*] included

mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, clinical pathology (hematology, chemistry and urinalysis), gross pathology, organ weight and histopathology. Plasma levels of the test article were measured in all studies. All the study reports are located in M4/4.2.3.2. The 52-week rat study was reviewed in an itemized format. Other studies in shorter durations were reviewed in a summary format.

**NP03169: S 16257-02: 4 week oral (gavage) toxicity in the rat**

Sprague-Dawley rats (8-week old, 10/gender/group) received once daily oral administration (gavage, 10 ml/kg) of 0 (control, water for injection), 14, 56 or 223 (base) mg/kg/d for 4 weeks. In addition to standard parameters described above, heart rate was monitored by lead-II ECG on Day 1, pre-dose, and 1, 3 and 24 h post-dose.

No mortality was observed at any dose level. Transient subdued behavior and closed eyes were observed at 223 mg/kg; a dose-related salivation was observed immediately after dosing. A decrease in body weight gain and food consumption (first week of treatment) as well as an increase in most clinical chemistry parameters (calcium, glucose, triglycerides, BUN, alkaline phosphatase, and ALT) were observed at 223 mg/kg; a slight increase in water consumption and an increase in calcium and ALT were observed at doses  $\geq$  56 mg/kg.

A dose-related HR reduction (up to -41% compared to the pre-dose value) was observed in all treated animals, mainly from 1-4 hours post dosing and the effect was still present at 24 hr post dose. Focal myocardial lesions with interstitial fibrosis were increased in incidence and severity in all treated animals in comparison with controls. The slight increase in heart weight was observed in females at doses  $\geq$  56 mg/kg. It is possible that the findings could be a consequence of the marked pharmacological bradycardia observed in all treated groups but unexpected considering prior findings of anti-ischemic activity, and reduction in cardiac index. Pathogenesis of the excess myocardial lesions, in the context of the 40% decrease in HR, is uncertain absent determinations of wall stress and oxygen consumption. Minimal adrenal cortical cell hypertrophy was observed in the high dosage females, which may be related to stress.

Mean plasma exposure levels to ivabradine in satellite animals (8/gender/group) are presented in the table below. The maximum plasma concentrations were observed in 10 minutes after low dosing and tend to increase to 1 h after administration of the highest dose. The exposure of females to the drug was markedly higher than that of the males. The exposure increased more than proportionally to the dose.

Dose (mg.kg <sup>-1</sup> .day <sup>-1</sup> )	Male rats		Female rats	
	Day 1	Day 28	Day 1	Day 28
15	1157	1362	3521	4680
60	4028	10594	18287	20841
240	29262	56408	62663	116293

In conclusion, after oral administration of ivabradine for 4 weeks to SD rats up to 223 mg/kg/d, the majority of treatment-related changes were observed at the high dose. Due to the excess myocardial lesions at all doses, NOAEL could not be defined.

**NP03144: S 16257-02: 4 week oral (gavage) toxicity in the rat**

This study was complementary to the 4-week rat study reviewed above, in which lower doses of ivabradine were assessed under similar experimental conditions in order to define a NOAEL.

Sprague-Dawley rats (8-week old, 10/gender/group) received ivabradine once daily via oral gavage (10 ml/kg) at 0 (control, water for injection), 2.8 or 7 mg/kg/d for 4 weeks. In addition to standard parameters, heart rate was monitored by lead-II surface ECG once during the pre-test period, and then pre-dose, and 1, 3 and 24 h after dosing on Day 1 and in Week 4.

No signs of toxicity were observed at either dose tested. A slight to moderate heart-rate reduction was observed 1 and 3 h after both doses. The NOAEL was identified at 7 mg/kg/day.

Mean plasma exposure levels to ivabradine in satellite animals (4/gender/group) at the end of the dosing period are presented in the table below (excerpted from M2/2.6.6. page 26). Tmax was observed 10 min or 1 h after dosing. Mean plasma exposure to ivabradine increased dose-proportionally and was 3.3- to 4.5-fold higher in females than in males at the end of the dosing period. Time-dependent effect was not observed.

Dose (mg/kg/d)	AUC <sub>24</sub> (ng.h/ml)			
	Males		Females	
	Day 28	Multiple of hAUC <sub>24</sub>	Day 28	Multiple of hAUC <sub>24</sub>
2.8	226	0.7	741	2
7	515	1	2321	7

n=2/gender/dose/sampling time. AUC<sub>24</sub> = Area under the concentration curve over 24 hours;

hAUC<sub>24</sub> = mean plasma exposure at steady state over 24 hours in patients at the highest therapeutic dose.

**NP05320: S 16257-2: subacute toxicity study four-week oral (gavage) administration in the Wistar rat**

Wistar rats (8-week old, 10/gender/group) received once daily oral administration (gavage, 10 ml/kg) of 0 (control, demineralized water), 6.5, 19, 58 or 175 (base) mg/kg/d for 4 weeks. The study focused on the histopathology of the heart due to the previous positive cardiac findings. In addition to standard parameters, an extensive investigation of the cardiac lesions was conducted by examining serial sections of the heart from all animals. Each heart was cut transversally (from base to apex) into 6 or 7 pieces depending on its size, and 4 serial sections (at 250 µm intervals) were cut from each piece. Thereby the cardiac tissue was examined for spontaneous or drug-induced lesions from 0.02 mm<sup>2</sup>, which is the minimal size of spontaneous inflammatory cell aggregates in the rat strain used.

The noteworthy findings were summarized below (excerpted from M2/2.6.6, page 27)

Noteworthy findings	Doses (mg/kg/d)							
	6.5		19		58		175	
	M	F	M	F	M	F	M	F
<b>Overt signs:</b>								
Decreased spontaneous activity (first two weeks of dosing)					x	x	x	x
Increased salivation			x	x	x	x	x	x
<b>Increased water consumption:</b> (from second week of dosing)				x		x	x	x
<b>Blood biochemistry:</b>								
Increased total cholesterol <sup>a</sup>		x		x		x		x
<b>Urinalysis:</b>								
Increased urinary volume	x	x	x	x	x	x	x	x
Increased sodium excretion	x		x		x		x	x
<b>Post-mortem findings:</b>								
Increased heart weight	x	x	x	x	x	x	x	x
Cardiomegaly			x		x		x	x
Treatment-related myocardial lesions			x		x		x	x

<sup>a</sup> Change not dose-related, quantitatively moderate and within the background range of the rat strain

Examination of the serial sections of the heart revealed focal inflammatory myocardial lesions, mostly in males. Mononuclear inflammatory infiltrates, described as "histiocytic mononuclear cells with few macrophages and without polynuclear cells", were classified as "microgranulomas" (smaller than 0.05 mm<sup>2</sup>, usually visible on a single level) or "granulomas" (between 0.05 and 0.25 mm<sup>2</sup>, visible on several successive levels). Granulomas sometimes included altered myocardial fibers and slight fibrosis. The lesions were mainly located in the sub-endocardial region of the left ventricle, often causing damage to a cardiac pillar. The atria were not affected. The incidence and severity of the inflammatory lesions, which occurred spontaneously in untreated rats, increased with treatment (being dose-related in males at  $\geq 19$  mg/kg/d and occurring only at 175 mg/kg/d in females). There was no sign of degenerative change. The sub--endocardial location is consistent with an ischemic origin, but that is speculative absent determinations of oxygen consumption, wall stress, blood flow, and cardiac adrenergic tone.

Mean plasma exposure levels to ivabradine in satellite animals (2/gender/group) were presented in the table below (page 1062 of the report). [note: The doses in the table were expressed in salt form which equivalent to doses of 6.5, 19, 58 or 175 mg/kg/day, respectively.]

DOSE (mg/kg.day)	AUC <sub>24</sub> (ng.h/ml) in MALE rats				AUC <sub>24</sub> (ng.h/ml) in FEMALE rats			
	Rat n°	day 1	day 28	Ratio 28 / 1	Rat n°	day 1	day 28	Ratio 28 / 1
7	116	247	474	1.9	124	1408	2485	1.8
7	117	211	317	1.5	125	1395	2673	1.9
21	118	1080	1439	1.3	126	5031	4534	0.9
21	119	928	1301	1.4	127	4290	5992	1.4
63	120	3456	4455	1.3	128	10941	17277	1.6
63	121	3545	4327	1.2	129	9097	16909	1.9
189	122	14507	24606	1.7	130	31792	54448	1.7
189	123	7547	11426	1.5	131	34325	45981	1.3

Overall, due to the myocardial lesions, the NOAEL were identified at 6.5 mg/kg/day for the males and 58 mg/kg/day for the females; the AUC<sub>24hr</sub> on Day 28 at the NOAEL is equivalent to 1x and 49x human AUC<sub>24hr</sub> at MRHD, respectively.

**NP05319: S 16257-2: subacute toxicity study thirteen-week oral (gavage) administration in the Wistar rat**

Wistar rats (6-week old, 15/gender/group) received once daily oral administration (gavage, 10 mL/kg) of 0 (control, demineralized water), 7, 35 or 175 mg/kg/d for 13 weeks. At the end of the dosing period, 10 rats/gender/group were euthanized and examined, while the remaining 5 rats/gender/group were retained for a further 6-week recovery period. In addition to standard parameters, an extensive investigation of the cardiac lesions by serial sectioning, as in the 4-week study (NP05320), was conducted. A Masson's Trichrome stain was added to explore fibrosis in control and high-dose groups at the end of the recovery period.

No mortality was observed. Clinical sign of hypersalivation was observed from the dose of 7 mg/kg/d in females and from 35 mg/kg/d in males, with a dose-related frequency. It lasted till the end of treatment period but resolved in the recovery period. Decrease in spontaneous locomotor activity in both sexes treated at 175 mg/kg/d during the first week of dosing and was reversible. Reduced body weight gain in males and moderate decreased in food consumption in both genders were observed at 175 mg/kg/d. Dose-related increase in water consumption was observed in females, treated from the dose of 7 mg/kg/d and in males from the dose of 35 mg/kg/d. The higher the dose, the earlier the increase appeared during the study; it lasted until the end of the treatment period.

The noteworthy clinical pathology, gross pathology and histopathology findings were summarized in the table below (excerpted from M2/2.6.7, page 42). An increased sodium excretion was not associated with an increase in urine volume in this case.

Daily dose (mg/kg) Number of animals (main groups)		0 (vehicle)		7		35		175	
		M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
<b>Hematology<sup>a,c</sup></b>									
White blood cell count (G/l)		-	4.66	-	4.47	-	5.15	-	5.91**
Neutrophils (G/l)		-	0.73	-	0.73	-	0.92	-	1.08*
<b>Blood biochemistry<sup>b</sup></b>									
ALT (U/l)		-	18.6	-	24.4	-	24.3**	-	26.1**
Glucose (mmol/l)		-	6.13	-	5.95	-	5.82	-	5.71
Total cholesterol (mmol/l)		-	1.07	-	1.14**	-	1.26**	-	1.28**
Triglycerides (mmol/l)		-	0.42	-	0.47	-	0.53	-	0.63**
Urea (mmol/l)		-	6.28	-	7.57	-	7.81	-	8.51
<b>Urinalysis<sup>d</sup></b>									
Urinary volume (ml/24 h)		-	-	-	-	-	-	-	-
Sodium excretion (mmol/24 h)		0.28	0.14	0.32	0.23	0.38	0.25	0.51	0.50**
<b>Organ weights (%)<sup>a</sup></b>									
Heart	Absolute	1.05 g	0.72 g	+3	+6	+10	+20**	+23**	+33**
	Relative to bodyweight	0.23%	0.29%	+7	+5	+11**	+13**	+40**	+34**
Adrenal glands	Absolute	-	0.0847 g	-	+0.5	-	-8.7	-	+12
	Relative to bodyweight	-	0.0338%	-	-0.3	-	-14	-	+14
<b>Gross pathology<sup>a</sup></b>									
Number examined <sup>b</sup>		10	10	10	10	10	10	10	10
. Cardiomegaly		0	0	2	0	6	4	9	8
<b>Histopathology<sup>a</sup></b>									
Number examined <sup>b</sup>		10	10	10	10	10	10	10	10
. Heart lesions:									
Animals without heart lesions		1	8	4	6	1	2	0	1
Animals with one microgranuloma (<0.05 mm <sup>2</sup> )		8	1	3	3	5	3	0	2
Animals with ≥2 microgranulomas (<0.05 mm <sup>2</sup> )		1	1	3	0	4	4	10	7
Animals with one granuloma (>0.05 and <0.20 mm <sup>2</sup> )		0	0	1	1	4	1	4	2
Animals with ≥2 granulomas (>0.05 and <0.20 mm <sup>2</sup> )		0	0	0	0	1	1	2	2
Animals with granulomas (>0.20 and <0.50 mm <sup>2</sup> )		0	0	0	0	0	2	0	1
Animals with fibrosis (focal, mild to moderate)		0	0	1	0	2	0	7	4

The primary histopathology of note was an exacerbation of spontaneous focal myocardial lesions. The histological aspect and topography of inflammatory lesions were the same as previously described in the 4-week study. Areas of interstitial fibrosis were observed in the sub-endocardial region, mainly for high-dose males. At the end of the off-dose period, cardiac changes were observed though with markedly lower incidence and severity. Fibrous scars were detected by Masson's trichrome in control and high-dose males with a similar incidence.

All changes except body weight gain, glucose and urea were resolved in end of the recovery period. The magnitude of clinical chemistry change was relatively small and was not considered toxicologically significant. The NOAEL was identified at 7 mg/kg/day due to the cardiac lesions.

Dose (mg/kg/d)	AUC <sub>24</sub> (ng.h/ml)							
	ivabradine				S 18982			
	Males		Females		Males		Females	
	Day 88	Multiple of hAUC <sub>24</sub>	Day 88	Multiple of hAUC <sub>24</sub>	Day 88	Multiple of hAUC <sub>24</sub>	Day 88	Multiple of hAUC <sub>24</sub>
7	640	2	1750	5	20	0.2	17	0.1
35	2650	8	9850	28	149	1	143	1
175	16000	46	46000	133	1588	12	977	8

n=2/gender/dose/sampling time. AUC<sub>24</sub> = Area under the concentration curve over 24 hours;  
hAUC<sub>24</sub> = mean plasma exposure at steady state over 24 hours in patients at the highest therapeutic dose.

Mean plasma exposures of ivabradine and S 18982 in satellite animals (2/sex/group) on Day88 are presented in the table above (excerpted from M2/2.6.6, page 30). T<sub>max</sub> for ivabradine was observed 10 min after dosing. Mean plasma exposure to ivabradine increased dose-proportionally and was higher in females than in males (by 2.7- to 3.7-fold at the end of the dosing period). There was no time-dependent effect on exposure

to ivabradine between Days 28 and 88. Exposure to S 18982 represented less than 10% of that to ivabradine, with a similar plasma kinetic profile.

### NP06240: S 16257-2: chronic toxicity study twenty-six week oral (gavage) administration in the Wistar rat

Wistar rats (8-week old, 20/gender/group) received oral administration (gavage, 10 ml/kg, 7-hour interval between doses) of 0 (control, demineralized water), 3, 16 or 90 mg/kg twice daily (i.e. 6, 32 or 180 mg/kg/d, respectively) for 26 weeks. At the end of the dosing period, main study rats (n=15/sex/group) were sacrificed for examination, while recovery animals (n=5/sex/group) were sacrificed and examined after a further 6-week treatment-free period.

In addition to standard parameters, an extensive investigation of histological cardiac lesions by serial sectioning, was conducted, as in the 4- and 13-week studies. Serum ANP levels were determined to investigate the elevated urinary volume and sodium excretion seen in earlier studies. In addition, because of the slight increase in liver weight observed at 90 mg/kg b.i.d. in the absence of enzyme induction, transmission electron microscopy (TEM) was conducted on the liver of some females.

No mortality was observed in the study. Clinical signs included partial blepharoptosis (Week 1 at  $\geq 2 \times 16$  mg/kg/d, from week 6 or 7 at  $2 \times 90$  mg/kg/d), increased salivation (from Week 2) in all treated females and males at doses  $\geq 2 \times 16$  mg/kg/d, and piloerection (Week 1 and from Week 6) in both sexes at  $2 \times 90$  mg/kg/d. Water consumption was increased in all treated females and  $2 \times 90$  mg/kg/d males.

Noteworthy changes for clinical pathology including ANP values, gross pathology, organ weights and histopathology were summarized in the table below (excerpted from M2/2.6.6, page 45). The markedly increased ANP levels at the high dose suggested that increased sodium excretion may be mediated through the release of this factor. However, there was no significant increase in urinary volume and sodium excretion in treated males.

Daily dose (mg/kg)	2 x 0 (vehicle)		2 x 3		2 x 16		2 x 90	
	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Number of animals (main groups)								
Hematology <sup>a</sup>	-	-	-	-	-	-	-	-
Blood biochemistry <sup>a</sup>								
Atrial natriuretic peptide (ng/l) <sup>c</sup>	485	425	513	527	341	634	789**	1493**
Urinalysis <sup>a</sup>								
Urinary volume (ml/24 h)	-	12.0	-	14.9	-	13.8	-	17.5
Sodium excretion (mmol/24 h)	-	0.171	-	0.255	-	0.230	-	0.427**
Organ weights (%) <sup>a</sup>								
Heart								
Absolute	1.126 g	0.752 g	-2.4	+5.0	+5.9	+19**	+28**	+41**
Relative to bodyweight	0.223%	0.284%	+2.2	+5.3	+13**	+14**	+33**	+42**
Liver								
Absolute	-	6.571 g	-	+2.5	-	+10*	-	+15**
Relative to bodyweight	2.34%	2.49%	+1.3	+2.0	+6.4*	+5.6*	+8.1**	+15**
Adrenal glands								
Absolute	-	0.075 g	-	+2.3	-	+7.9	-	+20**
Relative to bodyweight	-	0.028%	-	+2.1	-	+3.2	-	+20**
Gross pathology								
Number examined	15	15	15	15	15	15	15	15
Heart, enlarged or dilated appearance	0	0	0	0	0	0	1	2
Glandular stomach, erosions	0	1	6	3	6	3	8	6

Similar to the previous 4-week and 13-week studies, primary histopathology finding was an exacerbation of spontaneous myocardial lesions. As shown in the table below (excerpted from M2/2.6.6, page 46), the histological aspect and topography of

inflammatory lesions and interstitial fibrosis were similar to those previously described. Chondroid metaplasia was observed on left ventricle endocardial pillars, mainly for high-dose males. Glandular stomach erosions were observed with higher incidence at high dose groups. The GI safety pharmacology studies compared single oral dose with an iv infusion study (28 mg/kg) in rats, which suggested that the ulcerogenic effect observed following a single oral dose of 30 mg/kg could be a local effect due to oral administration rather than a systemic drug effect. TEM on liver sections did not reveal architectural, morphologic nor ultrastructural changes in hepatocytes from some high dose female rats when compared to some control rats.

Daily dose (mg/kg)	2 x 0 (vehicle)		2 x 3		2 x 16		2 x 90	
	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
<b>Number of animals (main groups)</b>								
Histopathology								
Number examined	15	15	15	15	15	15	15	15
Heart lesions:								
Animals without heart lesions	11	14	10	13	3	6	0	2
Animals with one microgranuloma (<0.05 mm <sup>2</sup> )	1	0	2	2	7	7	2	3
Animals with ≥2 microgranulomas (<0.05 mm <sup>2</sup> )	3	1	3	0	5	2	13	9
Animals with granulomas (>0.05 and <0.20 mm <sup>2</sup> )	0	0	1	0	1	0	9	1
Animals with granulomas (>0.20 and <0.50 mm <sup>2</sup> )	0	0	0	0	0	0	0	0
Animals with granulomas (>0.50 mm <sup>2</sup> )	0	0	0	0	1	0	0	0
Animals with interstitial fibrosis	0	0	1	0	0	1	5	1
Animals with chondroid metaplasia (left ventricle, endocardial pillars)	0	0	0	0	0	0	5	2
Glandular stomach, erosions	0	1	1	0	2	2	8	5

At the end of the 6-week treatment-free period, myocardial changes were markedly decreased in both incidence and severity. All other changes, except ANP and sodium excretion in females, were fully reversed by the end of the treatment-free period.

Based on cardiac lesions, the NOAEL was identified at 2x3 mg/kg/day (associated AUC<sub>24h</sub> of 1648 ng.hr/mL [females] on Day 183, equivalent to 5x human AUC<sub>24hr</sub> at MRHD).

Mean plasma exposures (AUC<sub>24hr</sub> and C<sub>max</sub>) levels to ivabradine and S 18982 and their concentrations in the liver were summarized in the table below (excerpted from M2/2.6.7, page 44). Mean plasma exposure to ivabradine increased dose-proportionally for females between 2x3 and 2x90 mg/kg/day, for males between 2x3 and 2x16 mg/kg/day, and more than dose-proportionally for males between 2x16 and 2x90 mg/kg/day. The exposure was 1.9- to 2.3-fold higher in females than in males at the end of the dosing period. There was no time effect on exposure to ivabradine between Days 27, 90 and 183. A similar plasma profile was observed for S 18982, that represented less than 9% of ivabradine.

Daily dose (mg/kg)	2 x 0 (vehicle)		2 x 3		2 x 16		2 x 90	
	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
<b>Number of animals (main groups)<sup>a</sup></b>								
<b>Number of animals (satellite TK groups)</b>	M: 0	F: 0	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
<b>Toxicokinetics (on Day 183 - no time-effect)</b>								
S 16257								
· C <sub>max,1</sub> (ng/ml) (mean t <sub>max,1</sub> : 10 min)			152	373	1188	1363	5361	8179
· C <sub>max,2</sub> (ng/ml) (mean t <sub>max,2</sub> : 10 min)			96	231	1237	1491	10198	5919
· AUC <sub>24</sub> (ng.h/ml)			nd	1648	4621	10817	32867	62216
S 18982								
· C <sub>max,1</sub> (ng/ml) (mean t <sub>max,1</sub> : 10 min)			14 <sup>o</sup>	6.3 <sup>o</sup>	86	28	492	317
· C <sub>max,2</sub> (ng/ml) (mean t <sub>max,2</sub> : 10 min)			5.7	3.5 <sup>b</sup>	94	31	703	196
· AUC <sub>24</sub> (ng.h/ml)			nd	nd	nd	nd	2356	2392
<b>Mean liver concentrations (ng/g) 15 h after the last dosing with S 16257-2 (liver to plasma ratio)</b>								
S 16257			np	np	np	np	4217 (20)	10926 (24)
S 18982			np	np	np	np	602 (34)	897 (55)

**Study title: S 16257-2 - 52 week oral (gavage) toxicity study in the rat.**

Study no.: NP07026 (contract lab study No. 303/591)  
Study report location: M4/4.2.3.2  
Conducting laboratory and location:  (b) (4)  
Date of study initiation: May 21, 1996  
GLP compliance: Statement included  
QA statement: Statement included  
Drug, lot #, and % purity: S 16257-2, batch# 47734, purity:100.5%

**Key Study Findings**

Test article-related unscheduled deaths occurred on one male treated with 16 mg/kg bid as well as 8/40 males and 16/40 females treated with 90 mg/kg bid, and mainly attributed to the cardiac lesions observed in these animals.

Clinical signs of salivation observed in all treated groups. Reduced body weight gains up to -13% observed in high dose groups. Slightly increased AP level observed in high dose groups.

Increased the heart weight, along with gross pathology findings, was associated with histopathology changes observed in the heart, which included:

mucification, cartilaginous and/or osseous metaplasia in the chordae tendinae at doses  $\geq$  16 mg/kg bid;  
increased incidence and severity of ventricular degeneration (characterized by cardiomyocyte vacuolation, contraction bands and myoblasts), fibrosis and/or necrosis in males at doses  $\geq$  16 mg/kg bid and in females at doses  $\geq$  3 mg/kg bid.  
atrial or ventricular thrombosis and atrial hypertrophy/dilation at 90 mg/kg bid;  
ventricular hypertrophy/dilation in males at doses  $\geq$  3 mg/kg bid and in females at doses  $\geq$  16 mg/kg bid.

Mucification and cartilaginous metaplasia of pulmonary arteries in the lungs at 90 mg/kg bid.

Increased incidence of stomach erosion/ulceration in males at  $\geq$  3 mg/kg bid and in females at doses  $\geq$  16 mg/kg bid

Increased liver and adrenal glands weights observed in the both males and females at doses  $\geq$  16 mg/kg bid

NOAEL cannot be identified due to the findings observed at low dosage. The  $AUC_{24h}$  at LOAEL 3 mg/kg bid was 935 ng.hr/mL for males and 2385 ng.hr/mL for females at Week 52.

## Methods

Doses: 0, 3, 16, 90 mg/kg (expressed in terms of base material)

Frequency of dosing: BID

Route of administration: Oral gavage

Dose volume: See study design below

Formulation/Vehicle: solution in the vehicle at concentrations of 0.6, 3.2 and 18.0 mg/ml./water for injection

Species/Strain: Rat/Wistar, Ico : WI (IOPS Han)

Number/Sex/Group: See study design below

Age: ~ 6 weeks

Weight: Male: 171 to 214 g; female: 131 to 182 g.

Satellite groups: See study design below

Unique study design: n/a

Deviation from study protocol: deviations were not considered to have affected the outcome of the study objectives.

Group number	Group designation	Dose level (mg/kg/day)	Dose volume (ml/kg/day)	Dose concentration (mg/ml)	Number of animals	
					Males	Females
1	Control	2 x 0	2 x 5	0	40	40
2	Low dose	2 x 3	2 x 5	0.6	40	40
3	Intermediate dose	2 x 16	2 x 5	3.2	40	40
4	High dose	2 x 90	2 x 5	18.0	40	40

## Observations and Results

## Mortality

All animals were observed twice daily.

No animal died before Day 188. The following animals were found dead or moribund sacrificed after Day 188 and before the scheduled termination. The incidence is summarized in the table below.

Daily dose (mg/kg)	2 x Vehicle		2 x 3		2 x 16		2 x 90	
	M	F	M	F	M	F	M	F
Unscheduled Death related to cardiac lesions	-	-	-	-	1	-	8	16
Unscheduled Death with other lesions	-	1 <sup>a</sup>	2 <sup>b</sup>	1 <sup>c</sup>	-	-	-	-
Unscheduled Death related to anesthesia/blood sampling	1 <sup>d</sup>	-	-	2	-	1	-	-

- Animal No. 183, Killed moribund (ulcerated mass on glandular stomach at necropsy)
- Animal Nos. 51 and 54, Death related to lymphoma or pancreatic adenocarcinoma
- Animal No. 220, Killed moribund (pituitary adenoma at necropsy)
- Animal No. 3, Death without evident cause.

Treatment related mortality was observed in one male (no. 83) given 2x16 mg/kg/day and all animals given 2 x 90 mg/kg/day (nos. 123,127, 128, 147, 149, 150, 151, 155 and 281, 282, 283, 288, 289,293,294,295, 298, 301,308, 313, 315, 318, 319, 320). Histopathologic changes in the heart were noted in these animals, which probably were the origin of the poor general condition/death. Before dying, some of these animals (nos. 282, 288, 293,315,319,320) showed signs including respiratory difficulties, subdued behavior, pallor, piloerection, and edema.

## **Clinical Signs**

Animals were observed at least once after each dosing for any clinical signs and reaction to treatment. A full clinical examination was performed weekly.

Increased incidences of salvation were observed from Week 2 after dosing at all treated animals in a dose-dependent pattern.

## **Body Weights**

Individual body weights were recorded weekly for 17 weeks and then generally every two weeks from the first week of treatment.

Mean body weight gain was significantly reduced up to -11% and -13% in high dose males and females, respectively, when compared to the controls.

## **Feed Consumption**

Feed consumption was measured weekly for 16 weeks and then for a period of one week in every 2 thereafter, for each cage of animals and reported in g/animal/day.

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

## **Ophthalmoscopy**

Not performed.

## **ECG**

Not performed.

## **Hematology**

Blood samples were taken from 10 rats/sex/group fasted at least 15 hours at the scheduled termination (26 or 52 weeks of treatment). Where possible, samples were also taken from animals killed moribund.

There were no significant test article-related effects on measured hematology parameters.

### Clinical Chemistry

Blood samples were taken from 10 rats/sex/group fasted at least 15 hours at the scheduled termination (26 or 52 weeks of treatment). Where possible, samples were also taken from animals killed moribund.

The mean alkaline phosphatase (AP) level was increased at the end of Week 26 (1-2 fold of control value) and Week 52 (< 2-fold of control value) in high dose males and females. The mean calcium level in high dose females was slightly increased when compared to the control value (107 mg/L vs. 102 mg/L), whereas that increase was not observed in high dose males.

### Urinalysis

Not performed

### Gross Pathology

All designated animals were necropsied at the end of treatment period. A complete gross pathology examination was conducted for all animals subjected to unscheduled death or scheduled termination.

The treatment related macroscopic findings are summarized in the table below. The primary findings were limited to the heart. Dark coloration/dark area with relative higher incidence was observed in the high dose groups subject to unscheduled death.

Daily dose (mg/kg)	2 x Vehicle		2 x 3		2 x 16		2 x 90	
	M	F	M	F	M	F	M	F
<b>Unscheduled terminated animals examined (N)</b>	1	1	2	3	1	1	8	16
Heart:								
Enlarged	0	0	0	0	0	0	5	12
Pale areas	0	0	0	0	0	0	5	6
Raised areas	0	0	0	0	0	0	0	3
Soft	0	0	0	0	0	0	0	1
Lung: dark coloration/areas	1	0	1	2	1	0	4	5
<b>Scheduled termination animals examined (N)</b>	39	39	38	37	39	39	32	24
Heart:								
Enlarged	1	0	3	0	4	0	23	15
Pallor or pale areas	0	0	0	0	1	0	0	3
Dark discoloration/dark areas	1	1	0	0	0	3	3	11
Mass	0	0	0	0	0	0	1	0

### Organ Weights

The organs listed below were weighed for all necropsied animals.

adrenal glands	ovaries
brain	pituitary gland
epididymides	prostate
heart	spleen
kidneys	testes
liver	thyroid gland (including parathyroid gland)

The increased mean organ weight of heart, liver and adrenal glands was observed in treated groups as shown in the table below (excerpted from M2/2.6.7, page 50). The increased weight of the liver or adrenal glands was observed in the mid- and high dose groups and was not associated with any histopathological findings.

Daily dose (mg/kg)	2 x 0 (vehicle)		2 x 3		2 x 16		2 x 90		
	M: 40	F: 40	M: 40	F: 40	M: 40	F: 40	M: 40	F: 40	
<b>Noteworthy findings (Cont'd)</b>									
<b>Organ weights (%)</b>									
Heart	Absolute	1.35 g	1.00 g	+5.2*	+9.0**	+19**	+29**	+47**	+86**
	Relative to bodyweight	0.225%	0.273%	+3.6	+9.9**	+13**	+27**	+64**	+116**
Liver	Absolute	14.6 g	9.4 g	+3.4	+4.3	+15**	+8.5*	+2.7	+5.3
	Relative to bodyweight	2.42%	2.55%	+2.5	+5.5	+9.9**	+7.8**	+15**	+24**
Adrenal Glands	Absolute	0.073 g	0.081 g	-4.1	+7.4	+12**	+7.4	+16**	+27**
	Relative to bodyweight	0.012%	0.022%	-3.3	+6.3	+8.3**	+5.4	+31**	+46**

For controls, group means (organ weight) are shown. For treated group, percent differences from controls are shown. Statistical significance is based on actual data (not on percent difference). \*p<0.05, \*\* p<0.01

### Histopathology

The listed organs/tissues sampled from all animals were fixed and preserved in 10% neutral formalin with the exceptions for eyes which was fixed in Davidson’s fluid and for bone marrow smears that were fixed in methanol. All sections were stained with haematoxylin and eosin (except bone marrow smears). Microscopic examinations of the listed organ/tissues were performed for the following animals:

- all animals found dead or killed moribund during the study,
- all animals in groups 1 (control) and 4 (high dose) killed after 52 weeks of treatment,
- heart for all animals in groups 2 and 3 killed after 52 weeks of treatment

List of tissues/organs were collected:

adrenal glands	oesophagus
bone (sternum) with bone marrow	optic nerves
bone marrow smears	ovaries
bronchi (mainstem)	pancreas
brain	parathyroid glands
caecum	pituitary gland
colon	prostate
duodenum	salivary gland (submaxillary)
epididymides	skin
eyes	spinal cord (cervical, thoracic, lumbar)

heart	spleen
ileum	stomach
jejunum	testes
kidneys	thymus (where identified)
liver	thyroid gland
lungs	trachea
lymph node (submaxillary)	urinary bladder
lymph node (mesenteric)	uterus (horns + cervix)
mammary gland	all gross lesions

Adequate Battery: yes. Peer Review: yes.

### Histological Findings

The histological changes of the animals subject to unscheduled termination were similar to the findings of the animals at scheduled termination. Treatment related findings were observed primarily in the heart, and also in the lungs and stomach.

The noteworthy findings are summarized in the table below. Findings of the heart (excerpted from page 34 of the report) were correlated with the increased heart weight and gross pathology changes.

Dose level (mg/kg/day)	Males				Females			
	2x0	2x3	2x16	2x90	2x0	2x3	2x16	2x90
Number examined	40	40	40	40	40	40	40	40
<b>Heart</b>								
Changes to the chordae tendinae (mucification, cartilaginous and/or osseous metaplasia)	0	0	9	30	0	0	6	37
Left ventricular degeneration and/or necrosis	3	3	1	38	2	0	0	38
<i>minimal- slight</i>	3	3	1	10	2	0	0	16
<i>moderate - marked</i>	0	0	0	28	0	0	0	22
Left ventricular fibrosis	21	29	<b>38</b>	<b>37</b>	3	<b>17</b>	<b>37</b>	<b>30</b>
<i>minimal- slight</i>	20	25	<b>17</b>	<b>12</b>	3	<b>16</b>	<b>24</b>	<b>15</b>
<i>moderate - marked</i>	1	4	<b>21</b>	<b>25</b>	0	<b>1</b>	<b>13</b>	<b>15</b>
Right ventricular fibrosis, degeneration and/or necrosis	7	7	<b>16</b>	<b>18</b>	1	1	2	<b>12</b>
Atrial or ventricular thrombosis	0	0	0	<b>8</b>	0	0	0	<b>16</b>
Ventricular hypertrophy/dilatation	0	<b>2</b>	<b>11</b>	<b>33</b>	0	0	1	<b>38</b>
Atrial hypertrophy/dilatation	0	0	0	<b>21</b>	0	0	0	<b>26</b>
<b>Lungs</b>								

Mineralization of major arteries	17	11	10	28	21	10	3	22
Mucification+/- cartilaginous metaplasia of major arteries	0	0	0	0	0	0	0	2
<b>Stomach, glandular erosion or ulceration</b>	<b>1</b>	<b>5</b>	<b>4</b>	<b>9</b>	<b>2</b>	<b>2</b>	<b>5</b>	<b>7</b>
<b>Duodenum, erosion</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>

In the heart, the changes of chordae tendinae included mucification (deposition of a basophilic ground substance among the cardiomyocytes, cartilaginous metaplasia (chondrocytes formation) and/or osseous metaplasia (mineralization of the ground substance) at doses  $\geq 16$  mg/kg bid. Atrial or ventricular thrombosis and atrial hypertrophy/dilation was observed at 90 mg/kg bid and ventricular hypertrophy/dilation in males at doses  $\geq 3$  mg/kg bid and in females at doses  $\geq 16$  mg/kg bid.

Increased incidence and severity of ventricular degeneration (characterized by cardiomyocyte vacuolation, contraction bands and myoblasts), fibrosis and/or necrosis in males at doses  $\geq 16$  mg/kg bid and in females at doses  $\geq 3$  mg/kg bid. Although myocardial degeneration with fibrosis is a common spontaneous observation in aged rats, especially in males, this study showed a higher incidence of myocardial degeneration with fibrosis in treated male and female rats with a dose-dependent increase in the severity of the change. The extensive lesions, particularly in the high dose animals, are considered beyond background finding. It might reflect the exacerbation of a spontaneous cardiomyopathy. Some lesions, such as contraction bands and endocardial necrosis, also recall cardiac toxicities secondary to excess adrenergic stimulation.

The lesions observed in pulmonary arteries may be pathogenetically related to the toxicological changes in the heart due to the treatment. The other changes in the lungs (e.g. agonal congestion, interstitial pneumonia, atelectasia, mineralization) cross groups might be agonal in origin or part of aging process.

The increased incidence of stomach erosion/ulceration in treatment groups, especially in high dose groups and a single finding of duodenum ulceration, each in the high dose male and female groups might be secondary to the cardiac lesions or related to a local effect due to oral administration.

Pituitary hyperplasia and adenoma were observed in control and treated groups, which were considered age-related lesions. Other changes, especially hyperplastic and neoplastic changes, which had a low incidence, were not considered treatment-related.

## Special Evaluation

### Toxicokinetics

TK samples were taken during Week 4, 26 and 52 at time points listed in the table below.

	Time after				
	First dosing			Second dosing	
	+ 10 min.	+2 h	+7 h <sup>(1)</sup>	+10 min. <sup>(2)</sup>	+17 h
2 males and 2 females	+			+	
2 males and 2 females		+			+
2 males and 2 females			+		

<sup>(1)</sup> : before the second daily dosing.

<sup>(2)</sup> : between 9 and 13 minutes after the second dosing.

Mean plasma exposures of ivabradine and its N-demethylated metabolite, S 18982, at the end of dosing period are summarized in the table below (excerpted from M2, Section 2.6.7, page 48, NP07026). Exposure ( $AUC_{24}$ ) of ivabradine increased more than dose-proportionally and was higher in female rats than in males (by 1.4-2.8 fold at the end of dosing period). There was no time-dependent effect on exposure to ivabradine, except for accumulation in high dose males for which the AUC ratio between Week 52 and Week 4 was 4.1. The exposure to S 18982 represented less than 13% of the exposure to ivabradine at highest dosage.

Daily dose (mg/kg)	2 x 0 (vehicle)		2 x 3		2 x 16		2 x 90	
	M: 40	F: 40	M: 40	F: 40	M: 40	F: 40	M: 40	F: 40
Number of animals	M: 0	F: 0	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
Number of animals (TK evaluation)								
Toxicokinetics (in Week 52)								
S 16257								
- $C_{max,1}$ (ng/ml) (median $t_{max,1}$ : 12 min)			78	199	390	808	4793	6024
- $C_{max,2}$ (ng/ml) (median $t_{max,2}$ : 12 min)			94	226	452	904	5456	6699
- $AUC_{24}$ (ng.h/ml)			935	2366	4364	12426	64479	86276
S 18982								
- $C_{max,1}$ (ng/ml) (median $t_{max,1}$ : 10 min or 2 h)			nd	nd	nd	nd	786	158
- $C_{max,2}$ (ng/ml) (median $t_{max,2}$ : 10 min)			nd	nd	nd	nd	868	280
- $AUC_{24}$ (ng.h/ml)			nd	nd	nd	nd	8608	2632

nd: Not determined due to plasma levels below the limit of quantitation

## Stability and Homogeneity

Stability of S 16257-2 was tested at concentrations of 0.5, 10 and 100 mg base/ml in demineralized water after 25 days storage at room temperature and resulted in 99.6%, 101.1% and 98.5 % of the nominal concentrations, respectively. The test article is soluble in vehicle (water) at the highest tested concentration.

### NP05181: S 16257-2: 4 week intravenous toxicity study in the rat

Wistar rats (8-week old, 10/gender/group) received once daily iv bolus administration (tail vein, 6 ml/kg, 1 ml/min) of 0 (control, 0.9% (w/v) sodium chloride injection), 2.3, 9.3 or 37 mg/kg/d for 4 weeks. In addition to the standard parameters stated above, the hearts were examined by serial sectioning.

No mortality was observed. Clinical signs included subdued behavior (within one hour of dosing) at all treated doses, and mydriasis, closed eyes, tremor and/or ventral decubitus (within one hour of dosing) at  $\geq 9.3$  mg/kg/d. Reduced body weight associated with decrease in food consumption were observed at  $\geq 9.3$  mg/kg/d. Increased water consumption and urine volume were observed at high dose. The treatment-related myocardial lesions observed in high dose males were described as degenerative

myocytes with an infiltration of mononuclear cells and occasionally some polymorphs, termed "microgranulomas" or "granulomas" by the reviewing pathologist. Inflammatory lesions were mainly located in the sub-epicardial region of the right ventricle. No signs of irritation with the test article were seen at the injection sites.

Plasma exposure levels to ivabradine in satellite animals (n=2/sex/group) were determined. TK parameters are listed below (excerpted from page 489 of the report). In most samples, the S 18982 plasma concentrations were below the limit of quantitation (i.e. 0.5 ng/ml) and the TK parameters of S 18982 could not be determined.

AUC<sub>t</sub>, AUC and other pharmacokinetic parameters of S 16257 in Wistar rats obtained after the first (day 1) and the last (day 28) intravenous administration of S 16257-2

DOSE (mg/kg/day)	Rat n <sup>o</sup>	AREAS (ng.h/ml) on day 1			CL (ml/min/kg)	V <sub>ss</sub> (l/kg)
		AUC <sub>t</sub>	C <sub>t(t<sub>1/2</sub>/ln2)</sub>	AUC		
2.5	MALE n <sup>o</sup> 81	613	5 (0.9)	618	63	3.2
2.5	MALE n <sup>o</sup> 82	544	40 (0.4)	584	66	2.3
2.5	FEMALES*	971	38 (1.4)	1009	38	3.3
10	MALE n <sup>o</sup> 83	2238	23 (1.0)	2261	68	3.7
10	MALE n <sup>o</sup> 84	2268	13 (0.8)	2281	68	3.4
10	FEMALES*	3187	226 (1.8)	3413	45	4.9
40	MALE n <sup>o</sup> 85	10165	154 (1.1)	10319	60	3.6
40	MALE n <sup>o</sup> 86	8670	57 (0.8)	8727	71	3.7
40	FEMALES*	18023	1604 (1.9)	19627	32	3.8

Overall, following iv administration for 4 weeks up to 37 mg/kg/d, the changes were similar to those observed during the 4-week oral study. The heart was the main target organ. Ivabradine was well tolerated locally. The NOAEL was determined at 2.3 mg/kg/d.

## Dog

Repeat-dose 4, 13, 26 and 52 week oral toxicity studies and a 4-week intravenous study were conducted in dogs. For the subacute studies, daily oral doses up to 42 mg/kg/day (4-week study) or 30 mg/kg/day (13-week) were applied. For the chronic studies, twice daily dose up to 2x20 mg/kg/day (26-week study) or 2x12 mg/kg/day (52-week study) were applied in order to mimic clinical dosing regimen. Parameters generally measured in these studies [standard parameters] included mortality, clinical signs, body weight, food consumption, ophthalmoscopy, ECG, blood pressure, body temperature, fecal occult blood, clinical pathology (hematology, chemistry and urinalysis), gross pathology, organ weight and histopathology. Plasma levels of the test article were measured in all

studies. The study reports are located in M4/4.2.3.2. The 52-week rat study was reviewed in an itemized format. Other studies in shorter durations were reviewed in a summary format.

### NP03162: Toxicology study of S 16257-2 following repeated administration for 4 weeks by oral route in the beagle dog

Beagle dogs (8- to 8.5-month old, 3/gender/group) were given once daily oral administration (gavage, 1 ml/kg) of 0 (control, demineralized water), 4.6, 14 or 42 mg/kg/d for 4 weeks. Standard parameters described above were assessed. Six-lead surface ECG (heart rate, PR-interval) was performed once during the pre-test period, and then prior to daily dosing, and 3 and 5 h after dosing in Weeks 1 and 4.

No mortality was observed. Clinical signs included vomiting observed at all treated groups, neuromuscular symptoms (ataxia, tremor, hypertonia or opisthotonos within 1 h of dosing, mainly from Days 1 to 4) observed at 14 or 42 mg/kg/d with the severity and duration correlated with dose, and occasional convulsive seizures (within 30 min after dosing, on Days 1, 4 and 8), without loss of consciousness, as well as nodding head (within 1 h after dosing), observed in some animals at 42 mg/kg/day.

Heart rate reduction was mainly observed in low and mid-dosage groups as the effect was less clear in high dosage group due to the presence of neuromuscular signs. Treatment-related ECG findings consisted of sinus bradycardia (at 4.6 and 42 mg/kg), sinoatrial block (at all doses) or arrest (at 42 mg/kg), first-degree (at 4.6 and 14 mg/kg) or second-degree atrioventricular block (at 4.6 and 42 mg/kg). ECG changes occurred within 3-5 hours post dose.

Reduced thymus weight in 14 and 42 mg/kg/d dose groups was histologically confirmed by an increase in the frequency of thymic atrophy at these two dose levels. The cardiac lesions observed in rat studies were not found in the current dog study.

Overall, at 4.6 mg/kg/day, besides minimal emesis, the only effects were ECG changes including heart rate reduction and sinoatrial block, first-degree or second-degree AVB, which might be the result of exaggerated pharmacological action. The NOAEL cannot be identified due to the ECG findings. [the sponsor identified the NOAEL at 4.6 mg/kg/day (associated AUC<sub>24hr</sub> in males and females, equivalent to 10x or 5x human AUC<sub>24hr</sub> at MRHD, respectively), which was not consistent with the determination for the later studies.]

Mean exposure to ivabradine was summarized in the table below (excerpted from M2/2.6.7, page 55).

Daily dose (mg/kg)	0 (vehicle)		4.6		14		42	
	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Toxicokinetics (S 16257 - on Day 28 - no time-effect)								
. C <sub>max</sub> (ng/ml) [median t <sub>max</sub> : 1 h]								
. AUC <sub>24</sub> (ng·h/ml)								
			1500	1083	3997	2767	8385 <sup>a</sup>	7277
			3492	1750	9912	6361	33889 <sup>a</sup>	28437

For ivabradine,  $T_{max}$  was observed 1 h after dosing (first sampling time). Mean plasma exposure increased dose-proportionally for males between 4.6 and 42 mg/kg/d and for females between 4.6 and 14 mg/kg/d, and more than dose-proportionally for females between 14 and 42 mg/kg/d. Mean plasma exposure was 1.2- to 2.0-fold higher in males than in females at the end of the dosing period. There was no time-dependent effect on exposure to ivabradine.

**NP05574: Toxicity study of S-16257-2 by repeated oral administration for thirteen weeks in the beagle dog**

Beagle dogs (6.5- to 7.5-month old, 5/sex/group) were given once daily oral administration (gavage, 1 ml/kg) of 0 (control, demineralized water), 3, 10 or 30 mg/kg/d for 13 weeks. Main study dogs (n=3/sex/group) and recovery dogs (n=2/sex/group) were sacrificed for examination at the end of dosing period and at the end of 6-week treatment-free period. Standard parameters described above were assessed. Six-lead surface ECG (heart rate, PR-interval) was performed once during the pre-test period, then prior to daily dosing, 3 and 5 h after dosing in Weeks 4, 8 and 13, and once in Week 6 of the treatment-free period.

No mortality was observed. Overt clinical signs included emesis (within 1 hr after dosing) and dose-dependent neuromuscular disturbances (tremor/ stiff hind limbs (within 4 h after dosing) observed at 10 or 30 mg/kg/d, and convulsions (within 10 min of dosing, Days 1 to 4), observed at 30 mg/kg/day. These manifestations were transient.

A progressive and dose-related decrease in body weight (statistically significant for the females from the highest treated group) was observed throughout the treatment period.

Treatment-related ECG findings consisted of sinus bradycardia (at all doses), sinoatrial block or arrest, first- and second-degree AVB, and ventricular escape complexes (at 10 and 30 mg/kg/d) and ventricular fusion complexes (at 30 mg/kg/d). Bradycardia persisted over 24 h at higher doses. Other ECG changes occurred mainly 3 or 5 h after dosing.

The histological examination revealed an increased incidence and severity of thymus atrophy in animals (mainly the females) from groups at 10 or 30 mg/kg/d. Such atrophies, associated with the body-weight loss, may have been related to an effect of the compound resulting in a "stress phenomenon". There were no histopathological changes associated with the neurobehavioral or ECG changes.

All treatment-related changes were reversible on the cessation of treatment. Based on ECG changes, the NOAEL was identified at 3 mg/kg/day (associated  $AUC_{24hr}$  in males and females, equivalent to 5x or 3x human  $AUC_{24h}$  at MRHD, respectively).

Mean plasma maximum concentrations and exposure levels to ivabradine and S 18982 at the end of dosing period were summarized in the table below (excerpted from M2/2.6.7, page 58). For ivabradine,  $t_{max}$  was observed 1 h after dosing (first sampling time). Mean plasma exposure increased dose-proportionally and was 1.1- to 1.6-fold

higher in males than in females. There was no time-dependent effect. Exposure to S 18982 ranged between 13 and 22% of that to ivabradine, with a similar plasma kinetic profile.

Daily dose (mg/kg) Number of animals <sup>a</sup>	0 (vehicle)		3		10		30	
	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Toxicokinetics (on Day 91 - no time-effect)								
S 16257								
. C <sub>max</sub> (ng/ml) [median t <sub>max</sub> : 1 h]			784	608	2069	2511	7015	6659
. AUC <sub>24</sub> (ng.h/ml)			1600	990	6800	6000	29000	21000
S 18982								
. C <sub>max</sub> (ng/ml) [median t <sub>max</sub> : 1 or 3 h]			73	62	248	381	1141	1009
. AUC <sub>24</sub> (ng.h/ml)			210	140	1000	900	6500	2900

### NP06245: Toxicity study of S 16257-2 by repeated oral administration for twenty-six weeks to the beagle dog

Beagle dogs (5- to 8-month old, 6/gender/group) received (by oral gavage, 1 mL/kg) 0 (control, demineralized water), 2, 6.3 or 20 mg/kg of ivabradine twice daily (7 hour-interval between doses: 4, 12.6 or 40 mg/kg/d) for 26 weeks. At the end of the dosing period, 4 dogs/sex/group were euthanized and examined, while the remaining 2 dogs/sex/group were terminated at the end of an 8-week treatment-free period. Six-lead surface ECG (heart rate, PR-interval) was performed once during the pre-test period, then before and 1, 4 and 6 h after first daily dosing on Weeks 12 and 25, and once on Week 8 of the treatment-free period. In addition to the standard parameters stated above, serum ANP was measured to detect possible changes as those seen in the rat; serum and urinary cortisol were measured and thyroid immunocytochemistry was conducted using calcitonin- and thyroglobulin-specific staining, to monitor the spread and proportion of C-cells in thyroidal parenchyma.

No mortality was observed. Overt clinical signs are listed in the table below (from M2/2.6.6, page 42).

Noteworthy findings	Doses (mg/kg/d)					
	2 x 2		2 x 6.3		2 x 20	
	M	F	M	F	M	F
<b>Overt signs:</b>						
Emesis (mostly within 1 h after dosing)					x	x
Tremor (within 1 h after dosing, Weeks 1 to 17)			x	x	x	x
Hind limb hypertonia (within 1 h after dosing, Weeks 1 to 17)					x	x
Convulsions (1 h after dosing on Day 2)						x

The heart rate reduction was not in a dose-dependent manner and occurred within 4-6 hr post dosing. Treatment-related electrocardiographic findings (incidences listed below, excerpted from M2/2.6.7, page 43) consisted of sinus bradycardia and SA block at all doses, or SA arrest (at 2x20 mg base/kg), 1<sup>st</sup> degree AVB (at all doses), and atrial or ventricular premature complexes at 2x20 mg base/kg. These changes might be related to exaggerated pharmacological effect of S 16257, and were no longer observed at the end of the recovery phase. The 2nd degree AVB was only observed at 2x2 mg base/kg which may not be considered toxicologically significant in this case. [note: the 2<sup>nd</sup> degree

AVB was observed in the 52-week dog study at  $\geq 2 \times 3.5$  mg/kg/d. see the study review below. ]. No changes for serum ANP and urine parameters were reported.

There was no evidence of thymic atrophy. There were no treatment-related effects either on adrenal and thyroid structure or function. There were no drug-related adverse histopathologic findings and other measured parameters.

Daily dose (mg/kg) Number of animals	2 x 0 (vehicle)		2 x 2		2 x 6.3		2 x 20	
	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
<b>Noteworthy findings (Cont'd)</b>								
<b>Electrocardiography (Cont'd)</b>								
- Sinus bradycardia (Weeks 12 or 25; 1 h after dosing)	-	-	1/6	-	1/6	-	-	1/6
- Sinoatrial block (Weeks 12 or 25; 1, 4 or 6 h after dosing)	-	-	1/6	-	1/6	-	5/6	2/6
- Sinoatrial arrest (Weeks 12 or 25; 4 h after dosing)	-	-	-	-	-	-	1/6	1/6
- First-degree atrioventricular block (Weeks 12 or 25; 1, 4 or 6 h after dosing)	-	-	2/6	2/6	1/6	1/6	2/6	2/6
- Second-degree atrioventricular block (Week 25; 1 h after dosing)	-	-	1/6	1/6	-	-	-	-
- Atrial premature complexes (Week 12; 4 h after dosing)	-	-	-	-	-	-	1/6	-
- Ventricular premature complexes (Week 12; 4 h after dosing)	-	-	-	-	-	-	1/6	-

The TK parameters were summarized in the table below (excerpted from M2/2.6.7, page 61). S 16257 was rapidly absorbed with maximum plasma concentrations ( $C_{max}$ ) of parent drug and S 18982 occurring approximately within the hour after dosing.  $C_{max}$  values increased with doses and were slightly higher after the morning dose than after the evening one (feed was offered after the morning dose). S 16257 and S 18982 AUC values were slightly higher for males than for females and increased in a dose-proportional manner without any substantial time-related effect. AUC values obtained for S 18982 represented approximately 20% of those of the parent drug.

Daily dose (mg/kg) Number of animals <sup>a</sup>	2 x 0 (Vehicle)		2 x 2		2 x 6.3		2 x 20	
	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
<b>Toxicokinetics (on Day 176)<sup>b</sup></b>								
<b>S 16257</b>								
- $C_{max,1}$ (ng/ml) (median $t_{max,1}$ : 1 h)			693	479	1886	1740	3870	3253
- $C_{max,2}$ (ng/ml) (median $t_{max,2}$ : 1.5 or 2 h)			513	324	1194	992	2827	2737
- $AUC_{24}$ (ng.h/ml)			3993	2086	9056	7976	28175	20302
<b>S 18982</b>								
- $C_{max,1}$ (ng/ml) (median $t_{max,1}$ : 35 min or 1 h)			69	55	243	224	584	500
- $C_{max,2}$ (ng/ml) (median $t_{max,2}$ : 2 to 3 h)			38	25	163	94	508	450
- $AUC_{24}$ (ng.h/ml)			451	241	1445	1022	5908	3917

No NOAEL could be identified due to the ECG changes noted at the lowest dose.

### Study title: S 16257-2 – One year oral (gavage) toxicity study in the beagle dog followed by a 12 week treatment-free period

Study no.: NP15280(contract lab study No. 303/648)  
 Study report location: M4/4.2.3.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: July 22, 1998  
 GLP compliance: Statement included  
 QA statement: Statement included  
 Drug, lot #, and % purity: S 16257-2 (hydrochloride) or Ivabradine, batch# 49652, purity:100.5%

## Key Study Findings

One high dose female was found dead on day 341. Cause of death could not be determined. The relationship to the test article treatment cannot be excluded.

Clinical signs of hypersalivation, emesis and liquid feces were observed in high dose groups, mainly in the first 3 months of treatment period.

ECG findings in dogs treated at doses  $\geq 2 \times 3.5$  mg/kg/day included heart rate reduction (starting from Week 1) up to -44%, sinus bradycardia (<60 bpm) from Week 13, isolated sinoatrial block or arrest (defined as an R-R interval greater than 1.5 or 2 seconds, respectively) or second-degree AV blocks (P wave not followed by QRS complex). The findings were not observed after the 1st week of recovery period. No changes on QTc, PR, and QRS complex were observed. No test article-related effect on BP was observed.

ERG findings included a dose-dependent increase in a-wave slope reduction of b-wave amplitude, and negative ERG dominated by the PIII component observed in all treated groups for cone system response assessment; negative EGR and delayed appearance of positive b-wave in kinetics of dark adaptation in dogs at doses  $\geq 2 \times 3.5$  mg/kg/day. The findings were reversed after the 1<sup>st</sup> week of recovery period.

No changes were observed in standard ophthalmoscopic examinations, indirect ophthalmoscopic exam (eyes fundus morphology), and transmission electron microscopy (TEM) exam.

NOAEL could not be determined due to ERG findings at lowest dose.

## Methods

Doses:	0, 1, 3.5, 12 mg/kg (expressed in terms of base material, salt/base ratio: 1.08)
Frequency of dosing:	BID
Route of administration:	Oral gavage
Dose volume:	See study design below
Formulation/Vehicle:	solution in the vehicle at concentrations of 0.5, 1.75 and 6 mg/ml./water for injection
Species/Strain:	Dog/Beagle, lco : WI (IOPS Han)
Number/Sex/Group:	See study design below
Age:	6-7 months at initiation
Weight:	Male: 7.3-10.4 kg; female: 6.4 to 8.8 kg.
Satellite groups:	See study design below
Unique study design:	n/a
Deviation from study protocol:	Deviations were not considered to have affected the outcome of the study objectives.

Group/ Treatment	Dose level* (mg/kg/day)	Dose volume (ml/kg/administration)	Dose concentration (mg/ml)	Number of animals			
				Week 54 (1)		Week 66 (2)	
				Males	Females	Males	Females
1. Control	2 x 0	2	0	4	4	2	2
2. Low dose	2 x 1	2	0.5	4	4	2	2
3. Intermediate dose	2 x 3.5	2	1.75	4	4	2	2
4. High dose	2 x 12	2	6.0	4	4	2	2

\* Interval between the first and the second dosing: approximately 8 hours.

## Observations and Results

### Mortality

All animals were observed twice daily.

One high dose female was found dead on Day 341 about 6 hours post the 1<sup>st</sup> daily dosing. No clinical signs were observed during the days preceding the death. Throughout the study, this animal showed occasional periods of liquid feces, sometimes with blood, and occasional vomiting. No body weight gain was affected. ECG evaluation of this animal showed bradycardia with a heart rate of 40-60 bpm observed from Week 26/27 without any adverse effects on QTc, PR, QRS and rhythm. The clinical pathology, gross and histopathological examinations did not reveal any changes that could determine the cause of death. A possible test article-related effect could not be excluded.

### Clinical Signs

Animals were examined before and at least once after each dosing for any clinical signs and reaction to treatment. A full clinical examination was performed prior to the initiation of treatment and during weeks 4, 13, 26, 52 and 66.

During the treatment period, increased incidence of hyper-salivation was observed before, during and after dosing in the high dose males and females, with more frequent in the first 3 months of the study. Slightly higher incidence of vomiting and liquid feces (sometimes with blood) was observed in high dose groups during the first 3 months of the study.

One female animal (No. 147) on Day 90, showed prostration and pallor of mucous membrane before the 1<sup>st</sup> dosing, then about 30 min post dosing, hyperthermia (40.8 C) and vomiting were noted; and about 3 hour post dosing, subdued behavior, hyperthermia (40.1 C) and a HR of 70 bpm with systolic murmur and a few extrasystoles were observed in the clinical examination. The HR measured next day (Day 91) about 5 hours post dosing was 50 bpm without clear associated clinical signs. This animal also showed frequent liquid feces (often with blood).

In the recovery period, there was no significant treatment-related clinical sign.

## Body Weights

Individual body weights were recorded at least once weekly for 17 weeks. Data were presented starting two weeks before the initiation of treatment.

There was no significant treatment-related effect on body weight.

## Feed Consumption

Feed consumption was measured daily and reported as weekly mean in g/animal/day. Data were presented starting two weeks before the initiation of treatment.

There was no significant test article-related effect on food consumption.

## Cardiovascular Examination

Cardiovascular examinations including blood pressure (BP), heart rate (HR) and ECG were performed to all survived animals twice pretest; prior to the 1<sup>st</sup> daily dosing during Weeks 1, 13, 26/27 and 39; prior to and 2 hours post the 1<sup>st</sup> dosing during Week 41/42, prior to, 2 and 4 hours post the 1<sup>st</sup> dosing during Week 53 and once each during Weeks 61 and 66 (recovery period).

Systolic, diastolic and mean arterial blood pressure was measured in unanesthetized animals with tail cuff method. ECG (leads I, II and III) traces were recorded. HR, rhythm, QRS duration, PR and QT intervals were analyzed. QTc was calculated using Fridericia's formula.

There was no significant treatment-related effect on arterial BP.

Heart rate reduction up to -44% was observed in animals treated at doses  $\geq 2 \times 3.5$  mg/kg/day, with male high dose group from Week 1 and other groups from Week 13 (summarized in the table below, excerpted from Section 2.6.7. page 66). Sinus bradycardia (<60 bpm) was observed from Week 13 at doses  $\geq 2 \times 3.5$  mg/kg/day, generally at 2 or 4 hours post dosing, and sporadically before dosing at  $2 \times 12$  mg/kg/day.

Daily dose (mg/kg)	2 x 0 (vehicle)		2 x 1		2 x 3.5		2 x 12	
	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6*
Number of animals								
Noteworthy findings (Cont'd)								
Blood pressure								
Electrocardiography								
Heart rate (beats/min):								
Pretest (2 measures)	123/123	125/115	145/138	143/153**	125/118	120/123	107/120	132/137
Week 1	120	113	137	132	102	110	93	103
Week 13	108	118	122	133	107	93	80	82
Week 26/27	95	120	110	133	98	110	83	82
Week 39	95	118	110	137	102	103	85	80
Week 41/42								
Before dosing	110	100	122	130	107	108	83	87
2 h after dosing	105	112	102	110	87	78*	78*	68**
Week 53								
Before dosing	100	123	117	115	108	93	75*	90*
2 h after dosing	100	105	103	107	85	73*	92	72
4 h after dosing	113	115	125	122	90	88	78*	64**
Heart-rate change [mean decrease (%) vs. vehicle]								
Week 53			+10	+6	-21	-23	-32	-44

Isolated sinoatrial block or arrest (defined as an R-R interval greater than 1.5 or 2 seconds, respectively) or second-degree AV blocks (P wave not followed by QRS complex) were noted with higher incidence in groups at doses  $\geq 2 \times 3.5$  mg/kg/day than the controls (see table below, excerpted from page 54 of the report). No similar changes were observed in the recovery period.

Daily dose (mg/kg)	2 x 0 (vehicle)		2 x 1		2 x 3.5		2 x 12	
Number of animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
<b>Noteworthy findings (Cont'd)</b>								
<b>Electrocardiography (Cont'd)</b>								
- Sinus bradycardia	-	-	-	-	1/6	-	2/6	3/6
- Sinoatrial block or arrest (generally 2 or 4 h after first daily dosing, in Weeks 13, 42 or 53)	-	-	-	-	1/6	-	3/6	3/6
- Second-degree atrioventricular block (2 h after first daily dosing, in Weeks 41/42 or 53)	-	1/6	-	-	2/6	2/6	2/6	1/6

Sinoatrial block or arrest was generally observed 2 or 4 hours post dosing and was not observed during the 1<sup>st</sup> part of the study probably because, until Week 39, ECGs were performed only prior to the 1<sup>st</sup> daily dosing. It might be considered as an accentuation of sinus arrhythmia which is a normal variation of the canine cardiac rhythm influenced by an inherent high degree of vagal tone.

Second degree AV block is occasionally observed in the healthy beagle dog without associated cardiac pathology. However, incidence of this change in the dose groups  $\geq 2 \times 3.5$  mg/kg/day was much higher than the control, indicating a test article-related effect.

There was no treatment-related effect on PR interval, QRS complex and QTc.

## Ophthalmoscopy and ERG

All animals were examined at pretest and during Weeks 27, 52, 62 and 66. Examination of adnexa and optic media was performed using a slit lamp or an indirect ophthalmoscope. The fundus was examined with a Kowa camera.

An additional examination (indirect ophthalmoscopy) was performed during Week 28 for group 1 male nos. 101 and 102, female nos. 107 and 108, group 4 animals and group 3 female no. 131.

As the ERG was flat, the vision and oculomotor reflexes were evaluated for the group 3 female no. 131 in comparison with group 4 animals and with 4 controls. No abnormality was detected.

ERG was recorded for all animals at pretest, after 6 months of treatment (Week 26/27), and at the end of the treatment period (week 50/51), two days after the first week of the treatment-free period (week 55), one day after the seventh week of the treatment-free period (week 61), two days after the eleventh week of the treatment-free period (week 65). Examination was performed in animals anesthetized with an intramuscular injection of medetomidine and ketamine. A mydriatic agent tropicamide (Mydriaticum®, MSD-Chibret) was instilled into the eyes before examination. Evaluation included the

cone-system responses (under photopic condition), the rod-system responses (during dark adaptation under scotopic conditions), and mixed cone-and rod-system responses.

There were no treatment-related changes in standard ophthalmological examinations.

On each examination of indirect ophthalmoscopy, eye fundus was morphologically normal for all animals. Neither hyperreflectivity nor hypo-reflectivity was observed. The aspect of the papillar, arteries and veins were normal. There were no indications of abnormal surface reflectivities problematic vascular perfusion or retinal or optic nerve atrophy. Representative photographs of eye fundus performed during the study period in a control beagle dog (M106) and in a treated beagle dog (M141) with the highest dose of ivabradine (2 x 12 mg/kg/day) were shown in the study report.

For ERG morphology, photopic ERG of control animals had typical a- and b-waves on all occasions. For treated groups, a dose-dependent altered ERG morphology was observed, i.e. from the reduced b-wave amplitude to the negative ERG dominated by the PIII component. Following one week recovery period, ERG recovered normal morphology for all animals examined.

Cone system responses (photopic condition) including a-wave slope, b-wave amplitude and b-wave implicit time were evaluated. As shown in the table below, a dose-dependent effect on a-wave slope and b-wave amplitude was observed. Such an effect was not observed after the 1<sup>st</sup> week of recovery. Slight variations in b-wave implicit time observed in both males and females were within the range of those observed in the control groups. There was no dose-, time- or gender-related effect on this parameter.

**Table 1: Photopic ERG: a-wave slope ( $\mu\text{V}/\text{ms}$ )**

<b>Females</b>	<b>Control</b>	<b>2x1 mg/kg/d</b>	<b>2x3,5 mg/kg/d</b>	<b>2x12 mg/kg/d</b>
Pre-test	4.1 $\pm$ 0.9	4.4 $\pm$ 1.3	3.6 $\pm$ 0.5	4.5 $\pm$ 0.6
Week 26/27	5.3 $\pm$ 1.5	5.7 $\pm$ 1.7	5.4 $\pm$ 1.8*	7.0 $\pm$ 2.1
Week 50/51	6.1 $\pm$ 1.2	5.1 $\pm$ 0.9	7.1 $\pm$ 1.5	9.0 $\pm$ 2.6
TC1	5.3 (6.2-4.5)	4.8 (4.2-5.4)	6.0 (6.1-5.9)	6.8*
TC7	4.9 (6.0-3.7)	4.0 (2.9-5.1)	6.1 (6.5-5.7)	6.1
TC11	4.1 (5.0-3.2)	3.8 (4.0-3.6)	6.5 (5.9-7.0)	6.3

Pre-test, week 26 and week 50/51: mean  $\pm$  sd ; n = 6 except for group 2x3.5 and on week 51 for the group 2 x 12 mg/kg/d n=5

Treatment-free period: n = 2 except for 2 x 12 mg/kg group n = 1

TC1: 1 week, TC7: 7 weeks, TC11: 11 weeks after treatment cessation

\*: only one eye measured for the female n°135 (ERG of the other eye unreadable) on Week 26 and for female n°147 on TC1 (other eye not dilated)

<b>Males</b>	<b>Control</b>	<b>2x1 mg/kg/d</b>	<b>2x3,5 mg/kg/d</b>	<b>2x12 mg/kg/d</b>
Pre-test	4.6 $\pm$ 0.8	5.5 $\pm$ 1.2	3.9 $\pm$ 1.3	4.4 $\pm$ 1.0
Week 26/27	4.8 $\pm$ 0.9	4.4 $\pm$ 1.0	5.6 $\pm$ 1.0	6.8 $\pm$ 1.7
Week 50/51	5.4 $\pm$ 1.8	5.2 $\pm$ 2.0	8.7 $\pm$ 1.4	7.0 $\pm$ 1.2
TC1	4.2 (5.0-3.4)	5.1 (6.4-3.9)	4.4 (5.0-3.8)	5.8 (5.7-5.9)
TC7	4.6 (5.3-3.9)	4.1 (4.8-3.5)	5.6 (5.3-5.9)	4.6 (4.9-4.4)
TC11	4.1 (4.5-3.7)	4.0 (4.6-3.4)	4.4 (5.0-3.8)	5.7 (5.5-5.9)

Weeks 26/27 and 50/51: mean  $\pm$  sd; n = 6 - Recovery periods: individual values; n = 2

TC1: 1 week, TC7: 7 weeks, TC11: 11 weeks after treatment cessation

**Table 2: Photopic ERG: b-wave amplitude (µV)**

**Females**

	Control	2x1 mg/kg/d	2x3,5 mg/kg/d	2x12 mg/kg/d
Pre-test	175 ± 55	189 ± 29	180 ± 24	193 ± 43
Week 26/27	169 ± 38	126 ± 23	45 ± 11*	55 ± 11
Week 50/51	176 ± 35	98 ± 16	60 ± 15*	45 ± 15*
TC1	193 (191-195)	184 (143-226)	189 (178-199)	134
TC7	194 (219-169)	158 (130-185)	168 (161-176)	197
TC11	182 (200-164)	179 (153-204)	206 (181-231)	160

Pre-test, week 26/27 and week 50/51: mean ± sd ; n = 6 except for group 2x3.5 and on week 51 for the group 2 x 12 mg/kg/d n=5

Recovery periods: n = 2 except for 2 x 12 g/kg group n = 1

TC1: 1 week, TC7: 7 weeks, TC11: 11 weeks after treatment cessation

\*: only one eye measured for females n°143 and 147 on Week 26, females n° 134, 135 143 on Week 50/51 (ERG of the other eye unreadable) and for female n°147 on TC1 (other eye not dilated)

**Males**

	Control	2x1 mg/kg/d	2x3,5 mg/kg/d	2x12 mg/kg/d
Pre-test	190 ± 51	192 ± 20	165 ± 21	172 ± 45
Week 26/27	146 ± 33	92 ± 28	58 ± 21	24 ± 7
Week 50/51	156 ± 26	64 ± 22	47 ± 11	31 ± 13
TC1	158 (187-130)	169 (172-166)	121 (147-95)	106 (102-111)
TC7	177 (223-130)	160 (148-171)	167 (165-169)	124 (117-130)
TC11	167 (209-126)	182 (146-217)	134 (107-162)	145 (145-145)

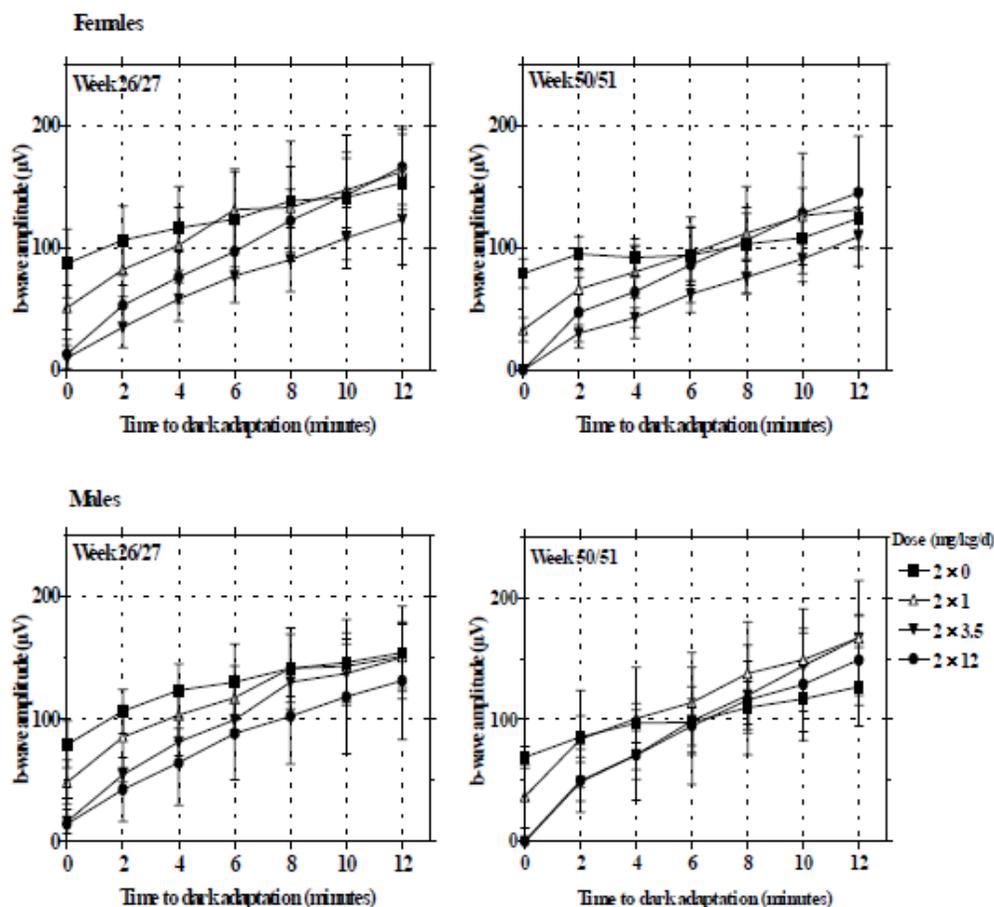
Weeks 26/27 and 50/51: mean ± sem; n = 6 - Recovery periods: individual values; n = 2

TC1: 1 week, TC7: 7 weeks, TC11: 11 weeks after treatment cessation

Rod system responses (scotopic conditions) including scotopic ERG, b-wave amplitude and implicit time during dark adaption up to 12 min were assessed. Scotopic ERG b-wave appeared normal on all occasions through the dark adaption process for the low dose groups. Animals treated at doses ≥2x 3.5 mg/kg/day showed negative ERG and emerging positive b-wave through the dark adaption process in a dose-dependent pattern. As shown in the table (excerpted from Section 2.6.7 page 65) and graph (page 41 of ERG report in Volume V of the study report) below, a delayed appearance of b-wave in the kinetics of dark adaption was observed in the dogs treated at doses ≥2x 3.5 mg/kg/day. Responses of the rod system recorded after 12 minutes of dark adaptation process (i.e. half-maximal response in beagle dogs) were not affected by treatment. Treatment effect on b-wave amplitude was fully reversed after one week recovery period.

Daily dose (mg/kg)	2 x 0 (Vehicle)		2 x 1		2 x 3.5		2 x 12			
	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6		
Rod system responses (scotopic conditions):										
b-wave amplitude (µV)										
- Week 26	Dark onset (B0)		79±19	87±28	-39%	-41%	-80%	-89%	-82%	-85%
	2-min dark adaptation (B2)		106±18	106±28	-20%	-23%	-49%	-67%	-60%	-50%
	4-min dark adaptation (B4)		123±22	116±34	-16%	-12%	-34%	-50%	-48%	-34%
	From 6- to 12-min dark adaptation (B6 to B12)		-	-	-	-	-	-	-	-
- Week 50/51	Dark onset (B0)		69±9	79±12	-46%	-58%	-100%	-100%	-100%	-100%
	2-min dark adaptation (B2)		86±17	95±14	-2.3%	-31%	-43%	-68%	-42%	-51%
	4-min dark adaptation (B4)		97±16	92±15	+4.1%	-13%	-27%	-53%	-27%	-30%
	From 6- to 12-min dark adaptation (B6 to B12)		-	-	-	-	-	-	-	-
b-wave implicit time (ms)										
			-	-	-	-	-	-	-	-

Figure 16: Scotopic ERG: b-wave amplitude over 12 minutes dark adaptation process during the treatment period



Values are mean  $\pm$  sd; n=6 for all groups, except for females at 2 × 3.5 mg/kg b.i.d. at all times and for females at 2 × 12 mg/kg/d on W50/51, where n=5.

For mixed cone and rod responses, on all occasions, all animals that were examined exhibited single flash ERG with typical negative a-wave followed by a positive b-wave.

## Hematology

Blood samples were taken from all animals once pretest, and during Weeks 12/13, 25/26, 52/53, 61/62 and 65/66.

There were no significant test article-related effects on measured hematology parameters.

## Clinical Chemistry

Blood samples were taken from all animals once pretest, and during Weeks 12/13, 25/26, 52/53, 61/62 and 65/66. The animals were fasted for at least 15 hours before sampling.

There were no treatment-related adverse effects on the measured parameters.

### Urinalysis

Urine samples were taken from all animals once pretest, and during Weeks 12/13, 25/26, 52/53, 61/62 and 65/66. Urine was collected in metabolism cages (for about 15 hours) from animals deprived of feed and water before the beginning of the collection period.

There were no treatment-related adverse effects on the measured parameters.

### Gross Pathology

All designated animals were necropsied at the scheduled termination. A complete gross pathology examination was conducted for all animals subjected to unscheduled death or scheduled termination.

For the female found dead on day 341, black areas in the lungs, most prominent in one lobe, was associated with congestion and edema in the lungs. The direct relationship with test article could not be determined.

For the animals necropsied at the scheduled termination, there were no test article-related adverse changes in the macroscopic observations.

### Organ Weights

The organs listed below were weighed for all necropsied animals.

adrenal glands	ovaries
brain	pituitary gland
heart	spleen
kidneys	testes
liver	thyroid glands

There were no significant test article-related changes in the measured organ weights.

### Histopathology

The listed organs/tissues sampled from all animals, as shown below, were fixed and preserved in 10% neutral formalin with the exceptions for partial left eyes which was fixed in 4% glutaraldehyde for transmission electron microscopy (TEM) and for bone marrow smears that were fixed in methanol. A standard microscopic examination of the listed organ/tissues and gross lesions was performed for all animals.

Seven sections were prepared for the heart (2) except for female no. 148 for which slides of left ventricular free wall and dorsal papillary muscle were not prepared as this

animal died before it was decided to perform non-standard preparation of the heart. The slides obtained were examined for all animals in the study.

A section through the primary visual cortex was performed and examined in the brain of controls and group 4 animals killed at the end of the study and after the treatment-free period.

All slides were stained with haematoxylin and eosin (except bone marrow smears). In addition, a von Kossa and an Alizarin stain were performed on heart samples corresponding to aortic valve, aortic outflow tract and aorta from animals Nos. 107, 111 (group 1 females) and 140 (group 4 male) and on heart samples corresponding to right ventricular free wall, atrioventricular valve and atrium from animal No. 138 (group 4 male).

adrenal glands	ovaries
bone (sternum) with bone marrow	pancreas
bone marrow smears (1)	parathyroid glands
bronchi (mainstem)	pituitary gland
brain	prostate
caecum	rectum
colon	salivary gland (submaxillary)
duodenum	skeletal muscle
epididymides	skin
eyes	spinal cord (cervical, thoracic, lumbar)
gall bladder	spleen
heart	stomach
ileum	testes
jejunum	thymus
kidneys	thyroid glands
liver	tongue
lungs	trachea
lymph node (submaxillary)	urinary bladder
lymph node (mesenteric)	uterus (horns + cervix)
mammary gland	vagina
oesophagus	all gross lesions.
optic nerves	

Adequate Battery: yes. Peer Review: yes.

### Histological Findings

There were no significant test article-related adverse findings in the microscopic examinations. The following observations listed in the table below were not considered toxicologically significant due to low incidence, minimal severity, comparable cross groups at the end of recovery period, inconsistent in the topography, or consistent with background findings in beagle dogs.

		Males				Females			
Dose level (mg/kg/day)		2x0	2x1	2x3	2x12	2x0	2x1	2x3	2x12
Number examined (treatment/recovery)		4/2	4/2	4/2	4/2	4/2	4/2	4/2	4/2
Focal changes in the Heart									
Papillary muscle, minimal focus of fibrosis with mineralization at the end of <b>treatment</b>		0	0	0	1 <sup>a</sup>	0	0	0	0
Left papillary muscle, slight focus of fibrosis with mineralization at the end of <b>recovery</b>		0	1	0	0	0	0	0	0
Left ventricle, minimal focus of chronic inflammation at the end of <b>treatment</b>		0	0	0	1 <sup>a</sup> +1	0	0	0	0
Base of aortic valve, moderate focus of fibrosis or vacuolated myocytes at the end of <b>recovery</b>		0	1	0	0	0	0	0	0
Left and Right ventricles, slight fibrosis with mineralization at <b>recovery</b> period		0	0	0	0	0	0	0	1
<b>Aorta or pulmonary artery</b>									
dark granules (calcium mineralization)	Treatment phase	0	0	0	3	1	0	0	4
	Minimal	0	0	0	1	1	0	0	4
	Slight				2				
	Recovery phase, minimal	0	2	0	0	1	1	2	0

a. the same animal

### Special Evaluation: TEM

An ultrastructural evaluation of the left eye was performed on:

2 males and 2 females from groups 1 and 4 killed during week 54 (8 animals),

Group 1: male nos. 101 and 102, female nos. 107 and 108.

Group 4: male nos. 137 and 138, female nos. 143 and 144.

1 male and 1 female from groups 1 and 4 killed during week 66 (4 animals).

Group 1: male no. 105 and female no. 111.

Group 4: male no. 142 and female no. 147.

(As no treatment-related changes were noted, no additional animals were examined).

TEM examination of retina did not reveal any adverse findings.

### Toxicokinetics

TK samples were taken from all animals including controls, during Weeks 4 (day 23), 26 (day 178) and 53 (day 366) at the following time points:

before the first daily dosing,

approximately 15 minutes, 1 hour and 4 hours after the first daily dosing, before the second daily dosing (i.e. 8 hours after the first daily dosing), 1 hour, 2 hours and 3 hours after the second daily dosing.

For tissue distribution in the eyes and the heart, the right eye was weighed and collected as 3 separate parts: vitreous body, anterior part (including cornea, iris and lens), and posterior part (including retina, choroïd and sclera). One piece of the cardiac apex (2 to 7 g) was collected. Additional blood samples were taken from all animals sacrificed at the scheduled termination.

Mean plasma exposures of ivabradine and its N-demethylated metabolite, S 18982, at the end of dosing period are summarized in the table below (excerpted from page 57 of the report). For ivabradine,  $t_{max}$  ranged between 15 min and 1 h after first daily dosing and was observed 1 or 1.5 h after second daily dosing. Mean plasma exposure increased more than dose-proportionally between 1 and 3.5 mg/kg b.i.d. and dose-proportionally between 3.5 and 12 mg/kg b.i.d. Mean plasma exposure to ivabradine was slightly higher in males than in females (by 1.2- to 1.8-fold at the end of the dosing period). There was no time-dependent effect. Exposure to S 18982 represented ~10 to 20% of that to ivabradine, with a similar plasma kinetic profile.

dose (mg/kg/day)	S 16257						S 18982							
	2 x 1		2 x 3.5		2 x 12		2 x 1		2 x 3.5		2 x 12			
	M	F	M	F	M	F	M	F	M	F	M	F		
$C_{min,1,ss}$ (ng/ml) :	BLQ	BLQ	BLQ	BLQ	28.7	27.1	BLQ	BLQ	BLQ	BLQ	9.09	6.52	week 4	
$C_{min,2,ss}$ (ng/ml) :	BLQ	BLQ	3.88	BLQ	76.7	35.7	BLQ	BLQ	BLQ	BLQ	20.9	8.35		
$C_{max,1,ss}$ (ng/ml) :	170	180	1373	1299	3341	4812	16.1	12.7	171	144	600	694		
$C_{max,2,ss}$ (ng/ml) :	79.1	54.5	631	607	2365	3824	3.52	2.61	51.1	38.5	407	673		
$t_{max,1}$ (h)	0.250	0.250	0.250	1.00	1.00	0.625	0.250	0.250	0.625	0.250	1.00	1.00		
$t_{max,2}$ (h)	1.00	1.00	1.00	1.00	2.00	1.00	1.00	1.00	1.00	1.50	1.00	1.00		
$AUC_{24,ss}$ (µg.h/ml) :	0.385	0.306	3.89	3.80	18.1	10.7	-	-	0.446	0.431	3.62	3.46		
Cl / f :	-	-	-	-	-	-	-	-	-	-	-	-		
T 1/2 lambda i-z :	-	-	-	-	-	-	-	-	-	-	-	-		
Accumulation factor :	-	-	-	-	-	-	-	-	-	-	-	-		
$C_{min,1,ss}$ (ng/ml) :	BLQ	BLQ	5.76	8.68	20.0	17.7	BLQ	BLQ	BLQ	BLQ	5.97	4.17		week 28
$C_{min,2,ss}$ (ng/ml) :	BLQ	BLQ	16.7	15.9	94.2	104	BLQ	BLQ	BLQ	3.97	29.0	13.5		
$C_{max,1,ss}$ (ng/ml) :	172	137	2172	2359	4636	4286	15.6	15.9	294	243	665	491		
$C_{max,2,ss}$ (ng/ml) :	64.0	57.8	1158	1042	2167	2053	5.39	BLQ	73.8	94.7	386	437		
$t_{max,1}$ (h)	0.250	0.250	0.250	0.250	0.625	0.250	0.250	0.250	0.625	0.250	1.00	1.00		
$t_{max,2}$ (h)	1.00	1.00	1.00	1.00	1.00	1.50	1.00	-	1.50	1.00	1.00	1.50		
$AUC_{24,ss}$ (µg.h/ml) :	0.415	0.304	7.59	6.63	19.9	17.5	-	-	0.781	0.770	3.73	2.52		
Cl / f :	-	-	-	-	-	-	-	-	-	-	-	-		
T 1/2 lambda i-z :	-	-	-	-	-	-	-	-	-	-	-	-		
Accumulation factor :	1.16	1.01	1.90	1.71	1.12	0.893	-	-	1.70	1.81	1.04	0.815		
$C_{min,1,ss}$ (ng/ml) :	BLQ	BLQ	2.84	BLQ	16.6	9.25	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	week 53	
$C_{min,2,ss}$ (ng/ml) :	BLQ	BLQ	33.9	4.37	105	49.0	BLQ	BLQ	6.67	BLQ	32.8	12.9		
$C_{max,1,ss}$ (ng/ml) :	94.5	88.0	1252	1174	3678	3241	8.94	11.1	146	166	565	617		
$C_{max,2,ss}$ (ng/ml) :	55.2	32.0	615	583	1845	1751	BLQ	BLQ	39.8	46.8	308	334		
$t_{max,1}$ (h)	0.250	0.250	0.625	0.625	0.625	1.00	0.250	0.250	0.250	0.625	1.00	1.00		
$t_{max,2}$ (h)	1.00	1.00	1.00	1.50	1.00	1.00	-	-	0.250	0.625	1.00	1.00		
$AUC_{24,ss}$ (µg.h/ml) :	0.361	0.205	4.65	3.89	17.3	14.3	-	-	0.481	0.536	3.24	2.58		
Cl / f :	-	-	-	-	-	-	-	-	-	-	-	-		
T 1/2 lambda i-z :	-	-	-	-	-	-	-	-	-	-	-	-		
Accumulation factor :	0.926	0.671	1.17	0.954	1.00	0.703	-	-	1.04	1.18	0.940	0.712		

As shown in the table below (excerpted from M2/2.6.7, page 67), tissue concentrations of S16257 and S18982 in the heart after one year of treatment were below the limit of quantitation. In the eyes, a dose-related increase in concentrations of S16257 and S18982 was measured in the posterior and anterior ocular structures. After the three-month treatment-free period, ocular concentrations of S16257 were reduced by 14 to 86%, indicating reversible binding.

Daily dose (mg/kg)		2 x 0 (vehicle)		2 x 1		2 x 3.5		2 x 12	
Number of animals		M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6 <sup>a</sup>
<b>Tissue concentrations (mean values in ng/g)</b>									
Heart									
. S 16257		blq		blq		blq		blq	
. S 18982		blq		blq		blq		blq	
Eye									
. S 16257		Anterior part		12688	8874	59616	47491	128819	151487
		Posterior part		27472	10619	95561	81680	228757	292910
		Vitreous body		blq	blq	163	blq	122	36
. S 18982		Anterior part		891	872	10218	8152	40680	30922
		Posterior part		2629	1269	22612	19595	63125	64755
		Vitreous body		blq	blq	blq	blq	blq	blq

Daily dose (mg/kg)		2 x 0 (vehicle)		2 x 1		2 x 3.5		2 x 12	
Number of animals (postdose evaluation)		M: 2	F: 2	M: 2	F: 2	M: 2	F: 2	M: 2	F: 1
<b>Tissue concentrations (individual values in ng/g)</b>									
Heart									
. S 16257		blq, blq		blq, blq		blq, blq		blq, blq	
. S 18982		blq, blq		blq, blq		blq, blq		blq, blq	
Eye									
. S 16257		Anterior part		2642,	9147,	14500,	21343,	77822,	5386 <sup>a</sup>
		Posterior part		8276,	6161,	27749,	13136,	33904,	
		Vitreous body		2273,	7269,	43694,	14964, 7169	65274,	8089 <sup>a</sup>
				5690,	5360	74005		17034	
				blq, blq	blq, blq	blq, blq	60, blq	53, blq	blq <sup>a</sup>
. S 18982		Anterior part		1234, 538	1604, 567	4338, 6934	7307, 4202	25476,	16613 <sup>a</sup>
		Posterior part		1825, 963	2075,	14806,	9603, 7049	24567,	
		Vitreous body		1147		23470		29415,	24064 <sup>a</sup>
				blq, blq	blq, blq	blq, blq	blq, blq	18258	
								33, blq	blq <sup>a</sup>

## Stability and Homogeneity

Stability of S 16257-2 was tested at concentrations of 0.5, 10 and 100 mg base/mL in demineralized water after 25 days storage at room temperature and resulted in 99.6%, 101.1% and 98.5 % of the nominal concentrations, respectively (the stability report was available in rat study NP07026). The test article is soluble in vehicle (water) at the highest tested concentration.

## NP05108: Toxicity study on S 16257-2 by repeated intravenous administration for four weeks in the beagle dog

Beagle dogs (7-month old, 3/sex/group) were surgically prepared by location of a cannula into the jugular vein and given continuous iv infusion (1 ml/kg/h) of 0 (control, 5% (w/v) aqueous glucose injection), 3, 15 or 70 mg/kg/d for 4 weeks. The standard parameters stated above were measured, except for rectal temperature and fecal occult blood. Six-lead surface ECG (heart rate, PR-interval, QRS-complex duration, QT-interval, QTc) was performed once during the pre-test period and in Week 4.

No mortality was observed. The noteworthy findings are summarized below (excerpted from M2/2.6.6, page 47). ECG changes were noted in 1 out of 3 animals of each group, within the first 3 h after dosing.

Noteworthy findings	Doses (mg/kg/d)					
	1.9		3.7		7.4	
	M	F	M	F	M	F
<b>Overt signs:</b> (dose-related incidence and severity, decreased severity from Day 14)						
Tremor, generalised stiffness, arched back, splayed legs	x		x	x	x	x
Barking, growling or biting of the cage	x				x	x
Head nodding or paddling movements			x			
<b>Electrocardiography:</b>						
Sinus bradycardia						x
Sinoatrial block				x	x	x
Atrial premature complexes					x	x

The TK parameters are listed below (excerpted from M2/2.6.7, page 69). Mean plasma exposure to ivabradine increased dose-proportionally, was similar in males and females, and there was no time-dependent effect.

Daily dose (mg/kg)	0 (vehicle)		1.9		3.7		7.4	
	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Toxicokinetics (S 16257 - on Day 28, no time effect)								
C <sub>5min</sub> (ng/ml) <sup>a</sup>			1773	1930	3550	4060	5217	4853
AUC <sub>24</sub> (ng.h/ml)			2108	1856	4777	5191	8635	8083

Overall, after iv (bolus) administration of ivabradine for 4 weeks up to 7.4 mg/kg/d, the changes were generally similar to those observed during the 4-week oral study. The NOAEL was determined at 1.9 mg/kg/d.

## 7 Genetic Toxicology

See a complete review in Appendix 1.

## 8 Carcinogenicity

See a complete review in Appendix 2.

## 9 Reproductive and Developmental Toxicology

See a complete review in Appendix 3.

## 10 Special Toxicology Studies

**NP32768: Acute Eye Irritation/Corrosion study with S 16257-2 (Ivabradine hydrochloride) in the New Zealand White rabbit**

Single samples of approximately 32 mg of S 16257-2 (0.1 mL) were instilled into the conjunctival sac of one eye of each of three rabbits, the other eye remained untreated and served as reference control. Observations of the eyes (cornea, iris and conjunctivae) were made 1, 24, 48 and 72 hours after instillation.

No symptoms of systemic toxicity were observed. Instillation of S 16257-2 resulted in slight and transitory irritation of the conjunctivae, which consisted of redness, chemosis and discharge (mean score for conjunctivae redness=0.7, remaining score=0). The chemosis and discharge completely resolved within 24 hours and the redness completely resolved within 72 hours after treatment in all animals. No iridial irritation or corneal opacity were observed as well as no corneal epithelial damage after fluorescein treatment. There was no evidence of ocular corrosion.

**NP15279: Determination of the potential immunomodulating effects of S 16257-2 by means of the Plaque-Forming Cell assay and Lymphocyte Subset Analysis after 4 week oral dosing of male and female Wistar rats**

Wistar rats (8- to 9-week old, 8/gender/group) received oral administration (gavage, 5 ml/kg, 7-hour interval between doses) of 0 (control, demineralized water), 3, 16 or 90 mg/kg twice daily (6, 32 or 180 mg/kg/d) for 4 weeks. Endpoints measured included clinical signs, bodyweight, food consumption, hematology and plasma levels of ivabradine and S 18982. At necropsy, the lymphoid organs were sampled and weighed. The primary antibody response to a T-cell dependent antigen was assessed by a Plaque-Forming Cell (PFC) assay on spleen cells after sensitization with sheep red blood cells. In addition, lymphocyte subset analysis by flow cytometry was conducted in peripheral blood mononuclear cells. The positive control was cyclophosphamide.

The positive control (cyclophosphamide) showed marked effects on most parameters measured, and almost complete absence of PFCs, demonstrating the sensitivity of the model to immunomodulation. Reduced bodyweight with decreased feed intake (F) was observed at 90 mg/kg b.i.d. There were no relevant changes in lymphocyte subset analysis on peripheral blood cells and no changes in the PFC assay on spleen cells (i.e., spleen cell viability, Plaque Forming Cells per million spleen cells and per whole spleen) up to 90 mg/kg b.i.d. Mean plasma concentrations were consistent with those expected in Wistar rats after oral administration for 4 weeks at the same dosage levels.

Overall, at doses up to 90 mg/kg bid x 4 weeks, no immune functional disturbance was observed, as demonstrated by the absence of effect on the T-cell dependent primary antibody response and on lymphocyte subsets.

**NP08592: CYTOTOXICITY ASSAY IN VITRO WITH BALBIC3T3 CELLS:NEUTRAL RED (NR) TEST WITH S 16257-2 AT SIMULTANEOUS IRRADIATION WITH ARTIFICIAL SUNLIGHT**

The study information was presented in the table below (excerpted from M2/2.6.7, page 150). Cultured mouse Balb/c 3T3 fibroblasts cell line was incubated with ivabradine (8 concentrations). The positive control chlorpromazine induced phototoxicity in the

expected range after irradiation with artificial sunlight. The EC<sub>50</sub> values were calculated in the absence (200 µg/mL) and in the presence (4 µg/mL) of radiation. All acceptance criteria were met. No cytotoxic effect was observed after treatment of cells with ivabradine, either in the presence or in the absence of irradiation with artificial sunlight, indicating the absence of phototoxic potential.

Species/ strain or test system	Method of administration	Duration of treatment	Doses (µg/ml)	Gender and No. per group	Noteworthy findings
Other studies					
- Phototoxicity:					
Balb/c 3T3 fibroblasts	Neutral red uptake Test	1 h of pre-incubation (dark) + 50 min of irradiation	0.012, 0.06, 0.3, 1.5, 7.5, 37.5, 187, 937	n=6/dose	- No relevant cytotoxic effect after incubation with ivabradine, either with or without irradiation by artificial sunlight Conclusion: Absence of phototoxic potential

## 11 Integrated Summary and Safety Evaluation

Ivabradine has been developed as a heart rate (HR) lowering agent for treatment of chronic heart failure (b) (4). It was originally approved in EU for treatment of chronic stable angina pectoris in Oct. 2005 and later approved for treatment of chronic heart failure in EU.

*Hyperpolarization-activated current, general designated as  $I_f$  (funny current) in the heart (pacemaker cells) and  $I_h$  in the neurons, is a mixed  $Na^+/K^+$  inward current which is activated by hyperpolarizing steps to potentials negative to -55 mV and regulated by cAMP. The channels underlie  $I_h/I_f$  have been discovered and termed as HCN (hyperpolarization-activated cyclic nucleotide-gated) channels. In mammals, four different members of the HCN family (HCN1-4) have been cloned and heterogeneous expression of the cDNAs of HCN1-4 displays the typical features of native  $I_h/I_f$ . These four HCNs are different in tissue distribution, activation kinetics and response to cAMP. In the heart, all four isoforms have been detected with HCN4 predominant in sinus atrial node in most species (e.g. human, rabbit, dog and mouse) and other part of cardiac conducting system. [Ref. 1]*

Ivabradine is considered the first for the class of HCN channel blocker. In vitro data demonstrated that ivabradine reduced the slope of spontaneous diastolic depolarization without affecting maximal diastolic potential or threshold potential of activation. In pacemaker cells from rabbit SAN, ivabradine selectively inhibited  $I_f$  with an IC<sub>50</sub> of 1.5-3 µM in a concentration-dependent and use-dependent pattern. It also blocked the equivalent  $I_f$  in human atrial cells (where HCN2 was the major form) from patients undergoing cardiac surgery and  $I_h$  in mouse retina rods (where HCN1 was expressed) with comparable potency (IC<sub>50</sub> of 3 µM). However, the specific mechanism for current blockage by ivabradine may be different according to the type of HCN isoforms. In vivo, a dose-related heart rate reduction (HRR) was demonstrated in different species with peak effect generally reached within 3 to 4 h and lasting 8 to 12 h after oral administration in rats and dogs. The HRR by ivabradine was generally not associated with changes of mean blood pressure or negative inotropic effect, except at very high dose (10 mg/kg iv in rats). The major metabolite, S18982, was similar to ivabradine in the pharmacologic effect. The beneficial effects of ivabradine were shown by improving

cardiac function in ischemic heart failure models (rat and dog) but could not be demonstrated in two CHF models of non-ischemic origin.

In hERG assay, ivabradine and S18982 blocked  $I_{Kr}$ , with an  $IC_{50}$  of 4.85 and 15.8  $\mu\text{M}$ , respectively, i.e. a concentration >200-fold human  $C_{\text{max}}$  at MRHD with corrected protein binding. Ivabradine had no effect on  $I_{Ks}$ . In a 5-day dog telemetry study, ivabradine had no significant effect on QTc (Fridericia's correction), QT/RR-relationship (Sarma's analysis) or mean blood pressure, diastolic pressure, up to the dose associated with mean plasma  $C_{\text{max}}$  134-fold greater than in patients at MRHD. The relevant findings on other organ systems in the safety pharmacology studies will be discussed together with the target organ of toxicities in section of toxicology below.

Ivabradine was rapidly absorbed after oral administration with peak plasma concentrations achieved within 0.5 hr in the rat, and between 0.53 and 1.1 h in the dog after oral dosing. The oral bioavailability averaged at approximately 40% in rat and dog, reflecting appreciable first-pass clearance. In rats, the terminal half-life was up to 17.6 hr in males and 18.2 hr in females at 2x90 mg/kg/day over 52 weeks. In dogs, the first exponential phase half-life ( $t_{1/2,\alpha}$ ) was up to 1.39 h and the second elimination phase half-life ( $t_{1/2,\beta}$ ) was up to 22 hr based on a dose regimen of 2x20 mg/kg/day calculated from 26- and 52-week studies. For a given dose of ivabradine, the exposure of female rats is higher than male rats while there is no significant gender difference in exposure of dogs. All the metabolites observed in human were also detected in animals. The plasma exposure of ivabradine and its main primary metabolites following oral doses to animals and human was at least 2-fold higher in animals than that in human. The radioactivity levels in brain were below the LOQ after oral dose whereas the peak level of radioactivity in brain was 0.023% of dose/g brain tissue after iv dosing, although ivabradine showed high permeability in a bovine blood-brain barrier model. Ivabradine was mainly metabolized by CYP3A4.

Ivabradine has been evaluated in an extensive toxicology program which includes single dose to 52-week toxicity studies in mice, rats and dogs. The major findings with the exposure levels relative to human exposure at MRHD are listed in the table below. The primary target organs of toxicity are the heart and the eyes. Major findings in the heart and eyes and other minor findings are discussed below.

#### Heart

In rats following repeated oral doses up to 52 weeks, myocardial lesions including mucification and metaplasia in the chordae tendinae, ventricular degeneration characterized by cardiomyocyte vacuolation, contraction bands and myoblasts, fibrosis and/or necrosis, and atrial/ventricular hypertrophy, associated with increased heart weight, were observed. Although similar myocardial lesions were reported as spontaneous observations in aged rats, especially in males, the extensive lesions, particularly in the high dose animals are considered beyond background finding. The result may reflect the exacerbation of a spontaneous myocardial degeneration due to the treatment of ivabradine. The lesions were detected after 4-week exposure, got more significant through the long term studies and were not completely resolved at the end of

recovery period. The NOAEL could not be established due to the cardiac lesion. It should be noted that the cardiac findings are similar to the previously reported effects of beta blockers in rodents. Some lesions, such as endocardial necrosis and contraction bands, may also recall those of excess adrenergic stimulation. Similar lesions were not observed in dog studies.

In dogs following repeated dose up to 52 weeks, treatment related ECG findings included bradycardia, SA arrest or block, 1st and 2nd AV block and some isolated incidents of ventricular escape complexes as well as atrial or ventricular premature complexes, and a single episode of ventricular fusion complex. The ECG findings were resolved within one week of drug withdrawal. It was reported that SA block or arrest, and 1st and 2nd AV block can occur spontaneously in the beagle dog, in association with their inherently high vagal tone. Probably, in the dog studies, such effects could be exacerbated by the exaggerated pharmacodynamic effect of ivabradine at high exposure levels.

In the 52-week dog study, one female dog at 2x12 mg/kg/day was found dead on Day 341 without clinical signs preceding the death. ECG evaluation of this dog showed bradycardia with HR of 40-60 bpm without adverse effect on QTc, PR, QRS or rhythm. Gross and histopathologic examination could not reveal the cause of death. A relationship to ivabradine treatment may not be excluded.

### Eyes

Transient, reversible visual symptoms (primarily phosphene-like in nature) have been reported during clinical trials in some healthy volunteers and patients treated with ivabradine. Reversible dose-dependent ERG findings as listed in the table below were observed in dogs treated with ivabradine for 52 weeks. The ERG findings in dogs were not associated with any ophthalmologic and ultrastructure changes. Ivabradine accumulated in the eye upon repeated dosing in dogs. In isolated mouse rods, ivabradine selectively inhibits  $I_h$  ( $IC_{50}=2.7 \mu M$ ) and has no effect on other ionic currents, including  $I_{Kx}$ . In isolated mouse retina, using light as a more physiologically relevant stimuli than patch-clamp, ivabradine reduces the temporal response of the retina ( $IC_{50} = 30 \mu M$ ), which is consistent with an effect on  $I_h$ . After acute and chronic administration in both pigmented and albino rats, ivabradine decreases the temporal resolution of the ERG response and the effect is also reversible. No retinal cell morphology changes were noted in Wistar rats treated with ivabradine at 6-60 mg/kg/day for 4 weeks. Ocular distribution between pigmented and non-pigmented rats revealed high affinity of ivabradine for ocular melanin. However, the relationship between melanin binding and ERG changes was not clear as the drug levels in the eye did not correlate with the occurrence of ERG changes. The side-effect of ivabradine on visual system might be related to its pharmacologic effect on  $I_h$  at retina which, similar to  $I_f$  in the heart, is carried by HCN family.

Study	Dose mg/kg	Relevant Findings	Lowest dose with findings	Multiples of human AUC <sub>24h</sub>	
		<b>Heart</b>			
52-week rat  No NOAEL	3, 16, 90, bid	mucification, cartilaginous and/or osseous metaplasia in the chordae tendinae	16	13	36
		ventricular degeneration, fibrosis and/or necrosis	M:16, F: 3	13	7
		ventricular hypertrophy/dilation	M: 3, F: 16	3	36
		atrial or ventricular thrombosis and atrial hypertrophy/dilation	90	186	255
		Increased heart weight	3	3	7
		Mortality due to cardiac lesions	M: 16, F: 90	13	255
52-week dog	Oral 1, 3.5, 12, bid	One female unscheduled death (Day 341), cause undetermined	F: 12	n/a	41
		sinus bradycardia (<60 bpm), isolated sinoatrial block or arrest, and 2 <sup>nd</sup> degree AV blocks; HR reduction -21% to -44%	3.5	40x hC <sub>max</sub>	38x hC <sub>max</sub>
26-week Dog  No NOAEL	Oral 2, 6.3, 20, bid	sinus bradycardia (<60 bpm), isolated sinoatrial block, 1st degree and 2nd degree AV blocks;	2.0	22x hC <sub>max</sub>	15x hC <sub>max</sub>
		sinoatrial arrest, occasional atrial or ventricular premature complex,	20	120x hC <sub>max</sub>	n/a
<b>Eyes</b>					
52-week Dog  No NOAEL	Oral 1, 3.5, 12, bid	ERG cone response: a dose-dependent reduction of b-wave amplitude, and negative ERG dominated by the PIII component	1	3x hC <sub>max</sub>	3x hC <sub>max</sub>
		ERG cone response: increase in a-wave slop, ERG rod response: negative ERG and delayed appearance of b-wave in the 1 <sup>st</sup> 4 min after the dark onset	3.5	40x hC <sub>max</sub>	38x hC <sub>max</sub>
		No ophthalmoscopic changes, no pathological changes by light microscopy or TEM			
Rat, 3-week,	s.c. ~11 daily	Reduced temporal resolution in response to a sinusoidal light stimulus (acute and chronic); reversed after 1 week recovery; <i>No effect on cell morphology, HCN distribution and phototransduction</i>	11	3-5 x hC <sub>max</sub>	n/a
Mouse retinal rods	0.3 - 30 μM	Concentration- and use- dependent reduction of I <sub>h</sub> ; <i>(Concentration-dependent block of murine HCN1 with IC<sub>50</sub> of 1 μM)</i>	IC <sub>50</sub> : 2.7 μM;	41 x hC <sub>max</sub>	

#### Other noteworthy findings

In Irwin test, no adverse effects were observed up to 80 mg/kg. Moderate sedation and slight hypothermia were observed in rats given 119 or 238 mg/kg. In single- and repeated-dose studies in rats and dogs, there were indications of a dose-response relationship in type and severity of overt signs that included decreased activity, abnormal posture and behavior, tremor. Generally, the effects in the rats started to appear at mean C<sub>max</sub> levels 27 times higher than that in patients at MRHD, and at 57 times higher in dogs. Convulsions were observed in dog studies at mean C<sub>max</sub> at least

100-fold greater human  $C_{max}$  at MRHD and were only observed in single dose rat studies ( $\geq 56$  mg/kg, iv or po). The signs were usually developed within hours post dose and were transient. In rats, single oral dose of ivabradine up to 80 mg/kg was not proconvulsant.

Increases in urine volume and sodium excretion were detected in saline overloaded rats following a single oral dose of ivabradine up to 30 mg/kg. Increased water intake, urine volume and/or urinary sodium excretion were occasionally observed in rat repeated dose toxicity studies, but not in dogs. In the 26-week rat study, increase in excretion of sodium in the high dose females was associated with increase in ANP, suggesting that effects on sodium might be mediated through the release of ANP. Overall, there were no associated adverse changes in renal function and/or renal histopathology.

Slight increases in liver weight and/or enzymes (aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) without any histopathologic correlate were noted across the rodent studies at exposures  $>23$  times of human  $AUC_{24h}$  at MRHD. In mice, histopathology showed signs of hepatic congestion after 2 years dietary treatment at exposure  $\sim 40$ -fold of human  $AUC_{24h}$  at MRHD.

Genotoxicity assays and results are summarized in the table below. Ivabradine did not result in gene mutation in bacteria *in vitro* but was associated with a weak induction of unscheduled DNA synthesis in primary rat hepatocytes *ex vivo* and a weak induction of tk gene mutation in mouse lymphoma cells *in vitro*. The genotoxic responses were observed at dose concentrations  $> 15,000$  fold of human  $C_{max}$  at MRHD in these assays. The chromosomal aberration test in human lymphocytes was equivocal. *In vivo*, ivabradine did not show genotoxicity in three separate tests in mice and rats. The negative results were achieved at dosages up to 464 mg/kg (base) in mouse micronucleus test and at plasma exposures  $> 100$  fold of human  $C_{max}$  at MRHD in rat chromosome aberrations test and rat liver UDS assay. Given the uniformly negative *in vivo* results, the weak *in vitro* genotoxic responses observed at concentrations about 15,000 fold of human  $C_{max}$ , ivabradine is unlikely to pose a genotoxic risk in the proposed clinical use.

Genotoxicity Assays	Concentration range $\mu\text{g/mL}$	Results	Multiples of human $C_{max}$
Chromosomal Aberration	46*, 79, 116, 139	Equivocal -S9	1500 x
Mouse Lymphoma-1	87 to 928*, 1160	Positive-S9	2,800-30,000*
Mouse Lymphoma-2	58 to 464*, 928*, 1160*, 1392	Positive+S9/inconclusive	1,900-15,000*
UDS in rat hepatocytes	0, 55, 100, 180, 300, 400, 550*	Positive	1,800-18,000*
All other assay results are negative including: Ames test, <i>In vivo</i> mouse micronucleus test, <i>In vivo</i> chromosomal aberration and UDS assays in rats			

Ivabradine was not tumorigenic in the 104-Week carcinogenicity studies with dietary administration in CD-1 mice and Wistar rats at exposures at least 23 times of human  $AUC_{24h}$  at MRHD, as shown in the table below.

Species/ Duration	Route/Dose range mg/kg	Results <b>Carcinogenicity</b>	Highest dose tested (mg/kg)	Multiples of human AUC <sub>24h</sub>	
				M	F
Mouse 104-wk	Diet, 20-405/180	Negative: concurred by Executive CAC	405/180	23	41
Rat 104-wk	Diet, 120/60	Negative: concurred by Executive CAC	120/60	23	24

The major findings of reproductive toxicity studies are summarized in the table below.

There were no treatment-related adverse effects on male and female fertility or early stage of embryonic development in the rats treated at up to 175 mg/kg/day.

Study	Route/Dose range mg/kg	Results <b>Reproductive Toxicology</b>	Lowest dose with findings (mg/kg)	Multiples of human AUC <sub>24h</sub>	
				M	F
Rat fertility	Oral, daily 7,35, 175 14d pre-G6	Negative for male and female fertility NOAEL=175 mg/kg	175	NA	NA
Rat Embryo- Fetal, Pivotal + suppl.	Oral, daily 2.3, 4.6, 9.3, 19, GD6-15 0.5 1.5, 9.3 GD6-17	Reduced fetal weight, one fetal death with a delay in ossification and a spina bifida oculata (5th & 7th cervical vertebra )	19	-	14
		Teratogenic: interventricular septal defect and complex anomalies of primary arteries	4.6	-	3
		F1: external abnormal shape of the heart	2.3	-	2
		NOAEL= 1.5		-	1
Rabbit Embryo- Fetal	Oral daily 7, 15, 28, GD6-18	Increased post-implantation loss	7	-	5
		Reduced fetal and placental weight ectrodactylia	28	-	34
		NOAEL for embryotoxicity <7		-	5
Rat, post- natal	Oral daily 2.5-20 GD6-LD20	F1: Increased postnatal mortality (dead pups: misshapen heart, interventricular septal defect); adult: abnormal shape of the heart	20	-	15
		F1: enlarged heart	7		4

In pregnant rats treated during organogenesis, external abnormal shape of the heart (dysplasia) with or without simple anomalies of the major proximal arteries was observed at 2.3 mg/kg/day (close to human AUC<sub>24h</sub> at MRHD) and above. Teratogenic effects include interventricular septal defect and complex anomalies of the major proximal arteries observed at dosages  $\geq$  4.6 mg/kg/day (approximately 3 times human AUC<sub>24h</sub> at MRHD). In pregnant rabbits treated during organogenesis, increased postimplantation loss was observed at lowest dosage of 7 mg/kg (AUC<sub>24h</sub> of 1664

ng.h/mL, approximately 5 times of human AUC<sub>24h</sub> at MRHD) and above. Reduced fetal and placental weights and a small number of fetuses with ectrodactylia were observed at 28 mg/kg (approximately 34 times human AUC<sub>24h</sub> at MRHD). In the rat pre-postnatal study, reduced postnatal survival associated with interventricular septum defect and abnormal shape of the heart was observed in the F1 pups delivered by dams treated at 20 mg/kg (AUC<sub>24h</sub> on LD5 = 5037 ng.h/mL); enlargement of the heart in adult F1 rats was observed at dosages  $\geq$  7 mg/kg.

Ivabradine is excreted into milk in maternal rats. Ratio for ivabradine concentration in amniotic liquid/plasma was larger than 1 in pregnant rats, indicating a placenta transfer. Taken together, as ivabradine was associated with lethal cardiac teratogenicity in rats, it should not be given to women during pregnancy, particularly during the embryogenesis of the heart and period of lactation.

Overall, there are no significant nonclinical safety issues for the proposed clinical use when the labeling is revised as recommended.

#### Reference

1. Biel M, et al, *Hyperpolarization-Activated Cation Channels: From Genes to Function. Physiol Rev* 80: 847-885, 2009

## 12 Appendix/Attachments

Appendix 1: Pharmacology/Toxicology NDA Review  
Evaluation of Genetic Toxicology

Appendix 2: Pharmacology/Toxicology NDA Review  
Evaluation of Carcinogenicity

Appendix 3: Pharmacology/Toxicology NDA Review  
Evaluation of Reproductive and Developmental Toxicology

**Appendix 1**  
**Pharmacology/Toxicology NDA Review**  
**Evaluation of Genetic Toxicology**

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW  
EVALUATION (Genetic Toxicology)

Application number: 206,143  
Supporting document/s: S016 (eCTD0014), S022 (eCTD0017)  
Applicant's letter date: April 30, 2014, June 27, 2014  
CDER stamp date: April 30, 2014, June 27, 2014  
Product: Ivabradine  
Indication: reduce the risk of [REDACTED] (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure [REDACTED] (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm), [REDACTED] (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated [REDACTED] (b) (4)  
Applicant: Amgen, Inc.  
Review Division: Division of Cardio-Renal Products  
Reviewer: Jean Q. Wu  
Supervisor/Team Leader: Albert DeFelice  
Division Director: Norman Stockbridge  
Project Manager: Alexis Childers

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# 1 Executive Summary

## 2 Drug Information

### 2.1 Drug

#### 2.1.1 CAS Registry Number (Optional)

Ivabradine: 155974-00-8

Ivabradine hydrochloride: 148849-67-6

#### 2.1.2 Generic Name

ivabradine

#### 2.1.3 Code Name

S 16257-2; AMG 998

#### 2.1.4 Chemical Name

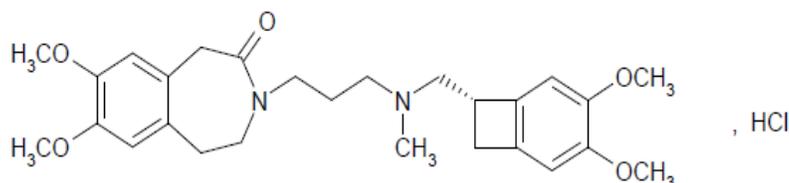
3-(3-(((7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl) methyl amino)propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride

#### 2.1.5 Molecular Formula/Molecular Weight

C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>, HCl/ 505.1 g/mol;

468.593 g/mol (base); Conversion factor from the salt to the base: 0.928

#### 2.1.6 Structure



#### 2.1.7 Pharmacologic class

hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel blocker

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND (b) (4)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

Ivabradine is provided as film-coated tablet in two strengths: 5 mg (oval) and 7.5 mg (triangular). The composition of the drug is listed in the table below (excerpted from Module 2.3. P, Table 1, Page 7)

Table 1. Composition of Ivabradine 5 mg and 7.5 mg Tablets

Component	5 mg		7.5 mg		Function	Reference to specifications
	Percentage (% w/w)	Quantity (mg/tablet)	Percentage (% w/w)	Quantity (mg/tablet)		
<b>Tablet</b>						
Ivabradine hydrochloride <sup>a</sup> (free base equivalent)	5.39 (5.00)	5.390 (5.000)	8.085 (7.50)	8.085 (7.500)	Drug substance	In-house
Lactose monohydrate	(b) (4)				(b) (4)	USP/NF, PhEur.
Maize starch	(b) (4)				(b) (4)	USP/NF
Maltodextrin	(b) (4)				(b) (4)	USP/NF, PhEur
Magnesium stearate	(b) (4)				(b) (4)	USP/NF, PhEur
Colloidal silicon dioxide (b) (4)	(b) (4)				(b) (4)	USP/NF, PhEur
	(b) (4)				(b) (4)	USP
<b>Core Tablet Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>		
<b>Film-Coating</b>						
(b) (4) salmon <sup>c</sup>	(b) (4)				(b) (4)	See 3.2.P.4.1
Polyethylene glycol 6000 (b) (4)	(b) (4)				(b) (4)	USP/NF, PhEur
	(b) (4)				(b) (4)	USP
<b>Total</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>		

<sup>a</sup> The molecular weights of Ivabradine hydrochloride anhydrous and ivabradine free base are 505.1 g/mol and 468.6 g/mol, respectively. The free base accounts for 92.77% of the salt.

(b) (4)

## 2.4 Proposed Clinical Population and Dosing Regimen

Ivabradine is indicated to reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm), (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)

The proposed starting dose of ivabradine is 5 mg twice daily. After 2 weeks of treatment, if heart rate is between 50 and 60 bpm, the dose of 5 mg twice daily should be maintained. The dose should be increased to 7.5 mg twice daily if resting heart rate is persistently above 60 bpm.

As listed in the table below (excerpted from Module 2, Section 2.4, Page 7), the human plasma exposures of ivabradine and its N-desmethylated metabolite (S 18982) were estimated at steady state in patients receiving the maximum recommended human dose (MRHD), 7.5 mg bid. These values were derived from a population pharmacokinetic analysis using Phases II and III clinical data [Summary of Clinical Pharmacology Studies, in Section 2.7.2 (2.1.2.2)], and are accepted as references when preclinical doses are expressed as multiples of human exposures in current review, unless otherwise indicated.

**Table 1. Ivabradine and S 18982 (Metabolite) Plasma C<sub>max</sub> and Estimated AUC<sub>24</sub> at Steady State in Patients at HTD**

	Ivabradine (n=492)	S 18982 (n=541)
Population C <sub>max</sub> (ng/ml)	31 ± 9.8	7.9 ± 2.3
Equivalent C <sub>max</sub> in μM	0.07	0.02 <sup>b</sup>
Population AUC <sub>24</sub> <sup>a</sup> (ng.h/ml)	346	128

Values are mean ± SD;

a: Calculated from AUC over 12 h period from [WS (2.7.2) 3.1/ Table 12; Table 34 and 35]

b: S 18982 MW = 454.6

## 2.5 Regulatory Background

A pre-NDA meeting was held between the sponsor and the Division on January 23, 2014. There was no IND opened in the US FDA [REDACTED] (b) (4)

## 3 Studies Submitted

### 3.1 Studies Reviewed

#### In Vitro

NP03142: Genotoxicity Study of S 16257-02: In Vitro Test for Gene Mutation on Salmonella Typhimurium in The Histidine Reversion System on Escherichia Coli in The Tryptophan Reversion System (Ames Test)

NP05576: In Vitro Genetic Toxicology Study For The Detection Of Chromosome Aberrations On Cultured Human Lymphocytes

NP05144: Mutation Assay At The TK Locus In L5178Y Mouse Lymphoma Cells Using A Microtiter Cloning Technique (Trifluorothymidine Resistance) With S 16257-2

NP05489: S 16257-2: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells using the Microtitre® Fluctuation Technique

NP05912: Genotoxicity Study With S 16257-2: Measurement of Unscheduled DNA Synthesis in Isolated Rat Hepatocytes

In Vivo

NP03141: Mutagenicity Study Using the Micronucleus Test In Mice With S 16257-02

NP05409: S16257: Measurement of Unscheduled DNA Synthesis in Rat Liver using an In Vivo/In Vitro Procedure

NP05504: S 16257-2: Induction of Chromosome Aberrations in the Bone Marrow of Treated Rats

## 4 Pharmacology

## 5 Pharmacokinetics/ADME/Toxicokinetics

## 6 General Toxicology

## 7 Genetic Toxicology

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

**Study title: Genotoxicity Study of S 16257-02: In Vitro Test for Gene Mutation on Salmonella Typhimurium in The Histidine Reversion System on Escherichia Coli in The Tryptophan Reversion System (Ames Test)**

Study no.: NP03142

Study report location: M4/4.2.3.3.1

Conducting laboratory and location: (b) (4)

Date of study initiation: April 26, 1993

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: S 16257-02, Batch# EF558, purity=99.8%

### Key Study Findings

The results did not show mutagenic activity of S 16257-02 under the experimental conditions.

Methods

Strains: TA100, TA1535, TA1537, TA98, WP2 uvrA, WP2 uvrA(pKM101)

Concentrations in definitive study: See tables below for the first test and second test.

**First test**

Group identification code	D0	D1	D2	D3	D4	D5	D+
Compound administered	-	S 16257-02					①
Vehicle	Water for injection						
Dose ( $\mu\text{g}/\text{plate}$ )	0	50	150	500	1500	5000	
Volume per plate ( <i>ml</i> )	0.1						
Without metabolic activation	0.5 ml of S9 mix phosphate buffer/plate						
With metabolic activation	0.5 ml of S9 mix/plate						

**Second test**

Group identification code	D0	D1	D2	D3	D4	D5	D+
Compound administered	-	S 16257-02					①
Vehicle	Water for injection						
Dose ( $\mu\text{g}/\text{plate}$ )	0	500	1000	1500	2500	5000	
Volume per plate ( <i>ml</i> )	0.1						
Without metabolic activation	0.5 ml of S9 mix phosphate buffer/plate						
With metabolic activation	0.5 ml of S9 mix/plate						

**Basis of concentration selection:**

Preliminary toxicity with or without metabolic activation was tested at 0, 5, 15, 50, 150, 500, 1500 and 5000  $\mu\text{g}/\text{plate}$ . Microscopic examination of the density and uniformity of this bacterial lawn indicates the degree of toxicity to bacterial growth. Toxicity may also be reflected by a reduction in the size or number of revertant colonies. The narrative of the report did not discuss the toxicity with the preliminary test result. The tabulated study report in the appendix stated no cytotoxic effects. The concentrations up to 5000  $\mu\text{g}/\text{plate}$  were selected in the definitive assays.

Negative control: vehicle, see the table of "acceptance limits for negative control"

Positive control: See the table below.

Strain	Without metabolic activation	With metabolic activation
TA100	sodium azide 8 µg/plate in physiological solution	2-anthramine 2.5 µg/plate in DMSO
TA1535		
TA1537		
TA98	4-nitroquinoline N-oxide 0.5 µg/plate in DMSO	2-anthramine 10 µg/plate in DMSO
WP2 <i>uvrA</i>	4-nitroquinoline N-oxide 2 µg/plate in DMSO	
WP2 <i>uvrA</i> ( <i>pKM101</i> )	4-nitroquinoline N-oxide 0.5 µg/plate in DMSO	2-anthramine 2.5 µg/plate in DMSO

Formulation/Vehicle: stock solution, 50 mg/mL/Water for injection

Incubation & sampling time:

The day before treatment, samples of each strain stored in liquid nitrogen were inoculated into Oxoid nutrient culture medium. Plates were analyzed after 3 days' (72 hours) incubation at 37°C. The number of revertant colonies was counted visually or using an electronic counter (*Artek 880*).

### Study Validity

The spontaneously revertant colony counts in the mutagenicity test negative controls were within the acceptance limits for each strain. The acceptance limits for negative control without metabolic activation was shown in the table below. After metabolic activation, a 15% deviation from these limits was tolerated.

Strain	Acceptance limits for negative control
TA100	150 - 250
TA1535	5 - 25
TA1537	3 - 15
TA98	20 - 55
WP2 <i>uvrA</i>	20 - 50
WP2 <i>uvrA</i> ( <i>pKM101</i> )	40 - 100

Each positive control group demonstrated the sensitivity of the test: the numbers of revertant colonies induced by the positive control agents were significantly higher than the spontaneous reversion values.

## Results

The summary of assay results with and without metabolic activation is listed in the tables below (excerpted from pages 41 and 42 of the report).

Small but statistically significant increase in the number of revertant colonies (highlighted) in the presence of S9 were observed at 500 µg/plate against WP2 *uvrA* (pKM101) and TA98 in the 1<sup>st</sup> assay, and at 5000 µg/plate against TA98 in the 2<sup>nd</sup> assay. A minimal but marginally statistical significant ( $p=0.049$ ) increase in the number of revertant colonies in the absence of S9 was observed at 2500 µg/plate against WP2 *uvrA* (pKM101) in the 2<sup>nd</sup> assay only.

The increase in the number of revertant colonies reached 1.7-fold of its control value at 500 µg/plate against WP2 *uvrA* (pKM101) in the presence of S9, whereas the other increases were about 1.2-fold of their respective control values. These increases were neither appreciable, nor exposure-dependent or reproducible, hence, were not considered biologically significant.

The results can be interpreted as negative for any unequivocal mutagenic activity of S 16257-02 under the experimental conditions.

## Genetic assays without metabolic activation

REVERTANT COLONIES COUNT		GROUP						
		D0	D1	D2	D3	D4	D5	D+
DOSE ( $\mu\text{g}/\text{plate}$ )	ASSAY							
	1	0.0	50.0	150.0	500.0	1500.0	5000.0	*
	2	0.0	500.0	1000.0	1500.0	2500.0	5000.0	*
STRAIN	ASSAY							
	1	150.7	153.3	214.7	202.3	163.3	177.3	719.0
	2	179.0	189.3	209.3	216.7	202.3	189.3	612.7
TA 1535	1	22.0	20.7	18.7	22.7	29.0	21.0	941.0
	2	17.0	15.0	18.0	16.3	18.0	22.0	1016.7
TA 1537	1	8.7	10.7	6.3	9.3	8.0	10.3	666.3
	2	8.7	8.3	8.0	6.3	10.7	6.3	725.0
TA 98	1	32.0	26.3	32.3	31.7	28.0	24.3	280.7
	2	48.7	46.7	46.3	45.0	50.0	57.7	397.3
WP2 uvr A	1	37.0	40.7	40.3	44.0	44.3	46.0	857.7
	2	32.7	39.7	39.7	33.7	38.0	37.7	864.0
WP2 uvr A (pKM 101)	1	99.3	100.0	94.0	81.7	103.3	83.3	878.3
	2	43.3	35.0	37.0	50.0	52.3	43.3	584.7

D0: negative control; D+: positive control; D1, D2, D3, D4 and D5: treated dose groups;  
 For WP2 uvrA (pKM101) in absence of S9, p value for assay 2 = 0.049 (the highest number at  
 2500  $\mu\text{g}/\text{plate}$  was approximately 1.2-fold of the control value)

## Genetic assays with metabolic activation

REVERTANT COLONIES COUNT		GROUP						
		D0	D1	D2	D3	D4	D5	D+
DOSE (µg/plate)	ASSAY							
	1	0.0	50.0	150.0	500.0	1500.0	5000.0	*
	2	0.0	500.0	1000.0	1500.0	2500.0	5000.0	*
STRAIN	ASSAY							
TA 100	1	253.3	238.7	255.7	242.7	254.7	268.7	1521.0
	2	231.7	234.3	242.7	247.0	229.3	258.3	2265.3
TA 1535	1	21.7	22.7	24.3	21.3	19.7	19.0	126.7
	2	20.3	23.7	28.3	32.7	28.3	27.3	158.0
TA 1537	1	18.0	17.3	14.7	17.3	17.7	20.7	287.3
	2	14.0	17.0	16.3	19.0	18.3	15.0	212.3
TA 98	1	50.3	42.7	57.0	60.0	56.7	57.3	1183.0
	2	58.0	63.3	66.3	69.3	60.3	71.3	1284.0
WP2 uvr A	1	46.7	50.0	52.7	50.0	40.7	40.3	643.0
	2	52.3	47.7	52.0	53.0	52.3	55.3	716.3
WP2 uvr A (pKM 101)	1	55.7	57.7	68.7	95.3	78.0	56.3	801.3
	2	114.7	96.3	99.0	87.7	108.3	82.0	854.0

D0: negative control; D+: positive control; D1, D2, D3, D4 and D5: treated dose groups;  
 For TA98, p value for assay 1 = 0.007, p value for assay 2 = 0.039;  
 For WP2 uvrA (pKM101), P value for assay 1 = 0.005.

## 7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

### Study title: *In Vitro* Genetic Toxicology Study For The Detection Of Chromosome Aberrations On Cultured Human Lymphocytes

Study no.: NP05576  
Study report location: M4/4.2.3.3.1  
Conducting laboratory and location: (b) (4)  
Date of study initiation: Sept. 6, 1994  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: S 16257-2, Batch # EG418, purity=101.3%

### Key Study Findings

Increase in aberration frequency and in number of breaks was observed at concentration of 50 µg/mL in the absence of S9 in both 1<sup>st</sup> and 2<sup>nd</sup> assays. However, the lack of exposure-related- activity made the results equivocal although the potential weak clastogenicity of ivabradine hydrochloride may not be excluded.

In the presence of S9, the 1<sup>st</sup> assay showed a small but significant increase in aberration frequency at 950 µg/mL (male donor) and pulverized chromosomes at 525 µg/mL (male donor). However, the finding was not exposure -dependent and reproducible (in the 2<sup>nd</sup> and 3<sup>rd</sup> assays), suggesting an overall negative result.

### Methods

Cell line: Human Lymphocytes (whole blood), replicate blood cultures were used (one derived from a male donor and one from a female donor).

Concentrations in definitive study:

- 1<sup>st</sup> assay: 50, 85, 125, 150 µg/mL without metabolic activation.  
280, 525, 950, 1800 µg/mL with metabolic activation.
- 2<sup>nd</sup> assay: 50, 85, 125 µg/mL without metabolic activation,  
525, 950, 1800, 2250 µg/mL with metabolic activation.
- 3<sup>rd</sup> assay: 525, 950, 1800 µg/mL with metabolic activation.

Basis of concentration selection:

The exposure levels were selected on the basis of the mitotic index reduction observed in the 1<sup>st</sup> assay.

Without metabolic activation (-S9):

The mitotic index reduction reached -52 % and -58% of the negative control in the 1<sup>st</sup> and the 2<sup>nd</sup> assay, respectively, at 125 µg/mL, which was the highest level chosen for metaphase analysis.

With metabolic activation (+S9):

At 1800 µg/mL, the highest level of the 1st assay, the mitotic index (MI) reduction was -37%. However, in the 2nd assay, the MI reduction was -52% at the same concentration and -66% at 2250 µg/mL. The highest exposure chosen for the metaphase analysis was 1800 µg/mL.

Negative control: vehicle

Positive control:

a) Bleomycin: 250 µg /mL (vehicle = saline)

b) Cyclophosphamide (vehicle = saline):

17.5 µg/mL (harvest at 52 hours); 12.5 µg/ml (harvest at 46 hours)

Formulation/Vehicle: S 16257-2 was dissolved in the treatment medium from 0.056 to 2.5 mg/mL, just prior to use. Vehicle=treatment medium

Incubation & sampling time:

a) 24 h treatment (-S9, 1<sup>st</sup> and 2<sup>nd</sup> assay)

Recovery Time: 4 h

Sampling time: 52 hours after stimulation

b) 2 h treatment (+S9, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> assay)

Recovery time: 24 h (1<sup>st</sup> assay) and 18 h (2<sup>nd</sup> assay)

Sampling time: 52 hours after stimulation (1<sup>st</sup> and 3<sup>rd</sup> assay); 46 hours after stimulation for the 2<sup>nd</sup> assay

For the 1<sup>st</sup> assay, the treatment is started 24 hours (26 hours with metabolic activation) after mitogenic stimulation of lymphocytes.

The second assay consists of the repetition of the first assay, at a sampling time of 46 hours with metabolic activation, with a readjusted range of concentrations selected on the basis of the results of the first assay.

The third assay was the reiteration of the 52 hours sampling time with metabolic activation at three exposures.

## Study Validity

The acceptance criteria:

- The mean number of abnormal metaphases should be less than 5%.
- The mean number of abnormal metaphases in the positive control groups must be statistically significantly increased over the negative control group.
- A minimum of three exploitable concentrations is required to analyze the results.

A combination of different criteria used to evaluate a positive response:

- a statistical increase in one group over the negative control group.
- a concentration-response effect.
- an unusual number of aberrations, especially exchanges, even in the absence of a statistically significant increase.
- the reproducibility of the above criteria.

All the acceptability criteria were met except that the cells from the female donor group of the 1<sup>st</sup> assay, when exposed to 17.5 µg/mL of cyclophosphamide, failed to show a statistically significant increase in the number of abnormal metaphases. However, the number of abnormal metaphases and the number of primary breaks were over the usual negative control range and clearly over the negative control of the assay. For these reasons, and with respect to the results obtained in the treated groups, the assay was considered acceptable.

## Results

The results are summarized in the table below excepted from pages 43-44 of the report. In the absence of S9, the 1<sup>st</sup> assay showed a total of 5 structural aberrations found in the male-donor cell culture at 50 µg/mL and the 2<sup>nd</sup> assay showed 6 structural aberrations found at the same exposure in the culture from the female donor. An increased number of breaks was observed in both cases. However, the increase in aberration frequency was not exposure-dependent (i.e. not observed at the two higher exposures).

Dose (µg/ml)	WITHOUT METABOLIC ACTIVATION									
	ASSAY 1 Harvest time : 52 hours					ASSAY 2 Harvest time : 52 hours				
	Group	Number of primary breaks		Number of abnormal metaphase		Group	Number of primary breaks		Number of abnormal metaphase	
		①		①②			①		①②	
	M	F	M	F		M	F	M	F	
0	D(-)	2	3	2	2	D(-)	2	0	2	0
50	D1	8	2	5	1	D1	2	9	1	6
85	D2	0	3	0	2	D2	3	4	3	4
125	D3	0	5	0	3	D3	2	2	2	2
250	D(+) <i>Bleomycin</i>	108	79	37	27	D(+) <i>Bleomycin</i>	46	48	25	21

① Number for 100 metaphase plates, unless otherwise indicated.

② Without achromatic lesions.

Dose (µg/ml)	WITH METABOLIC ACTIVATION									
	ASSAY 1 Harvest time : 52 hours					ASSAY 2 Harvest time : 46 hours				
	Group	Number of primary breaks ①		Number of abnormal metaphase ①②		Group	Number of primary breaks ①		Number of abnormal metaphase ①②	
		M	F	M	F		M	F	M	F
0	D(-)°	0	2	0	2	D(-)°	2	0	2	0
525	D2°	23/96	6/104	3/96	3/104	D1°	0	0	0	0
950	D3°	5	2	5	2	D2°	4	1	4	1
1800	D4°	1/142	4/58	1/142	3/58	D3°	2	2	2	1
17.5 12.5	D(+) Cyclophos- phamide	15	9	14	7	D(+) Cyclophos- phamide	12	12	11	12

① Number for 100 metaphase plates, unless otherwise indicated.

② Without achromatic lesions.

Dose (µg/ml)	WITH METABOLIC ACTIVATION					
	ASSAY 3 Harvest time : 52 hours					
	Group	Number of primary breaks ①		Number of abnormal metaphase ①②		
		M	F	M	F	
0	D(-)°	0	1	0	1	
525	D1°	4	2	3	2	
950	D2°	4	5	4	4	
1800	D3°	1	0	1	0	
17.5	D(+) Cyclophos- phamide	23	19	11	15	

In the presence of S9, the 1<sup>st</sup> assay showed a small but significant increase in aberration frequency at 950 µg/mL and pulverized chromosomes at 525 µg/mL for the culture from the male donor. However, the findings were not exposure-related and could not be reproduced in the 2<sup>nd</sup> and 3<sup>rd</sup> assays.

In conclusion, due to the reproducible positive finding at 50 µg/mL in the absence of S9, the potential weak clastogenicity of ivabradine hydrochloride may not be excluded though the lack of an exposure relationship could make the results equivocal.

### 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

**Study title: Mutagenicity Study Using the Micronucleus Test In Mice With S 16257-02**

Study no.: NP03141  
Study report location: M4/4.2.3.3.2  
Conducting laboratory and location:  (b) (4)  
Date of study initiation: May 17, 1993  
GLP compliance: Yes  
QA statement: Included  
Drug, lot #, and % purity: S 16257-02 Hydrochloride, EF 558  
Purity=99.8%

**Key Study Findings**

Single dose of S16257-02 up to 500 mg/kg (464 base mg/kg) did not induce micronuclei in the polychromatic erythrocytes (PCEs) of mouse bone marrow under study conditions.

**Methods**

Doses in definitive study: 0 (negative control), 125, 250, 500 mg/kg  
equivalent base dosages: 0, 116, 232, 464 mg/kg  
Frequency of dosing: Single dose  
Route of administration: Oral  
Dose volume: 25 mL/kg  
Formulation/Vehicle: Solution in distilled water/water  
Species/Strain: Mouse/ OF1 IFFA CREDO (male and female)  
Number/Sex/Group: 5/sex/group  
Satellite groups: n/a  
Basis of dose selection: Preliminary toxicity test (n=2 male/group) showed 100% mortality after a single oral dosage of 1250 mg/kg. Confirmatory toxicity test (n=5/sex/group) showed 30% mortality at 24 hours following a single dosage of 800 mg/kg by oral intubation in mice. The dosage of 500 mg/kg which provoked no death was considered as the maximum tolerated dose (MTD). Under these conditions, the following dosages were selected for the definitive micronucleus test: 500 mg/kg (MTD), 250 mg/kg (1/2MTD), 125 mg/kg (1/4MTD).  
Negative control: Vehicle  
Positive control: Cyclophosphamide 50 mg/kg, i.p.

## Study Validity

The mean number of micronuclei observed in the negative control animals must be close to that of the historical values for control animals.

The mean number of micronuclei observed in the positive control animals treated with cyclophosphamide must show a statistically significant difference from the negative control animals.

If deaths observed at the test dosage, the mortality rate must be less than 20 % per time. The dead animals are replaced by those in parallel treatment.

The sampling time: 24 and 48 hours after treatment

Micronuclei were scored in 2 x 1000 PCEs for each animal.

The PCE:NCE (normochromatic erythrocytes) ratio were determined in 1000 cells to assess bone-marrow toxicity.

The study criteria were met. Historical controls were provided. The study is considered valid.

## Results

The mean number of micronuclei for 1000 PCEs was summarized in the tables below (excerpted from pages 36 and 37 in the report). There were no statistically significant increases in the number of PCEs with micronuclei in the treated groups when compared to the control. There was no difference in the PCE:NCE ratio between groups.

In conclusion, single dose of S16257-02 up to 500 mg/kg (464 base mg/kg) did not induce micronuclei in the PCEs of mouse bone marrow under study conditions.

SAMPLING TIME 24 HOURS	GROUP	SEX	MICRONUCLEI MEAN FOR 1000 PCE	Standard error	MIN	MAX	Mann-Whitney U rank test		p
							UA	UB	
NEGATIVE CONTROL	SOLVENT	♂	0.9	0.22	0.5	1			
		♀	0.9	0.89	0	2			
		♂ + ♀	0.9	0.61	0	2			
POSITIVE CONTROL	CYCLOPHOS- PHAMIDE 50 mg/kg  ROUTE : I.P.	♂	28.3	8.11	18	39.5	0	25	=0.01
		♀	20.5	7.57	13	33	0	25	=0.01
		♂ + ♀	24.4	8.46	13	39.5	0	100	<0.01
COMPOUND STUDIED	HIGH DOSE 500 mg/kg	♂	0.9	0.96	0	2.5	9	16	N.S.
		♀	0.4	0.42	0	1	8.5	16.5	N.S.
		♂ + ♀	0.65	0.75	0	2.5	34	66	N.S.
	MIDDLE DOSE 250 mg/kg	M	0.5	0.35	0	1	4.5	20.5	N.S.
		♀	1.8	0.57	1	2.5	5	20	N.S.
		♂ + ♀	1.15	0.82	0	2.5	43	57	N.S.
	LOW DOSE 125 mg/kg	♂	1	0.35	0.5	1.5	10.5	14.5	N.S.
		♀	1.1	0.89	0	2	10.5	14.5	N.S.
		♂ + ♀	1.05	0.64	0	2	44.5	55.5	N.S.

N.S. = non-significant at the threshold of p = 0.05

SAMPLING TIME 48 HOURS	GROUP	SEX	MICRONUCLEI MEAN FOR 1000 PCE	Standard error	MIN	MAX	Mann-Whitney U rank test		p
							UA	UB	
NEGATIVE CONTROL	SOLVENT	♂	1.1	0.65	0.5	2			
		♀	0.9	0.55	0	1.5			
		♂ + ♀	1	0.58	0	2			
COMPOUND STUDIED	HIGH DOSE 500 mg/kg	♂	0.8	0.57	0	1.5	9.5	15.5	N.S.
		♀	0.5	0	0.5	0.5	5	20	N.S.
		♂ + ♀	0.65	0.41	0	1.5	30.5	69.5	N.S.
	MIDDLE DOSE 250 mg/kg	♂	0.5	0.5	0	1	6	19	N.S.
		♀	0.4	0.22	0	0.5	4.5	20.5	N.S.
		♂ + ♀	0.45	0.37	0	1	21.5	78.5	<0.05
	LOW DOSE 125 mg/kg	♂	0.4	0.42	0	1	4.5	20.5	N.S.
		♀	0.5	0.5	0	1	7	18	N.S.
		♂ + ♀	0.45	0.44	0	1	23	77	= 0.05

N.S. = non-significant at the threshold of  $p = 0.05$

## 7.4 Other Genetic Toxicity Studies

### Study title: Mutation Assay At The TK Locus In L5178Y Mouse Lymphoma Cells Using A Microtiter Cloning Technique (Trifluorothymidine Resistance) With S 16257-2

Study no.

NP05144

Study report location:

M4/4.2.3.3.1

Conducting laboratory and location:

(b) (4)

Date of study initiation:

Aug 3, 1994

GLP compliance:

Yes

QA statement:

Yes

Drug, lot #, and % purity:

S 16257-2, Batch # 46762, 100.4%

### Key Study Findings

The result showed a weak mutagenicity of S16257-2 in the absence of S9 at concentration of 1000 µg/mL and a negative mutagenic activity in the presence of S9 under study conditions.

### Methods

Cell line: L5178Y (TK+/-) Mouse lymphoma cells

## Concentrations in definitive study:

Assay 1: 750, 375, 187.5 and 93.8 µg/mL without S9;  
1500, 750, 375, 187.5 and 93.8 µg/mL with S9  
Assay 2: 1000, 750, 375, 187.5 and 93.8 µg/mL without S9;  
1500, 750, 375, 187.5 and 93.8 µg/mL with S9;  
Assay 3: 1250, 1000, 800, 640 and 512 µg/mL without S9  
1750, 1500, 1250, 1000, and 750 µg/mL with S9;

## Basis of concentration selection:

The concentration selection was based on the cytotoxicity assay, in which concentrations of 0, 100, 300, 1000, 3000 and 5000 µg/mL were tested. The percent relative survival (%RS), and percent relative total growth (%RTG) were shown in the tables below.

Concentration (µg/mL)		0	100	300	1000	3000	5000
-S9	%RS	100	70.1	61.3	40.0	1.3	0.8
	%RTG	100	53.2	38.8	13.7	0	0
+S9	%RS	100	65.1	66.2	36.6	1.4	0
	%RTG	100	49.8	48.0	13.4	0	0

In the absence of S9, a high level of cytotoxicity (0.8 to 1.3% RS and 0% RTG) was observed at two highest concentrations (5000 and 3000 µg/mL). The intermediate concentration of 1000 µg/mL at which %RTG was 13.7% was chosen as the highest concentration for the definitive assay. However, due to a strong cytotoxicity in the definitive assay, the eventual concentrations retained for the first mutagenicity assay in the absence of S9 were 750, 375, 187.5 and 93.8 µg/mL.

In the presence of S9, a strong cytotoxicity (0 to 1.4% RS and 0% RTG) was observed at two highest concentrations. As the intermediate concentration of 1000 µg/mL provoked 36.6%RS and 13.4% RTG, the concentrations for the definitive assay with S9 were 1500 µg/mL (supposed having 10% RS), 750, 375, 187.5 and 93.8 µg/mL.

Negative control: vehicle

## Positive control:

In the absence of S9, Methyl Methanesulfonate (MMS), culture medium (Fischer) 0, 10 µg/mL;

In the presence of S9, Cyclophosphamide (CPA): culture medium 0, 2 µg/mL.

Formulation/Vehicle: S 16257-2 was dissolved in culture medium at 50 mg/mL.

Vehicle = culture medium.

## Exposure condition:

Cell cultures: duplicates except for positive control;

Treatment: 3 hours in the absence or the presence of S9

Expression period (recovery): 48 hours in the absence or the presence of S9

Plating for viability: 10-12 days.

### Study Validity

The sponsor's criteria for a valid assay:

1. the plating efficiency (PE) of the negative control is higher than 50% at T0 and T2 (Time 0 and 2 day after treatment).
2. the mutation frequency of the negative control is within a range of historical data of the Laboratory
3. the mutation frequency of the positive control is significantly increased compared with the solvent. The observed values must be close to those of historical positive controls.

The criteria were met and the study is considered valid. Historical data was provided in Table 1 of the report).

Criteria defined for a compound which was considered as mutagenic in the assays:

1. A multiplication by TWO of the number of spontaneous mutants has to be provoked by at least one dose.
2. A concentration/effect relationship that is an increase in the number of mutants with the exposure, must be observed, and does not necessarily imply a proportional increase (in the case of positive responses, a decrease in the number of mutants at the highest concentration, is frequently observed linked with cytotoxic effects).
3. The results have to be reproducible in an independent study, at least from a qualitative point of view.

### Results

The results from three assays are shown in the tables below (excerpted from Table 21, and Table 22 of study report, pages 36 and 37).

In the absence of S9, the 1st assay did not show any significant increase in the number of induced mutants. A slight increase (~1.6 fold) was observed at the highest concentration tested of 750 µg/mL. In the 2<sup>nd</sup> and 3<sup>rd</sup> assays, a significant increase (2.2 or 2.1 folds) in the number of mutants was observed at concentration of 1000 µg/mL (highest concentration tested in the 2<sup>nd</sup> assay) at which no strong cytotoxicity was observed. The highest concentration tested in the 3<sup>rd</sup> assay (1250 µg/mL) resulted in significant cytotoxicity (5% RS), hence, the marked increase in the number of mutants (3.8 folds) at this concentration should be taken into account with precaution for mutagenic activity. Under these conditions, the result showed that S16257-2 had a slight mutagenic activity at concentration of 1000 µg/mL.

In the presence of S9, no significant increase in the number of mutants was observed at all the concentrations tested in the 1<sup>st</sup> and the 3<sup>rd</sup> assays. A significant increase (2.6 folds) in the number of mutants noted at the highest concentration, 1500 µg/mL, in the

2<sup>nd</sup> assay was not reproducible in the 3<sup>rd</sup> assay at same and higher concentrations. Hence, there was no consistent evidence for mutagenicity of S16257-2 in the presence of S9 under study conditions.

In conclusion, the result showed weak mutagenicity of S16257-2 in the absence of S9 at concentration of 1000 µg/mL and a negative mutagenic activity in the presence of S9 under study conditions.

**RECAPITULATION OF THREE ASSAYS  
WITHOUT METABOLIC ACTIVATION**

ASSAY 1	TEST COMPOUND					MMS 10µg/ml
	DOSE µg/ml					
	0	93.8	187.5	375	750	
SURVIVAL RATE	100.0	91.5	84.4	82.5	61.2	102.8
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	91.8	64.2	86.9	106.2	144.0	718.2
<i>Ratio</i>		0.7	0.9	1.2	1.6	7.8

ASSAY 2	TEST COMPOUND						MMS 10µg/ml
	DOSE µg/ml						
	0	93.8	187.5	375	750	1000	
SURVIVAL RATE	100.0	108.8	98.7	86.7	73.0	70.6	96.7
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	68.7	86.6	81.7	96.0	75.1	152.4	678.4
<i>Ratio</i>		1.3	1.2	1.4	1.1	2.2	9.9

ASSAY 3	TEST COMPOUND						MMS 10µg/ml
	DOSE µg/ml						
	0	512	640	800	1000	1250	
SURVIVAL RATE	100.0	101.0	66.3	66.7	56.0	5.0	98.7
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	73.5	64.9	90.5	78.7	151.5	277.5	695.0
<i>Ratio</i>		0.9	1.2	1.1	2.1	3.8	9.5

**RECAPITULATION OF THREE ASSAYS  
WITH METABOLIC ACTIVATION**

ASSAY 1	TEST COMPOUND						CPA 2µg/ml
	DOSE µg/ml						
	0	93.8	187.5	375	750	1500	
SURVIVAL RATE	100.0	126.7	104.5	108.3	96.1	53.7	81.3
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	104.5	138.3	135.5	117.2	109.1	128.4	733.2
<i>Ratio</i>		1.3	1.3	1.1	1.0	1.2	7.0

ASSAY 2	TEST COMPOUND					CPA 2µg/ml
	DOSE µg/ml					
	0	187.5	375	750	1500	
SURVIVAL RATE	100.0	120.9	90.3	77.0	35.4	74.5
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	97.1	113.3	118.2	138.5	254.0	743.1
<i>Ratio</i>		1.2	1.2	1.4	2.6	7.7

ASSAY 3	TEST COMPOUND						CPA 2µg/ml
	DOSE µg/ml						
	0	750	1000	1250	1500	1750	
SURVIVAL RATE	100.0	96.2	91.4	79.6	64.9	40.1	79.0
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	100.2	90.9	106.3	102.0	112.5	120.3	922.7
<i>Ratio</i>		0.9	1.1	1.0	1.1	1.2	9.2

**Study title: S 16257-2: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells using the Microtitre® Fluctuation Technique**

Study no.

NP05489

Study report location:

M4/4.2.3.3.1

Conducting laboratory and location:

(b) (4)

Date of study initiation:

January 30, 1995

GLP compliance:

Yes

QA statement:

Yes

Drug, lot #, and % purity:

S 16257-2, Batch # 46762, 100.4%

**Key Study Findings**

Overall, three experiments suggested a negative result in the absence of S9 and weak mutagenicity in the presence of S9. The result was different from another mouse

lymphoma assay (NP05144). The large variability in cytotoxicity among three assays may affect the reproducibility of the assay results, which made the study rather inconclusive.

### Methods

Cell line: L5178Y (tk) Mouse lymphoma cells

Concentrations in definitive study:

Experiment 1: 125 -1250 µg/mL without S9 and 250-1500 µg/mL with S9;

Experiment 2: 500 -2000 µg/mL without S9 and 500-1500 µg/mL with S9;

Experiment 3: 125 -2000 µg/mL without S9 and 62.5-1500 µg/mL with S9.

Basis of concentration selection:

The concentration selection was based on the cytotoxicity in the range-finder assay, in which 6 concentrations were tested. The raw plate counts and relative survival values are shown in the table below (excerpted from page 20 of the report). The highest survival concentrations were 625 µg/mL in the absence of S9 with 58.7% relative survival (%RS), and **1250** µg/mL in the presence of S9 with 10.1% relative survival. The definitive Experiment 1 was tested concentrations up to 1250 µg/mL in the absence of S9 and up to 1500 µg/mL in the presence of S9.

Based on the results of Experiment 1, the test concentrations in Experiment 2 were modified to ensure they were up to limits of toxicity. Concentrations up to 2000 µg/mL in the absence of S9 and 1500 µg/mL in the presence of S9 were tested. As shown in the tables of the result section, the top two concentrations in the absence and presence of S9 were later rejected from analysis due to excessive toxicity (%RS <10%). It was noted that more toxicity was observed in Experiment 2 than may have been expected from the results of Experiment 1.

In view of the results from Experiments 1 and 2, concentrations in Experiment 3 were extended and eight doses were tested ranging 125 - 2000 µg/mL in the absence of S9 and from 62.5 -1500 µg/mL in the presence of S9. The top concentrations selected yielded 31.8% and 37.8% RS in the absence and presence of S9, respectively.

Treatment µg/mL	In the absence of S-9		In the presence of S-9	
	Survival <sup>1</sup> at day 0*	% Relative survival	Survival <sup>1</sup> at day 0*	% Relative survival
0	20	100.0	26	100.0
156.25	23	129.3	22	69.5
312.5	18	84.3	22	69.5
625	14	58.7	16	41.4
1250	0	0.0	5	10.1
2500	0	0.0	0	0.0
5000	0	0.0	0	0.0

\*= 32 wells scored

<sup>1</sup>= 1.6 cells/well plated

Negative control: vehicle

Positive control: prepared in dimethyl sulphoxide (DMSO)

In the absence of S9, 4-nitroquinoline 1-oxide (4-NQO): 0.05 and 0.1 µg/mL

In the presence of S9, benzo(a)pyrene (BaP): 2 and 3 µg/mL

Formulation/Vehicle: S 16257-2 was dissolved in water at concentrations up to 2000 µg/mL. Vehicle = purified water.

Exposure condition:

Cell cultures: duplicates except for positive control;

Treatment: 3 hours in the absence or the presence of S9

Expression period: 2 days for Experiments 1 and 2; 3 days for Experiment 3.

Plating for viability: 10-12 days without 5-trifluorothymidine (TFT); 12-14 days with TFT.

### Study Validity

The sponsor's criteria for a valid assay:

1) the mutant frequencies in the negative (vehicle) control cultures fell within the normal range (not more than three times the historical mean value);

2) at least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value);

3) the % RS following treatment was higher than 10%. Dose levels yielding less than 10% relative survival were eliminated from further calculations.

The criteria for a valid study were met.

Criteria defined for a compound which was considered mutagenic:

1. the assay was valid;
2. the mutant frequency at one or more doses was significantly greater than that of the negative control;
3. there was a significant concentration-dependence as indicated by the linear trend analysis;
4. the effects described above were reproducible.

### Results

The results from three independent experiments are summarized below (excerpted from page 32-33 of the study report).

In the absence of S9, no statistically significant increase in mutant frequency was observed at concentrations up to 1250 µg/mL in Experiment 1, up to 750 µg/mL in Experiment 2 and up to 2000 µg/mL in Experiment 3. The relative survival at top concentrations of experiments 1 and 3 was 51% and 32%, respectively. Cytotoxicity

should be taken into consideration in evaluating the statistically significant increase in mutant frequency observed in Experiment 2 at concentration of 1000 µg/mL with a %RS <10%.

In the presence of S9, in Experiment 1, no statistically significant increase in mutant frequency was observed at concentrations up to 1500 µg/mL. In Experiment 2, a significant increase in mutant frequency was observed at concentrations 500-1000 µg/mL with %RS of 32.8-11.8%, and excessive cytotoxicity (<10% RS) was observed at higher concentrations of 1250 and 1500 µg/mL (top concentration). In Experiment 3, a slight but statistically significant increase in mutant frequency was observed at concentration of 1250 µg/mL but not at others including the higher concentration of 1500 µg/mL. The top concentrations of the 1<sup>st</sup> and 3<sup>rd</sup> assays resulted in %RS of 15% and 38%, respectively.

Experiment 1

Treatment (µg/mL)	%RS	-S-9 Mutant frequency#	Treatment (µg/mL)	%RS	+S-9 Mutant frequency#
0	100.0	138.07	0	100.0	130.75
125 \$	79.2	NE	250 \$	58.0	NE
250	89.5	130.76 NS	500	46.7	183.89 NS
500	86.8	90.53 NS	750	38.8	171.01 NS
750	59.1	84.81 NS	1000	33.8	164.01 NS
1000	50.8	126.08 NS	1250	22.4	166.49 NS
1250	50.8	108.24 NS	1500	15.7	152.40 NS
Linear trend		NS	Linear trend		NS
NQO			BP		
0.05	86.8	683.72	2	59.3	865.09
0.1	74.3	817.18	3	55.0	1380.19

Experiment 2

Treatment (µg/mL)	%RS	-S-9 Mutant frequency#	Treatment (µg/mL)	%RS	+S-9 Mutant frequency#
0	100.0	185.36	0	100.0	167.31
500	74.4	160.28 NS	500	32.8	294.44 *
750	60.9	178.48 NS	750	21.8	366.84 *
1000	6.7	276.54 *	1000	11.8	374.66 *
1250 X	3.0	NE	1250 \$\$, X	2.8	NE
1500 \$	0.3	NE	1500 X	2.5	NE
2000 \$	0.0	NE			
Linear trend		NS	Linear trend		***
NQO			BP		
0.05	78.4	1535.98	2	70.5	1586.02
0.1	38.0	1504.64	3	32.4	2347.08

Experiment 3

Treatment (µg/mL)	%RS	-S-9 Mutant frequency#	Treatment (µg/mL)	%RS	+S-9 Mutant frequency#
0	100.0	142.47	0	100.0	104.09
125 \$	84.8	NE	62.5 \$	101.5	NE
250 \$	96.4	NE	125 \$	91.0	NE
500 \$	84.2	NE	250 \$	93.7	NE
750	81.2	117.21 NS	500	78.4	122.43 NS
1000	77.6	128.85 NS	750	77.9	137.02 NS
1250	73.0	97.49 NS	1000	61.5	122.70 NS
1500	63.3	120.04 NS	1250	50.3	150.64 *
2000	31.8	99.27 NS	1500	37.8	109.73 NS
Linear trend		NS	Linear trend		NS
NQO			BP		
0.05	86.8	387.57	2	96.4	745.27
0.1	87.7	517.68	3	56.7	1391.29

# Per 10<sup>6</sup> viable cells  
 %RS Percent relative survival  
 \$ Not plated for viability / 5-TFT resistance  
 \$\$ Treatment excluded due to excessive heterogeneity  
 X Treatment excluded from test statistics due to excessive toxicity  
 NS Not significant  
 \*, \*\*, \*\*\* Significant at 5%, 1% and 0.1% level respectively  
 NE Not evaluated  
 NQO 4-nitroquinoline 1-oxide  
 BP Benzo(a)pyrene

Overall, three experiments suggested a negative result in the absence of S9 and a weak mutagenicity in the presence of S9. The result was different from another mouse lymphoma assay (NP05144). The large variability in cytotoxicity among three assays may affect the reproducibility of the assay results, rendering the study rather inconclusive.

**Study title: Genotoxicity Study With S 16257-2: Measurement of Unscheduled DNA Synthesis in Isolated Rat Hepatocytes**

Study no. NP05912  
 Study report location: M4/4.2.3.3.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: Dec 23, 1994  
 GLP compliance: Compliant with (b) (4) guidelines  
 QA statement: Yes  
 Drug, lot #, and % purity: S 16257-2, Batch # EG418, 101.3%

**Key Study Findings**

No reproducible or dose-related increase in mean net nuclear grain counts was observed at up to 400 µg base/mL.

A slight increase in  $^3\text{H}$ -thymidine incorporation in the nuclei of rat hepatocytes in primary cultures was observed at 550  $\mu\text{g}/\text{mL}$ , suggesting a treatment induced unscheduled DNA synthesis.

### Methods

Cell: Rat hepatocytes in primary cultures

Induction: The unscheduled DNA synthesis was measured by autoradiography after the incorporation of  $^3\text{H}$ -thymidine.

Concentrations in definitive study:

1<sup>st</sup> Assay: 0 (culture medium), 55, 100, 180, 300, 550  $\mu\text{g}/\text{mL}$ ;

2<sup>nd</sup> Assay: 0 (culture medium), 100, 180, 300, 400, 550  $\mu\text{g}/\text{mL}$ ;

Basis of concentration selection:

The concentration selection was based on the preliminary cytotoxicity test, in which doses of 0 (culture medium), 3, 10, 30, 100, 300, 1000, 2000  $\mu\text{g}/\text{mL}$  were tested. As shown below, The relative survival (%) reached 11% at 1000  $\mu\text{g}/\text{mL}$ , indicating a cytotoxicity. The top dose for definitive assays was selected at 550  $\mu\text{g}/\text{mL}$ .

#### PRELIMINARY ASSAY

CYTOTOXICITY EFFECT ON RATS HEPATOCYTES	MEAN OF CELL NUMBERS	RELATIVE SURVIVAL (%)
GROUP AND DOSE		
D(-) (0 $\mu\text{g}/\text{ml}$ )	1144.3	100.0
D1 (3 $\mu\text{g}/\text{ml}$ )	1170.3	102.3
D2 (10 $\mu\text{g}/\text{ml}$ )	1082.7	94.6
D3 (30 $\mu\text{g}/\text{ml}$ )	930.7	81.3
D4 (100 $\mu\text{g}/\text{ml}$ )	910.0	79.5
D5 (300 $\mu\text{g}/\text{ml}$ )	942.0	82.3
D6 (1000 $\mu\text{g}/\text{ml}$ )	127.0	11.1
D7 (2000 $\mu\text{g}/\text{ml}$ )	11.0	1.0

Negative control: culture medium

Positive control: Solution of N-Acetylaminofluorene (2-AAF) in DMSO at 0.2  $\mu\text{g}/\text{mL}$  (2% v/v) in the medium

Formulation/Vehicle: Solution in water for injectable preparation (2% v/v).

Treatment/Recovery: 20 - 22 h treatment and recovery

Study Validity

The mean net nuclear grain counts of the medium negative controls were within the range of acceptability (1.0 and 0.2, in the first assay, and 1.9 and 2.6, in the second assay).

Positive controls induced at least a 4-fold increase in unscheduled DNA synthesis over the medium negative control, demonstrating the sensitivity of the test system.

Five usable concentrations of the tested compound were available.

The sponsor's criteria to evaluate a positive response:

- an increase above the solvent control value in the mean net nuclear grain count to at least 5 grains per nucleus, or in the number of cells with five or more net nuclear grains,
- a concentration-response effect,
- reproducibility of the above criteria.

The study was considered valid.

## Results

The results from three assays are shown in the tables below (summarized based on tables on page 36, 37, 39, and 40 of study report).

Assay 1, Dose ( $\mu\text{g/mL}$ )	0	55	100	180	300	550
Relative Survival (%)	100	98.9	85.4	89.3	85.7	41
Mean Net Nuclear Grain Counts culture 1/culture 2	1.0/ 0.2	0.8/ 0.3	0/ 0.9	0.4/ 1.1	0.3/ 2.0	7.8/ 1.9

Assay 2, Dose ( $\mu\text{g/mL}$ )	0	100	180	300	400	550
Relative Survival (%)	100	109.6	90.4	76.2	82.6	54.2
Mean Net Nuclear Grain Counts culture 1/culture 2	1.9/ 2.6	2.6/ 0.8	1.4/ 2.6	3.4/ 4.3	4.5/ 2.0	9.8/ 6.8

In conclusion, no reproducible or concentration-related increase in mean net nuclear grain counts was observed at up to 400  $\mu\text{g base/mL}$ . In both assays, a slight increase in  $^3\text{H}$ -thymidine incorporation in the nuclei of rat hepatocytes in primary cultures was observed at 550  $\mu\text{g base/mL}$ , at which relative survival was 41% to 54%, indicating a treatment induced unscheduled DNA synthesis. [note: *The in vivo unscheduled DNA synthesis study in rat did not show a positive activity (see review of study NP05409 below).*]

**Study title: S16257: Measurement of Unscheduled DNA Synthesis in Rat Liver using an In Vivo/In Vitro Procedure**

Study no.: NP05409  
 Study report location: M4/4.2.3.3.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: February 20, 1995  
 GLP compliance: (b) (4) compliance  
 QA statement: Included  
 Drug, lot #, and % purity: S 16257-2 Hydrochloride, 47734  
 Purity= 100.5%.

**Key Study Findings**

A single dose of S16257-02 up to 1000 mg/kg (males) or 650 mg/kg (females) did not induce unscheduled DNA synthesis in rat liver under study conditions.

At the high dosages, mean blood and liver, concentrations of S 16257-2 (expressed as the base) were between 48 and 17 µg/g for males and between 59 and 421 µg/g for females depending on the sampling time (2 or 12 hours after dosing).

**Methods**

Doses in definitive study: 0 (negative control), 300, 1000 mg/kg for males  
 0, 195, 650 mg/kg for females  
 Frequency of dosing: Single dose  
 Route of administration: Oral gavage  
 Dose volume: 20 mL/kg except 10 mL/kg for positive control  
 Formulation/Vehicle: Solution in purified water/purified water  
 Species/Strain: Rat/Wistar (male and female)  
 Number/Sex/Group: 5/sex/group  
 Satellite groups: See study design table below for blood samples  
 Basis of dose selection: A range-finding study was conducted in Wistar male and female rats treated with a single oral dose at dosage levels of 178.4, 274.6, 422.6, 650, 1000 mg/kg. One female animal died within 24 hours of dosing at 1000 mg/kg. Overt clinical signs of toxicity were observed at dosages of 650 mg/kg in females (eye closure, unsteady gait, tremors/shivers, and vocalization) and at 1000 mg/kg in males (eye closure, lethargy, unsteady gait, tremors, irregular breathing and uncoordinated movement). The maximum tolerated dosage (MTD) was determined at 650 mg/kg for females, and 1000 mg/kg for males,

which were selected as the maximum doses for main study.

In the main study, one male from the 1000 mg/kg and one female from the 650 mg/kg dosage groups died after dosing with S 16257-2, confirming the MTDs were achieved.

Negative control: Vehicle (purified water)

Positive control: For 2-4 hours sampling, dimethylnitrosamine (DMN), 10 mg/kg (in purified water), oral;  
For 12-14 hours sampling, 2-acetamidofluorene (2-AAF), 75 mg/kg (in corn oil), oral;

Sampling Time: 2-4 hours; 12-14 hours

Three rats/gender/group were euthanized 2 to 4 hours, or 12 to 14 hours, after dosing and livers were perfused with collagenase to provide primary hepatocytes for UDS analysis. After 4 h incubation in the presence of [<sup>3</sup>H]-thymidine, for 100 hepatocytes/rat, net nuclear grain counts were recorded by autoradiography. The net nuclear grain count (NNG), i.e. the number of grains present in the nucleus minus the mean number of grains in 3 equivalent areas of cytoplasm, was determined for each of 2 of the 3 slides, each animal and each dose.

Blood and liver samples were taken from satellite animals (3/sex/group/time point) at 2 hours and 12 hours post dosing for TK analysis.

### Study Validity

The criteria were set for a valid study:

1. The negative control animals had zero NNG counts or less (i.e a negative value).
2. The positive control treatments should have values of 5 or more NNG with 50% or more cells having NNG counts of 5 or greater.

The criteria for a positive response:

1. the test article yielded group mean NNG values greater than 0 and 20% or more of cells responding (mean NNG values  $\geq 5$ )
2. an increase was seen in both NNG and the percentage of cells in repair.

The study criteria were met.

As shown in the tables below in Results, male negative (vehicle) control animals gave a group mean NNG value of less than 0 with only 0.3 to 1.0% of cells in repair. Female negative (vehicle) control animals gave a group mean NNG value of less than 0 with only 1.3 to 1.7% of cells in repair. The vehicle control NNG value was consistent with historical control data.

Both positive control treatments produced NNG values greater than 5 with greater than 20% of cells in repair, indicating that the test system was sensitive to 2 known DNA damaging agents requiring metabolism for their action.

The study is considered valid.

## Results

As shown in the tables below (excerpted from pages 26 and 35 of the report), there were no increases in NNG counts or in the percentage of cells in repair in treated male and female groups when compared to their respective controls.

Group mean NNG values (Males)

### 12-14 hour experiment

Dose (mg/kg)	Net nuclear grain count (NNG)		Net grain count of cells in repair		Percent of cells in repair (NNG $\geq$ 5)	
	mean	SD	mean	SD	mean	SD
0 Water	-1.3	0.2	6.8	0.8	1.0	1.0
300	-1.6	0.4	5.7	0.9	1.3	1.5
1000	-1.6	0.4	5.0	0.0	0.3	0.6
75 2-AAF	13.0	1.6	14.1	1.5	89.0	3.5

### 2-4 hour experiment

Dose (mg/kg)	Net nuclear grain count (NNG)		Net grain count of cells in repair		Percent of cells in repair (NNG $\geq$ 5)	
	mean	SD	mean	SD	mean	SD
0 Water	-1.2	0.1	5.7	0.0	0.3	0.6
300	-1.7	0.0	6.7	0.0	0.3	0.6
1000	-2.0	0.5	5.0	0.0	0.3	0.6
10 DMN	11.2	0.6	12.4	0.7	87.0	1.7

Group Mean NNG Values (Females)

## 12-14 hour experiment

Dose (mg/kg)	Net nuclear grain count (NNG)		Net grain count of cells in repair		Percent of cells in repair (NNG $\geq$ 5)	
	mean	SD	mean	SD	mean	SD
0 Water	-1.3	0.2	5.4	0.1	1.7	1.5
195	-1.5	0.7	7.2	0.0	1.0	1.7
650	-1.6	0.3	5.5	0.7	0.7	0.6
75 2-AAF	9.2	0.1	10.5	0.3	81.3	4.0

## 2-4 hour experiment

Dose (mg/kg)	Net nuclear grain count (NNG)		Net grain count of cells in repair		Percent of cells in repair (NNG $\geq$ 5)	
	mean	SD	mean	SD	mean	SD
0 Water	-1.3	0.7	7.4	0.0	1.3	2.3
195	-1.4	0.8	8.1	3.0	1.7	2.1
650	-1.8	0.3	5.7	0.0	0.3	0.6
10 DMN	10.1	0.9	11.4	1.0	84.0	1.0

Mean plasma and liver exposure to ivabradine and its metabolite, S 18982, at 2 hr post-dose are shown in the table below (excerpted from table 34, Module 2, section 2.6.6. page 55). For the highest dosages, mean liver concentrations of S 16257-2 (expressed as the base) were between 48 and 17  $\mu\text{g/g}$  for males and between 59 and 421  $\mu\text{g/g}$  for females depending on the sampling time (2 or 12 hours after dosing).

**Table 34. Ivabradine and S 18982 Mean Plasma and Liver Concentrations in Wistar Rats 2 h After Single Oral Dosing**

Dose (mg/kg)	Plasma concentration (ng/ml)				Liver concentration (ng/g)	
	Males		Females		Males	Females
	2 h	Multiple of hC <sub>max</sub>	2 h	Multiple of hC <sub>max</sub>	2 h	2 h
<b>ivabradine</b>						
181			2100	68		28000
278	810	26			10000	
603			6300	203		59000
928	3400	110			48000	
<b>S 18982</b>						
181			34	4		750
278	42	5			640	
603			70	9		1500
928	190	24			2900	

n=3/gender/dose. hC<sub>max</sub> = Mean plasma maximum concentration at steady state in patients at the highest therapeutic dose.

In conclusion, a single dose of S16257-02 up to 1000 mg/kg (males) or 650 mg/kg (females) did not induce unscheduled DNA synthesis in rat liver under study conditions.

#### **Study title: S 16257-2: Induction of Chromosome Aberrations in the Bone Marrow of Treated Rats**

Study no.:

NP05504

Study report location:

M4/4.2.3.3.2

Conducting laboratory and location:

(b) (4)

Date of study initiation:

June 8, 1995

GLP compliance:

(b) (4) compliance

QA statement:

Included (reviewer scientist statement error in title, rats=mice

Drug, lot #, and % purity:

S 16257-2 Hydrochloride, 47734

Purity= 100.5%.

#### **Key Study Findings**

A single dose of S16257-02 up to 1000 mg/kg (males) or 650 mg/kg (females) did not induce chromosomal aberrations *in vivo* in rat bone marrow cells under study conditions.

#### **Methods**

Doses in definitive study:

See study design table below. Equivalent base dosages: 0, 232, 464, 928 for males; 0, 151, 302 or 603 mg/kg for females.

Frequency of dosing:

Single dose

Route of administration: Oral gavage  
 Dose volume: 20 mL/kg except 10 mL/kg for positive control  
 Formulation/Vehicle: Solution in purified water/purified water  
 Species/Strain: Rat/Wistar (male and female)  
 Number/Sex/Group: 5/sex/group  
 Satellite groups: See study design table below for TK samples  
 Basis of dose selection: The range-finding study 303/167 (i.e. NP05409, "Measurement of UDS in Rat Liver using an In Vivo/In Vitro Procedure") indicated that the maximum tolerated dose was determined at dosage of 1000 mg/kg in males, and 650 mg/kg in females. See "Basis of dose selection" in the review of study NP05409 in section 7.4.

In the main study, four females and three males died, and one male was sacrificed moribund in the high dosage groups and overt clinical signs of toxicity were observed in both male and female high dosage groups, confirming that MTDs were achieved.

Negative control: Vehicle  
 Positive control: Cyclophosphamide (CPA) 40 mg/kg, oral

Treatment (mg/kg)	Number of treated animals							
	18 hour sample		42 hour sample		Blood samples		Spares	
	♂	♀	♂	♀	♂	♀	♂	♀
Vehicle	5	5	5	5	2+2	2+2		
250	5				2+2			
500	5				2+2			
1000	5		5		2+2		5	
162.5		5				2+2		
325		5				2+2		
650		5		5		2+2		5
CPA, 40	5	5						

Study design including treatment groups and sampling times in main study was summarized in the table below (excerpted from page 15 of the report). Bone marrow from both femurs was collected. Bone marrow samples from rats treated at all three dosages were analyzed at the 18 hour post dosing and that from rats in the high dosage (1000 mg/kg for males, 650 mg/kg for females) and control groups were analyzed at the 42 hour post dosing. Slides were examined, uncoded, for mitotic index (MI) or percentage of cells in mitosis, based on 1000 cells scored per animal. Five hundred metaphases per dosage group (100 per rat) were analyzed. Blood samples for TK analysis were collected from satellite animals at 10 min and 2 hours post dosing.

**Study Validity**

The following criteria were set for an acceptable study:

1. the heterogeneity X2 test demonstrated acceptable variability between animals within groups, and
2. the proportion of cells with structural aberrations (excluding gaps) in negative control animals fell within the normal range, and
3. at least eight animals (males and females together) out of each group at each kill time and 400 cells out of an intended 500 were analyzable for analysis, and
4. the positive control chemical (CPA) induced a clear increase in the number of cells with structural aberrations.

The criteria for a positive response:

1. a statistically significant increase in the frequency of cells with structural aberrations occurred at one or more dosage and/or sampling time
2. the incidence of cells with aberrations at such data points exceeded the normal range;
3. corroborating evidence was obtained such as increased but insignificant increases in the incidence of structural aberrations at other doses or kill times, or dose response profiles.

Most study criteria were met. Less than 400 cells were available for analysis from the group of male rats treated with CPA, but as the cells analyzed contained a high frequency of aberrant cells, the slight lower number of cells was not considered to have significant impact on the validity of the assay. The study is considered acceptable.

**Results**

Mitotic indices from control and treated groups were comparable.

The frequencies of cells with structural chromosome aberrations from all treated groups were similar to and not significantly different from those observed in concurrent vehicle control groups at both sampling times. All group mean frequencies of aberrant cells were within the historical control range. The group data was summarized in the tables below (excerpted from pages 25 and 26 of the report).

**Table 1**

18 hour sample time: male animals

Treatment (mg/kg)	N <sup>o</sup> of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index % (group mean)
Vehicle	5	500	6	0		2.8
250	5	500	2	2	NS	4.7
500	5	500	3	1	NS	5.8
1000	5	500	4	2	NS	4.3
CPA, 40	5	319	147	131	p≤0.001	0.52

18 hour sample time: female animals

Treatment (mg/kg)	N <sup>o</sup> of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index % (group mean)
Vehicle	5	500	1	0		5.6
162.5	5	500	1	0	NS	5.2
325	5	500	3	1	NS	5.8
650	5	500	4	3	NS	3.7
CPA, 40	5	500	279	279	p≤0.001	0.98

**Table 2**

42 hour sample time: male animals

Treatment (mg/kg)	N <sup>o</sup> of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index % (group mean)
Vehicle	5	500	7	5		3.5
1000	5	483	1	0	p ≤ 0.05	5.6

Note: Statistical significance refers to a decrease in aberrant cell frequency

42 hour sample time: female animals

Treatment (mg/kg)	N <sup>o</sup> of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index % (group mean)
Vehicle	5	500	3	3		5.8
650	5	500	2	2	NS	5.4

Mean plasma exposure to ivabradine and its metabolite S 18982 were summarized in the table below (excerpted from Table 35 in Module 2, section 2.6.6, page 56).

*Note: The doses referred in the table below were equivalent base doses. The study report noted that the results for S 18982 are NOT validated because concentrations found in Quality Control samples were not within acceptability limits. The concentrations were given in the study report table just to indicate that S 18982 was present in plasma.*

**Table 35. Ivabradine and S 18982 Mean Plasma Concentrations in Wistar Rats 10 min and 2 h After Single Oral Dosing**

Dose (mg/kg)	Plasma concentration (ng/ml)							
	Males		Females		Males		Females	
	10 min	Multiple of hC <sub>max</sub>	10 min	Multiple of hC <sub>max</sub>	2 h	Multiple of hC <sub>max</sub>	2 h	Multiple of hC <sub>max</sub>
<b>ivabradine</b>								
151			4200	135			3000	97
232	1400	45			760	25		
302			3500	113			10000	323
464	4000	129			1200	39		
603			18000	581			4900	120
928	9100	294			6400	206		
<b>S 18982</b>								
151			64	8			37	5
232	58	7			17	2		
302			60	8			100	13
464	140	18			56	7		
603			270	34			nd	
928	390	49			230	29		

n=2/gender/dose. hC<sub>max</sub> = Mean plasma maximum concentration at steady state in patients at the highest therapeutic dose.

In conclusion, a single dose of S16257-02 up to 1000 mg/kg (males) or 650 mg/kg (females) did not induce chromosomal aberrations in rat bone marrow cells under study conditions.

## 8 Carcinogenicity

## 9 Reproductive and Developmental Toxicology

## 10 Special Toxicology Studies

## 11 Integrated Summary and Safety Evaluation

The results of genotoxicity assays are summarized in the table below.

**Table 1 Genotoxicity Summary**

Assay	+/- S9	Concentration/Dose Range <sup>c</sup> (µg/mL)	Results	<sup>a</sup> Multiples of $hC_{max}$
<b>In Vitro assays</b>				
Ames test	+/-	0, 46, 139, 464, 928, 1392, 2320, 4640 µg/plate	Negative	
Chromosomal Aberration in human lymphocytes	-	46*, 79, 116, 139	Equivocal	1,500*-4,500
	+	260, 487*, 882*, 1670, 2088	Negative	8,000-67,000
tk-gene mutation MLA (NP05144)	-	87 to 928*, 1160	Positive	2,800-30,000*
	+	87 to 1624	negative	2,800-52,000
tk-gene mutation MLA (NP05489)	-	116 to 1856	negative	5,200-60,000
	+	58 to 464*, 928*, 1160*, 1392	Positive (Inconclusive)	1,900-15,000*
UDS in rat hepatocytes	n/a	0, 55, 100, 180, 300, 400, 550*	Positive	1,800-18,000*
<b>In Vivo assays (single dose by oral gavage)</b>				
Micronucleus, mouse	n/a	0, 116, 232, 464 mg/kg	Negative	No TK data
Chromosomal Aberration, rat	n/a	M: 0, 232, 464, 928 mg/kg F: 0, 151, 302, 603 mg/kg	Negative	M: 294 <sup>b</sup> ; F: 581 <sup>b</sup>
UDS, rat	n/a	M: 0, 278, 928 mg/kg F: 0, 181, 603 mg/kg	Negative	M: 110 <sup>b</sup> ; F: 203 <sup>b</sup>

a.  $hC_{max}$  = mean plasma maximum concentration at steady state in patients at the highest therapeutic dose of 7.5 mg bid = 31 ng/mL.

b. Multiples of  $hC_{max}$  were based on the mean  $C_{max}$  from TK analysis in each rat study.

c. Ivabradine doses were expressed in terms of free base in this table, which could be calculated from dose of ivabradine hydrochloride  $\times$  0.928 (conversion factor).

+/-: with/without exogenous metabolic activation (rat liver S9 mix);

\* indicated that a significant finding was observed at that dose (exposure) level.

Ivabradine did not result in gene mutation in bacteria in vitro but was associated with a weak induction of unscheduled DNA synthesis in primary rat hepatocytes ex vivo and a weak induction of *tk* gene mutation in mouse lymphoma cells in vitro. The genotoxic responses were observed at dose concentrations > 15,000 fold of human  $C_{max}$  at maximum recommended human dose (MRHD), 7.5 mg bid, in these assays. The chromosomal aberration test in human lymphocytes produced an equivocal result for a possible weak clastogenic activity due to lack of dose-dependency.

In vivo, ivabradine did not show genotoxicity in three separate tests in mice and rats. The negative results were achieved at dosages up to 464 mg/kg (base) in mouse micronucleus test and at plasma exposures > 100 fold of human  $C_{max}$  at MRHD in rat chromosome aberrations test and rat liver UDS assay.

Given the uniformly negative in vivo results, the weak in vitro genotoxic responses observed at concentrations about 15,000 fold of human  $C_{max}$ , ivabradine is unlikely to pose a genotoxic risk in the proposed clinical use. The conclusion is substantiated by the results of 2-year carcinogenicity studies in rats and mice which showed no evidence

of tumorigenic potential after dietary administration of ivabradine at dosages up to 120/60 mg/kg/day (rats) and 405/180 mg/kg/day (mice), respectively.

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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JEAN Q WU  
11/19/2014

ALBERT F DEFELICE  
11/19/2014

**Appendix 2**  
**Pharmacology/Toxicology NDA Review**  
**Evaluation of Carcinogenicity**

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW  
EVALUATION (Carcinogenicity)

Application number: 206,143

Supporting document/s: S016 (eCTD0014), S022 (eCTD0017), S023  
(eCTD0022)

Applicant's letter date: April 30, 2014, June 27, 2014, July 11, 2014

CDER stamp date: April 30, 2014, June 27, 2014, July 11, 2014

Product: Ivabradine

Indication: reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq$  70 beats per minute (bpm), (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)

Applicant: Amgen, Inc.

Review Division: Division of Cardio-Renal Products

Reviewer: Jean Q. Wu

Supervisor/Team Leader: Albert DeFelice

Division Director: Norman Stockbridge

Project Manager: Alexis Childers

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# 1 Executive Summary

## 2 Drug Information

### 2.1 Drug

#### 2.1.1 CAS Registry Number (Optional)

Ivabradine: 155974-00-8

Ivabradine hydrochloride: 148849-67-6

#### 2.1.2 Generic Name

ivabradine

#### 2.1.3 Code Name

S 16257-2; AMG 998

#### 2.1.4 Chemical Name

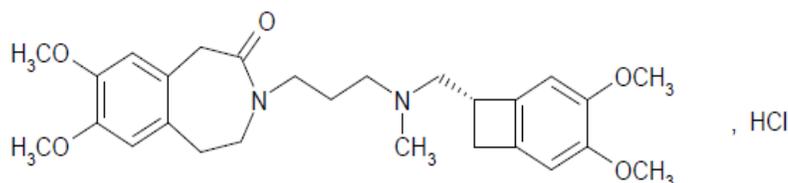
3-(3-(((7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl) methyl amino)propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride

#### 2.1.5 Molecular Formula/Molecular Weight

C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>, HCl/ 505.1 g/mol;

468.593 g/mol (base); Conversion factor from the salt to the base: 0.928

#### 2.1.6 Structure



#### 2.1.7 Pharmacologic class

hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND (b) (4)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

Ivabradine is provided as film-coated tablet in two strengths: 5 mg (oval) and 7.5 mg (triangular). The composition of the drug is listed in the table below (excerpted from Module 2.3. P, Table 1, Page 7)

Table 1. Composition of Ivabradine 5 mg and 7.5 mg Tablets

Component	5 mg		7.5 mg		Function	Reference to specifications
	Percentage (% w/w)	Quantity (mg/tablet)	Percentage (% w/w)	Quantity (mg/tablet)		
<b>Tablet</b>						
Ivabradine hydrochloride <sup>a</sup> (free base equivalent)	5.39 (5.00)	5.390 (5.000)	8.085 (7.50)	8.085 (7.500)	Drug substance	In-house
Lactose monohydrate	(b) (4)				(b) (4)	USP/NF, PhEur.
Maize starch	(b) (4)					USP/NF
Maltodextrin	(b) (4)					USP/NF, PhEur
Magnesium stearate	(b) (4)					USP/NF, PhEur
Colloidal silicon dioxide	(b) (4)					USP/NF, PhEur
(b) (4)	(b) (4)					USP
<b>Core Tablet Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>		
<b>Film-Coating</b>						
(b) (4)	(b) (4)				(b) (4)	See 3.2.P.4.1
salmon <sup>c</sup>	(b) (4)					USP/NF, PhEur
Polyethylene glycol 6000	(b) (4)					USP
(b) (4)	(b) (4)					
<b>Total</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>		

<sup>a</sup> The molecular weights of ivabradine hydrochloride anhydrous and ivabradine free base are 505.1 g/mol and 468.6 g/mol, respectively. The free base accounts for 92.77% of the salt.

(b) (4)

## 2.4 Proposed Clinical Population and Dosing Regimen

Ivabradine is indicated to reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm), (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)

The proposed starting dose of ivabradine is 5 mg twice daily. After 2 weeks of treatment, if heart rate is between 50 and 60 bpm, the dose of 5 mg twice daily should be maintained. The dose should be increased to 7.5 mg twice daily if resting heart rate is persistently above 60 bpm.

As listed in the table below (excerpted from Module 2, Section 2.4, Page 7), the human plasma exposures of ivabradine and its N-desmethylated metabolite (S 18982) were estimated at steady state in patients receiving the maximum

recommended human dose (MRHD), 7.5 mg bid. These values were derived from a population pharmacokinetic analysis using Phases II and III clinical data [Summary of Clinical Pharmacology Studies, in Section 2.7.2 (2.1.2.2), and are accepted as references when preclinical doses are expressed as multiples of human exposures in current review, unless otherwise indicated.

**Table 1. Ivabradine and S 18982 (Metabolite) Plasma C<sub>max</sub> and Estimated AUC<sub>24</sub> at Steady State in Patients at HTD**

	Ivabradine (n=492)	S 18982 (n=541)
Population C <sub>max</sub> (ng/ml)	31 ± 9.8	7.9 ± 2.3
Equivalent C <sub>max</sub> in μM	0.07	0.02 <sup>b</sup>
Population AUC <sub>24</sub> <sup>a</sup> (ng.h/ml)	346	128

Values are mean ± SD;

a: Calculated from AUC over 12 h period from [WS (2.7.2) 3.1/ Table 12; Table 34 and 35]

b: S 18982 MW = [REDACTED]

## 2.5 Regulatory Background

A pre-NDA meeting was held between the sponsor and the Division on January 23, 2014. There was no IND opened in the US FDA [REDACTED] (b) (4). Hence, no carcinogenicity study protocols were submitted to the Division and ECAC for comments prior to study initiation or during the study.

## 3 Studies Submitted

### 3.1 Studies Reviewed

NP07944: S 16257-2: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice

NP07946: Toxicokinetics of S 16257 in male and female CD-1 mice after dietary administration of S 16257-2 for at least 104 weeks (TK report for study NP07944)

NP07943: S 16257-2: Potential Tumorigenic Effects in Prolonged Dietary Administration to Rats

NP07945: Toxicokinetics of S 16257 in male and female Wistar rats after dietary administration of S 16257-2 for at least 104 weeks (TK report for study NP07943)

*The following studies were briefly reviewed, and relevant findings are discussed in the reviews of the 2-year carcinogenicity studies:*

NP05454: Palatability Study in Mice by Dietary Administration for 4 Weeks

NP06393: S 16257-2: Subacute Toxicity Study to Mice by Dietary Administration for 13 Weeks.

NP05455: Palatability Study in Rats by Dietary Administration for 6 Weeks  
NP06450: S 16257-2: Subacute Toxicity Study to Rats by Dietary Administration for 13 Weeks.

#### **4 Pharmacology**

#### **5 Pharmacokinetics/ADME/Toxicokinetics**

#### **6 General Toxicology**

#### **7 Genetic Toxicology**

#### **8 Carcinogenicity**

##### **Study title: S 16257-2: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice (Study No. NP07944)**

Conducting laboratory/location:

(b) (4)

Date of study initiation:

November 12, 1996

GLP compliance:

Yes

QA statement:

Included

Drug, lot #, and % purity:

S 16257-2, batch# 49 652, Purity: 100.4%  
(the conversion factor from free base to salt: 1.078)

CAC concurrence:

Study protocol was not discussed in ECAC.

#### **Key Study Findings**

##### **Adequacy of Carcinogenicity Study**

S16257-2 was administered by diet to CD-1 mice at doses of 20, 90, and 405/180 mg/kg/day for up to 104 weeks (93 weeks for high dose male group). The study protocol was not submitted for discussion/concurrence by the ECAC (see regulatory history).

During the first 80 weeks, a significantly higher mortality was observed in the high dose (405 mg/kg/day) males and females when compared to the controls. During Weeks 81-93 and/or Weeks 84-104, the incidence of mortality in the high dose (reduced to 180

mg/kg/day) groups was comparable to the control groups. The high dose male group was terminated early at the end of Week 93 due to the low survival rate of 20%.

A body weight gain reduction in the 1<sup>st</sup> 80 weeks was observed in high dose males (-36%) and in all treated female groups in a dose-dependent manner (up to -33%). Body weight gain reduction was up to -38% over the treatment period (Week 1-93) for high dose males. The overall body weight gain for all treated females during Week 1-104 was reduced about -13% to -23% without dose-dependency.

The treatment-related non-neoplastic histopathology findings, observed in the high dose males and females, were primarily cardiac, which included atrial thrombus, dilated chambers, minimal to moderate myocardial degeneration, vacuolation and fibrosis, and minimal to slight myocardial cell hypertrophy, epicardial inflammation and fibroblast proliferation. A lesser degree of increased incidence of myocardial degeneration and vacuolation was also observed in the decedent mid-dose males. The pathology changes in the lungs, liver, spleen and thymus of the decedent mice were associated with cardiac toxicity.

Mortality and body weight gain reduction in the high dose males and females suggest that a MTD was achieved and possibly exceeded in this study. The duration of treatment, 93-week for high dosage males and 104-week for other groups, is considered acceptable.

The high dosage was associated with AUC<sub>24h</sub> of 7920 (23 X human AUC<sub>24h</sub>) and 14331 ng.h/mL (41 X human AUC<sub>24h</sub>) for male mice at Week 94 and female mice at the week of 104, respectively.

### **Appropriateness of Test Models**

A dietary administration of test article to CD-1 mice for up to 104 weeks is considered appropriate for tumorigenic potential evaluation.

### **Evaluation of Tumor Findings**

There were no treatment-related tumorigenic effects in ivabradine treated mice.

The statistically significant increase in incidence of lymphoid histiocytic sarcoma in the female low dose group was not dose-dependent, similar to the incidence in the male control group, and not observed in any other tissues/organs, hence, was not considered toxicologically significant.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 28, 2014 in Appendix 1).

## Methods

Doses:	20, 90, 405 (base) mg/kg/day <sup>a</sup> (week 1-80)/ 20, 90,180 (base) mg/kg/day <sup>a</sup> (week 81-104)
Frequency of dosing:	Daily in diet
Dose volume:	diet
Route of administration:	diet
Formulation/Vehicle:	Test article mixed with the diet <sup>b</sup> /Normal untreated diet for mice
Basis of dose selection:	The 13-week study (SVA 217/961267, NP06393) with dietary administration at dosages of 30, 100, 300 or 600 mg/kg/day showed a body weight gain decrease and an increase in heart weight at dosages $\geq 100$ mg/kg/day and $\geq 300$ mg/kg/day in males and females, respectively. Therefore, the high dosage of 405 mg/kg/day was chosen to elicit signs of minimal toxicity and to provide at least 30 times the human AUC <sub>24h</sub> estimated to be 346 ng/h.mL. The low dose of 20 mg/kg/day was chosen as no adverse effects were expected at this level and it provided at least 3 times the human therapeutic exposure.
Species/Strain:	CrI:CD-1(ICR)BR Mouse
Number/Sex/Group:	50
Age:	~5 weeks
Animal housing:	2 mice of the same sex/cage
Paradigm for dietary restriction:	All mice had free access to tap water and ground SDS Rat and Mouse No. 1 modified maintenance diet.
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	26/sex/group for TK profiling
Deviation from study protocol:	No deviations from the study protocol and amendments were considered to affect the integrity of the study
a.	<i>Food consumption and test article control data indicated that the actual doses received were 20, 91 and 403/179 mg/kg/day for males and 21, 91 and 408/184 mg/kg/day for females.</i>
b.	<i>Concentration of test article in the diet were changed as necessary to preserve the dosage levels (i.e. in line with body weight changes and food consumption)</i>

## Observations and Results

### Mortality and Clinical Signs

Animals were observed at least once weekly for mortality and moribundity. A detailed palpation of each mouse was performed once weekly for any palpable masses.

The distribution of unscheduled deaths during the study period was summarized below (excerpted from summary table of the report). During the first 80 weeks, a significantly higher mortality was observed in the high dose (405 mg/kg/day) males and females when compared to the control. Due to this high mortality, the high dose was reduced to 180 mg/kg/day from Week 81. During the week 81-93, the mortality incidence appeared similar between high dose and control groups. However, at the end of Week 93, the surviving number of high dose male reached 10, i.e. 20% survival point; thus, this group was terminated at Week 94 to ensure that there were enough animals to be examined to allow for a meaningful scientific interpretation of the terminal data.

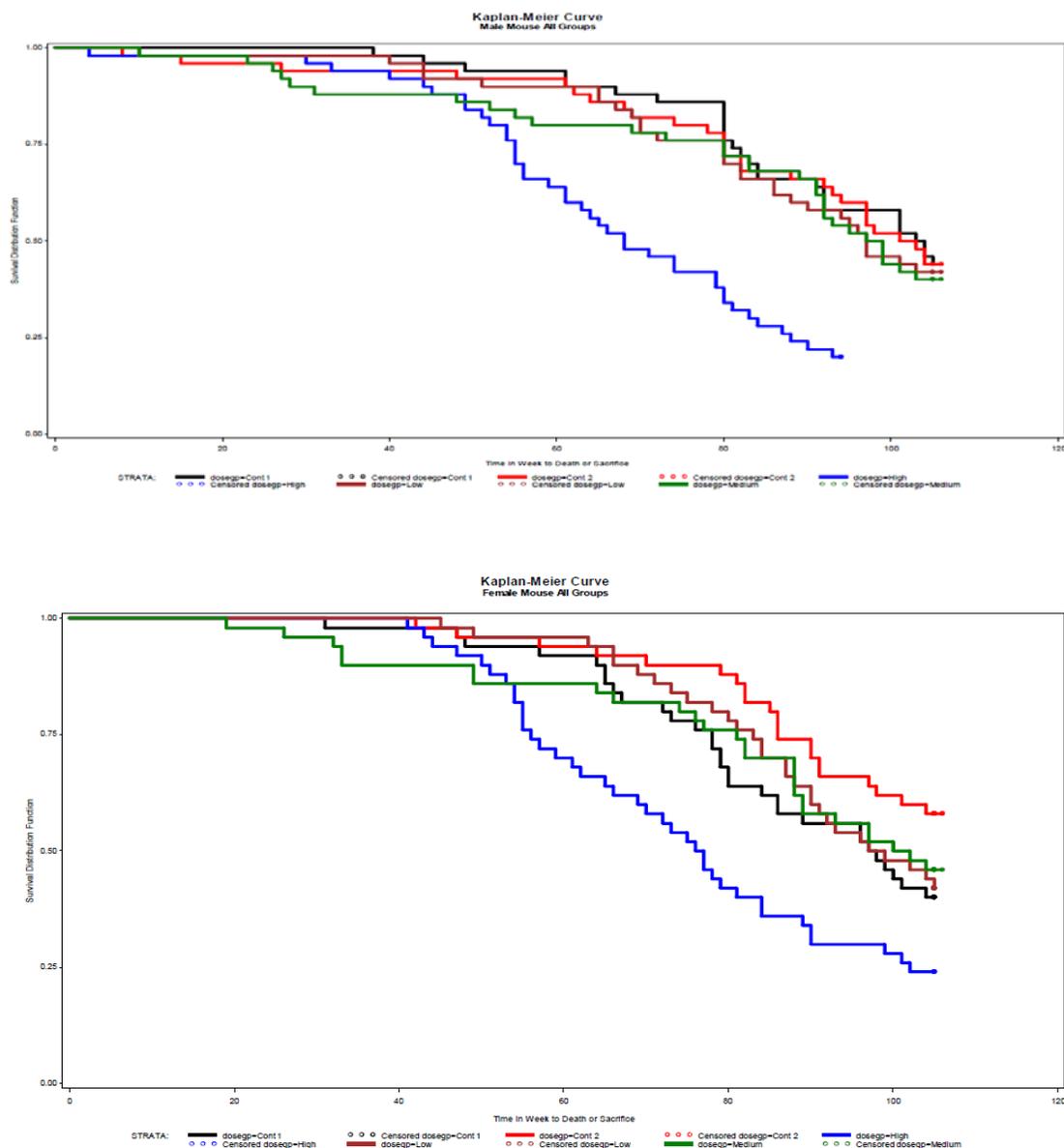
Number of decedents Weeks	Group/dosage (mg base/kg/day)									
	1M Control	2M Control	3M 20	4M 90	5M 405/180†	1F Control	2F Control	3F 20	4F 90	5F 405/180†
1 - 80	12	14	15(5)	14(6)	33(15)	18	6	11(8)	12(4)	29(16)
81 - 93	9	5	6(2)	9(3)	7(3)	4	11	12(3)	10(4)	6(1)
94 - 104	6	9	8(3)	7(3)	-	8	4	5(4)	5(5)	3(3)
1 - 104	27	28	29(10)	30(12)	40*(18)	30	21	28(15)	27(13)	38(20)
Main group mortality (%)	54	56	58	60	80	60	42	56	54	76
Main group survival (%)	46	44	42	40	20	40	58	44	46	24

( ) Satellite group animals

† Dosage was lowered from 405 mg base/kg/day to 180 mg base/kg/day from Week 81

\* Surviving Group 5 males were sacrificed in Week 94 due to high mortality

The survival test conducted by FDA statistical reviewer, Dr. A. Rahman, showed a statistically significant dose response relationship in mortality across the combined control and treated groups in both genders of mice. The pairwise comparison showed statistically significant increased mortality in the high dosage group in both genders when compared to their respective combined control groups (see the figures below, excerpted from Statistical Review and Evaluation for this NDA, by Dr. A. Rahman).



Histopathology examination of the main group decedents during the 1st 80 weeks revealed an increased incidence and severity of heart lesions that included myocardial degeneration, myocardial vacuolation, myocardial fibrosis, dilated chambers, myocardial cell hypertrophy, atrial thrombi and epicardial inflammation with edema or fibroblast proliferation, predominantly in the high dosage (405 mg/kg/day) males and females when compared to the controls.

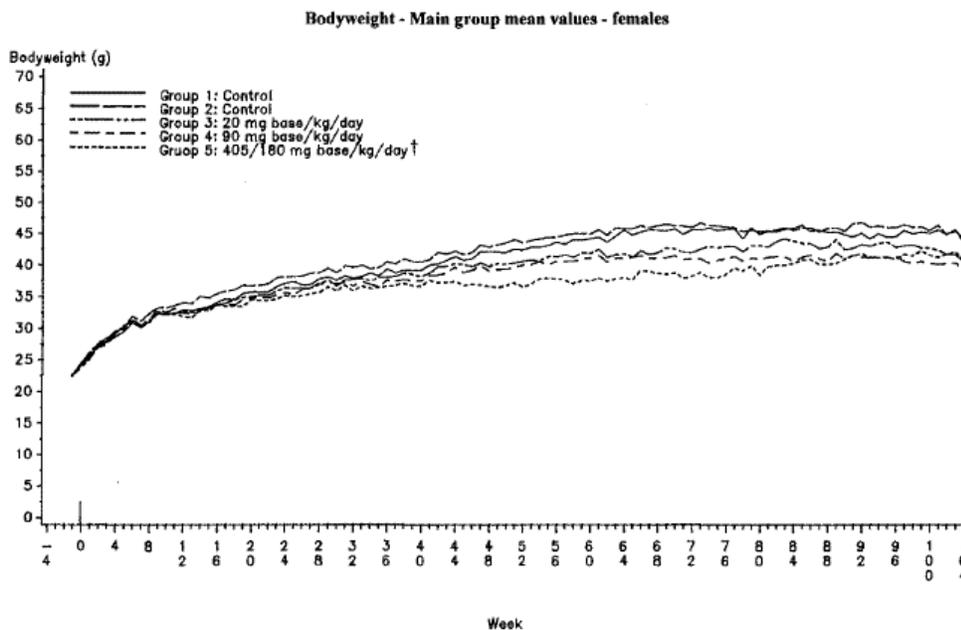
There were no other test article-related adverse clinical observations.

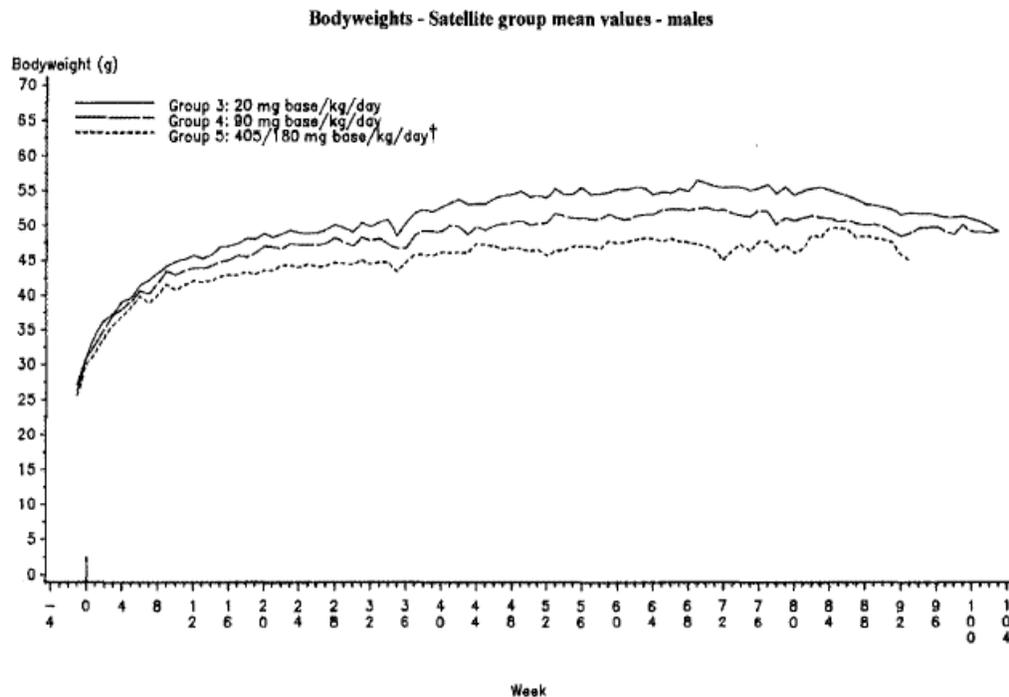
## Body Weights

Body weight was collected at randomization, one week prior to and on the day of initial treatment, and once a week thereafter.

Body weight gain g (% of combined control)	Dose Mg/kg/day	Male					Female				
		0	0	20	90	405/180	0	0	20	90	405/180
Week 0-80		22.7	24.0	21.9 (94)	24.3 (104)	14.9 (64)	21.1	20.7	19.3 (92)	16.5 (79)	13.9 (67)
Week 0-93		23.0	22.3	21.5 (95)	21.7 (96)	13.5 (62)	20.4	21.3	19.1 (92)	16.5 (79)	17.4 (83)
Week 0-104		21	17	19 (0)	19 (0)	N/A	20.8	18.6	16.7 (86)	14.8 (77)	17.0 (87)

As shown in the table above and figures below (excerpted from page 51-52 of the report), a dose-related reduction in group mean bodyweight gain was noted in the first 80 weeks among all treated female groups up to -33% and in high dose males (-36%) when compared with concurrent controls. Following the reduction of the high dosage from 405 to 180 mg/kg/day, female bodyweight gain exhibited a slight improvement between Weeks 81 to 104. This resulted in an overall weight gain for high dose females that was comparable to that for low dose females while the reduction for the mid-dose females reached -23% over the period of 104 weeks. The overall bodyweight gain for high dose males was reduced about -38% during the Week 1-93 treatment period while that for low and mid-dose males was similar to the concurrent controls during the 104-week treatment period.





## Food Consumption

The quantity of food consumed by each cage of mice was recorded weekly. Individual mouse food intake per week was calculated as  $[(\text{total food given} - \text{total food left}) / \text{number of animal days}] \times 7$ .

Food conversion ratios were calculated, where possible, over the period Weeks 1 to 24, from the bodyweight and food consumption data as weight of food consumed per unit gain in bodyweight.

Daily monitoring by visual appraisal of the water bottles was maintained throughout the study. Weekly water consumption was monitored and measured during selected intervals.

There were no significant test article-related effects on food consumption through the 93 week (high dosage males) or 104 week treatment period. Marginally lower food consumption was observed in the mid-dosage females.

The overall efficiency of food utilization during the 1<sup>st</sup> 24 weeks was inferior for all treated animals when compared to that of control. It was not in a dose-related trend in male groups and was severe in only high dosage males.

The overall water intake for high dosage males was higher than that of controls through the first half of the study up to Week 47 whereas the water intake for high dosage females was lower than that of concurrent controls between Weeks 27 to 55. The overall water intake at low and mid- dosages was generally unaffected.

## Hematology

Blood samples were taken from all animals, where possible, at sacrifice or at scheduled termination.

A marginal but statistically significant increase in total RBC was observed in the males and females treated at 405 mg/kg/day in Week 80. Such a change was not observed at termination. There was no significant test article-related effect on total WBC.

## Gross Pathology

The surviving high dosage males were sacrificed at the end of 93 weeks of treatment due to high mortality in this group. All other survivors were sacrificed on completion of at least 104 weeks of treatment. A complete gross pathology examination was conducted for all animals that were subject to unscheduled death or scheduled termination.

Daily Dose (mg base/kg/day)	0 (Control)		0 (Control)		20		90		405/180*	
	M:50	F:50	M:50	F:50	M:50	F:50	M:50	F:50	M:50	F:50
<b>Macroscopic pathology</b>										
<b>Lesions (all animals examined)</b>	50	50	50	50	50	50	50	50	50	50
Heart - Enlarged	2	0	4	0	2	0	7	1	22	18
Thrombus – left atrium	2	0	6	0	1	1	3	0	23	10
Pale areas – ventricle/s	0	0	3	0	0	0	2	0	1	5
Mediastinal Lymph Nodes – Enlarged	3	3	0	1	1	3	1	2	5	7
<b>Lesions (Decedent animals only) number examined</b>	28	30	28	21	29	29	30	27	40	38
Heart - Pale area/s – atrium/a	1	0	0	0	0	0	2	0	5	0
Lungs - Congested	7	5	4	1	6	9	5	7	17	18
Oedematous	0	0	1	1	2	0	0	0	4	6
Thoracic Cavity – Contained fluid	2	2	2	0	2	6	2	2	10	15
Adipose Tissue – Minimal	7	8	7	6	8	11	16	9	17	17

The macroscopic findings were summarized above (excerpted from summary table of the report). Most treatment related findings were observed in the high dosage male and female groups, which included an increased incidence of the heart enlargement, thrombus in the left atrium and pale areas on the atrium (males) and ventricles (females); an increased incidence of congestion and edema in the lungs; excess fluid present in the thoracic cavity; and enlargement of the mediastinal lymph nodes, and a reduction of the adipose tissue. The histopathology changes associated with these findings are discussed below.

A slightly increased incidence of the heart enlargement and a reduction of the adipose tissue were also noted in the mid-dose males.

## Histopathology

Samples of any lesions, and all the tissues listed below from all animals were preserved in buffered 10% formalin (except eyes, which were preserved in Davidson's fixative and testes/ epididymides which were fixed in Bouin's solution and then transferred to 70% alcohol). Tissues for microscopic examination were as specified in the list below. All

specified tissues from all mice in the control and high dosage groups, all specified tissues from unscheduled death or moribund sacrificed mice in the low and mid-dosage groups, and all tissues with lesions from mice in the low and mid-dosage groups at the termination were examined microscopically.

Peer Review: Yes

adrenal glands*	heart*	sciatic nerve
alimentary tract*	kidneys*	seminal vesicles*
(oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum)	larynx and pharynx	skeletal muscle
aorta*	liver*	skin
brain* (medullary, cerebellar and cerebral sections)	lungs* (all lobes and mainstem bronchi)	spinal cord* (cervical region)
clitoral gland	lymph nodes* (cervical and mesenteric)	spleen*
eyes*	mammary gland*	sternum* (for bone and marrow)
femur (with joint)*	other macroscopic abnormalities*	testes* (with epididymides)
gall bladder*	ovaries*	thymus* (where present)
Harderian gland*	pancreas*	thyroids* (with parathyroid glands)
head (to preserve nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lachrymal gland and Zymbal's gland)	pituitary gland*	tongue*
	preputial gland*	trachea*
	prostate*	urinary bladder*
	salivary glands* (submandibular)	uterus* (corpus and cervix)
		vagina*

\* Tissues required for histopathological examination

Neoplastic

There were no treatment-related neoplastic findings.

Based on Statistical Review and Evaluation by Dr. A. Rahman, there was no statistically significant dose response relationship in any of the observed tumor types in either gender. The pairwise comparison showed statistically significant increased incidence of lymphoid histiocytic sarcoma in the female low dosage group when compared to the combined control. The incidence of histiocytic sarcoma is shown below. Apparently, it was not dose-dependent and the incidence of histiocytic sarcoma observed in the male control group approached that in the low dosage female cohort. There was no histiocytic sarcoma observed in any other organs/tissues. The NTP carcinogenicity studies in rats and mice (TR-551, CAS No. 97-54-1) indicated that a historical control incidence range for histiocytic sarcoma (all tissues) in B6C3F1 female mice by all routes exposure is 0%-8% or 2.5%±2.5%. Taken together, the increased incidence of histiocytic sarcoma in the female low dose group was considered random, and not toxicologically significant.

	Male					Female				
Dose (mg/kg/day)	0	0	20	90	405/180	0	0	20	90	405/180
Incidence of histiocytic sarcoma in lymphoid	5	1	0	0	2	1	2	8	2	3

## Non Neoplastic

The noteworthy non-neoplastic histopathology changes were summarized below (excerpted from summary table of the report).

Daily Dose (mg base/kg/day)	0 (Control) <sup>1</sup>		0 (Control) <sup>2</sup>		20		90		405/180 <sup>a</sup>	
	M:50	F:50	M:50	F:50	M:50	F:50	M:50	F:50	M:50	F:50
<b>Histopathology – Non-Neoplastic Lesions</b>										
<b>Heart</b>										
Number hearts examined:	28	30	28	21	29	29	30	27	40	38
Atrial thrombus	1	0	3	0	2	1	7*	0	31***	28***
Dilated chambers	1	0	4	1	5	3	5	0	24***	24***
Myocardial degeneration	5	7	13*	2	11	12+	13*	11+	37***	36***
Myocardial vacuolation	18	13	18	6	18	11	21	10	37***	36***
Myocardial fibrosis	23	13	22	7	19	16	26	8	33	35***
Myocardial cell hypertrophy	0	0	2	0	3	0	5*	0	24***	19***
Epicardial inflammation and fibroblast proliferation	2	1	4	0	2	2	1	1	11*	23***
<b>Lungs</b>										
Number lungs examined:	28	30	28	21	29	29	30	27	40	38
Alveolar septal fibrosis	0	3	3	0	4	1	7**	0	17***	17***
Alveolar septal thickening and/or degeneration	0	2	3	1	2	3	5*	1	24***	25***
Alveolar eosinophilic material	1	1	2	0	3	2	4	1	20***	16***
Foamy alveolar macrophages	5	6	5	3	5	8	8	5	28***	26***
<b>Thymus</b>										
Number thymus examined	20	28	20	16	21	25	22	25	28	29
Involution/atrophy	10	11	11	4	12	9	16	7	24***	19**
<b>Spleen</b>										
Number spleens examined	28	30	28	21	29	29	30	27	40	38
Haemosiderosis	8	9+	3	1	6	6	4	2	15+	21**
<b>Liver</b>										
Number livers examined	28	30	28	21	29	29	30	27	40	38
Centrilobular hepatocyte vacuolation	12	11	9	6	12	12	7	8	18	31***
Sinusoidal dilatation/congestion	2	1	4	0	3	2	2	0	13*	12***

\*  $p < 0.05$ , \*\*  $p < 0.01$ , Fisher's Exact test compared to control<sup>1</sup>  
+  $p < 0.05$ , ++  $p < 0.01$ , Fisher's Exact test compared to control<sup>2</sup>  
<sup>a</sup> Dosage level was lowered from 405 mg base/kg/day to 180 mg base/kg/day from Week 81

Treatment related non-neoplastic microscopic findings, noted principally among decedent high dosage male and female mice, consisted of atrial thrombus, dilated chambers, minimal to moderate myocardial degeneration, minimal to moderate myocardial vacuolation, minimal to moderate myocardial fibrosis, minimal to slight myocardial cell hypertrophy, and minimal to slight epicardial inflammation and fibroblast proliferation. A lesser degree of increased incidence of myocardial degeneration and vacuolation was also observed in the decedent males treated at dose of 90 mg/kg/day. The significant changes observed in the lungs, liver, spleen and thymus of the decedent mice were associated with the myocardial toxicity and could be a consequence of heart failure. No other treatment related microscopic findings were observed.

## Toxicokinetics

Blood samples were collected from animals in the satellite groups on one day during Weeks 13, 26, 52, 80 and at termination. Samples were withdrawn at approximately 7, 10, 16 and 21 hours with different animals used at each time point.

The TK analysis was reported in a separate report (NP07946). The plasma exposure data of ivabradine (S16257) and its major metabolite (S18982) are summarized in the

table below (excerpted from report NP07946, pages 25-26).

20 mg base form/kg/day															
	Week 13			Week 26			Week 52			Week 80			Week 104		
	M	F	F/M	M	F	F/M	M	F	F/M	M <sup>(1)</sup>	F <sup>(2)</sup>	F/M	M <sup>(3)</sup>	F <sup>(4)</sup>	F/M
<b>C<sub>min,ss</sub></b> (ng/ml)	35.2	39.2	-	46.0	49.5	-	45.8	68.4	-	31.0	71.5	-	12.3	13.4	-
<b>C<sub>max,ss</sub></b> (ng/ml)	120	99.4	-	113	91.7	-	118	103	-	71.5	83.4	-	47.3	78.4	-
<b>t<sub>max</sub></b> (h)	21:00	07:00	-	21:00	21:00	-	21:00	21:00	-	21:00	10:00	-	21:00	21:00	-
<b>AUC<sub>24,ss</sub></b> (ng.h/ml)	2112	1789	0.847	1940	1641	0.846	1782	2037	1.14	1182	1845	1.56	730	1189	1.63
<b>% S 18982 (AUC<sub>24,ss</sub>)</b>	18.3	13.7	-	21.5	13.5	-	20.9	11.1	-	21.8	9.81	-	23.5	11.7	-

- (1) depending on the sampling time only 5 or 6 animals were sampled instead of 6
- (2) depending on the sampling time only 4 or 5 animals were sampled instead of 6
- (3) depending on the sampling time only 3 to 5 animals were sampled instead of 6
- (4) at each sampling time only 3 animals were sampled instead of 6

90 mg base form/kg/day															
	Week 13			Week 26			Week 52			Week 80			Week 104		
	M	F	F/M	M	F	F/M	M	F	F/M	M <sup>(1)</sup>	F <sup>(2)</sup>	F/M	M <sup>(3)</sup>	F <sup>(3)</sup>	F/M
<b>C<sub>min,ss</sub></b> (ng/ml)	185	146	-	278	153	-	176	209	-	232	213	-	107	93.3	-
<b>C<sub>max,ss</sub></b> (ng/ml)	806	380	-	466	375	-	478	493	-	359	421	-	380	526	-
<b>t<sub>max</sub></b> (h)	10:00	21:00	-	10:00	10:00	-	21:00	21:00	-	21:00	21:00	-	21:00	21:00	-
<b>AUC<sub>24,ss</sub></b> (ng.h/ml)	8588	7277	0.759	9208	6689	0.724	7827	8109	1.04	6802	7348	1.08	6010	8670	1.44
<b>% S 18982 (AUC<sub>24,ss</sub>)</b>	24.2	18.1	-	25.3	18.6	-	22.8	14.9	-	26.7	16.5	-	23.4	13.6	-

- (1) at each sampling time only 5 animals were sampled instead of 6
- (2) depending on the sampling time only 5 or 6 animals were sampled instead of 6
- (3) depending on the sampling time only 3 or 4 animals were sampled instead of 6

405 mg base form/kg/day												180 mg/kg/day			
	Week 13			Week 26			Week 52			Week 80			Week 104		
	M	F	F/M	M	F	F/M	M	F	F/M	M <sup>(1)</sup>	F <sup>(1)</sup>	F/M	M <sup>(1)</sup>	F <sup>(2)</sup>	F/M
<b>C<sub>min,ss</sub></b> (ng/ml)	908	737	-	759	1022	-	747	1041	-	820	1094	-	172	254	-
<b>C<sub>max,ss</sub></b> (ng/ml)	1534	1544	-	2884	2444	-	1482	2062	-	1290	2232	-	425	1085	-
<b>t<sub>max</sub></b> (h)	21:00	21:00	-	10:00	10:00	-	21:00	21:00	-	21:00	10:00	-	21:00	10:00	-
<b>AUC<sub>24,ss</sub></b> (ng.h/ml)	29195	27410	0.939	38698	40762	1.05	25489	36980	1.45	25022	43160	1.72	7920	14331	1.81
<b>% S 18982 (AUC<sub>24,ss</sub>)</b>	37.1	24.5	-	35.5	20.8	-	32.9	14.6	-	36.6	16.3	-	-	-	-

- (1) depending on the sampling time only 2 or 3 animals were sampled instead of 6 ; last week of treatment = week 93 instead of 104
- (2) at each sampling time only 3 animals were sampled instead of 6
- M : Male F : Female
- % S 18982 (AUC<sub>24,ss</sub>) : ratio of AUC<sub>24,ss</sub> S 18982/S 18257 expressed as percentage
- : not determined

Mean plasma exposure to ivabradine increased dose-proportionally in females, and slightly less than dose-proportionally in males. In females, AUC<sub>24h</sub> for ivabradine did not change over time. In males, mean plasma exposure decreased progressively over time, by up to 18% and 65% in Weeks 52 and 104, respectively, at low and mid-dose levels,

and to a lesser extent at the high dose level. Up to Week 26, there was no gender difference. At the end of the dosing period, mean plasma exposure was lower in males than in females. Exposure to metabolite S 18982 was about 15% and 25% that to ivabradine in female and male mice, respectively.

### Stability and Homogeneity

Analysis of samples taken at 3 monthly intervals from the diets administered to the animals indicated that the achieved concentrations were within -10%/+7.3% of nominal.

### Study title: S 16257-2: Potential Tumorigenic Effects in Prolonged Dietary Administration to Rats (Study No. NP07943)

Conducting laboratory/location: (b) (4)

Date of study initiation: November 12, 1996

GLP compliance: Yes

QA statement: Included

Drug, lot #, and % purity: S 16257-2, batch# 49 652, Purity: 100.4%  
(the conversion factor from free base to salt: 1.078)

CAC concurrence: Study protocol was not discussed in ECAC.

### Key Study Findings

#### Adequacy of Carcinogenicity Study

S16257-2 was administered by diet to Wistar Rats at dosages of 7.5, 30, and 120/60 mg/kg/day for up to 104 weeks. The study protocol was not submitted for a discussion/concurrence of the ECAC (see regulatory history).

There was no treatment-related effect on survival.

A body weight gain reduction in the 1<sup>st</sup> 52 weeks was observed in high dosage males up to -26% and in all treated female groups in a dose dependent manner up to -36% when compared with the combined controls. Overall for the period of 104 weeks, the group mean bodyweight gain was reduced up to -29% for treated males and -42% for treated females (in a dose-dependent manner) when compared with the combined controls.

Treatment-related non-neoplastic findings were observed in the heart and the lungs. There were increased incidence of heart enlargement in both mid-dose males and females, thrombus in the left atrium and fenestration of the ventricles in high dose males, and the ventricle fenestration in the high dose females. The associated histopathological changes included increased incidence/severity of myocardial fibrosis and systrophic mineralization/chondroid metaplasia in the chordae tendineae at doses  $\geq$  30 mg/kg/day. There were increased incidences of pale focus/i or areas in the lungs of

all treated male and female rats, most evident in the mid- and high dosage groups. The associated histopathological changes in the lung included increased incidence of aggregation of alveolar macrophages with focal septal thickening in the high dose males.

Significant body weight gain reduction and cardiac toxicity findings in the high dosage males and females indicate that a MTD was achieved..

The high dosage was associated with AUC<sub>24h</sub> of 7950 (23xhuman AUC<sub>24h</sub>) and 8436 ng.h/mL (24xhuman AUC<sub>24h</sub>) for male and female rats, respectively, at the week of 104.

### **Appropriateness of Test Models**

Dietary administration of test article to Wistar rats for up to 104 weeks is considered appropriate for tumorigenic potential evaluation.

### **Evaluation of Tumor Findings**

There were no treatment-related tumorigenic effects in ivabradine treated rats.

The statistically significant increased incidence of tubulostromal adenoma in ovaries of mid-dosage female rats was not dose-dependent pattern and within the published historical control incidence range in Wistar rats, hence, was not considered toxicologically significant.

The marginally increased incidence of uterine epithelial tumors, mainly uterine adenocarcinoma, in the treated female rats, associated with increased incidence of uterine masses, was not in a dose-dependent pattern and did not reach statistical significance whether incidence of individual tumor types or combined uterine epithelial tumors was analyzed.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 28, 2014 in Appendix 1).

## Methods

Doses: 7.5, 30, 120 (base) mg/kg/day<sup>a</sup> (week 1-52)/  
 7.5, 30, 60 (base) mg/kg/day<sup>a</sup> (week 53-104)

Frequency of dosing: Daily in diet  
 Dose volume: diet  
 Route of administration: diet  
 Formulation/Vehicle: Test article mixed with the diet<sup>b</sup>  
 /Normal untreated diet for rats

Basis of dose selection: The 13-week study (NP06450) with dietary administration at dosages of 20, 50, 100, 150 or 200 mg/kg/day showed a body weight gain decrease at doses  $\geq 100$  mg/kg/day and increased heart weight as well as provoking myocardial lesions at dosages  $\geq 20$  mg/kg/day. Therefore, a dosage of 120 mg/kg/day was chosen to elicit signs of toxicity and provide approximately 14-25 fold rat/human AUC ratio (estimated human AUC<sub>24h</sub> of 346 ng/h.mL) in males and >25-fold in females. The low dose of 7.5 mg/kg/day was chosen to achieve an AUC approximately equal to the AUC at MRHD. The high dosage was reduced to 60 mg/kg/day after 1 year of treatment due to the decrease in overall body weight gain in males (-26%) and females (-36%) when compared to the combined controls. In addition, a separate 52-week oral gavage rat study (NP07026) showed increased heart weight and myocardial lesions at dosage  $\geq 3$  (females) and  $\geq 16$  mg/kg bid (males). Mortalities were observed after Day 188 and all deaths in high dosage groups and 1 out of 2 deaths in the mid-dosage males were attributed to severe cardiac toxicity. The AUC<sub>24h</sub> at dose of 16 mg/kg bid in Week 52 was 4384 ng.h/mL (male) and 12426 ng.h/mL (female) which was lower than or similar to the AUC of high dosage male or female rats, respectively, in Week 52 of this 2-year carcinogenicity study.

Species/Strain: HanIbm Wistar Rat  
 Number/Sex/Group: 50  
 Age/Weight: ~8 weeks/male:134-179 g; female:101-140 g  
 Animal housing: 5 rats of the same sex/cage for main group  
 3 rats of the same sex/cage for satellite group  
 Paradigm for dietary restriction: All rats had free access to tap water and ground SDS Rat and Mouse No. 1 modified maintenance diet.

Dual control employed: Yes  
 Interim sacrifice: No  
 Satellite groups: 6/sex/group for TK profiling  
 Deviation from study: No deviations from the study protocol and

protocol: amendments were considered to affect the integrity of the study

- a. Food consumption and test article control data indicated that the actual doses received were 7.4, 30 and 119/59 mg/kg/day for males and 7.5, 30 and 119/60 mg/kg/day for females.
- b. Concentration of test article in the diet were changed as necessary to preserve the dosage levels (i.e. in line with body weight changes and food consumption)

## Observations and Results

### Mortality and Clinical Signs

Animals were observed at least once weekly for mortality and morbidity. A detailed palpation of each rat was performed once weekly for any palpable masses.

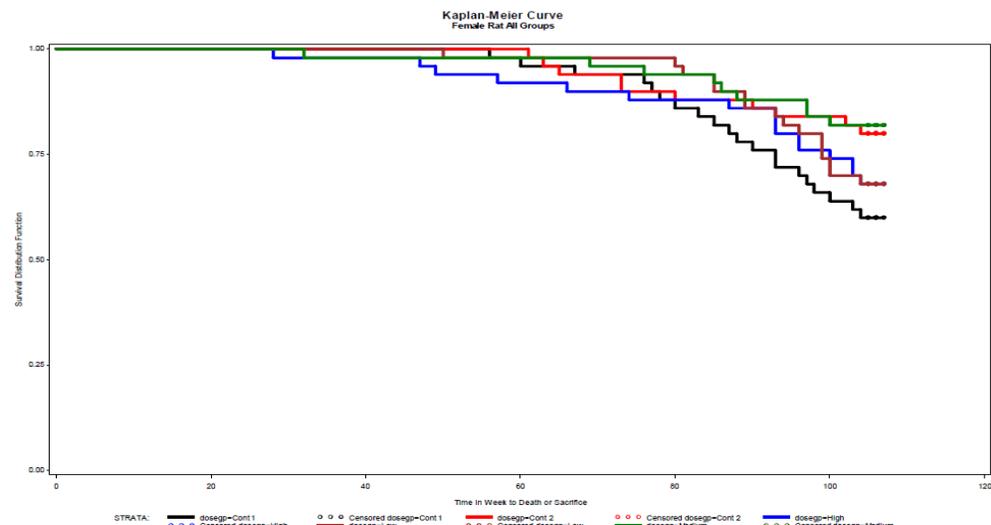
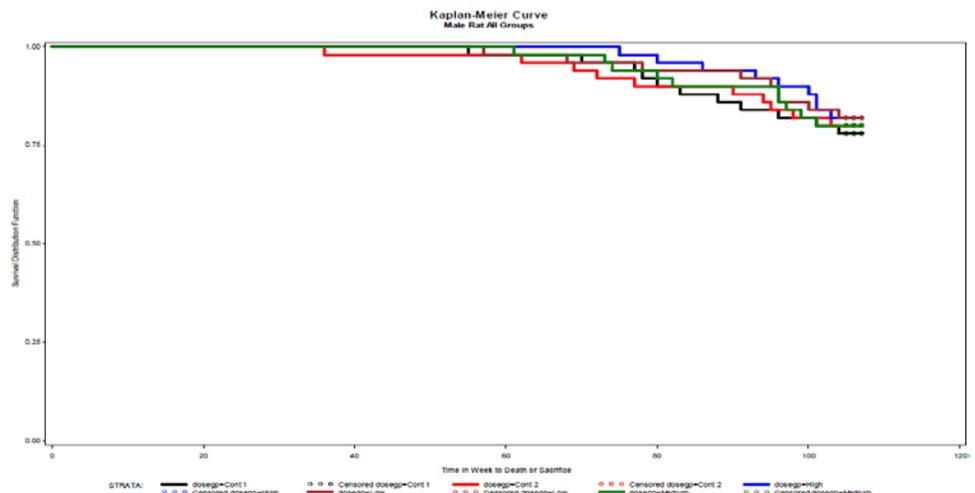
The distribution of unscheduled deaths during the study period was summarized below (excerpted from page 32 of the report). There were no treatment-related effects on the incidence or distribution of unscheduled deaths between control and treated groups over the 104 week treatment period.

Number decedents weeks	Group/dosage (mg base/kg/day)									
	1M Control	2M Control	3M 7.5	4M 30	5M 120/60†	1F Control	2F Control	3F 7.5	4F 30	5F 120/60†
1 - 52	0	1	0	0	0	0	0	1	1	3
53 - 104	11	9	9	10	9	20	10	15	8	13
1 - 104	11	10	9 (2)	10 (2)	9 (3)	20	10	16 (6)	9 (4)	16 (4)
Main group mortality (%)	22	20	18	20	18	40	20	32	18	32
Main group survival (%)	78	80	82	80	82	60	80	68	82	68

( ) Satellite group animals

† Dosage was lowered from 120 mg base/kg/day to 60 mg base/kg/day from Week 53

The survival analysis, as shown in the figure below (excerpted from Statistical Review by Dr. Rahman), did not reveal statistically significant dose response relationship in mortality across the treated and the combined control groups and the pairwise comparison did not show statistically significant increased mortality in treated groups when compared to the combined control.



There were no test article-related adverse clinical observations.

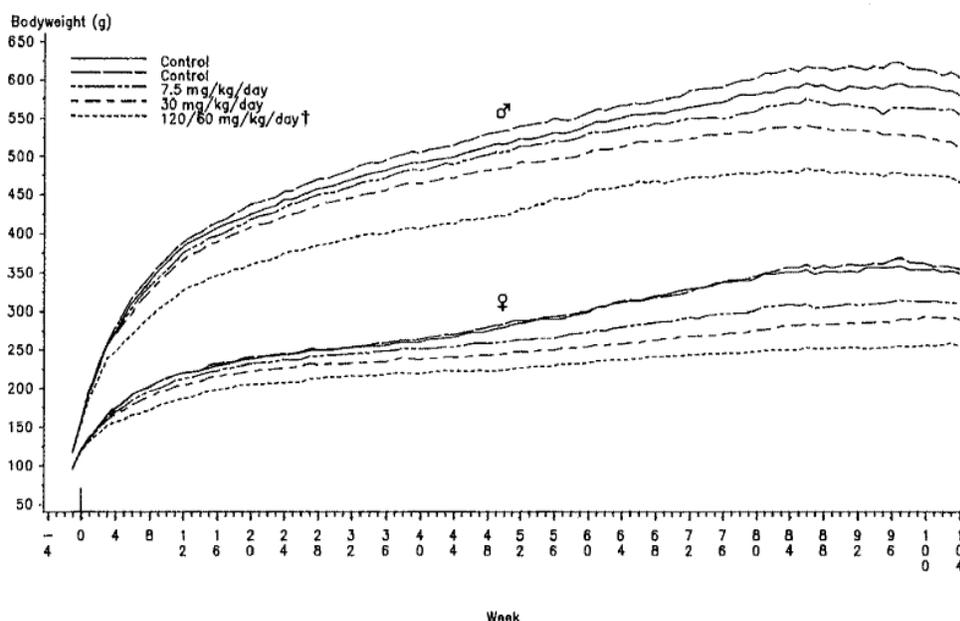
### Body Weights

Body weight was collected at randomization, one week prior to and on the day of initial treatment, and once a week thereafter.

*Body weight gain (g) (% of combined control)	Dose Mg/kg/day	Male					Female				
		0	0	7.5	30	120/60	0	0	7.5	30	120/60
Week 0-52		368	385	362 (96)	338 (90)	277 (74)	166	168	145 (87)	124 (74)	107 (64)
Week 53-104		56	63	42 (95)	17 (31)	34 (66)	64	67	45 (70)	49 (59)	28 (39)
<b>Week 0-104</b>		424	448	404 (92)	355 (81)	311 (71)	230	235	190 (82)	173 (74)	135 (58)

\*The body weight gain was calculated based on the mean body weight at Week 0, Week 52 and Week 104.

As shown in the table above and figures (excerpted from page 43) below, during the first 52 weeks of treatment, a reduction in group mean bodyweight gain was noted in all treated female groups in a dose-related pattern up to -36% and in mid- and high dose males up to -26% when compared to that of combined controls. From Week 53, the high dose level was decreased from 120 to 60 mg/kg/day. Overall (Weeks 52 to 104) body weight gains in the treated groups remained lower than that of the combined controls. Group mean bodyweight gain for the treated female groups, over this period, also remained lower than that of concurrent combined controls, with the values following a dose-related trend. Overall in the period of 104 weeks, the group mean bodyweight gain among all male and female treated groups was reduced up to -29% for males and -42% for females in a dose-dependent manner when compared with the combined controls.



## Feed Consumption

The quantity of food consumed by each cage of rats was recorded weekly. Food intake per rat (g/rat/week) was calculated as [(total food given-total food left)/number of animal days] x 7. Food conversion ratios were calculated, where possible, over the period Weeks 1 to 26, from the bodyweight and food consumption data as weight of food consumed per unit gain in bodyweight. Daily monitoring by visual appraisal of the water bottles was maintained throughout the study. Weekly water consumption was monitored in the selected intervals.

During the first 52 weeks of treatment a dosage related reduction in food consumption was noted among male and female treated groups when compared to that of combined controls. From Week 53, when the high dosage was reduced to 60 mg/kg/day, there was a slight improvement in food consumption for both sexes at this dosage (Weeks 53 to 60), although the mean food intakes remained significantly lower than that of the

concurrent combined controls. Food consumption for both sexes remained significantly lower among rats receiving mid and low dosages during this period.

Over the 104 week treatment, mean food consumption for male and female treated groups was lower than that of their respective combined controls in a dose-dependent pattern and with all the differences attaining statistical significance.

The overall efficiency of food utilization during the 1<sup>st</sup> 26 weeks was marginally inferior for males and females treated at 120 mg/kg/day, which reflected the lower body weight gain for these animals without a commensurately lower food consumption.

The mean water intake in the high dose males and females as well as that in the mid-dose females was generally lower than their respective controls. The water intake in the other treated groups was comparable to the respective controls over the study period.

## **Hematology**

Blood samples were taken from all animals, where possible, at sacrifice or at scheduled termination.

A slight but statistically significant decrease in total WBC was observed in the mid- and high dosage males in Week 104 (5.33, and 4.77 x10<sup>9</sup>/L, respectively), when compared to the control (5.70-6.30x10<sup>9</sup>/L). There were no significant changes of the measured parameters in other treated groups.

## **Gross Pathology**

All surviving main group animals were sacrificed on completion of at least 104 weeks of treatment. A complete gross pathology examination was conducted for all animals with unscheduled death or scheduled termination. The major organ weights of the rats subject to unscheduled termination were recorded at discretion of the pathologist. Organ weights were reported when necessary to clarify macroscopic findings.

The macroscopic findings are summarized below. There were treatment related findings observed in the heart, lung and uterus. The histopathology changes associated with these findings are discussed in the next section.

In the heart, there were increased incidences of the heart enlargement observed in the mid- and high dosage males and females, thrombus in the left atrium in the high dose males, and fenestration of the ventricles observed in both genders at mid- and high dosages as well as in the low dosage males.

In the lung, increased incidences of pale focus/i or areas were observed in all treated groups, especially in the mid- and high dose groups.

In the uterus, excess mass/es were observed in the high dosage rats.

In the spleen, the marginal increased incidence of the enlargement was observed in mid- and high dosages but without associated histopathologic changes.

Males on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	50		50		50		50		50	
Animals completed	Decedent	Terminal								
	12	38	10	40	9	41	10	40	9	41
<b>Lungs</b>										
Mass/es	0	0	0	1	0	0	0	1	0	0
Raised focus/i	0	0	0	1	0	0	0	0	0	1
Pale area/s or focus/i	4	6	2	6	5	7	6	11	4	21
Petechiae	0	19	1	23	0	17	1	5	0	5
Congested	4	1	3	2	4	2	3	8	5	3
Dark focus/i	1	0	0	2	0	0	0	0	1	0
<b>Heart</b>										
Enlarged	0	0	1	0	0	0	1	5	5	14
Thrombus - left atrium	0	0	0	0	0	0	0	0	1	1
Fenestrated - ventricle/s	0	0	0	1	0	2	0	3	0	12
Pale	1	0	0	1	0	0	0	2	1	3
Pale area/s - atrium	0	0	0	0	0	0	0	0	0	1
<b>Spleen</b>										
Enlarged	2	8	2	6	2	9	3	8	3	11
Pale area/s	1	0	0	0	0	0	0	0	0	0

Females on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	50		50		50		50		50	
Animals completed	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal
	20	30	10	40	16	34	9	41	16	34
<b>Lungs</b>										
Mass/es	0	0	0	0	0	0	0	0	1	0
Nodule/s	0	1	0	1	0	0	0	0	0	0
Raised focus/i	0	0	0	0	0	0	0	0	1	0
Pale area/s or focus/i	6	12	3	12	8	13	2	24	8	17
Petechiae	0	18	0	18	0	20	0	13	0	2
<b>Lungs</b>	(continued)									
Congested	5	3	3	4	6	4	3	12	6	5
Dark focus/i	1	0	0	0	3	0	0	0	0	0
<b>Heart</b>										
Enlarged	1	2	0	2	1	1	0	7	4	10
Fenestrated - ventricle/s	0	0	0	0	0	0	0	0	0	3
Pale	0	1	0	2	0	1	0	1	1	1
<b>Spleen</b>										
Enlarged	4	6	1	7	2	4	5	11	8	3
Pale	1	0	0	0	0	1	1	0	0	0
<b>Uterus</b>										
Mass/es	3	5	3	5	4	6	1	6	3	9
Pale raised area/s	1	0	0	0	1	0	0	0	0	0
Swelling/s	1	4	0	2	0	3	0	1	2	4
Fluid swelling/s	2	4	0	5	3	8	1	7	2	7
Fluid distension	1	1	0	1	1	1	0	2	1	2
Thickened	1	0	0	1	0	0	0	0	0	0

### Histopathology

Samples of any lesions, and all the tissues listed below (excerpted from page 29) from all animals were preserved in buffered 10% formalin (except eyes, which were preserved in Davidson's fixative and testes/ epididymides which were fixed in Bouin's solution and then transferred to 70% alcohol). All specified tissues (marked as\* below) from all rats in the control and high dose groups, all specified tissues from unscheduled death or moribund sacrificed rats in the low and mid-dose groups, and all tissues with lesions from rats in the low and mid-dose groups at the termination, were examined microscopically.

Peer Review: Yes

adrenal glands*	heart*	sciatic nerve
alimentary tract*	kidneys*	seminal vesicles*
(oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum)	larynx and pharynx	skeletal muscle
aorta*	liver*	skin
brain* (medullary, cerebellar and cerebral sections)	lungs* (all lobes and mainstem bronchi)	spinal cord* (cervical region)
clitoral gland	lymph nodes* (cervical and mesenteric)	spleen*
eyes*	mammary gland*	sternum* (for bone and marrow)
femur (with joint)*	other macroscopic abnormalities*	testes* (with epididymides)
gall bladder*	ovaries*	thymus* (where present)
Harderian gland*	pancreas*	thyroids* (with parathyroid glands)
head (to preserve nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lachrymal gland and Zymbal's gland)	pituitary gland*	tongue*
	preputial gland*	trachea*
	prostate*	urinary bladder*
	salivary glands* (submandibular)	uterus* (corpus and cervix)
		vagina*

\* Tissues required for histopathological examination

Neoplastic

There were no treatment-related neoplastic findings.

Based on the statistical review by Dr. A. Rahman, there was no statistically significant dose response relationship in any observed tumor types in either gender. However, the pairwise comparison showed statistically significant increased incidence of tubulostromal adenoma in ovaries of mid-dose female rats when compared to the combined control (see the table below). According to the literature report (<sup>ii</sup>Ref. *Carlus M et al 2013*) with eight 2- year carcinogenicity studies in Wistar rats, the ovary tubulostromal adenoma could be observed in control rats at incidence of 3% with a range of 0-6.7%. Given that the incidence of this finding was not dose-dependent and was within the range of 0-6.7%, it was not considered toxicologically significant.

<b>Female rats, Dose (mg/kg/day)</b>	<b>0</b>	<b>0</b>	<b>7.5</b>	<b>30</b>	<b>120/60</b>
<b>Ovaries:</b> tubulostromal adenoma	0	0	1	3	0
<b>Uterine:</b>					
Endometrial adenoma	0	0	1	0	1
Endometrial adenocarcinoma	3	5	6	4	8
Uterine carcinoma (anaplastic)	1	0	0	0	0
<i>Combined uterine epithelial tumor</i>	4	5	6	4	9

As listed in the table above, uterine epithelial tumors, mainly adenocarcinomas, were observed in control and treated females, and associated with the uterine masses reported in the gross pathology evaluation. The historical control data from the CRO is

listed below (excerpted from page 35 of the report). The incidence of uterine epithelial tumors in both control and treated groups fell at the upper limit or outside of the historical range. Based on FDA statistical analysis of uterine tumors, pairwise comparison showed that incidence of endometrial adenocarcinoma alone or combined epithelial tumors including endometrial adenoma, endometrial adenocarcinoma and uterine carcinoma (anaplastic) of the high dose females was not statistically significantly different from that of the combined control.

**Uterine epithelial tumours in HAN Wistar control group rats**  
at (b) (4)

Study code	9220	9221	93A	93B	9402
Endometrial adenoma	0	0	0	0	1
Endometrial adenocarcinoma	4	1	2	2	1
Uterine carcinoma (anaplastic)	0	0	0	0	0
Number of uteri examined	50	50	55	55	55

**Uterine epithelial tumours in HAN Wistar control group rats**  
at (b) (4)

Study code	017	019a	019b	20a	20b	20c	22a	22b	23a	23b
Endometrial adenoma	1	0	2	0	0	0	0	1	0	1
Endometrial adenocarcinoma	1	1	0	3	0	0	2	3	1	4
Uterine carcinoma (anaplastic)	0	0	0	0	0	0	0	0	0	0
Number of uteri examined	50	50	50	55	55	55	50	50	60	60

Non-Neoplastic

The noteworthy non-neoplastic histopathology changes are summarized below.

An increased incidence of minimal to moderate myocardial fibrosis was observed at high dosage in both genders as well as at mid-dosage in females. Excess dystrophic mineralization/chondroid metaplasia in chordae tendineae was observed in all treated males and at mid- and high dosage in females. These findings were consistent with gross pathology lesions in the heart.

Group	Male					Female				
	1	2	3	4	5	1	2	3	4	5
Dose level (mg base/kg/day)	0	0	7.5	30	120/60A	10	0	7.5	30	120/60A
Myocardial fibrosis										
Total	35	39	31	37	45*	20	10	19	32*	35**
Minimal	21	28	22	28	22	18	10	18	24	16
Slight	10	10	8	8	19*	2	0	1	8*	15**
Moderate	4	1	1	0	4	0	0	0	0	4
Marked	0	0	0	1	0	0	0	0	0	0
Dystrophic mineralisation/chondroid metaplasia in chordae tendineae	1	0	2	4	16**	0	0	0	6*	15**
Number of hearts examined	50	50	50	50	50	50	50	50	50	50

\*  $p < 0.05$  \*\*  $p < 0.01$  with Fisher's Exact test when compared to Group 1  
A- dose level reduced to 60 mg base/kg/day at Week 53

An increased incidence of aggregations of alveolar macrophages with focal septal thickening was observed in the lungs of males at mid- and high dosages, consistent

with the gross pathology findings in the lungs. There were no significant findings in the lungs of the treated females.

Group	1	2	Male 3	4	5
Dose level (mg base/kg/day)	0	0	7.5	30	120/60A
Aggregations of alveolar macrophages with focal septal thickening	14	13	11	17	25*
Number of lungs examined	50	50	50	50	50

\*  $p < 0.05$  with Fisher's Exact test  
 A- dose level reduced to 60 mg base/kg/day at week 53

There were no other significant gross or microscopic non-neoplastic lesions..

### Toxicokinetics

Blood samples were collected from animals in the satellite groups on one day during Weeks 13, 26, 52, 78 and 105 (satellite group animals continued to receive the test article I diet until the day of termination). Samples were withdrawn at approximately 7, 16 and 22 hours.

The plasma exposure data of ivabradine (S16257) is summarized in the table below (excerpted from study report NP07945, page 28). Mean plasma exposure increased dose-proportionally in both genders, and was higher in females than in males throughout the study. No time-effect was observed in Week 13 to Week 105 interval for males. Exposure in low-dosage females was gradually decreased by approximately 42% in this interval. Most of S18982 concentrations were below the limit of quantitation (i.e. 2.5 ng/ml).

7.5 mg/kg/day (expressed as base form)																		
Week 13			Week 25			Week 39			Week 52			Week 78			Week 104			
M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	
$C_{min,ss}$ (ng/ml)	18.8	40.7	-	18.1	47.6	-	12.3	24.3	-	11.2	41.3	-	15.4	23.2	-	27.4	20.86	-
$C_{max,ss}$ (ng/ml)	33.7	88.0	-	29.7	85.5	-	23.4	73.8	-	20.4	58.5	-	29.9	48.0	-	47.5	48.1	-
$t_{max}^*$ (h)	7:00	22:00	-	7:00	22:00	-	16:00	14:30	-	7:00	11:30	-	7:00	16:00	-	7:00	7:00	-
$AUC_{24,ss}$ (ng.h/ml)	628	1470	2.34	581	1595	2.75	452	1282	2.84	380	1249	3.28	544	816	1.50	888	859	0.968

30 mg/kg/day (expressed as base form)																		
Week 13			Week 26			Week 39			Week 52			Week 78			Week 104			
M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	
$C_{min,ss}$ (ng/ml)	42.9	102	-	30.9	157	-	35.4	95.8	-	26.5	160	-	39.5	84.0	-	196	86.0	-
$C_{max,ss}$ (ng/ml)	98.5	345	-	116	251	-	114	371	-	102	226	-	212	259	-	342	246	-
$t_{max}^*$ (h)	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-
$AUC_{24,ss}$ (ng.h/ml)	1733	5537	3.20	1781	4776	2.71	1711	5321	3.11	1505	4545	3.02	2766	4026	1.46	6376	3984	0.625

	120 mg/kg/day (expressed as base form)												60 mg base form/kg/day					
	Week 13			Week 26			Week 39			Week 52			Week 78			Week 104		
	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M
$C_{min,ss}$ (ng/ml)	247	329	-	231	671	-	125	286	-	119	316	-	108	79.4	-	201	176	-
$C_{max,ss}$ (ng/ml)	470	854	-	515	1153	-	432	1279	-	424	744	-	338	426	-	480	594	-
$t_{max}^*$ (h)	7:00	7:00	-	7:00	18:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-
$AUC_{24,ss}$ (ng.h/ml)	8680	14870	1.71	8860	22181	2.50	6466	16616	2.57	6329	12823	2.03	5151	5987	1.16	7950	8435	1.06

## Stability and Homogeneity

Analysis of samples taken at 3 monthly intervals from the diets administered to the animals indicated that the achieved concentrations were within -18%/+3% of nominal.

## 10 Special Toxicology Studies

### 11 Integrated Summary and Safety Evaluation

The carcinogenicity potential of ivabradine (S16257-2) was assessed in 104-Week dietary studies in CD-1 mice and Wistar rats.

#### Mouse

S16257-2 was administered by diet to CD-1 mice at dosages of 20, 90, and 405/180 mg/kg/day for up to 104 weeks. The high dose was reduced to 180 mg/kg/day due to the high mortality observed in the high dose males and females during the first 80 weeks of treatment. The high dose male group was terminated early at the end of Week 93 when survival reached 20%.

Significantly reduced survival rate was observed in the high dose males and females.

A body weight gain reduction in the 1<sup>st</sup> 80 weeks was observed in high dose males (-36%) and in all treated female groups with a dose-dependent manner (up to -33%). Body weight gain reduction was up to -38% over the treatment period (Week 1-93) for high dose males. The overall body weight gain for all treated females during Week 1-104 was reduced about -13% to -23% without dose-dependence.

Higher mortality and body weight gain reduction in the high dose males and females indicated that at least an MTD was achieved. The route (diet) and the duration of 104 weeks (93 weeks for high dose male group) are considered acceptable.

Treatment-related non-neoplastic findings, primarily cardiac, included atrial thrombus, dilated chambers, minimal to moderate myocardial degeneration, vacuolation and fibrosis, and minimal to slight myocardial cell hypertrophy, epicardial inflammation and fibroblast proliferation observed in the high dose males and females. A lesser degree of

increased incidence of myocardial degeneration and vacuolation was also observed in the decedent mid-dosage males. The pathology changes in the lungs, liver, spleen and thymus of the decedent mice were associated with cardiac toxicity. The increased mortality was attributed to the cardiac toxicity.

There were no treatment-related tumorigenic effects in ivabradine treated mice.

The statistically significant increased incidence of lymphoid histiocytic sarcoma in the female low dosage group only was not dose-dependent, similar to the incidence in the male control group, and not observed in any other tissues/organs, hence, was not considered toxicologically significant.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 28, 2014).

The plasma exposures of ivabradine and the human exposure multiples in the 104-week mouse study are summarized in the tables below (excerpted from Section 2.6.6. page 59, Table 38).

**Table 38. Ivabradine and S 18982 Mean Plasma AUC<sub>24</sub> in CD-1 Mice After 104-week Oral Dosing**

Dose (mg/kg/d)	AUC <sub>24</sub> (ng.h/ml)							
	ivabradine				S 18982			
	Males		Females		Males		Females	
	Week 104	Multiple of hAUC <sub>24</sub>	Week 104	Multiple of hAUC <sub>24</sub>	Week 104	Multiple of hAUC <sub>24</sub>	Week 104	Multiple of hAUC <sub>24</sub>
20	730	2	1189	3	172	1	140	1
90	6010	17	8670	25	1406	11	1179	9
405 <sup>a</sup>	2502 2	72	43160	125	9164	72	7033	55
180 <sup>b</sup>	7920	23	14331	41	nd		nd	

n=6/gender/dose/sampling time <sup>a</sup> week 80 <sup>b</sup> week 93. AUC<sub>24</sub> = Area under the concentration curve over 24 hours; hAUC<sub>24</sub> = mean plasma exposure at steady state over 24 hours in patients at the highest therapeutic dose.

### Rat

Ivabradine (S16257-2) was administered by diet to Wistar Rats at doses of 7.5, 30, and 120/60 mg/kg/day for up to 104 weeks. The high dosage was reduced to 60 mg/kg/day from Week 53 due to significant body weight gain reduction in the first 52 weeks and mortality with severe cardiac toxicity observed in a separate 52-week rat oral gavage study in which the exposures at a dosage of 16 mg/kg bid were lower than or similar to the AUC of the high dose male or female rats, respectively, by Week 52 in this carcinogenicity study.

There were no treatment-related effects on survival.

In the first 52 weeks, significant body weight gain reduction was observed in the high dosage males (-26%) and in all treated female groups in a dose-dependent manner up to -36%. Overall (Weeks 0 to 104), a dose-dependent mean bodyweight gain reduction was up to -29% for treated males and -42% for treated females when compared to the respective combined controls.

Treatment-related non-neoplastic findings were observed in the heart and the lungs. There were increased incidences of heart enlargement in both mid-dose males and females, thrombus in the left atrium and fenestration of the ventricles in high dose males, and ventricle fenestration in high dose females. The associated histopathological changes of the heart included increased incidence/severity of myocardial fibrosis and systrophic mineralization/chondroid metaplasia in the chordae tendineae in the rats treated at dosages  $\geq 30$  mg/kg/day. There were increased incidences of pale focus/i or areas in the lungs of all treated cohorts, especially in the mid- and high dose groups. The associated histopathological changes included increased incidence of aggregation of alveolar macrophages with focal septal thickening in the high dose males.

Significant body weight gain reduction and cardiac toxicity findings in the high dose rats suggest that a MTD was achieved in this study. The route (diet) and the duration of 104 weeks are considered acceptable.

There were no treatment-related tumorigenic effects in ivabradine treated rats.

The statistically significant increased incidence of tubulostromal adenoma in ovaries of mid-dose female rats was not dose-dependent, and within the published control incidence range in Wistar rats, hence was not considered toxicologically significant.

The marginally increased incidence of uterine epithelial tumors, mainly uterine adenocarcinoma, in the treated female rats, associated with increased incidence of uterine masses, was not dose-dependent and did not reach statistical significance whether incidence of individual tumor types or combined uterine epithelial tumors was analyzed.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 28, 2014).

The plasma exposures of ivabradine and the human exposure multiples in the 104-week rat study are summarized in the tables below (excerpted from Section 2.6.6. page 61, Table 40)

**Table 40. Ivabradine Mean Plasma AUC<sub>24</sub> in Wistar Rats After 104-week Oral Dosing**

Dose (mg/kg/d)	AUC <sub>24</sub> (ng.h/ml)			
	Males		Females	
	Week 104	Multiple of hAUC <sub>24</sub>	Week 104	Multiple of hAUC <sub>24</sub>
7.5	888	3	859	2
30	6376	18	3984	12
120 <sup>a</sup>	6329	18	12823	37
60	7950	23	8436	24

n=5/gender/dose/sampling time <sup>a</sup> week 52. AUC<sub>24</sub> = Area under the concentration curve over 24 hours;  
hAUC<sub>24</sub> = mean plasma exposure at steady state over 24 hours in patients at the highest therapeutic dose.

### Conclusion

Ivabradine was not tumorigenic in the 104-Week carcinogenicity studies with dietary administration in CD-1 mice and Wistar rats. The statistically significant increase in the incidences of lymphoid histiocytic sarcoma in the low dose female mice and tubulostromal adenoma in ovaries of mid-dose female rats are not considered toxicologically significant.

## 12 Appendix/Attachments

### Appendix 1: EXEC CAC MINUTES

<sup>i</sup> TR-551 CAS No. 97-54-1: Toxicology and Carcinogenesis Studies of Isoeugenol in F344/N Rats and B6C3F1 Mice (Gavage Studies)

<sup>ii</sup> *Marin Carlus et al: Historical Control Data of Neoplastic Lesions in the Wistar Hannover Rat among Eight 2-year Carcinogenicity Studies. Experimental and Toxicologic Pathology 65, 2013: 243-253*

Appendix 1 is 3 pages of the Duplicate Executive CAC Meeting Minutes dated 8/28/14 that can be found in the Administrative Correspondence Section of this Approved NDA. Please refer to this section for these Meeting Minutes.

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/s/  
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ADELE S SEIFRIED  
08/28/2014

PAUL C BROWN  
08/28/2014

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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JEAN Q WU  
11/18/2014

ALBERT F DEFELICE  
11/18/2014

**Appendix 3**  
**Pharmacology/Toxicology NDA Review**  
**Evaluation of Reproductive and Developmental Toxicology**

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW  
EVALUATION (Reproductive and Development Toxicology)

Application number: NDA 206,143  
Supporting document/s: S016 (eCTD0014), S022 (eCTD0017)  
Applicant's letter date: April 30, 2014, June 27, 2014  
CDER stamp date: April 30, 2014, June 27, 2014  
Product: Ivabradine  
Indication: To reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm), (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)  
Applicant: Amgen, Inc.  
Review Division: Division of Cardio-Renal Products  
Reviewer: Jean Q. Wu  
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Division Director: Norman Stockbridge  
Project Manager: Alexis Childers

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# 1 Executive Summary

## 2 Drug Information

### 2.1 Drug

#### 2.1.1 CAS Registry Number (Optional)

Ivabradine: 155974-00-8

Ivabradine hydrochloride: 148849-67-6

#### 2.1.2 Generic Name

ivabradine

#### 2.1.3 Code Name

S 16257-2; AMG 998

#### 2.1.4 Chemical Name

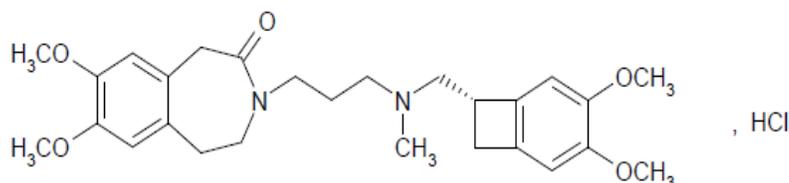
3-(3-(((7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl) methyl amino)propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride

#### 2.1.5 Molecular Formula/Molecular Weight

$C_{27}H_{36}N_2O_5$ , HCl/505.1 g/mol;

468.593 g/mol (base); Conversion factor from the salt to the base: 0.928

#### 2.1.6 Structure



#### 2.1.7 Pharmacologic class

hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND (b) (4)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

Ivabradine is provided as film-coated tablet in two strengths: 5 mg (oval) and 7.5 mg (triangular). The composition of the drug is listed in the table below (excerpted from Module 2.3. P, Table 1, Page 7)

Table 1. Composition of Ivabradine 5 mg and 7.5 mg Tablets

Component	5 mg		7.5 mg		Function	Reference to specifications
	Percentage (% w/w)	Quantity (mg/tablet)	Percentage (% w/w)	Quantity (mg/tablet)		
Tablet						
Ivabradine hydrochloride <sup>a</sup> (free base equivalent)	5.39 (5.00)	5.390 (5.000)	8.085 (7.50)	8.085 (7.500)	Drug substance	In-house
Lactose monohydrate	(b) (4)				(b) (4)	USP/NF, PhEur.
Maize starch	(b) (4)				(b) (4)	USP/NF
Maltodextrin	(b) (4)				(b) (4)	USP/NF, PhEur
Magnesium stearate	(b) (4)				(b) (4)	USP/NF, PhEur
Colloidal silicon dioxide	(b) (4)				(b) (4)	USP/NF, PhEur
(b) (4)	(b) (4)				(b) (4)	USP
Core Tablet Total	100.0	100.0	100.0	100.0		
Film-Coating						
(b) (4) salmon <sup>c</sup>	(b) (4)				(b) (4)	See 3.2.P.4.1
Polyethylene glycol 6000 (b) (4)	(b) (4)				(b) (4)	USP/NF, PhEur USP
Total	102.0	102.0	102.0	102.0		

<sup>a</sup> The molecular weights of Ivabradine hydrochloride anhydrous and ivabradine free base are 505.1 g/mol and 468.6 g/mol, respectively. The free base accounts for 92.77% of the salt

(b) (4)

## 2.4 Proposed Clinical Population and Dosing Regimen

Ivabradine is indicated to reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm) (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)

The proposed starting dose of ivabradine is 5 mg twice daily. After 2 weeks of treatment, if heart rate is between 50 and 60 bpm, the dose of 5 mg twice daily should be maintained. The dose should be increased to 7.5 mg twice daily if resting heart rate is persistently above 60 bpm.

As listed in the table below (excerpted from Module 2, Section 2.4, Page 7), the human plasma exposures of ivabradine and its N-desmethylated metabolite (S 18982) were estimated at steady state in patients receiving the maximum recommended human dose (MRHD), 7.5 mg bid. These values were derived from a population pharmacokinetic analysis using Phases II and III clinical data [Summary of Clinical Pharmacology Studies, in Section 2.7.2 (2.1.2.2)], and are accepted as references when preclinical doses are expressed as multiples of human exposures in current review, unless otherwise indicated.

**Table 1. Ivabradine and S 18982 (Metabolite) Plasma C<sub>max</sub> and Estimated AUC<sub>24</sub> at Steady State in Patients at HTD**

	Ivabradine (n=492)	S 18982 (n=541)
Population C <sub>max</sub> (ng/ml)	31 ± 9.8	7.9 ± 2.3
Equivalent C <sub>max</sub> in μM	0.07	0.02 <sup>b</sup>
Population AUC <sub>24</sub> <sup>a</sup> (ng.h/ml)	346	128

Values are mean ± SD;

a: Calculated from AUC over 12 h period from [WS (2.7.2) 3.1/ Table 12; Table 34 and 35]

b: S 18982 MW = 454.6

## 2.5 Regulatory Background

There was no IND opened in the US FDA

(b) (4)

(b) (4)

## 3 Studies Submitted

### 3.1 Studies Reviewed

NP06673: Oral reproduction toxicity study in the Wistar rat (fertility and early embryonic development)

NP05135: Oral reproduction toxicity study in the Wistar rat (embryofetal development)

NP26980: Oral reproduction toxicity study in the Wistar rat (embryofetal development)-supplementary

NP05134 Oral reproduction toxicity study in the New Zealand White rabbit (embryofetal development)

NP08073: Oral reproduction toxicity study in the rabbit (complementary investigation on embryofetal development)—nonGLP

NP07356: S 16257-2: oral reproduction toxicity study in the Wistar rat (pre- and postnatal development)

(b) (4)

*The following non-pivotal studies were cursory reviewed along with the relevant pivotal studies:*

NP08074: Oral preliminary reproduction toxicity study in the Wistar rat (male and female fertility/pre- and postnatal development)

NP08075: Oral preliminary reproductive toxicology study in the rat, Segment II: embryo-fetotoxicity and teratogenicity

NP08076: Oral preliminary reproductive toxicology study in the rabbit, Segment II: embryo-fetotoxicity and teratogenicity

(b) (4)

(b) (4)

**3.2 Studies Not Reviewed**

none

**3.3 Previous Reviews Referenced**

N/A

## 4 Pharmacology

## 5 Pharmacokinetics/ADME/Toxicokinetics

## 6 General Toxicology

## 7 Genetic Toxicology

## 8 Carcinogenicity

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

Study title: Oral reproduction toxicity study in the Wistar rat (fertility and early embryonic development)

Study no.:	NP06673
Study report location:	EDR M4/4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Dec 12, 1995
GLP compliance:	Yes, statement included
QA statement:	Included
Drug, lot #, and % purity:	S16257-2, batch # 46762, 100.4 %

### Key Study Findings

Clinical signs including ptialism, hypoactivity, and blepharoptosis were sporadically observed in treated males, mainly in the high dosage group. The NOAEL for paternotoxicity is identified at high dosage, 175 mg/kg.

Clinical signs including hypoactivity, blepharoptosis, piloerection and ptialism were frequently observed in treated females, most markedly in the high dosage group. Significantly reduced body weight gain associated with reduced feed consumption was observed in the high dosage females in pre-mating and gestation periods. The reduced body weight gain in the mid-dosage females was observed in the 1<sup>st</sup> day of gestation period and was not associated with reduced feed consumption. Increased water

consumption was observed in all treated groups, significantly in mid- and high dosage groups. The NOAEL for maternal toxicity is identified at low dosage, 7 mg/kg.

There was no treatment-related effect on reproductive organ weights, sperm analysis parameters (males), mating/fertility index, reproductive performance and macro-, microscopic examinations of reproductive organs. The NOAEL for male and female fertility and the early stages of embryonic development is identified as 175 mg/kg.

Direct plasma exposures at the dosages in this study are not available as TK samples were not collected.

## Methods

Doses: 0 (D0), 7 (D1), 35 (D2) or 175 (D3) base mg/kg  
 Frequency of dosing: daily  
 Dose volume: 10 mL/kg  
 Route of administration: Oral gavage  
 Formulation/Vehicle: Solution/Demineralized water  
 Species/Strain: Rats/Wistar (male: 7-week, female: 11-week)  
 Number/Sex/Group: 20  
 Satellite groups: n/a  
 Study design: See table below (excerpted from page 280 of the report)  
 Deviation from study protocol: Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

Study group identification	D0		D1		D2		D3	
Subgroup identification ①	A	B	A	B	A	B	A	B
No. of F0 animals per group and per sex	20							
Female numbering	1 to 20	21 to 40	41 to 60	61 to 80	81 to 100	101 to 120	121 to 140	141 to 160
Male numbering	201 to 220	/	241 to 260	/	281 to 300	/	321 to 340	/
Administration route and method	Oral: oesophageal intubation							
Vehicle	Demineralised water							
Volume (ml/kg)	10							
Dose (mg/kg) administered once daily	0		7		35		175	
- expressed as the base	0		7.55		37.7		189	
- expressed as the salt	0		7.55		37.7		189	
Dosing frequency and period	Males: daily for 63 days before pairing, then until sacrifice for all males in group A Females: daily for 14 days before pairing, then up to day 6 of gestation inclusive for all females in group B							

① The females in group A and the males in group B will not be treated and will undergo no physical examination. After the mating period, the males will be eliminated without examination. The females will undergo pregnancy examination on day 14 of gestation. The animals will enable study of the potential effects of the test substance administered separately on male fertility and on female fertility. The mates will be determined as per the order of numerical identification for the animals in group A.

The high dose 175 mg/kg was selected based on the preliminary reproductive toxicity study (NP08074) in which the high dosage induced general toxic effects in the parental F0 generation. The general toxic effects at 175 mg/kg were characterized by clinical signs and a reduction in mean body weight gain for the animals of both sexes, as well as by a decrease in mean feed consumption and an increase in mean water consumption for the F0 females. A cardiomegaly in several animals was considered as an exaggerated pharmacological activity of the test article.

## Observations and Results

### Mortality and Clinical Signs

Untreated animals were subjected to a daily mortality check only. Treated F0 males and females were observed once daily during acclimation and then prior to and after treatment each day during treatment period. During gestation the F0 females were observed at least once daily and any sign of abortion was recorded.

No animal died through the study.

For F0 males, ptyalism after treatment was observed in 19 out of 20 males from high dosage group with a frequency varying from occasional to very frequent, in 16 out of 20 males from mid-dose group with variable occurrence, and in only two males from low dosage group with one to two occasions. Ptyalism the next morning just before treatment was observed sporadically in 5 males from high dosage group and in 11 males from mid-dose group. Hypoactivity after treatment only was observed sporadically in half of the males from high dosage group, once to twice in two males from mid-dose group and once in one male from low dosage group. Blepharoptosis after treatment only was observed with a very low occurrence in seven males from high dosage group.

For F0 females, during the pregestation period, hypoactivity, blepharoptosis, piloerection and ptyalism were observed frequently in more than half to all females in high dosage group and sporadically in a very few females in the mid-dosage group. These clinical signs were observed in a smaller number of females in both groups with similar occurrence, except for ptyalism affecting more than half of the females in the mid-dosage group. Mastication was sporadically observed in a few females in the high and mid-dosage groups during the pre mating period but was only observed in high dosage females during gestation. Ventral decubitus was observed very sporadically in a few high dosage females during pre mating period but not in the gestation. In low dosage females, no particular signs were observed during pre mating period but occasional to frequent salivation was observed in almost half of females. Some of these findings, mainly piloerection, hypoactivity and blepharoptosis, were still observed next morning prior to the treatment in several females, mainly from high dosage group.

### Body Weight

During the pre mating period males from subgroup A and females from subgroup B were weighed once weekly.

Each female from subgroup B effectively mated (sperm positive vaginal smear) was weighed daily during gestation, from day 0 to day 14 inclusive. The body weights of females found not to be pregnant at Caesarean section, and at subsequent uterus staining, were not included in the calculation of group mean values.

For F0 males, there was no treatment-related adverse effect on body weight gains.

For F0 females, mean body weight gains are listed in the table below.

<b>Dosage mg/kg/day</b>	<b>control</b>	<b>7</b>	<b>35</b>	<b>175</b>
Premating – (14-7 day)	6.8	8.4	6.8	0.8*
Premating – (7-1 day)	9.9	12.8	12.6	13.7
Gestation 1 <sup>st</sup> week	29.1	25.0	22.8	18.6
Gestation 0-3	4.96	3.76	3.22*	2.04*
Gestation 2 <sup>nd</sup> week	32.3	30.3	29.1	26.7
Gestation 6-9	3.72	3.36	2.92	2.02*

\*statistically significant when compared to the control group

During the pre-mating period, after the first week of treatment, the mean body weight gain was lower in the high dosage group compared to control with statistically significance. During the second week of treatment, the mean body weight gain was higher in all treated groups than in the control group, but the high dosage females did not recover entirely.

During the first week of gestation, the mean body weight gain was dose dependently reduced in all treated groups when compared with that in the control group. The statistical analysis of mean body weight gain over a series of three-day periods showed significant differences between the mid- and high dosage groups and the control group for GD 0-3. During the second week of gestation, after withdrawal of treatment, the mean body weight gain remained dose dependently reduced in all treated group with a statistically significant difference for GD 6-9 between high dosage and control groups.

The effect on body weight gain was considered test article-related and the significant decrease in body weight gain for the high dosage group was consistent with significantly reduced feed consumption observed in the same group. The biological significance of this finding for the low dosage cannot be established due to the small magnitude of the change.

## Feed Consumption

Feed and water consumption were calculated for the two treatment weeks of the pre-mating period and for the first two weeks of gestation. The results were expressed in g/day.

The result was summarized in the table below (excerpted from module 2, section 2.6.7, page 120). The mean feed consumption from high dosage group was consistently lower than that of the controls during the treatment period, which was most marked during the first week of the pre-mating period (control: 20.5 g/day, high dose: 14.3 g/day).

Differences between these two groups were statistically significant in pre-mating weeks one and two, and gestation week one ( $p < 0.01$ ). After the withdrawal of treatment,

mean feed consumption did not show any statistically significant difference between groups.

Mean water consumption was dose- dependently higher in all treated groups than in the control group throughout the study, even after withdrawal of treatment. It is consistent with the repeated-dose general toxicity studies which showed an increase in urinary volume and modifications of electrolyte balance. These findings were possibly related to the pharmacodynamic activity of the test article.

Daily dose (mg/kg) (Cont'd)	0 (control)	7	35	175
<b>Females:</b>				
Preparing food consumption (% <sup>a</sup> )	-			
1 <sup>st</sup> week of dosing	20.50 g/d	-2	-6	-30**
2 <sup>nd</sup> week of dosing	20.89 g/d	+1	+2	-6**
Gestation food consumption (% <sup>a</sup> )				
1 <sup>st</sup> week of dosing	23.99 g/d	-1	-4	-12**
2 <sup>nd</sup> week of dosing	-	-	-	-
Preparing water consumption (% <sup>a</sup> )				
1 <sup>st</sup> week of dosing	25.63 g/d	+13	+17	+20
2 <sup>nd</sup> week of dosing	26.61 g/d	+16	+27*	+32**
Gestation water consumption (% <sup>a</sup> )				
1 <sup>st</sup> week of dosing	32.58 g/d	+18	+22	+25
2 <sup>nd</sup> week of dosing	36.33 g/d	+8	+10	+11

*Incidence of clinical signs (number of animals affected and/or frequency): +: Low to average ++: High  
a: For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on percent differences)*

*Two-way analysis of variance, Dunnett's Test \*: p<0.05 \*\*: p<0.01*

## Toxicokinetics

Neither TK sampling nor TK evaluation was performed in the study.

## Stability and Homogeneity

Stability was verified at concentrations of 0.5, 10 and 100mg/mL of S 16257-2 in demineralized water. The solutions were stable 25 days storage at room temperature exclusively.

## Necropsy

Untreated F0 males (subgroup B) were euthanized after successful pairing or at the end of the monogamous mating period, and not necropsied.

Treated F0 males (subgroup A) were euthanized after examination of the uterine content of their corresponding F0 females. A detailed autopsy was carried out.

F0 females from subgroups A and B, with sperm positive vaginal smear, were euthanized on GD14. F0 females from subgroups A and B, with a sperm negative vaginal smear at the end of the 3-week mating period, were euthanized at least ten days after the last mating day. An examination of F0 females' uterine content was carried out and also a detailed autopsy of the treated F0 females from subgroup B.

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

**Mating:** During the mating period (three weeks), monogamous pairs were formed each evening by introducing a male into the cage of a female. Treated males were paired with untreated females and *vice versa*. The next morning the male was removed from the cage of the female and returned to its own cage. Effective mating was checked by vaginal smear. A female was considered effectively mated when light microscopic examination of the vaginal smear detected the presence of spermatozoa (gestation day zero), or when the examination of uterine content at terminal sacrifice revealed pregnancy, despite a sperm negative vaginal smear during the entire mating period.

**Uterine content:** After hysterectomy, corpora lutea, implantation sites, live embryos and resorptions were counted. Dead embryos were considered as late resorptions. An external macroscopic examination was performed on the embryos, followed by an evaluation of their viability on the basis of heartbeats and a functional blood circulation.

**Necropsy:** A detailed autopsy was carried out on each treated F0 male from subgroup A and each treated F0 female from subgroup B. Organs with macroscopic anomalies were removed and preserved for a possible histopathological evaluation according to the decision of the Study Director. Corresponding organs of sufficient controls were preserved for comparison. Only F0 animals with organ anomalies confirmed by histopathological evaluation are presented in the report.

The testes and epididymides from the treated F0 males were systematically removed for light microscopic examination after recording of the respective organ weights and after sperm analysis (number and viability of spermatozoa) in the left cauda epididymis.

The complete male genital tract was systematically removed for light microscopic examination from the treated F0 males which did not fertilize their corresponding untreated female.

The ovaries and oviducts from treated F0 females which were not pregnant were systematically removed for a possible light microscopic examination. If no implantation site was detected after uterus staining, a histopathological examination was performed on the ovaries and oviducts.

### **Histopathology:**

The organs with macroscopic findings were fixed, dehydrated and then embedded in paraffin wax. Sections were stained with hemalum-eosin-saffron prior to light microscopic examination. Two different stains were used for the testes, i.e. PAS and hemalum-eosin. Uterus staining to count the implantation sites was performed according to (b) (4)'s method. For the systematic histopathological examination of the male and female reproductive organs, only those of the F0 animals with anomalies are presented.

Treatment Effects on F0 Males

## Mating/Fertility Index/Reproductive Performance

As shown in the table below (excerpted from page 93 of the report), all treated F0 males, succeeded in mating their corresponding untreated females. The mean time necessary for successful copulation, expressed in days, was slightly increased in all treated groups.

The number of pregnant females was slightly lower in all treated groups than in the control group, without a dose-response relationship. Consequently, the fertility index for treated males was also slightly lower than for control males. Given the small magnitude and lack of dose-dependency, it is not considered to be toxicologically significant.

	D0	D1	D2	D3
Number of F0 males to be paired	20	20	20	20
Number of F0 males which mated a female	20	20	20	20
Number of F0 males which fertilized a female (female was pregnant)	19	17	17	18
Effective mating rate of F0 males	1.00	1.00	1.00	1.00
Fertility index of F0 males	0.95	0.85	0.85	0.90
Mean time necessary for successful copulation (days)	2.80	3.20	(*) 3.05	4.00

(\*) F0 male 286 D2 excluded because of unknown mating date: the corresponding untreated female showed a sperm negative vaginal smear during the entire mating period but were eventually pregnant.

As shown in the table below (excerpted from page 3 of the report), evaluation of the uterine content in the untreated females on gestation day fourteen did not reveal any biologically or statistically significant difference between the control group and the treated groups for any of the parameters examined.

Dosage (mg base/kg)	0	7	35	175
Males Producing Live Embryos	18	16	17	18
Number of Pregnant Females	19	17	17	18
Corpora Lutea/Female	13.5	13.8	14.1	15.1
Implantations/Female	12.5	11.9	11.8	13.7
Live Embryos/Female	11.6	11.1	11.0	12.4
Pre-implantation Loss $\geq 3$ (80 <sup>th</sup> percentile)	2	4	6	3
Post-implantation Loss $\geq 2$ (80 <sup>th</sup> percentile)	6	3	2	6

Reproductive organ weights and sperm analysis showed (see the table below, excerpted from page 3 of the report) that mean weights of the testes and the epididymis as well as the sperm vitality (% motile sperm) per cauda epididymis were similar between treated groups and controls. The mean number of spermatozoa ( $\times 10^6$ ) per gram cauda epididymis revealed a slightly lower value in high dosage group than in the other groups, which did not reach a statistical significance. This result was due to male No. 339 with a bilateral testicular atrophy, resulting in only  $50.4 \times 10^6$  spermatozoa. i.e. about eight percent of the mean value for the remaining males from this group. There was no generalized decrease with regard to the individual values for the number of spermatozoa observed in high dosage group.

Testis Weight (absolute, g)	3.69	3.64	3.61	3.58
Testis Weight (relative to body weight, %)	0.78	0.73	0.75	0.78
Epididymis Weight (absolute, g)	1.27	1.26	1.24	1.26
Epididymis Weight (relative to body weight, %)	0.27	0.25	0.26	0.27
Number Spermatozoa/Gram Cauda Epididymis ( $\times 10^6$ ) (cf. suppl. sheet 1)	614	613	632	575
Epididymal Sperm Viability (%)	80.5	81.4	81.9	79.1

### Necropsy and Histopathology

At necropsy, one F0 male from low dosage group showed an enlarged right cauda epididymis with a yellowish mass, and another F0 male from high dosage group showed small testes and epididymis and also a yellowish mass on the left cauda epididymis. Both males were diagnosed with spermatocele by microscopic examination of the epididymal masses. Atrophy of both testes, characterized by a perturbation of spermatogenesis with clonal desquamation of immature cells as well as subatrophied epididymis was also detected for the male from the high dosage group.

Systematic histopathological examination of the testes and epididymis from all treated F0 males revealed that one male from mid-dose group showed about ten percent of involuted tubules with regression of Sertoli cells in one testis. Histopathological examination of the complete genital tract of the F0 males that did not fertilize their corresponding female did not detect any anomaly attributable to treatment.

The above findings in one male from each of low, mid and high dosage groups were not considered toxicologically significant. The incidence was very low and such findings were observed spontaneously in controls from various studies.

### Effects on F0 Female Fertility

#### Mating/Fertility Index/Reproductive Performance

As shown in the table below (excerpted from page 119 of the report), the number of mated F0 females was similar among all groups. The mean time necessary for successful copulation, expressed in days, was slightly increased in all treated groups. The number of pregnant F0 females was higher in all treated groups than in the control group. Hence, the fertility index was not adversely affected by the treatment.

	D0	D1	D2	D3
Number of F0 females to be paired	20	20	20	20
Number of F0 females effectively mated	20	20	20	19
Number of pregnant F0 females	17	18	18	19
Effective mating rate of F0 females	1.00	1.00	1.00	0.95
Fertility index of F0 females	0.85	0.90	0.90	1.00
Mean time necessary for successful copulation (days)	1.95	2.80	(*) 3.53	3.16

(\*) F0 female 108 D2 excluded because of unknown mating date: this female showed a sperm negative vaginal smear during the entire mating period but were eventually pregnant.

As shown in the table below (excerpted from M2/2.6.7. page 121), evaluation of the uterine content in the F0 females on GD14 did not reveal any biologically or statistically significant difference between the control group and the treated groups for any of the parameters examined. In particular, the external macroscopic examination of the embryos did not reveal any morphological anomaly.

Daily dose (mg/kg) (Cont'd)	0 (control)	7	35	175
<b>Females (Cont'd):</b>				
Mean No. of days prior to mating	2.0	2.8	3.5 <sup>a</sup>	3.2
No. of females sperm positive	20	20	20	20
No. of pregnant females	17	18	18	19
Mean No. of Corpora Lutea	14.2	14.6	13.8	13.4
Mean No. of implantations	12.8	13.6	12.5	12.2
Mean % of preimplantation loss	12.3	6.9	9.7	9.5
Mean No. of live conceptuses	11.7	12.1	11.6	11.1
Mean No. of resorptions	1.1	1.5	0.8	1.1
No. of dead conceptuses	0	0	0	0
Mean % of postimplantation loss	13.2	11.1	6.8	11.1

a: One female excluded because of unknown date of mating

## Necropsy and Histopathology

At the terminal sacrifice, autopsy revealed no macroscopic organ anomalies which made histopathological examination unnecessary. Furthermore, the systematic histopathological examination of the ovaries in the non-pregnant females did not reveal any anomaly attributable to the test article.

## 9.2 Embryonic Fetal Development

Study title: Oral Reproduction Toxicity Study in the Wistar Rat (Embryofetal Development)

Study no: NP05135  
 Study report location: EDR M4/4.2.3.5.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: Jan. 5, 1994  
 GLP compliance: Statement included  
 QA statement: Statement included  
 Drug, lot #, and % purity: Ivabradine hydrochloride, S 16257-2, batch# EF558, purity: 99.8 %

## Key Study Findings

There was no significant maternal toxicity observed in the F0 females treated at doses up to 20 mg/kg/day.

External abnormal shape of the heart (dysplasia) associated with or without simple anomalies of the major proximal arteries were observed at dosages  $\geq 2.3$  base mg/kg/day (AUC<sub>24h</sub> on GD15=565 ng.h/mL);

Teratogenic effects including interventricular septal defect and complex anomalies of the major proximal arteries were observed at dosages  $\geq 4.6$  base mg/kg/day (AUC<sub>24h</sub> on GD15=980 ng.h/mL).

One fetal death with a delay in ossification and a spina bifida occulta situated between 5<sup>th</sup> and the 7<sup>th</sup> cervical vertebra as well as reduced mean fetal weight were observed at 19 base mg/kg/day.

S 16257 was detected in amniotic fluid on GD15.

NOAEL for embryofetal development toxicity cannot be identified.

*Note: the dosage in salt form was referred in the following review of this study.*

#### Methods

Doses:	0, 2.5, 5, 10, 20 mg/kg (base: 2.3, 4.6, 9.3, 19)
Frequency of dosing:	daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	Solution/Demineralized Water,
Species/Strain:	Rat/Wistar
Number/Sex/Group:	25 females/group
Satellite groups:	N=5 females/treated group for TK evaluation
Study design:	Female Wistar rats aged 10 to 11 weeks were paired in a 1:1 ratio with untreated male Wistar rats (SPF) of the same age. The effective mating was checked by vaginal smear and the day that a sperm positive vaginal smear was observed, was considered day zero of gestation (GD0). The pregnant females were treated from GD6 to GD15 and C-section was conducted at GD20.
Deviation from study protocol:	Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

Dose selection: The dose selection was based on a preliminary rat embryo-fetal development study (NP08075). In the study, the doses of 120 and 240 mg/kg resulted in 97 and 100% resorptions, respectively. At the dose of 120 mg/kg, all existing fetuses showed cardiovascular anomalies. The dose of 60 mg/kg was less abortive (27% of resorptions) but resulted in a clearly teratogenic effect on the cardiovascular system. The dose of 15 mg/kg did not increase the spontaneous frequency of resorptions observed in the rat strain used (12%) and did not induce real malformations but minor cardiovascular anomalies (dysplasia of the heart and variations of the major cardiac vessels). The doses chosen in the present study was to analyze if a dose-response relationship exists for the effects observed and to define a NOAEL.

## Observations and Results

### Mortality and Clinical Signs

The F0 females were observed once daily during acclimatization. During gestation, the F0 females were observed at least once daily. During the treatment period, clinical monitoring was carried out prior to and after treatment. Any sign of abortion was recorded during gestation.

No mortality was observed during the study.

No treatment-related effects on clinical signs were observed.

### **Body Weight**

Each female effectively mated (sperm positive vaginal smear) was weighed daily during the entire gestation period from GD0 to GD20 inclusive. The body weight of the females found not to be pregnant at Caesarean section and subsequent uterus staining was excluded from the groups' mean value calculation.

The overall mean body weight of the F0 females was similar among all groups during the treatment period. The statistical analysis of the mean daily body weight and the mean daily weight gain during gestation did not reveal any significant difference between the treated groups and the control group.

A reduced weight gain on the first treatment day only was observed at 20 mg/kg which was related to precocious and ephemeral decrease of food consumption.

### **Feed and Water Consumption**

These parameters were calculated only for the pregnant females. The results were expressed in g/day. Four different periods were defined as follows: GD0-5, GD6-10, GD11-15 and GD16-19 inclusive.

The mean food consumption was similar among all groups for the different periods. The increase in the mean food consumption was smaller in the 20 mg/kg group than the other groups only during the first week of treatment.

The mean water consumption of the F0 females increased in a dosage range from 5 to 20 mg/kg and reached maximum values already at 10 mg/kg.

### **Toxicokinetics**

Blood samples were taken from five F0 females in each treatment group at 10 min, 1 and 6 hours after treatment on GD6 and GD15 for quantification of the unchanged test article in plasma. Only the samples collected from the first three females, for which the Caesarean section had confirmed pregnancy, were used for toxicokinetics.

The amniotic liquid was taken on GD15 from the embryos of the F0 females used for toxicokinetics at 6.5 to 7.5 hours after treatment. Quantification of the unchanged test article was performed on the amniotic fluid taken.

As shown in the table below (M2/ 2.6.7 page 122), for each dose group, the exposure was similar on GD6 (single administration) and GD15. The AUC<sub>24</sub> mean values

increased in a dose-proportional way in the range from 2.5 to 20 mg/kg. The maximal plasma concentrations were reached ten minutes after treatment in most of the animals.

Daily dose (mg/kg)		0 (control)	2.3	4.6	9.3	19
<b>Dams:</b>						
Toxicokinetics of S 16257:						
- C <sub>max</sub> (ng/ml) <sup>a</sup>	G6		80	222	517	1337
	G15		117	154	364	1039
- AUC <sub>24</sub> (ng.h/ml)	G6		469	1060	1874	5592
	G15		565	980	1857	4759
- Amniotic liquid/plasma concentration ratio (mean on G15) <sup>a</sup>			2.17	1.80	1.77	1.30

Note: the dosage in base form was used in the table.

Like the AUC<sub>24h</sub> mean values in plasma, the mean concentrations of S 16257 in the amniotic liquid increased in a dose-proportional way, and they were higher than the plasma concentrations in the corresponding F0 females measured on the same day 6 hours after treatment. However, the concentration ratio of amniotic liquid/plasma seemed to decrease when the dose increased.

### Stability and Homogeneity

Stability was verified at concentrations of 0.5, 10 and 100mg/mL of S 16257-2 in demineralized water. The solutions were stable 25 days storage at room temperature exclusively.

### Necropsy

The F0 males were euthanatized after the end of the mating period without necropsy.

The F0 females were euthanatized on GD20. An examination of their uterine contents and a detailed autopsy were carried out.

#### Uterine content:

After hysterectomy, the corpora lutea, the implantation sites, the fetuses and the resorptions were counted. An external macroscopic examination was performed on the fetuses and the placentas which were then weighed individually. Half of the fetuses from each litter were allocated to skeletal examination and half of them were allocated to soft tissue inspection. Only fetuses with anomalies were presented. The sex of each fetus was determined either during the organ inspection or during the evisceration of those aimed at skeletal examination.

A detailed autopsy was carried out on each F0 female. Organs with macroscopic anomalies were removed and preserved for a possible histopathological evaluation according to the decision of the Study Director. Corresponding organs of sufficient controls were preserved for comparison. Only F0 females with organ anomalies were presented.

The organs with macroscopic findings were fixed, dehydrated and then embedded in paraffin wax. Sections were stained with hemalum-eosin-saffron prior to their light microscopic examination. Special staining included the followings:

Uterus staining in order to count the implantation sites according to (b) (4)'s Method;

Skeletal examination of the fetuses by a double staining method drawn from that of (b) (4);

Soft tissue inspection of the fetuses according to the method of BARROW and TAYLOR modified by the dissection of the abdominal cavity.

At the terminal sacrifice on GD20, the autopsy did not reveal any organ anomaly.

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

As shown in the table below (excerpted from M2/2.6.7 page 123), the mean fetal body weight (3.16 g) observed in the high dosage group was slightly lower than the control, and the relationship to the test article cannot be excluded. One dead fetus was observed in the high dosage group. The other parameters including number of corpora lutea, number of implantations, pre- and postimplantation loss, number of resorptions, live fetuses and placental weight were similar between treated and control groups.

Daily dose (mg/kg)	0 (control)	2.3	4.6	9.3	19
No. pregnant	20	22	21	23	21
Mean No. Corpora Lutea	15.2	14.1	14.3	15.1	15.6
Mean No. implantations	13.1	12.4	12.1	12.1	13.1
Mean % preimplantation loss	13	11	15	19	16
Litters:					
No. litters evaluated	20	22	21	23	21
Mean No. live fetuses	11.6	11.1	10.7	10.9	12.0
Mean No. resorptions (early and late)	1.6	1.3	1.5	1.2	1.1
No. dead fetuses	0	0	0	0	1 <sup>a</sup>
Mean % postimplantation loss	11	10	12	12	9
Mean Fetal bodyweight (g)	3.41	3.32	3.33	3.31	3.16
Mean placental weight (g)	0.45	0.47	0.48	0.44	0.44
Fetal sex ratios (% males)	49	49	54	50	54

Note: the dosage in base form was used in the table.

### Offspring (Malformations, Variations, etc.)

Skeletal inspections of the fetuses:

In the 20 mg/kg group, the only dead fetus showed a general delay in ossification and a spina bifida occulta situated between the fifth and the seventh cervical vertebra. There were no other significant skeletal variations or malformations observed in the 20 mg/kg or 10 mg/kg groups, when compared to those from the control fetuses and those spontaneously observed in the rat strain. With these negative findings, the fetuses from the two low dose groups were not further examined for skeletal anomalies.

Daily dose (mg/kg) (Cont'd)	0 (control)	2.3	4.6	9.3	19
Dams (Cont'd):					
Fetal anomalies:					
- Visceral anomalies					
Heart: external abnormal shape <sup>c</sup>					
No. fetuses (%)	1 (0.8)	13 (10.3)	16 (13.6)	75 (56.4)	79 (60.3)
No. litters (%)	1 (5)	8 (36.4)	8 (38.1)	22 (95.7)	21 (100)

Heart : external abnormal shape <sup>a</sup> with anomalies of the major proximal arteries:					
Inominate artery lengthened or absent and/or juxtaposition of the two common carotid arteries or of the left common carotid artery and the left subclavian artery					
No. fetuses (%)	0 (0)	4 (3.2)	2 (1.7)	29 (21.8)	39 (29.8)
No. litters (%)	0 (0)	4 (18.2)	2 (9.5)	15 (65.2)	15 (71.4)
Ascending aorta and pulmonary trunk lengthened					
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.8)	0 (0)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)
Complex anomaly <sup>b</sup>					
No. fetuses (%)	0 (0)	0 (0)	1 (0.8)	1 (0.8)	2 (1.5)
No. litters (%)	0 (0)	0 (0)	1 (4.8)	1 (4.3)	2 (9.5)
Heart : external abnormal shape <sup>a</sup> with interventricular septum defect					
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.8)	3 (2.3)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (4.3)	2 (9.5)
Heart : external abnormal shape <sup>a</sup> with interventricular septum defect and anomalies of the major proximal arteries					
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.8)	4 (3.1)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (4.3)	3 (14.3)
- Skeletal anomalies					
	-	np	np	-	-

a: Described as dysplasia in the study report

b: Complex anomalies of the major proximal arteries including retro-oesophageal arch of the aorta, left common carotid arteries arising from ascending part of aorta, left subclavian artery arising from ducters arteriosus, persistent truncus arteriosus

Note: the dosage in base form was used in the table.

Soft tissue inspections of the fetuses:

As shown in the table (excerpted from section 2.6.7 page 124) above, the test article-related adverse effects on the development of the cardiovascular system from the dose of 2.5 mg/kg were observed which included:

- mild external shape anomaly of the heart (dysplasia) combined or not with simple variations of the major proximal arteries in the 2.5 mg/kg group (13.3% fetuses affected);
- 16.1% of the fetuses affected in 5 mg/kg group; dysplasia of the heart in one of them associated with malformations of the major proximal arteries (arch of the aorta retro-esophageal, absence of innominate artery, right and left common carotid arteries arising from the ascending part of the aorta, left subclavian artery arising from the ducters arteriosus). All the other fetuses from this group showed the same types of cardiovascular anomalies as those mentioned for the fetuses from the 2.5 mg/kg group;
- anomalies of the cardiovascular system in 81.4% of the fetuses in the 10 mg/kg group, among which two malformations (1.6%) of the heart (interventricular septum defects) associated with dysplasia of the heart and in one case also with anomalies of the major proximal arteries;
- anomalies of the cardiovascular system in 97.0% of the fetuses in the 20 mg/kg group, among which nine fetuses (6.9%) showed malformations either of the heart alone (e.g. interventricular septum defect) or of the major proximal arteries only (e.g. right subclavian artery retro-esophageal) or of both (e.g. interventricular septum defect and persistent trunks arteriosus).
- Minor anomalies (variations) of the major arteries close to the heart were seen in all groups including the control group, without treatment-related increase in their incidence. The substantially increased incidence associated with cardiac dysplasia in the treated groups observed at the doses of 10 and 20 mg/kg.

No cardiovascular malformation was detected in the control group. One isolated case of dysplasia of the heart was observed but the morphology of this heart was quite different from that observed in all the fetuses affected from the treated groups. In the treated groups, the shape of the dysplasia of the heart was all the more pronounced as the dose was high.

In conclusion, the treatment of the F0 females with S 16257-2 affected embryonic development in the cardiovascular region (cardiac dysplasia associated or not with variations of the major proximal arteries) at doses  $\geq 2.5$  mg/kg. Teratogenic effects on the cardiovascular system appeared at doses  $\geq 5$  mg/kg.

**Study title: Oral Reproduction Toxicity Study in the Wistar Rat (Embryofetal Development) --- supplement**

Study no:	NP26980
Study report location:	EDR M4/4.2.3.5.2
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	Nov 16, 2005
GLP compliance:	Statement included
QA statement:	Statement included
Drug, lot #, and % purity:	Ivabradine hydrochloride, S 16257-2, batch# 55 294, purity: 100.5 %

**Key study findings**

External abnormal shape of the heart and teratogenic effect including interventricular septal defect and complex anomalies of the major proximal arteries at the high dosage of 9.3 mg/kg (base)

The NOAEL for fetal cardiovascular morphological changes was determined at 1.5 mg/kg (base), corresponding to AUC<sub>24h</sub> value of 482 ng.h/mL on GD15.

## Methods

Doses: 0, 0.5, 1.5, 9.3 base mg/kg  
Frequency of dosing: daily  
Dose volume: 10 mL/kg  
Route of administration: Oral  
Formulation/Vehicle: Solution/purified Water,  
Species/Strain: Rat/Wistar  
Number/Sex/Group: 24 females/group  
Satellite groups: N=5 females/group for TK evaluation  
Study design: Initial age: approximately 13-week  
Day of mating: Day 0  
Duration of dosing: GD6 to GD17 inclusive.  
Day of C-section: GD20

Deviation from study protocol: Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

In the rat embryo-fetal development study NP05135 (reviewed above), fetal cardiovascular morphological changes were observed at the lowest dosage (2.3 mg base/kg/day). The NOAEL could not be identified for this parameter. This present supplementary study was performed to determine a NOAEL.

Pregnant female rats were treated by oral administration once daily from GD6 to GD17. A detailed necropsy, including the heart, on all F0 females of the main study and an examination of uterine contents after Caesarean section on GD20 were performed. , Only detailed examination of fetal cardiovascular system was conducted. Plasma levels of S 16257 and its main active metabolite S 18982 were determined at 10 min, 1, 4, 8 and 24 hours after dosing on GD15 in three pregnant satellite females for toxicokinetic evaluations.

There were no maternal mortalities.

There were no treatment-related adverse effects on clinical signs, bodyweight and feed and water consumption.

At the terminal sacrifice, there was no treatment-related effect on the uterine parameters (as shown in the table below, excerpted from page 3 of the report).

Daily Dose (mg base/kg)	0 (Control)	0.5	1.5	9.3
<b>Dams:</b>				
Necropsy Observations:				
Mean No. Corpora Lutea	13.2	13.7	13.9	13.5
Mean No. Implantations	11.5	12.4	11.5	11.7
Mean % Preimplantation Loss	11.7	9.3	18.0	13.7
<b>Litters:</b>				
No. Litters Evaluated	23 <sup>c</sup>	20	22	22
Mean No. Live Foetuses	10.2	11.1	10.2	10.8
Mean No. Early Resorptions	0.1	0.3	0.4	0.1
Mean No. Late Resorptions	1.2	1.0	0.9	0.8
Mean No. Dead Foetuses	0	0	0	0
Mean Postimplantation Loss Rate	0.18	0.11	0.13	0.08
Mean Foetal Bodyweight (g)	3.4	3.2	3.4	3.2
Mean Placental Weight (g)	0.43	0.45	0.46	0.42
Foetal Sex Ratios (% males)	52	43	46	45

As shown in the table below (excerpted from page 3 of the report), examination of the cardiovascular system of the fetuses revealed increased incidence of abnormal external shape of the heart, and complex anomalies of major proximal arteries as well as malformation of ventricular septum defect in the high dosage group.

Daily Dose (mg base/kg)	0 (Control)	0.5	1.5	9.3
Foetal Anomalies of the Cardiovascular System (# foetuses examined / # litters examined)	235/20	222/20	225/22	237/22
<b>Cardiovascular Variations</b>				
[No. Foetuses (%) / No. Litters (%)]				
Two carotid arteries juxtaposed <sup>d</sup>	14 (6) / 9 (45)	7 (3.2) / 4 (20)	16 (7.1) / 6 (27)	32 (13.5) / 11 (50)
Presence of an artery parallel to the left subclavian artery on the aortic arch <sup>e</sup>	0	0	0	1 (0.4) / 1 (5)
Abnormal external shape of the heart <sup>f</sup>	0	2 (0.9) / 2 (10)	5 (2.2) / 4 (18)	56 (23.6) / 18 (82)
<b>Cardiovascular Malformations</b>				
[No. Foetuses (%) / No. Litters (%)]				
Single malformation:				
<i>Situs inversus</i>	1 (0.4) / 1 (5)	0	0	0
Ventricular septum defect	0	1 (0.5) / 1 (5)	0	2 (0.8) / 1 (4.5)

The toxicokinetic results are shown in the table below (excerpted from page 2 of the report). The systemic exposure (AUC<sub>24h</sub>) for both S 16257 and its major metabolite, S 18982, increased slightly less than proportionally to dose and the maximal concentrations (C<sub>max</sub>), increased dose proportionally. The metabolic exposure ratio remained similar at all dose levels.

Daily Dose (mg base/kg)	0 (Control)	0.5	1.5	9.3	
<b>Dams:</b>					
<b>Toxicokinetics<sup>a</sup>:</b>					
<b>S 16257:</b>					
C <sub>max</sub> (ng/ml)	GD15 (Day 10)	BLQ	38	115	573
AUC <sub>24</sub> (ng.h/ml)	GD15 (Day 10)	BLQ	198	482	2551
<b>S 18982:</b>					
C <sub>max</sub> (ng/ml)	GD15 (Day 10)	BLQ	1.2	4.1	20
AUC <sub>24</sub> (ng.h/ml)	GD15 (Day 10)	BLQ	6.7	18	80
R <sub>m</sub> S 18982/S 16257	na	0.034	0.040	0.033	

<sup>a</sup> For all parameters, group means are shown.

GD: gestation day; R<sub>m</sub>: molar exposure ratio; BLQ: Below the Limit of Quantitation (0.25 ng/ml); na: not applicable.

In conclusion, the NOAEL for fetal cardiovascular morphological changes was 1.5 mg base/kg, corresponding to on GD15 mean  $AUC_{24hr}$  values of 482 and 18 ng.h/mL and to mean  $C_{max}$  values of 115 and 4.1 ng/mL for S 16257 and S 18982, respectively.

**Study title: Oral Reproduction Toxicity Study in the New Zealand White Rabbit (Embryofetal Development)**

Study no:	NP05134
Study report location:	EDR M4/4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Feb. 15, 1994
GLP compliance:	Statement included
QA statement:	Statement included
Drug, lot #, and % purity:	Ivabradine hydrochloride, S 16257-2, batch# EG418, purity: 101.3 %

**Key Study Findings**

There was no significant maternal toxicity observed in the F0 females treated at doses up to 28 mg/kg/day (base form).

The NOAEL for embryotoxicity cannot be identified as increase in the mean postimplantation-loss rate was observed at lowest dosage of 7 mg/kg ( $AUC_{24h}=1664$  ng.h/mL on GD18) and above.

The NOAEL for fetal toxicity is identified at 14 mg/kg due to the reduced mean fetal and placental weights observed at 28 mg/kg.

The NOAEL for teratogenicity is identified at 14 mg/kg due to a teratogenic effect on the paws in a small number of rabbits (two cases of ectrodactylia) at the dose of 28 mg/kg ( $AUC_{24h}=11603$  ng/h/mL on GD18).

## Methods

Doses:	0, 7, 14, 28 (base) mg/kg
Frequency of dosing:	daily
Dose volume:	1 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	Solution/Demineralized Water,
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	18/female/control, 19/female/treated group
Satellite groups:	N=3 females/treated group for TK evaluation
Study design:	Age at initiation: at least 15-week Mating: GD 0 (The effective mating was checked visually and the mating day was considered as day zero of gestation.) Treatment duration:GD6 to GD18 C-section: GD30
Deviation from study protocol:	Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

The dose selection was based on the results of a preliminary rabbit embryo-fetal development study (NP08076). In this preliminary study, S 16257-2 was orally administered to pregnant females during organogenesis at daily doses of 30, 60, 120, 180 and 240 mg (salt)/kg (equivalent doses in base form: 28, 56, 111, 167 and 223 mg/kg). The results showed a reduction of the mean weight gain and food consumption of the F0 females at dosage of 30 mg/kg. This dose level was also embryotoxic (increase in the mean number of early resorptions) and fetotoxic (decrease in the mean fetal weight). At the dosages  $\geq$  60 mg/kg, S 16257-2 provoked lethality among the F0 females. In consequence, the high dose of the present study was limited to 28 mg/kg in base form (equivalent to dose of 30.2 mg/kg in salt form). To identify the NOAEL, two lower doses were chosen using a geometrical progression with a factor 2 which resulted in 7 and 14 mg/kg in base form (equivalent to doses of 7.55 and 15.1 mg/kg in salt form, respectively).

## Mortality and Clinical Signs

During gestation, the F0 females were observed at least once daily and during the treatment period, clinical monitoring was carried out prior to and after treatment. Any sign of abortion was recorded during gestation.

No mortality was observed during the study.

Ptosis was observed in the F0 females at the doses of 14 and 28 mg/kg. No other clinical signs were noted.

The one abortion observed on GD30 at the low dosage was considered incidental.

## Body Weight

Each female was weighed daily from GD3 to GD30 inclusive. The body weight of the females found not to be pregnant at Caesarean section and subsequent uterus staining was excluded from the groups' mean value calculation.

Overall during the whole treatment period, there was no significant difference in mean body weight gain between the treated groups and the control group.

A reduced body weight gain on the first treatment day only was observed at 20 mg/kg which was related to precocious and ephemeral decrease of food consumption.

## Feed and Water Consumption

These parameters were calculated daily from GD3 to GD30 for the pregnant females. The results were expressed as g/day.

There was no significant treatment-related effect on the mean food consumption of the F0 females, though the values of high dosage group were slightly lower than those of the other groups.

There was no significant treatment-related effect on the mean water consumption.

## Toxicokinetics

Blood samples were taken from three F0 females in each treatment group at 30 min, 4 and 7 hours after treatment on GD6 and GD18 for quantification of the unchanged test article in plasma.

The TK parameters are shown in the table below (M2/2.6.7 page 129). The maximal plasma concentrations were reached thirty minutes after treatment in most of the animals. For each dose group, the AUC<sub>24h</sub> value was higher on GD18 than that on GD6. In the tested dose range, the mean AUC<sub>24h</sub> values increased more rapidly than the dose after the single administration on GD6, but on GD18, the increase was proportional to the dose after repeated administration.

Daily dose (mg/kg)		0 (control)	7	14	28
Dams/does:					
Toxicokinetics of S 16257 <sup>a</sup> :					
C <sub>max</sub> (ng.h/ml)	G6		192	679	2021
	G18		858	1603	3479
AUC (ng.h/ml)	G6		455	1920	5049
	G18		1664	6979	11603

G: Gestation day

a: Mean values not specified in study report and calculated from individual data

## Stability and Homogeneity

Stability was verified at concentrations of 0.5, 10 and 100mg/mL of S 16257-2 in demineralized water. The solutions were stable 25 days storage at room temperature exclusively.

## Necropsy

The F0 females were euthanatized on GD30. Examinations of uterine contents and detailed autopsies were carried out.

### Uterine content:

After hysterectomy, the corpora lutea, the implantation sites, the fetuses and the resorptions were counted. An external macroscopic examination was performed on the fetuses and the placentas which were then weighed individually.

All fetuses were then examined for organ and skeletal anomalies. Only fetuses with anomalies were presented. The sex of each fetus was determined either during the organ inspection.

A detailed autopsy was carried out on each F0 female. Organs with macroscopic anomalies were removed and preserved for a possible histopathological evaluation according to the decision of the Study Director. Corresponding organs of sufficient controls were preserved for comparison. Only F0 females with organ anomalies were presented.

The organs with macroscopic findings were fixed, dehydrated and then embedded in paraffin wax. Sections were stained with hemalum-eosin-saffron prior to their light microscopic examination. Special staining included the followings:

Uterus staining in order to count the implantation sites according to (b) (4) s Method;

Soft tissue inspection of the foetuses using a fresh microdissection technique

Skeletal examination of the fetuses using X-ray photography for live fetuses and double staining for dead fetuses. The latter technique was drawn from that of (b) (4).

At the terminal sacrifice on GD20, the autopsy did not reveal any organ anomaly.

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

As shown in the table below (excerpted from M2, section 2.6.7 pages 129-130), the mean postimplantation loss was higher in all the treated groups than that in the control group, which did not show a dose-related pattern. In the high dosage group, the mean fetal and placental weights were slightly reduced, but were not associated with any relevant ossification delays in the fetuses. There were no significant treatment-related adverse effects on the other uterine parameters.

Daily dose (mg/kg)	0 (control)	7	14	28
<b>Dams/does:</b>				
No. pregnant	17	18 <sup>a</sup>	19	18
Mean No. Corpora Lutea	11.4	11.9	11.1	11.5
Mean No. implantations	8.9	10.1	8.2	9.5
Mean % preimplantation loss	22	17	25	18
<b>Litters:</b>				
No. litters evaluated	17	17	19	18
Mean No. live fetuses	8.2	8.2	7.0	8.2
Mean No. resorptions	0.8	1.2	1.1	1.2
No. dead fetuses	0	0.6	0.1	0
Mean % postimplantation loss	7	18	13	15
Mean Fetal bodyweight (g)	42.4	41.8	43.0	39.7
Mean placental weight (g)	5.1	5.2	5.2	4.9
Fetal sex ratio (% males)	47	48	51	51

G: Gestation day

a: Mean values not specified in study report and calculated from individual data

b: One female given 7 mg/kg/d aborted (dead fetuses and late resorptions) on the day of sacrifice

c: Ptosis was sometimes noted at 14 and 28 mg/kg/d

## Offspring (Malformations, Variations, etc.)

As shown in the table (excerpted from M2/2. 6.7, page 130) below, skeletal inspections of the fetuses revealed ectrodactylia in the fetuses from the high dosage dams. The teratogenic effect on the paws at the high dosage cannot be excluded.

Daily dose (mg/kg) (Cont'd)	0 (control)	7	14	28
<b>Fetal anomalies:</b>				
- External and visceral anomalies	-	-	-	-
- Skeletal anomalies				
- Ectrodactylia involving absence of two thumbs, two right and three left toes and two left metatarsal bones				
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.7)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (5.6)
- Ectrodactylia involving absence of five fingers on the left forepaw and fusion of four sternebrae				
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.7)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (5.6)
- Total number of ectrodactylia				
No. fetuses (%)	0 (0)	0 (0)	0 (0)	2 (1.4)
No. litters (%)	0 (0)	0 (0)	0 (0)	2 (11.1)

The organ inspection of the fetuses did not reveal any treatment-related anomaly.

### Study title: Oral Reproduction Toxicity Study in the New Zealand White Rabbit (Embryofetal Development) ---Complementary (non-GLP)

Study no: NP08073  
 Study report location: EDR, M4/4.2.3.5.2  
 Conducting laboratory and location: (b) (4)

Date of study initiation: Jan 10, 1995  
 GLP compliance: no  
 QA statement: no  
 Drug, lot #, and % purity: Not available

This complementary study was to further investigate the teratogenic finding of ectrodactylia observed at 28 mg/kg/d in the pivotal embryo/fetal development study in the rabbit (NP05134, reviewed above). In this study, the group sizes were increased and the higher dose of 42 mg/kg/d was administered. Pregnant New-Zealand White rabbits (16-week old or more, 25 or 50 females/group) received once daily oral via oral gavage (1 ml/kg) of 0 (control, purified water) or 42 mg/kg/d from GD6 through GD18.

No mortality was observed in F0 females. In the treated group, clinical signs including half closed eyes, hypoactivity and tachypnea as well as the reduced body weight gain, food and water consumption were observed. Increased number of early resorptions was observed in the treated group. Ectrodactylia occurred in one fetus each of the control group and the treated group. However, the control one had multiple additional defects and amniotic bands whereas the treated one had no other defects. So it is not clear whether these cases were strictly comparable. Therefore, no firm conclusion can be drawn regarding the potential teratogenic effect observed in the pivotal rabbit study.

### 9.3 Prenatal and Postnatal Development

Study title: Oral Reproductive Toxicity Study in the Wistar Rat---Pre and Postnatal Development

Study no:	NP07356
Study report location:	EDR, M4/4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 13, 1998 (first delivery of animals)
GLP compliance:	Statement Included, compliant to (b) (4)
QA statement:	(1998)
Drug, lot #, and % purity:	Statement Included S 16257-2, batch # 46762, purity: 99.9%

### Key Study Findings

F0:

Enlargement of the heart was observed at dosages  $\geq 2.5$  mg/kg (lowest dose) which might be related to the bradycardia effect of the test article.

F1:

Enlargement of the heart was observed at dosages  $\geq 7$  mg/kg ( $AUC_{24h}$  on LD5 = 1396 ng.h/mL);

Increased postnatal mortality associated with interventricular septum defect and abnormal shape of the heart was observed in the pups from dam dosage group of 20 mg/kg ( $AUC_{24h}$  on LD5 = 5037 ng.h/mL);

The NOAEL for pre- and postnatal development of the F1 generation is 2.5 mg/kg ( $AUC_{24h}$  on LD5 = 608 ng.h/mL).

F2:

The NOAEL for pre- and early postnatal development of the F2 generation is 20 mg/kg.

## Methods

Doses:	2.5, 7, 20 base mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/Demineralised water
Species/Strain:	Rat/Wistar
Number/Sex/Group:	25/females/group
Satellite groups:	5/females/treated group (3/group analyzed)
Study design:	Approximately 190 males and females, aged 14-week, were paired to obtain about 115 females (F0) effectively mated. Day of Mating = Day 0 of Pregnancy (sperm positive vaginal smears); Day of Spontaneous Delivery = Day 0 of Lactation Day (LD0) For main study, pregnant females (F0) were treated from GD6 to LD20 inclusive; for TK sampling F0 females were treated from GD6 to LD5; Pups weaned on Day 21
Deviation from study protocol:	Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

## Observations

For F0 females, mortality, clinical observations (prior to and after treatment during treatment period), body weight (daily), feed and water consumption (GD0-GD5, GD6-GD13, GD14 to end of gestation, LD0-LD6, LD7-LD13) were recorded. Blood samples were taken from five F0 females designated for TK evaluation on GD17 and on LD5 at time points of 10 min, 1, 6 and 24 hours after treatment for quantitation of the unchanged test article and its N-demethylated metabolite in plasma. F0 females which delivered a litter were sacrificed on LD21 for a detailed autopsy. Those from the same subgroup with a sperm positive vaginal smear, but without delivery, were sacrificed from twenty-five days later for verification of pregnancy and detailed autopsy. Uterine examination for F0 reproductive performance and necropsy were conducted. Macroscopic anomalies were collected for possible histopathologic evaluation.

For F1 generation rats, mortality, clinical observations, and body weights were recorded.

For the F1 generations rats chosen for continued evaluation, the mortality, clinical observations, body weights, and food consumption were recorded. Pre-weaning landmarks of physical development (incisor eruption on LD11, eye opening on LD15 and auditory meatus opening on LD20) and reflex tests (surface righting on LD6,

prehensile traction on LD13, pupillary reflex on LD19 and auditory startle on LD20) were checked for all live F1 pups. Sexual maturation (postweaning landmark of development) including cleavage of the balanopreputial gland from day 40 post partum (p.p.) and vaginal opening from day 30 p.p. was also checked for all live pups.

For all F1 breeders (one male and one female per litter where possible), behavior assessment included locomotor activity in an open field at the age of 6-7 weeks, learning ability in a water maze at the age of 9-10 weeks, and memorizing ability in a water maze at the age of 10-11 weeks. At approximately 11-13 week old, F1 breeders were paired in a period of 1-2 weeks. F1 male breeders were sacrificed for a detailed autopsy after littering of their corresponding F1 females, or after terminal sacrifice of apparently non-pregnant F1 females for verification of pregnancy. The complete male genital tract (testes, epididymides, prostate, seminal vesicles and deferent ducts), from F1 males which did not fertilize their corresponding females were collected for histopathologic evaluation. The ovaries and oviducts from F1 females, that were apparently not pregnant at terminal sacrifice, were collected for histopathologic evaluation. F1 females, which delivered a litter and showed no total litter loss, were sacrificed between LD3-5 for a detailed autopsy. At the same time, their F2 pups were weighed and examined for any gross external anomaly.

F1 males and females not selected for behavior testing and breeding were sacrificed for a detailed autopsy after achievement of sexual maturation of all littermates from the same sex in a litter.

## **Results**

### F0 Dams

Survival: No mortality was recorded among the F0 females.

Clinical Signs:

Increased salivation (mainly in lactation) and decreased activity in gestation were observed in mid- and high dosage F0 females and additional signs including ventral posture, irregular breathing, partial blepharoptosis, and piloerection during gestation were observed in the high dosage F0 females.

Body weight:

There was no treatment-related effect on body weight and body weight gain during gestation and lactation period.

Feed and water consumption:

There was no treatment-related effect on food consumption during gestation and lactation period

During gestation, mean water consumption was dose dependently increased in all treated groups when compared with that of the control group. During lactation, mean water consumption was similar between control and treated groups.

#### Uterine content:

There was no treatment-related adverse effect on the progress of gestation and parturition. Mean duration of gestation, gestation index (number of females with a live litter / number of pregnant females) and mean live birth index (number of live newborn pups / number of implantation sites) were unaffected. There was no treatment-related effect on mean body weight of the F1 pups at birth and the sex-ratio of F1 pups. Data is shown in the table below (excerpted from M2.6.7 page 134-135).

Daily dose (mg/kg) (Cont'd)	0 (control)	2.5	7	20
<b>F0 females:</b>				
No. pregnant	24	24	22	22
Mean No. implantations	12.4	12.8	11.4	12.4
Mean postimplantation loss (%)	0.09	0.06	0.10	0.10
Mean duration of gestation (days)	22.5	22.7	22.4	22.6
Abnormal parturition	0	0	1 <sup>a</sup>	0
Gestation index <sup>b</sup>	1.00	1.00	0.95	1.00
No. female with total litter loss	0	0	0	0
<b>F1 litters: (preweaning)</b>				
No. litters evaluated	24	24	21	22
Mean No. pups/litter	11.3	12.2	10.6	11.3
Mean No. live newborn pups/litter	11.2	12.1	10.6	11.2
Mean No. dead newborn pups/litter	0.0	0.0	0.0	0.0
Mean No. stillborn pups/litter	0.1	0.1	0.0	0.0
Pup sex ratios (% males) (at birth)	54	48	52	53
Live birth index	0.90	0.94	0.90	0.90

#### Necropsy observation:

An enlarged heart was observed in one female of each of the 2.5 and 7 mg/kg dose groups and in two females of the 20 mg/kg dose group. No histopathologic evaluation of the heart was performed as the finding was considered similar to that observed in the previous general toxicity studies and related to bradycardia.

#### Toxicokinetics:

The TK parameters are presented in the table below (excerpted from M2, section 2.6.7. page 133). Mean systemic exposure ( $AUC_{24h}$ ), increased slightly more than dose on GD17 and proportionally to dose on LD5.  $AUC_{24h}$  values of S 16257 were similar on GD17 and LD5, except for the high dose. Mean systemic exposures to S 18982 (N-demethylated metabolite) could not be accurately determined, because plasma concentrations were below the limit of quantitation (LOQ) for most of the samples.

Daily dose (mg/kg)		0 (control)	2.5	7	20
<b>F0 females:</b>					
Toxicokinetics of S 16257 (mean values) <sup>a</sup> :					
- $C_{max}$ (ng/ml)	G17		100	209	729
	L5		140	222	1096
- $AUC_{24}$ (ng.h/ml)	G17		622	1460	7719
	L5		608	1396	5037

F1 pups (data was summarized in the table below, excerpted from M2.6.7 page 135)

Survival: Mortality of F1 pups from birth to weaning was increased at 20 mg/kg when compared with controls. Consequently, the weaning index was slightly decreased in the high dose group.

Clinical signs: No significant treatment-related clinical signs were observed.

Body weight: Mean body weight of the F1 pups from birth to weaning was similar for treated and control groups.

Physical development: There were no treatment-related anomalies (incisor eruption on LD11, eye opening on LD15 and the auditory meatus opening on LD20).

Reflex test: There were not treatment-related anomalies of reflex test (surface righting on LD6, prehensile traction on LD13, pupillary reflex on LD19 and auditory startle on LD20).

Examination of found dead pups before LD9:

Skeletal examination did not reveal treatment-related findings.

Examination of the heart from 6 out of 20 found dead pups from the high dosage group was performed to verify cause of death. Four hearts showed an interventricular septum defect, which is considered to be treatment-related and the potential cause of death of the four corresponding pups. All six hearts showed an abnormal external shape.

Daily dose (mg/kg) (Cont'd)	0 (control)	2.5	7	20
F1 litters: (preweaning)				
Postnatal mortality before weaning (birth to L20) (%)	0.8	1.0	1.3	8.1**
Weaning index (L21)	0.99	0.99	0.99	0.92
Mean pup bodyweights (g) (at birth)	6.5	6.5	6.4	6.3
Mean pup bodyweights (g) (at weaning)	42.3	40.7	41.8	41.5
Pup clinical signs	-	-	-	-
Physical landmarks:				
Incisor eruption, eye opening, auditory meatus opening	-	-	-	-
Reflex tests:				
Surface righting, prehensile traction, pupillary reflex, auditory startle	-	-	-	-
Skeletal examination of dead F1 pups <sup>a</sup>	-	-	-	-
Pup necropsy observations <sup>b</sup>				
Heart:				
External abnormal shape	np	np	np	6/6
Septum defect	np	np	np	4/6

### F1 (post-weaning)

Data was summarized in the table below (excerpted from M2/2.6.7 page 136)

Mortality: none.

Body weight: No treatment-related effect on body weight gain was observed. Mean body weight gain of F1 male and female breeders, randomly selected at weaning (three weeks old), was similar between the 3rd and 10th week p.p. for treated and control groups.

Sexual maturation: Sexual maturation of F1 animals was not affected by treatment of the parental F0 generation. The onset and accomplishment of F1 male and female sexual maturation was similar in all groups.

Behavior assessment (F1 breeders): There were no treatment-related adverse effects on F1 males and females' locomotor activities in an open field as well as learning and memorizing abilities in a water maze.

#### Necropsy observation:

Necropsy of F1 animals at terminal sacrifice (including F1 breeders) or found dead after LD9 revealed an enlarged or misshapen heart in one mid-dosage male and three high dosage males; and abnormal shape of the heart was also detected in one high dosage female. Other findings with single cases in different groups (F1 breeders) were not considered treatment-related.

#### Histopathology evaluation:

Evaluation of ovaries and oviducts from non-pregnant F1 females, as well as that of the complete genital tract from the corresponding F1 males, did not reveal any change. Evaluation of mammary glands of two F1 females from the 2.5 mg/kg dose group, which lost their litter, did also not reveal any change.

Daily dose (mg/kg) (Cont'd)	0 (control)	2.5	7	20
<b>F1 males: (postweaning)</b>				
No. evaluated postweaning	24	24	21	22
No. found dead or killed <i>in extremis</i>	0	0	0	0
Clinical observations	-	-	-	-
Necropsy observations (terminal kill)				
Heart:				
. Enlarged or misshapen	-	-	1	3
. Depressed whitish area on apex of left ventricle	-	-	-	1
Bodyweight change (from 21pp to 70pp)	-	-	-	-
Balanopreputial gland cleavage	-	-	-	-
Motor activity (open field)	-	-	-	-
Learning and memory (water maze)	-	-	-	-
Mean No. days prior to mating	3.3	3.0	3.0	2.5
No. of males that mated	24	24	21	22
No. of fertile males	23	22	21	22
<b>F1 females: (postweaning)</b>				
No. evaluated postweaning	24	24	21	22
No. found dead or killed <i>in extremis</i>	0	0	0	0
Clinical observations	-	-	-	-
Necropsy observations (terminal kill)				
Heart: misshapen	0	0	0	1
Premating bodyweight change	-	-	-	-
Bodyweight change (from 21pp to 70pp)	-	-	-	-
Vaginal opening	-	-	-	-
Motor activity (open field)	-	-	-	-
Learning and memory (water maze)	-	-	-	-
Mean No. days prior to mating	3.3	3.0	3.0	2.5
No. of females sperm positive	24	24	21	22
<b>F1 females: (postweaning) (Cont'd)</b>				
No. of pregnant females	23	22	21	22
Fertility index	0.96	0.92	1.00	1.00
Mean No. implantations	12.6	11.4	11.6	13.5
Mean No. postimplantation loss	0.08	0.14	0.10	0.04
Gestation index	1.00	1.00	0.95	1.00
No. female with total litter loss	0	1	0	0

#### Fertility, gestation and parturition for F1 breeders

There were not treatment-related effects on F1 breeders' fertility and progress from gestation to parturition. The results of the parameters were summarized in the tables above and below (excerpted from M2/2.6.7, pages 136-137).

#### F<sub>2</sub> Generation:

Survival: No treatment-related effect on mortality. No significant clinical signs observed.

Body weight: Mean body weight and mean body weight gain from birth to LD3 were similar for treated and control groups.

External evaluation: One live newborn pup of mid-dosage group with a shortened tail due to maternal cannibalism was not considered toxicologically significant. No other treatment-related findings.

Male/Female ratio: Not affected by the treatment of F0 females

External examination: No treatment-related findings were observed in pups found dead before terminal sacrifice or at terminal sacrifice between LD3-5.

Daily dose (mg/kg) (Cont'd)	0 (control)	2.5	7	20
F2 Litters:				
Number of F1 females with litter	23	22	20	22
Mean No. live conceptuses/litter	11.6	10.3	11.0	12.9
No. dead newborn <sup>3</sup> pups	1	2	0	0
No. stillborn pups	0	0	0	3
Mean % Postimplantation loss	0.08	0.14	0.10	0.04
Pup bodyweights (birth) (g)	6.3	6.3	6.3	6.1
Pup bodyweights (L3) (g)	9.2	9.1	9.2	8.7
Pup sex ratios (% males)	53.3	47.8	56.0	52.9
Live birth index	0.91	0.86	0.89	0.96
Viability index (L3)	1.00	0.95	1.00	0.99
External and skeletal examination of dead pups	-	-	-	-

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## 10 Special Toxicology Studies

## 11 Integrated Summary and Safety Evaluation

Reproductive and developmental toxicity was evaluated in the rat and rabbits and the relevant findings were summarized in the table below.

There were no treatment-related adverse effects on male and female fertility or early stage of embryonic development in the rats treated at up to 175 mg/kg/day.

In pregnant rats treated during organogenesis, external abnormal shape of the heart (dysplasia) with or without simple anomalies of the major proximal arteries was observed at 2.3 mg/kg/day and above. One fetal death with a delay in ossification and a spina bifida occulta situated between 5th and the 7th cervical vertebra as well as reduced mean fetal weight were observed at 19 mg/kg/day (approximately 14 times human AUC<sub>24h</sub> at MRHD). Teratogenic effects include interventricular septal defect and complex anomalies of the major proximal arteries observed at dosages  $\geq$  4.6 mg/kg/day (AUC<sub>24h</sub> of 1980 ng.h/mL, approximately 3 times human AUC<sub>24h</sub> at MRHD).

In pregnant rabbits treated during organogenesis, increased postimplantation loss was observed at lowest dosage of 7 mg/kg (AUC<sub>24h</sub> of 1664 ng.h/mL, approximately 5 times human AUC<sub>24h</sub> at MRHD) and above. Reduced fetal and placental weights and a small number of fetuses with ectrodactylia were observed at 28 mg/kg (AUC<sub>24h</sub> of 11603 ng.h/mL, approximately 34 times human AUC<sub>24h</sub> at MRHD).

Study	Route/Dose range mg/kg	Results <b>Reproductive and Development Toxicology</b>	Lowest dose with findings (mg/kg)	Multiples of human AUC	
				M	F
Rat fertility	Oral, daily 7,35, 175 14d pre-G6	Negative for male and female fertility NOAEL=175 mg/kg	175	NA	NA
Rat Embryo-Fetal, Pivotal + suppl.	Oral, daily 2.3, 4.6, 9.3, 19, GD6-15 0.5 1.5, 9.3 GD6-17	Reduced fetal weight, one fetal death with a delay in ossification and a spina bifida occulta (5th & 7th cervical vertebra )	19	-	14
		Teratogenic: interventricular septal defect and complex anomalies of primary arteries	4.6	-	3
		F1: external abnormal shape of the heart	2.3	-	2
		NOAEL= 1.5 mg/kg/day		-	1
Rabbit Embro-Fetal	Oral daily 7, 15, 28, GD6-18	Increased post-implantation loss	7	-	5
		Reduced fetal and placental weight ectrodactylia	28	-	34
		NOAEL for embryotoxicity <7		-	5
Rat, post-natal	Oral daily 2.5-20 GD6-LD20	F1:Increased postnatal mortality (dead pups: misshapen heart, interventricular septal defect); adult: abnormal shape of the heart	20	-	15
		F1 adult: enlarged heart	7	-	4

(b) (4)

NA: not available

In the rat pre-postnatal study, reduced postnatal survival associated with interventricular septum defect and abnormal shape of the heart was observed in the F1 pups delivered by dams treated at 20 mg/kg (AUC<sub>24h</sub> on LD5 = 5037 ng.h/mL, approximately 15 times

human  $AUC_{24h}$  at MRHD ). Enlargement of the heart in adult F1 rats was observed at dosages  $\geq 7$  mg/kg. No anomalies were observed for F2 generation from F0 dams treated at doses up to 20 mg/kg/day.

Taken together, as ivabradine was associated with lethal cardiac teratogenicity in rats, it should not be given to women during pregnancy, particularly during the embryogenesis of the heart and period of lactation.

(b) (4)



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11/28/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW  
EVALUATION (Reproductive and Development Toxicology)

Application number: NDA 206,143  
Supporting document/s: S016 (eCTD0014), S022 (eCTD0017)  
Applicant's letter date: April 30, 2014, June 27, 2014  
CDER stamp date: April 30, 2014, June 27, 2014  
Product: Ivabradine  
Indication: To reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm), (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)  
Applicant: Amgen, Inc.  
Review Division: Division of Cardio-Renal Products  
Reviewer: Jean Q. Wu  
Supervisor/Team Leader: Albert DeFelice  
Division Director: Norman Stockbridge  
Project Manager: Alexis Childers

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# 1 Executive Summary

## 2 Drug Information

### 2.1 Drug

#### 2.1.1 CAS Registry Number (Optional)

Ivabradine: 155974-00-8

Ivabradine hydrochloride: 148849-67-6

#### 2.1.2 Generic Name

ivabradine

#### 2.1.3 Code Name

S 16257-2; AMG 998

#### 2.1.4 Chemical Name

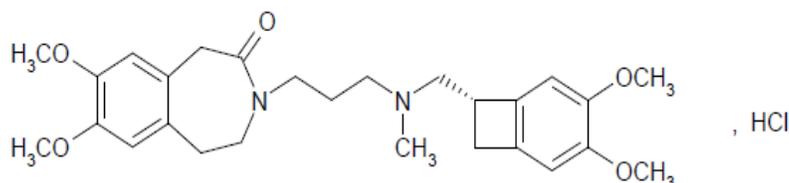
3-(3-(((7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl) methyl amino)propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride

#### 2.1.5 Molecular Formula/Molecular Weight

C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>, HCl/505.1 g/mol;

468.593 g/mol (base); Conversion factor from the salt to the base: 0.928

#### 2.1.6 Structure



#### 2.1.7 Pharmacologic class

hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND (b) (4)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

Ivabradine is provided as film-coated tablet in two strengths: 5 mg (oval) and 7.5 mg (triangular). The composition of the drug is listed in the table below (excerpted from Module 2.3. P, Table 1, Page 7)

Table 1. Composition of Ivabradine 5 mg and 7.5 mg Tablets

Component	5 mg		7.5 mg		Function	Reference to specifications
	Percentage (% w/w)	Quantity (mg/tablet)	Percentage (% w/w)	Quantity (mg/tablet)		
<b>Tablet</b>						
Ivabradine hydrochloride <sup>a</sup> (free base equivalent)	5.39 (5.00)	5.390 (5.000)	8.085 (7.50)	8.085 (7.500)	Drug substance	In-house
Lactose monohydrate	(b) (4)				(b) (4)	USP/NF, PhEur.
Maize starch	(b) (4)				(b) (4)	USP/NF
Maltodextrin	(b) (4)				(b) (4)	USP/NF, PhEur
Magnesium stearate	(b) (4)				(b) (4)	USP/NF, PhEur
Colloidal silicon dioxide	(b) (4)				(b) (4)	USP/NF, PhEur
(b) (4)	(b) (4)				(b) (4)	USP
<b>Core Tablet Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>		
<b>Film-Coating</b>						
(b) (4) salmon <sup>c</sup>	(b) (4)				(b) (4)	See 3.2.P.4.1
Polyethylene glycol 6000	(b) (4)				(b) (4)	USP/NF, PhEur
(b) (4)	(b) (4)				(b) (4)	USP
<b>Total</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>		

<sup>a</sup> The molecular weights of Ivabradine hydrochloride anhydrous and ivabradine free base are 505.1 g/mol and 468.6 g/mol, respectively. The free base accounts for 92.77% of the salt

(b) (4)

## 2.4 Proposed Clinical Population and Dosing Regimen

Ivabradine is indicated to reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm) (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)

The proposed starting dose of ivabradine is 5 mg twice daily. After 2 weeks of treatment, if heart rate is between 50 and 60 bpm, the dose of 5 mg twice daily should be maintained. The dose should be increased to 7.5 mg twice daily if resting heart rate is persistently above 60 bpm.

As listed in the table below (excerpted from Module 2, Section 2.4, Page 7), the human plasma exposures of ivabradine and its N-desmethylated metabolite (S 18982) were estimated at steady state in patients receiving the maximum recommended human dose (MRHD), 7.5 mg bid. These values were derived from a population pharmacokinetic analysis using Phases II and III clinical data [Summary of Clinical Pharmacology Studies, in Section 2.7.2 (2.1.2.2)], and are accepted as references when preclinical doses are expressed as multiples of human exposures in current review, unless otherwise indicated.

**Table 1. Ivabradine and S 18982 (Metabolite) Plasma  $C_{max}$  and Estimated  $AUC_{24}$  at Steady State in Patients at HTD**

	Ivabradine (n=492)	S 18982 (n=541)
Population $C_{max}$ (ng/ml)	31 ± 9.8	7.9 ± 2.3
Equivalent $C_{max}$ in $\mu$ M	0.07	0.02 <sup>b</sup>
Population $AUC_{24}$ <sup>a</sup> (ng.h/ml)	346	128

Values are mean ± SD;

a: Calculated from AUC over 12 h period from [WS (2.7.2) 3.1/ Table 12; Table 34 and 35]

b: S 18982 MW = 454.6

## 2.5 Regulatory Background

There was no IND opened in the US FDA

(b) (4)

(b) (4)

## 3 Studies Submitted

### 3.1 Studies Reviewed

NP06673: Oral reproduction toxicity study in the Wistar rat (fertility and early embryonic development)

NP05135: Oral reproduction toxicity study in the Wistar rat (embryofetal development)

NP26980: Oral reproduction toxicity study in the Wistar rat (embryofetal development)-supplementary

NP05134 Oral reproduction toxicity study in the New Zealand White rabbit (embryofetal development)

NP08073: Oral reproduction toxicity study in the rabbit (complementary investigation on embryofetal development)—nonGLP

NP07356: S 16257-2: oral reproduction toxicity study in the Wistar rat (pre- and postnatal development)

(b) (4)

*The following non-pivotal studies were cursory reviewed along with the relevant pivotal studies:*

NP08074: Oral preliminary reproduction toxicity study in the Wistar rat (male and female fertility/pre- and postnatal development)

NP08075: Oral preliminary reproductive toxicology study in the rat, Segment II: embryo-fetotoxicity and teratogenicity

NP08076: Oral preliminary reproductive toxicology study in the rabbit, Segment II: embryo-fetotoxicity and teratogenicity

(b) (4)

(b) (4)

**3.2 Studies Not Reviewed**

none

**3.3 Previous Reviews Referenced**

N/A

## 4 Pharmacology

## 5 Pharmacokinetics/ADME/Toxicokinetics

## 6 General Toxicology

## 7 Genetic Toxicology

## 8 Carcinogenicity

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

Study title: Oral reproduction toxicity study in the Wistar rat (fertility and early embryonic development)

Study no.:	NP06673
Study report location:	EDR M4/4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Dec 12, 1995
GLP compliance:	Yes, statement included
QA statement:	Included
Drug, lot #, and % purity:	S16257-2, batch # 46762, 100.4 %

### Key Study Findings

Clinical signs including ptialism, hypoactivity, and blepharoptosis were sporadically observed in treated males, mainly in the high dosage group. The NOAEL for paternotoxicity is identified at high dosage, 175 mg/kg.

Clinical signs including hypoactivity, blepharoptosis, piloerection and ptialism were frequently observed in treated females, most markedly in the high dosage group. Significantly reduced body weight gain associated with reduced feed consumption was observed in the high dosage females in pre-mating and gestation periods. The reduced body weight gain in the mid-dosage females was observed in the 1<sup>st</sup> day of gestation period and was not associated with reduced feed consumption. Increased water

consumption was observed in all treated groups, significantly in mid- and high dosage groups. The NOAEL for maternal toxicity is identified at low dosage, 7 mg/kg.

There was no treatment-related effect on reproductive organ weights, sperm analysis parameters (males), mating/fertility index, reproductive performance and macro-, microscopic examinations of reproductive organs. The NOAEL for male and female fertility and the early stages of embryonic development is identified as 175 mg/kg.

Direct plasma exposures at the dosages in this study are not available as TK samples were not collected.

## Methods

Doses: 0 (D0), 7 (D1), 35 (D2) or 175 (D3) base mg/kg  
 Frequency of dosing: daily  
 Dose volume: 10 mL/kg  
 Route of administration: Oral gavage  
 Formulation/Vehicle: Solution/Demineralized water  
 Species/Strain: Rats/Wistar (male: 7-week, female: 11-week)  
 Number/Sex/Group: 20  
 Satellite groups: n/a  
 Study design: See table below (excerpted from page 280 of the report)

Deviation from study protocol: Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

Study group identification	D0		D1		D2		D3	
Subgroup identification ①	A	B	A	B	A	B	A	B
No. of F0 animals per group and per sex	20							
Female numbering	1 to 20	21 to 40	41 to 60	61 to 80	81 to 100	101 to 120	121 to 140	141 to 160
Male numbering	201 to 220	/	241 to 260	/	281 to 300	/	321 to 340	/
Administration route and method	Oral: oesophageal intubation							
Vehicle	Demineralised water							
Volume (ml/kg)	10							
Dose (mg/kg) administered once daily	0		7		35		175	
- expressed as the base	0		7.55		37.7		189	
- expressed as the salt	0		7.55		37.7		189	
Dosing frequency and period	Males: daily for 63 days before pairing, then until sacrifice for all males in group A Females: daily for 14 days before pairing, then up to day 6 of gestation inclusive for all females in group B							

① The females in group A and the males in group B will not be treated and will undergo no physical examination. After the mating period, the males will be eliminated without examination. The females will undergo pregnancy examination on day 14 of gestation. The animals will enable study of the potential effects of the test substance administered separately on male fertility and on female fertility. The mates will be determined as per the order of numerical identification for the animals in group A.

The high dose 175 mg/kg was selected based on the preliminary reproductive toxicity study (NP08074) in which the high dosage induced general toxic effects in the parental F0 generation. The general toxic effects at 175 mg/kg were characterized by clinical signs and a reduction in mean body weight gain for the animals of both sexes, as well as by a decrease in mean feed consumption and an increase in mean water consumption for the F0 females. A cardiomegaly in several animals was considered as an exaggerated pharmacological activity of the test article.

## Observations and Results

### Mortality and Clinical Signs

Untreated animals were subjected to a daily mortality check only. Treated F0 males and females were observed once daily during acclimation and then prior to and after treatment each day during treatment period. During gestation the F0 females were observed at least once daily and any sign of abortion was recorded.

No animal died through the study.

For F0 males, ptyalism after treatment was observed in 19 out of 20 males from high dosage group with a frequency varying from occasional to very frequent, in 16 out of 20 males from mid-dose group with variable occurrence, and in only two males from low dosage group with one to two occasions. Ptyalism the next morning just before treatment was observed sporadically in 5 males from high dosage group and in 11 males from mid-dose group. Hypoactivity after treatment only was observed sporadically in half of the males from high dosage group, once to twice in two males from mid-dose group and once in one male from low dosage group. Blepharoptosis after treatment only was observed with a very low occurrence in seven males from high dosage group.

For F0 females, during the pregestation period, hypoactivity, blepharoptosis, piloerection and ptyalism were observed frequently in more than half to all females in high dosage group and sporadically in a very few females in the mid-dosage group. These clinical signs were observed in a smaller number of females in both groups with similar occurrence, except for ptyalism affecting more than half of the females in the mid-dosage group. Mastication was sporadically observed in a few females in the high and mid-dosage groups during the pre mating period but was only observed in high dosage females during gestation. Ventral decubitus was observed very sporadically in a few high dosage females during pre mating period but not in the gestation. In low dosage females, no particular signs were observed during pre mating period but occasional to frequent salivation was observed in almost half of females. Some of these findings, mainly piloerection, hypoactivity and blepharoptosis, were still observed next morning prior to the treatment in several females, mainly from high dosage group.

### Body Weight

During the pre mating period males from subgroup A and females from subgroup B were weighed once weekly.

Each female from subgroup B effectively mated (sperm positive vaginal smear) was weighed daily during gestation, from day 0 to day 14 inclusive. The body weights of females found not to be pregnant at Caesarean section, and at subsequent uterus staining, were not included in the calculation of group mean values.

For F0 males, there was no treatment-related adverse effect on body weight gains.

For F0 females, mean body weight gains are listed in the table below.

<b>Dosage mg/kg/day</b>	<b>control</b>	<b>7</b>	<b>35</b>	<b>175</b>
Premating – (14-7 day)	6.8	8.4	6.8	0.8*
Premating – (7-1 day)	9.9	12.8	12.6	13.7
Gestation 1 <sup>st</sup> week	29.1	25.0	22.8	18.6
Gestation 0-3	4.96	3.76	3.22*	2.04*
Gestation 2 <sup>nd</sup> week	32.3	30.3	29.1	26.7
Gestation 6-9	3.72	3.36	2.92	2.02*

\*statistically significant when compared to the control group

During the pre-mating period, after the first week of treatment, the mean body weight gain was lower in the high dosage group compared to control with statistically significance. During the second week of treatment, the mean body weight gain was higher in all treated groups than in the control group, but the high dosage females did not recover entirely.

During the first week of gestation, the mean body weight gain was dose dependently reduced in all treated groups when compared with that in the control group. The statistical analysis of mean body weight gain over a series of three-day periods showed significant differences between the mid- and high dosage groups and the control group for GD 0-3. During the second week of gestation, after withdrawal of treatment, the mean body weight gain remained dose dependently reduced in all treated group with a statistically significant difference for GD 6-9 between high dosage and control groups.

The effect on body weight gain was considered test article-related and the significant decrease in body weight gain for the high dosage group was consistent with significantly reduced feed consumption observed in the same group. The biological significance of this finding for the low dosage cannot be established due to the small magnitude of the change.

## Feed Consumption

Feed and water consumption were calculated for the two treatment weeks of the pre-mating period and for the first two weeks of gestation. The results were expressed in g/day.

The result was summarized in the table below (excerpted from module 2, section 2.6.7, page 120). The mean feed consumption from high dosage group was consistently lower than that of the controls during the treatment period, which was most marked during the first week of the pre-mating period (control: 20.5 g/day, high dose: 14.3 g/day).

Differences between these two groups were statistically significant in pre-mating weeks one and two, and gestation week one ( $p < 0.01$ ). After the withdrawal of treatment,

mean feed consumption did not show any statistically significant difference between groups.

Mean water consumption was dose- dependently higher in all treated groups than in the control group throughout the study, even after withdrawal of treatment. It is consistent with the repeated-dose general toxicity studies which showed an increase in urinary volume and modifications of electrolyte balance. These findings were possibly related to the pharmacodynamic activity of the test article.

Daily dose (mg/kg) (Cont'd)	0 (control)	7	35	175
<b>Females:</b>				
Preparing food consumption (% <sup>a</sup> )	-			
1 <sup>st</sup> week of dosing	20.50 g/d	-2	-6	-30**
2 <sup>nd</sup> week of dosing	20.89 g/d	+1	+2	-6**
Gestation food consumption (% <sup>a</sup> )				
1 <sup>st</sup> week of dosing	23.99 g/d	-1	-4	-12**
2 <sup>nd</sup> week of dosing	-	-	-	-
Preparing water consumption (% <sup>a</sup> )				
1 <sup>st</sup> week of dosing	25.63 g/d	+13	+17	+20
2 <sup>nd</sup> week of dosing	26.61 g/d	+16	+27*	+32**
Gestation water consumption (% <sup>a</sup> )				
1 <sup>st</sup> week of dosing	32.58 g/d	+18	+22	+25
2 <sup>nd</sup> week of dosing	36.33 g/d	+8	+10	+11

*Incidence of clinical signs (number of animals affected and/or frequency): +: Low to average ++: High  
a: For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on percent differences)*

*Two-way analysis of variance, Dunnett's Test \*: p<0.05 \*\*: p<0.01*

## Toxicokinetics

Neither TK sampling nor TK evaluation was performed in the study.

## Stability and Homogeneity

Stability was verified at concentrations of 0.5, 10 and 100mg/mL of S 16257-2 in demineralized water. The solutions were stable 25 days storage at room temperature exclusively.

## Necropsy

Untreated F0 males (subgroup B) were euthanized after successful pairing or at the end of the monogamous mating period, and not necropsied.

Treated F0 males (subgroup A) were euthanized after examination of the uterine content of their corresponding F0 females. A detailed autopsy was carried out.

F0 females from subgroups A and B, with sperm positive vaginal smear, were euthanized on GD14. F0 females from subgroups A and B, with a sperm negative vaginal smear at the end of the 3-week mating period, were euthanized at least ten days after the last mating day. An examination of F0 females' uterine content was carried out and also a detailed autopsy of the treated F0 females from subgroup B.

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

Mating: During the mating period (three weeks), monogamous pairs were formed each evening by introducing a male into the cage of a female. Treated males were paired with untreated females and *vice versa*. The next morning the male was removed from the cage of the female and returned to its own cage. Effective mating was checked by vaginal smear. A female was considered effectively mated when light microscopic examination of the vaginal smear detected the presence of spermatozoa (gestation day zero), or when the examination of uterine content at terminal sacrifice revealed pregnancy, despite a sperm negative vaginal smear during the entire mating period.

Uterine content: After hysterectomy, corpora lutea, implantation sites, live embryos and resorptions were counted. Dead embryos were considered as late resorptions. An external macroscopic examination was performed on the embryos, followed by an evaluation of their viability on the basis of heartbeats and a functional blood circulation.

Necropsy: A detailed autopsy was carried out on each treated F0 male from subgroup A and each treated F0 female from subgroup B. Organs with macroscopic anomalies were removed and preserved for a possible histopathological evaluation according to the decision of the Study Director. Corresponding organs of sufficient controls were preserved for comparison. Only F0 animals with organ anomalies confirmed by histopathological evaluation are presented in the report.

The testes and epididymides from the treated F0 males were systematically removed for light microscopic examination after recording of the respective organ weights and after sperm analysis (number and viability of spermatozoa) in the left cauda epididymis.

The complete male genital tract was systematically removed for light microscopic examination from the treated F0 males which did not fertilize their corresponding untreated female.

The ovaries and oviducts from treated F0 females which were not pregnant were systematically removed for a possible light microscopic examination. If no implantation site was detected after uterus staining, a histopathological examination was performed on the ovaries and oviducts.

**Histopathology:**

The organs with macroscopic findings were fixed, dehydrated and then embedded in paraffin wax. Sections were stained with hemalum-eosin-saffron prior to light microscopic examination. Two different stains were used for the testes, i.e. PAS and hemalum-eosin. Uterus staining to count the implantation sites was performed according to (b) (4) method. For the systematic histopathological examination of the male and female reproductive organs, only those of the F0 animals with anomalies are presented.

Treatment Effects on F0 Males

## Mating/Fertility Index/Reproductive Performance

As shown in the table below (excerpted from page 93 of the report), all treated F0 males, succeeded in mating their corresponding untreated females. The mean time necessary for successful copulation, expressed in days, was slightly increased in all treated groups.

The number of pregnant females was slightly lower in all treated groups than in the control group, without a dose-response relationship. Consequently, the fertility index for treated males was also slightly lower than for control males. Given the small magnitude and lack of dose-dependency, it is not considered to be toxicologically significant.

	D0	D1	D2	D3
Number of F0 males to be paired	20	20	20	20
Number of F0 males which mated a female	20	20	20	20
Number of F0 males which fertilized a female (female was pregnant)	19	17	17	18
Effective mating rate of F0 males	1.00	1.00	1.00	1.00
Fertility index of F0 males	0.95	0.85	0.85	0.90
Mean time necessary for successful copulation (days)	2.80	3.20	(*) 3.05	4.00

(\*) F0 male 286 D2 excluded because of unknown mating date: the corresponding untreated female showed a sperm negative vaginal smear during the entire mating period but were eventually pregnant.

As shown in the table below (excerpted from page 3 of the report), evaluation of the uterine content in the untreated females on gestation day fourteen did not reveal any biologically or statistically significant difference between the control group and the treated groups for any of the parameters examined.

Dosage (mg base/kg)	0	7	35	175
Males Producing Live Embryos	18	16	17	18
Number of Pregnant Females	19	17	17	18
Corpora Lutea/Female	13.5	13.8	14.1	15.1
Implantations/Female	12.5	11.9	11.8	13.7
Live Embryos/Female	11.6	11.1	11.0	12.4
Pre-implantation Loss $\geq 3$ (80 <sup>th</sup> percentile)	2	4	6	3
Post-implantation Loss $\geq 2$ (80 <sup>th</sup> percentile)	6	3	2	6

Reproductive organ weights and sperm analysis showed (see the table below, excerpted from page 3 of the report) that mean weights of the testes and the epididymis as well as the sperm vitality (% motile sperm) per cauda epididymis were similar between treated groups and controls. The mean number of spermatozoa ( $\times 10^6$ ) per gram cauda epididymis revealed a slightly lower value in high dosage group than in the other groups, which did not reach a statistical significance. This result was due to male No. 339 with a bilateral testicular atrophy, resulting in only  $50.4 \times 10^6$  spermatozoa. i.e. about eight percent of the mean value for the remaining males from this group. There was no generalized decrease with regard to the individual values for the number of spermatozoa observed in high dosage group.

Testis Weight (absolute, g)	3.69	3.64	3.61	3.58
Testis Weight (relative to body weight, %)	0.78	0.73	0.75	0.78
Epididymis Weight (absolute, g)	1.27	1.26	1.24	1.26
Epididymis Weight (relative to body weight, %)	0.27	0.25	0.26	0.27
Number Spermatozoa/Gram Cauda Epididymis ( $\times 10^6$ ) (cf. suppl. sheet 1)	614	613	632	575
Epididymal Sperm Viability (%)	80.5	81.4	81.9	79.1

### Necropsy and Histopathology

At necropsy, one F0 male from low dosage group showed an enlarged right cauda epididymis with a yellowish mass, and another F0 male from high dosage group showed small testes and epididymis and also a yellowish mass on the left cauda epididymis. Both males were diagnosed with spermatocele by microscopic examination of the epididymal masses. Atrophy of both testes, characterized by a perturbation of spermatogenesis with clonal desquamation of immature cells as well as subatrophied epididymis was also detected for the male from the high dosage group.

Systematic histopathological examination of the testes and epididymis from all treated F0 males revealed that one male from mid-dose group showed about ten percent of involuted tubules with regression of Sertoli cells in one testis. Histopathological examination of the complete genital tract of the F0 males that did not fertilize their corresponding female did not detect any anomaly attributable to treatment.

The above findings in one male from each of low, mid and high dosage groups were not considered toxicologically significant. The incidence was very low and such findings were observed spontaneously in controls from various studies.

### Effects on F0 Female Fertility

#### Mating/Fertility Index/Reproductive Performance

As shown in the table below (excerpted from page 119 of the report), the number of mated F0 females was similar among all groups. The mean time necessary for successful copulation, expressed in days, was slightly increased in all treated groups. The number of pregnant F0 females was higher in all treated groups than in the control group. Hence, the fertility index was not adversely affected by the treatment.

	D0	D1	D2	D3
Number of F0 females to be paired	20	20	20	20
Number of F0 females effectively mated	20	20	20	19
Number of pregnant F0 females	17	18	18	19
Effective mating rate of F0 females	1.00	1.00	1.00	0.95
Fertility index of F0 females	0.85	0.90	0.90	1.00
Mean time necessary for successful copulation (days)	1.95	2.80	(*) 3.53	3.16

(\*) F0 female 108 D2 excluded because of unknown mating date: this female showed a sperm negative vaginal smear during the entire mating period but were eventually pregnant.

As shown in the table below (excerpted from M2/2.6.7. page 121), evaluation of the uterine content in the F0 females on GD14 did not reveal any biologically or statistically significant difference between the control group and the treated groups for any of the parameters examined. In particular, the external macroscopic examination of the embryos did not reveal any morphological anomaly.

Daily dose (mg/kg) (Cont'd)	0 (control)	7	35	175
<b>Females (Cont'd):</b>				
Mean No. of days prior to mating	2.0	2.8	3.5 <sup>a</sup>	3.2
No. of females sperm positive	20	20	20	20
No. of pregnant females	17	18	18	19
Mean No. of Corpora Lutea	14.2	14.6	13.8	13.4
Mean No. of implantations	12.8	13.6	12.5	12.2
Mean % of preimplantation loss	12.3	6.9	9.7	9.5
Mean No. of live conceptuses	11.7	12.1	11.6	11.1
Mean No. of resorptions	1.1	1.5	0.8	1.1
No. of dead conceptuses	0	0	0	0
Mean % of postimplantation loss	13.2	11.1	6.8	11.1

a: One female excluded because of unknown date of mating

### Necropsy and Histopathology

At the terminal sacrifice, autopsy revealed no macroscopic organ anomalies which made histopathological examination unnecessary. Furthermore, the systematic histopathological examination of the ovaries in the non-pregnant females did not reveal any anomaly attributable to the test article.

## 9.2 Embryonic Fetal Development

Study title: Oral Reproduction Toxicity Study in the Wistar Rat (Embryofetal Development)

Study no: NP05135  
 Study report location: EDR M4/4.2.3.5.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: Jan. 5, 1994  
 GLP compliance: Statement included  
 QA statement: Statement included  
 Drug, lot #, and % purity: Ivabradine hydrochloride, S 16257-2, batch# EF558, purity: 99.8 %

### Key Study Findings

There was no significant maternal toxicity observed in the F0 females treated at doses up to 20 mg/kg/day.

External abnormal shape of the heart (dysplasia) associated with or without simple anomalies of the major proximal arteries were observed at dosages  $\geq 2.3$  base mg/kg/day (AUC<sub>24h</sub> on GD15=565 ng.h/mL);

Teratogenic effects including interventricular septal defect and complex anomalies of the major proximal arteries were observed at dosages  $\geq 4.6$  base mg/kg/day (AUC<sub>24h</sub> on GD15=980 ng.h/mL).

One fetal death with a delay in ossification and a spina bifida occulta situated between 5<sup>th</sup> and the 7<sup>th</sup> cervical vertebra as well as reduced mean fetal weight were observed at 19 base mg/kg/day.

S 16257 was detected in amniotic fluid on GD15.

NOAEL for embryofetal development toxicity cannot be identified.

*Note: the dosage in salt form was referred in the following review of this study.*

#### Methods

Doses:	0, 2.5, 5, 10, 20 mg/kg (base: 2.3, 4.6, 9.3, 19)
Frequency of dosing:	daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	Solution/Demineralized Water,
Species/Strain:	Rat/Wistar
Number/Sex/Group:	25 females/group
Satellite groups:	N=5 females/treated group for TK evaluation
Study design:	Female Wistar rats aged 10 to 11 weeks were paired in a 1:1 ratio with untreated male Wistar rats (SPF) of the same age. The effective mating was checked by vaginal smear and the day that a sperm positive vaginal smear was observed, was considered day zero of gestation (GD0). The pregnant females were treated from GD6 to GD15 and C-section was conducted at GD20.
Deviation from study protocol:	Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

Dose selection: The dose selection was based on a preliminary rat embryo-fetal development study (NP08075). In the study, the doses of 120 and 240 mg/kg resulted in 97 and 100% resorptions, respectively. At the dose of 120 mg/kg, all existing fetuses showed cardiovascular anomalies. The dose of 60 mg/kg was less abortive (27% of resorptions) but resulted in a clearly teratogenic effect on the cardiovascular system. The dose of 15 mg/kg did not increase the spontaneous frequency of resorptions observed in the rat strain used (12%) and did not induce real malformations but minor cardiovascular anomalies (dysplasia of the heart and variations of the major cardiac vessels). The doses chosen in the present study was to analyze if a dose-response relationship exists for the effects observed and to define a NOAEL.

## Observations and Results

### Mortality and Clinical Signs

The F0 females were observed once daily during acclimatization. During gestation, the F0 females were observed at least once daily. During the treatment period, clinical monitoring was carried out prior to and after treatment. Any sign of abortion was recorded during gestation.

No mortality was observed during the study.

No treatment-related effects on clinical signs were observed.

### **Body Weight**

Each female effectively mated (sperm positive vaginal smear) was weighed daily during the entire gestation period from GD0 to GD20 inclusive. The body weight of the females found not to be pregnant at Caesarean section and subsequent uterus staining was excluded from the groups' mean value calculation.

The overall mean body weight of the F0 females was similar among all groups during the treatment period. The statistical analysis of the mean daily body weight and the mean daily weight gain during gestation did not reveal any significant difference between the treated groups and the control group.

A reduced weight gain on the first treatment day only was observed at 20 mg/kg which was related to precocious and ephemeral decrease of food consumption.

### **Feed and Water Consumption**

These parameters were calculated only for the pregnant females. The results were expressed in g/day. Four different periods were defined as follows: GD0-5, GD6-10, GD11-15 and GD16-19 inclusive.

The mean food consumption was similar among all groups for the different periods. The increase in the mean food consumption was smaller in the 20 mg/kg group than the other groups only during the first week of treatment.

The mean water consumption of the F0 females increased in a dosage range from 5 to 20 mg/kg and reached maximum values already at 10 mg/kg.

### **Toxicokinetics**

Blood samples were taken from five F0 females in each treatment group at 10 min, 1 and 6 hours after treatment on GD6 and GD15 for quantification of the unchanged test article in plasma. Only the samples collected from the first three females, for which the Caesarean section had confirmed pregnancy, were used for toxicokinetics.

The amniotic liquid was taken on GD15 from the embryos of the F0 females used for toxicokinetics at 6.5 to 7.5 hours after treatment. Quantification of the unchanged test article was performed on the amniotic fluid taken.

As shown in the table below (M2/ 2.6.7 page 122), for each dose group, the exposure was similar on GD6 (single administration) and GD15. The AUC<sub>24</sub> mean values

increased in a dose-proportional way in the range from 2.5 to 20 mg/kg. The maximal plasma concentrations were reached ten minutes after treatment in most of the animals.

Daily dose (mg/kg)		0 (control)	2.3	4.6	9.3	19
<b>Dams:</b>						
Toxicokinetics of S 16257:						
- C <sub>max</sub> (ng/ml) <sup>a</sup>	G6		80	222	517	1337
	G15		117	154	364	1039
- AUC <sub>24</sub> (ng.h/ml)	G6		469	1060	1874	5592
	G15		565	980	1857	4759
- Amniotic liquid/plasma concentration ratio (mean on G15) <sup>a</sup>			2.17	1.80	1.77	1.30

Note: the dosage in base form was used in the table.

Like the AUC<sub>24h</sub> mean values in plasma, the mean concentrations of S 16257 in the amniotic liquid increased in a dose-proportional way, and they were higher than the plasma concentrations in the corresponding F0 females measured on the same day 6 hours after treatment. However, the concentration ratio of amniotic liquid/plasma seemed to decrease when the dose increased.

### Stability and Homogeneity

Stability was verified at concentrations of 0.5, 10 and 100mg/mL of S 16257-2 in demineralized water. The solutions were stable 25 days storage at room temperature exclusively.

### Necropsy

The F0 males were euthanatized after the end of the mating period without necropsy.

The F0 females were euthanatized on GD20. An examination of their uterine contents and a detailed autopsy were carried out.

#### Uterine content:

After hysterectomy, the corpora lutea, the implantation sites, the fetuses and the resorptions were counted. An external macroscopic examination was performed on the fetuses and the placentas which were then weighed individually. Half of the fetuses from each litter were allocated to skeletal examination and half of them were allocated to soft tissue inspection. Only fetuses with anomalies were presented. The sex of each fetus was determined either during the organ inspection or during the evisceration of those aimed at skeletal examination.

A detailed autopsy was carried out on each F0 female. Organs with macroscopic anomalies were removed and preserved for a possible histopathological evaluation according to the decision of the Study Director. Corresponding organs of sufficient controls were preserved for comparison. Only F0 females with organ anomalies were presented.

The organs with macroscopic findings were fixed, dehydrated and then embedded in paraffin wax. Sections were stained with hemalum-eosin-saffron prior to their light microscopic examination. Special staining included the followings:

Uterus staining in order to count the implantation sites according to (b) (4)'s Method;

Skeletal examination of the fetuses by a double staining method drawn from that of (b) (4);

Soft tissue inspection of the fetuses according to the method of BARROW and TAYLOR modified by the dissection of the abdominal cavity.

At the terminal sacrifice on GD20, the autopsy did not reveal any organ anomaly.

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

As shown in the table below (excerpted from M2/2.6.7 page 123), the mean fetal body weight (3.16 g) observed in the high dosage group was slightly lower than the control, and the relationship to the test article cannot be excluded. One dead fetus was observed in the high dosage group. The other parameters including number of corpora lutea, number of implantations, pre- and postimplantation loss, number of resorptions, live fetuses and placental weight were similar between treated and control groups.

Daily dose (mg/kg)	0 (control)	2.3	4.6	9.3	19
No. pregnant	20	22	21	23	21
Mean No. Corpora Lutea	15.2	14.1	14.3	15.1	15.6
Mean No. implantations	13.1	12.4	12.1	12.1	13.1
Mean % preimplantation loss	13	11	15	19	16
Litters:					
No. litters evaluated	20	22	21	23	21
Mean No. live fetuses	11.6	11.1	10.7	10.9	12.0
Mean No. resorptions (early and late)	1.6	1.3	1.5	1.2	1.1
No. dead fetuses	0	0	0	0	1 <sup>a</sup>
Mean % postimplantation loss	11	10	12	12	9
Mean Fetal bodyweight (g)	3.41	3.32	3.33	3.31	3.16
Mean placental weight (g)	0.45	0.47	0.48	0.44	0.44
Fetal sex ratios (% males)	49	49	54	50	54

Note: the dosage in base form was used in the table.

**Offspring (Malformations, Variations, etc.)**

Skeletal inspections of the fetuses:

In the 20 mg/kg group, the only dead fetus showed a general delay in ossification and a spina bifida occulta situated between the fifth and the seventh cervical vertebra. There were no other significant skeletal variations or malformations observed in the 20 mg/kg or 10 mg/kg groups, when compared to those from the control fetuses and those spontaneously observed in the rat strain. With these negative findings, the fetuses from the two low dose groups were not further examined for skeletal anomalies.

Daily dose (mg/kg) (Cont'd)	0 (control)	2.3	4.6	9.3	19
Dams (Cont'd):					
Fetal anomalies:					
- Visceral anomalies					
Heart: external abnormal shape <sup>c</sup>					
No. fetuses (%)	1 (0.8)	13 (10.3)	16 (13.6)	75 (56.4)	79 (60.3)
No. litters (%)	1 (5)	8 (36.4)	8 (38.1)	22 (95.7)	21 (100)

Heart : external abnormal shape <sup>a</sup> with anomalies of the major proximal arteries:					
Inominate artery lengthened or absent and/or juxtaposition of the two common carotid arteries or of the left common carotid artery and the left subclavian artery					
No. fetuses (%)	0 (0)	4 (3.2)	2 (1.7)	29 (21.8)	39 (29.8)
No. litters (%)	0 (0)	4 (18.2)	2 (9.5)	15 (65.2)	15 (71.4)
Ascending aorta and pulmonary trunk lengthened					
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.8)	0 (0)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)
Complex anomaly <sup>b</sup>					
No. fetuses (%)	0 (0)	0 (0)	1 (0.8)	1 (0.8)	2 (1.5)
No. litters (%)	0 (0)	0 (0)	1 (4.8)	1 (4.3)	2 (9.5)
Heart : external abnormal shape <sup>a</sup> with interventricular septum defect					
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.8)	3 (2.3)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (4.3)	2 (9.5)
Heart : external abnormal shape <sup>a</sup> with interventricular septum defect and anomalies of the major proximal arteries					
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.8)	4 (3.1)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (4.3)	3 (14.3)
- Skeletal anomalies					
	-	np	np	-	-

a: Described as dysplasia in the study report

b: Complex anomalies of the major proximal arteries including retro-oesophageal arch of the aorta, left common carotid arteries arising from ascending part of aorta, left subclavian artery arising from ducters arteriosus, persistent truncus arteriosus

Note: the dosage in base form was used in the table.

Soft tissue inspections of the fetuses:

As shown in the table (excerpted from section 2.6.7 page 124) above, the test article-related adverse effects on the development of the cardiovascular system from the dose of 2.5 mg/kg were observed which included:

- mild external shape anomaly of the heart (dysplasia) combined or not with simple variations of the major proximal arteries in the 2.5 mg/kg group (13.3% fetuses affected);
- 16.1% of the fetuses affected in 5 mg/kg group; dysplasia of the heart in one of them associated with malformations of the major proximal arteries (arch of the aorta retro-esophageal, absence of innominate artery, right and left common carotid arteries arising from the ascending part of the aorta, left subclavian artery arising from the ducters arteriosus). All the other fetuses from this group showed the same types of cardiovascular anomalies as those mentioned for the fetuses from the 2.5 mg/kg group;
- anomalies of the cardiovascular system in 81.4% of the fetuses in the 10 mg/kg group, among which two malformations (1.6%) of the heart (interventricular septum defects) associated with dysplasia of the heart and in one case also with anomalies of the major proximal arteries;
- anomalies of the cardiovascular system in 97.0% of the fetuses in the 20 mg/kg group, among which nine fetuses (6.9%) showed malformations either of the heart alone (e.g. interventricular septum defect) or of the major proximal arteries only (e.g. right subclavian artery retro-esophageal) or of both (e.g. interventricular septum defect and persistent trunks arteriosus).
- Minor anomalies (variations) of the major arteries close to the heart were seen in all groups including the control group, without treatment-related increase in their incidence. The substantially increased incidence associated with cardiac dysplasia in the treated groups observed at the doses of 10 and 20 mg/kg.

No cardiovascular malformation was detected in the control group. One isolated case of dysplasia of the heart was observed but the morphology of this heart was quite different from that observed in all the fetuses affected from the treated groups. In the treated groups, the shape of the dysplasia of the heart was all the more pronounced as the dose was high.

In conclusion, the treatment of the F0 females with S 16257-2 affected embryonic development in the cardiovascular region (cardiac dysplasia associated or not with variations of the major proximal arteries) at doses  $\geq 2.5$  mg/kg. Teratogenic effects on the cardiovascular system appeared at doses  $\geq 5$  mg/kg.

**Study title: Oral Reproduction Toxicity Study in the Wistar Rat (Embryofetal Development) --- supplement**

Study no:	NP26980
Study report location:	EDR M4/4.2.3.5.2
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	Nov 16, 2005
GLP compliance:	Statement included
QA statement:	Statement included
Drug, lot #, and % purity:	Ivabradine hydrochloride, S 16257-2, batch# 55 294, purity: 100.5 %

**Key study findings**

External abnormal shape of the heart and teratogenic effect including interventricular septal defect and complex anomalies of the major proximal arteries at the high dosage of 9.3 mg/kg (base)

The NOAEL for fetal cardiovascular morphological changes was determined at 1.5 mg/kg (base), corresponding to AUC<sub>24h</sub> value of 482 ng.h/mL on GD15.

## Methods

Doses: 0, 0.5, 1.5, 9.3 base mg/kg  
Frequency of dosing: daily  
Dose volume: 10 mL/kg  
Route of administration: Oral  
Formulation/Vehicle: Solution/purified Water,  
Species/Strain: Rat/Wistar  
Number/Sex/Group: 24 females/group  
Satellite groups: N=5 females/group for TK evaluation  
Study design: Initial age: approximately 13-week  
Day of mating: Day 0  
Duration of dosing: GD6 to GD17 inclusive.  
Day of C-section: GD20

Deviation from study protocol: Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

In the rat embryo-fetal development study NP05135 (reviewed above), fetal cardiovascular morphological changes were observed at the lowest dosage (2.3 mg base/kg/day). The NOAEL could not be identified for this parameter. This present supplementary study was performed to determine a NOAEL.

Pregnant female rats were treated by oral administration once daily from GD6 to GD17. A detailed necropsy, including the heart, on all F0 females of the main study and an examination of uterine contents after Caesarean section on GD20 were performed. , Only detailed examination of fetal cardiovascular system was conducted. Plasma levels of S 16257 and its main active metabolite S 18982 were determined at 10 min, 1, 4, 8 and 24 hours after dosing on GD15 in three pregnant satellite females for toxicokinetic evaluations.

There were no maternal mortalities.

There were no treatment-related adverse effects on clinical signs, bodyweight and feed and water consumption.

At the terminal sacrifice, there was no treatment-related effect on the uterine parameters (as shown in the table below, excerpted from page 3 of the report).

Daily Dose (mg base/kg)	0 (Control)	0.5	1.5	9.3
<b>Dams:</b>				
Necropsy Observations:				
Mean No. Corpora Lutea	13.2	13.7	13.9	13.5
Mean No. Implantations	11.5	12.4	11.5	11.7
Mean % Preimplantation Loss	11.7	9.3	18.0	13.7
<b>Litters:</b>				
No. Litters Evaluated	23 <sup>c</sup>	20	22	22
Mean No. Live Foetuses	10.2	11.1	10.2	10.8
Mean No. Early Resorptions	0.1	0.3	0.4	0.1
Mean No. Late Resorptions	1.2	1.0	0.9	0.8
Mean No. Dead Foetuses	0	0	0	0
Mean Postimplantation Loss Rate	0.18	0.11	0.13	0.08
Mean Foetal Bodyweight (g)	3.4	3.2	3.4	3.2
Mean Placental Weight (g)	0.43	0.45	0.46	0.42
Foetal Sex Ratios (% males)	52	43	46	45

As shown in the table below (excerpted from page 3 of the report), examination of the cardiovascular system of the fetuses revealed increased incidence of abnormal external shape of the heart, and complex anomalies of major proximal arteries as well as malformation of ventricular septum defect in the high dosage group.

Daily Dose (mg base/kg)	0 (Control)	0.5	1.5	9.3
Foetal Anomalies of the Cardiovascular System (# foetuses examined / # litters examined)	235/20	222/20	225/22	237/22
<b>Cardiovascular Variations</b>				
[No. Foetuses (%)/No. Litters (%)]				
Two carotid arteries juxtaposed <sup>d</sup>	14 (6) / 9 (45)	7 (3.2) / 4 (20)	16 (7.1) / 6 (27)	32 (13.5) / 11 (50)
Presence of an artery parallel to the left subclavian artery on the aortic arch <sup>e</sup>	0	0	0	1 (0.4) / 1 (5)
Abnormal external shape of the heart <sup>f</sup>	0	2 (0.9) / 2 (10)	5 (2.2) / 4 (18)	56 (23.6) / 18 (82)
<b>Cardiovascular Malformations</b>				
[No. Foetuses (%)/No. Litters (%)]				
Single malformation:				
<i>Situs inversus</i>	1 (0.4) / 1 (5)	0	0	0
Ventricular septum defect	0	1 (0.5) / 1 (5)	0	2 (0.8) / 1 (4.5)

The toxicokinetic results are shown in the table below (excerpted from page 2 of the report). The systemic exposure (AUC<sub>24h</sub>) for both S 16257 and its major metabolite, S 18982, increased slightly less than proportionally to dose and the maximal concentrations (C<sub>max</sub>), increased dose proportionally. The metabolic exposure ratio remained similar at all dose levels.

Daily Dose (mg base/kg)	0 (Control)	0.5	1.5	9.3	
<b>Dams:</b>					
<b>Toxicokinetics<sup>a</sup>:</b>					
<b>S 16257:</b>					
C <sub>max</sub> (ng/ml)	GD15 (Day 10)	BLQ	38	115	573
AUC <sub>24</sub> (ng.h/ml)	GD15 (Day 10)	BLQ	198	482	2551
<b>S 18982:</b>					
C <sub>max</sub> (ng/ml)	GD15 (Day 10)	BLQ	1.2	4.1	20
AUC <sub>24</sub> (ng.h/ml)	GD15 (Day 10)	BLQ	6.7	18	80
R <sub>m</sub> S 18982/S 16257	na	0.034	0.040	0.033	

<sup>a</sup> For all parameters, group means are shown.

GD: gestation day; R<sub>m</sub>: molar exposure ratio; BLQ: Below the Limit of Quantitation (0.25 ng/ml); na: not applicable.

In conclusion, the NOAEL for fetal cardiovascular morphological changes was 1.5 mg base/kg, corresponding to on GD15 mean  $AUC_{24hr}$  values of 482 and 18 ng.h/mL and to mean  $C_{max}$  values of 115 and 4.1 ng/mL for S 16257 and S 18982, respectively.

**Study title: Oral Reproduction Toxicity Study in the New Zealand White Rabbit (Embryofetal Development)**

Study no:	NP05134
Study report location:	EDR M4/4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Feb. 15, 1994
GLP compliance:	Statement included
QA statement:	Statement included
Drug, lot #, and % purity:	Ivabradine hydrochloride, S 16257-2, batch# EG418, purity: 101.3 %

**Key Study Findings**

There was no significant maternal toxicity observed in the F0 females treated at doses up to 28 mg/kg/day (base form).

The NOAEL for embryotoxicity cannot be identified as increase in the mean postimplantation-loss rate was observed at lowest dosage of 7 mg/kg ( $AUC_{24h}=1664$  ng.h/mL on GD18) and above.

The NOAEL for fetal toxicity is identified at 14 mg/kg due to the reduced mean fetal and placental weights observed at 28 mg/kg.

The NOAEL for teratogenicity is identified at 14 mg/kg due to a teratogenic effect on the paws in a small number of rabbits (two cases of ectrodactylia) at the dose of 28 mg/kg ( $AUC_{24h}=11603$  ng/h/mL on GD18).

## Methods

Doses:	0, 7, 14, 28 (base) mg/kg
Frequency of dosing:	daily
Dose volume:	1 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	Solution/Demineralized Water,
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	18/female/control, 19/female/treated group
Satellite groups:	N=3 females/treated group for TK evaluation
Study design:	Age at initiation: at least 15-week Mating: GD 0 (The effective mating was checked visually and the mating day was considered as day zero of gestation.) Treatment duration:GD6 to GD18 C-section: GD30
Deviation from study protocol:	Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

The dose selection was based on the results of a preliminary rabbit embryo-fetal development study (NP08076). In this preliminary study, S 16257-2 was orally administered to pregnant females during organogenesis at daily doses of 30, 60, 120, 180 and 240 mg (salt)/kg (equivalent doses in base form: 28, 56, 111, 167 and 223 mg/kg). The results showed a reduction of the mean weight gain and food consumption of the F0 females at dosage of 30 mg/kg. This dose level was also embryotoxic (increase in the mean number of early resorptions) and fetotoxic (decrease in the mean fetal weight). At the dosages  $\geq$  60 mg/kg, S 16257-2 provoked lethality among the F0 females. In consequence, the high dose of the present study was limited to 28 mg/kg in base form (equivalent to dose of 30.2 mg/kg in salt form). To identify the NOAEL, two lower doses were chosen using a geometrical progression with a factor 2 which resulted in 7 and 14 mg/kg in base form (equivalent to doses of 7.55 and 15.1 mg/kg in salt form, respectively).

## Mortality and Clinical Signs

During gestation, the F0 females were observed at least once daily and during the treatment period, clinical monitoring was carried out prior to and after treatment. Any sign of abortion was recorded during gestation.

No mortality was observed during the study.

Ptosis was observed in the F0 females at the doses of 14 and 28 mg/kg. No other clinical signs were noted.

The one abortion observed on GD30 at the low dosage was considered incidental.

## Body Weight

Each female was weighed daily from GD3 to GD30 inclusive. The body weight of the females found not to be pregnant at Caesarean section and subsequent uterus staining was excluded from the groups' mean value calculation.

Overall during the whole treatment period, there was no significant difference in mean body weight gain between the treated groups and the control group.

A reduced body weight gain on the first treatment day only was observed at 20 mg/kg which was related to precocious and ephemeral decrease of food consumption.

## Feed and Water Consumption

These parameters were calculated daily from GD3 to GD30 for the pregnant females. The results were expressed as g/day.

There was no significant treatment-related effect on the mean food consumption of the F0 females, though the values of high dosage group were slightly lower than those of the other groups.

There was no significant treatment-related effect on the mean water consumption.

## Toxicokinetics

Blood samples were taken from three F0 females in each treatment group at 30 min, 4 and 7 hours after treatment on GD6 and GD18 for quantification of the unchanged test article in plasma.

The TK parameters are shown in the table below (M2/2.6.7 page 129). The maximal plasma concentrations were reached thirty minutes after treatment in most of the animals. For each dose group, the AUC<sub>24h</sub> value was higher on GD18 than that on GD6. In the tested dose range, the mean AUC<sub>24h</sub> values increased more rapidly than the dose after the single administration on GD6, but on GD18, the increase was proportional to the dose after repeated administration.

Daily dose (mg/kg)		0 (control)	7	14	28
Dams/does:					
Toxicokinetics of S 16257 <sup>a</sup> :					
C <sub>max</sub> (ng.h/ml)	G6		192	679	2021
	G18		858	1603	3479
AUC (ng.h/ml)	G6		455	1920	5049
	G18		1664	6979	11603

G: Gestation day

a: Mean values not specified in study report and calculated from individual data

## Stability and Homogeneity

Stability was verified at concentrations of 0.5, 10 and 100mg/mL of S 16257-2 in demineralized water. The solutions were stable 25 days storage at room temperature exclusively.

## Necropsy

The F0 females were euthanatized on GD30. Examinations of uterine contents and detailed autopsies were carried out.

### Uterine content:

After hysterectomy, the corpora lutea, the implantation sites, the fetuses and the resorptions were counted. An external macroscopic examination was performed on the fetuses and the placentas which were then weighed individually.

All fetuses were then examined for organ and skeletal anomalies. Only fetuses with anomalies were presented. The sex of each fetus was determined either during the organ inspection.

A detailed autopsy was carried out on each F0 female. Organs with macroscopic anomalies were removed and preserved for a possible histopathological evaluation according to the decision of the Study Director. Corresponding organs of sufficient controls were preserved for comparison. Only F0 females with organ anomalies were presented.

The organs with macroscopic findings were fixed, dehydrated and then embedded in paraffin wax. Sections were stained with hemalum-eosin-saffron prior to their light microscopic examination. Special staining included the followings:

Uterus staining in order to count the implantation sites according to (b) (4);

Soft tissue inspection of the foetuses using a fresh microdissection technique

Skeletal examination of the fetuses using X-ray photography for live fetuses and double staining for dead fetuses. The latter technique was drawn from that of (b) (4).

At the terminal sacrifice on GD20, the autopsy did not reveal any organ anomaly.

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

As shown in the table below (excerpted from M2, section 2.6.7 pages 129-130), the mean postimplantation loss was higher in all the treated groups than that in the control group, which did not show a dose-related pattern. In the high dosage group, the mean fetal and placental weights were slightly reduced, but were not associated with any relevant ossification delays in the fetuses. There were no significant treatment-related adverse effects on the other uterine parameters.

Daily dose (mg/kg)	0 (control)	7	14	28
<b>Dams/does:</b>				
No. pregnant	17	18 <sup>a</sup>	19	18
Mean No. Corpora Lutea	11.4	11.9	11.1	11.5
Mean No. implantations	8.9	10.1	8.2	9.5
Mean % preimplantation loss	22	17	25	18
<b>Litters:</b>				
No. litters evaluated	17	17	19	18
Mean No. live fetuses	8.2	8.2	7.0	8.2
Mean No. resorptions	0.8	1.2	1.1	1.2
No. dead fetuses	0	0.6	0.1	0
Mean % postimplantation loss	7	18	13	15
Mean Fetal bodyweight (g)	42.4	41.8	43.0	39.7
Mean placental weight (g)	5.1	5.2	5.2	4.9
Fetal sex ratio (% males)	47	48	51	51

G: Gestation day

a: Mean values not specified in study report and calculated from individual data

b: One female given 7 mg/kg/d aborted (deaded fetuses and late resorptions) on the day of sacrifice

c: Ptosis was sometimes noted at 14 and 28 mg/kg/d

## Offspring (Malformations, Variations, etc.)

As shown in the table (excerpted from M2/2. 6.7, page 130) below, skeletal inspections of the fetuses revealed ectrodactylia in the fetuses from the high dosage dams. The teratogenic effect on the paws at the high dosage cannot be excluded.

Daily dose (mg/kg) (Cont'd)	0 (control)	7	14	28
<b>Fetal anomalies:</b>				
- External and visceral anomalies	-	-	-	-
- Skeletal anomalies				
- Ectrodactylia involving absence of two thumbs, two right and three left toes and two left metatarsal bones				
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.7)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (5.6)
- Ectrodactylia involving absence of five fingers on the left forepaw and fusion of four sternebrae				
No. fetuses (%)	0 (0)	0 (0)	0 (0)	1 (0.7)
No. litters (%)	0 (0)	0 (0)	0 (0)	1 (5.6)
- Total number of ectrodactylia				
No. fetuses (%)	0 (0)	0 (0)	0 (0)	2 (1.4)
No. litters (%)	0 (0)	0 (0)	0 (0)	2 (11.1)

The organ inspection of the fetuses did not reveal any treatment-related anomaly.

### Study title: Oral Reproduction Toxicity Study in the New Zealand White Rabbit (Embryofetal Development) ---Complementary (non-GLP)

Study no: NP08073  
 Study report location: EDR, M4/4.2.3.5.2  
 Conducting laboratory and location: (b) (4)

Date of study initiation: Jan 10, 1995  
 GLP compliance: no  
 QA statement: no  
 Drug, lot #, and % purity: Not available

This complementary study was to further investigate the teratogenic finding of ectrodactylia observed at 28 mg/kg/d in the pivotal embryo/fetal development study in the rabbit (NP05134, reviewed above). In this study, the group sizes were increased and the higher dose of 42 mg/kg/d was administered. Pregnant New-Zealand White rabbits (16-week old or more, 25 or 50 females/group) received once daily oral via oral gavage (1 ml/kg) of 0 (control, purified water) or 42 mg/kg/d from GD6 through GD18.

No mortality was observed in F0 females. In the treated group, clinical signs including half closed eyes, hypoactivity and tachypnea as well as the reduced body weight gain, food and water consumption were observed. Increased number of early resorptions was observed in the treated group. Ectrodactylia occurred in one fetus each of the control group and the treated group. However, the control one had multiple additional defects and amniotic bands whereas the treated one had no other defects. So it is not clear whether these cases were strictly comparable. Therefore, no firm conclusion can be drawn regarding the potential teratogenic effect observed in the pivotal rabbit study.

### 9.3 Prenatal and Postnatal Development

Study title: Oral Reproductive Toxicity Study in the Wistar Rat---Pre and Postnatal Development

Study no:	NP07356
Study report location:	EDR, M4/4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 13, 1998 (first delivery of animals)
GLP compliance:	Statement Included, compliant to (b) (4)
QA statement:	(1998)
Drug, lot #, and % purity:	S 16257-2, batch # 46762, purity: 99.9%

### Key Study Findings

F0:

Enlargement of the heart was observed at dosages  $\geq 2.5$  mg/kg (lowest dose) which might be related to the bradycardia effect of the test article.

F1:

Enlargement of the heart was observed at dosages  $\geq 7$  mg/kg ( $AUC_{24h}$  on LD5 = 1396 ng.h/mL);

Increased postnatal mortality associated with interventricular septum defect and abnormal shape of the heart was observed in the pups from dam dosage group of 20 mg/kg ( $AUC_{24h}$  on LD5 = 5037 ng.h/mL);

The NOAEL for pre- and postnatal development of the F1 generation is 2.5 mg/kg ( $AUC_{24h}$  on LD5 = 608 ng.h/mL).

F2:

The NOAEL for pre- and early postnatal development of the F2 generation is 20 mg/kg.

## Methods

Doses:	2.5, 7, 20 base mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/Demineralised water
Species/Strain:	Rat/Wistar
Number/Sex/Group:	25/females/group
Satellite groups:	5/females/treated group (3/group analyzed)
Study design:	Approximately 190 males and females, aged 14-week, were paired to obtain about 115 females (F0) effectively mated. Day of Mating = Day 0 of Pregnancy (sperm positive vaginal smears); Day of Spontaneous Delivery = Day 0 of Lactation Day (LD0) For main study, pregnant females (F0) were treated from GD6 to LD20 inclusive; for TK sampling F0 females were treated from GD6 to LD5; Pups weaned on Day 21
Deviation from study protocol:	Reported as neither affected the overall interpretation of study findings nor compromised the integrity of the study

## Observations

For F0 females, mortality, clinical observations (prior to and after treatment during treatment period), body weight (daily), feed and water consumption (GD0-GD5, GD6-GD13, GD14 to end of gestation, LD0-LD6, LD7-LD13) were recorded. Blood samples were taken from five F0 females designated for TK evaluation on GD17 and on LD5 at time points of 10 min, 1, 6 and 24 hours after treatment for quantitation of the unchanged test article and its N-demethylated metabolite in plasma. F0 females which delivered a litter were sacrificed on LD21 for a detailed autopsy. Those from the same subgroup with a sperm positive vaginal smear, but without delivery, were sacrificed from twenty-five days later for verification of pregnancy and detailed autopsy. Uterine examination for F0 reproductive performance and necropsy were conducted. Macroscopic anomalies were collected for possible histopathologic evaluation.

For F1 generation rats, mortality, clinical observations, and body weights were recorded.

For the F1 generations rats chosen for continued evaluation, the mortality, clinical observations, body weights, and food consumption were recorded. Pre-weaning landmarks of physical development (incisor eruption on LD11, eye opening on LD15 and auditory meatus opening on LD20) and reflex tests (surface righting on LD6,

prehensile traction on LD13, pupillary reflex on LD19 and auditory startle on LD20) were checked for all live F1 pups. Sexual maturation (postweaning landmark of development) including cleavage of the balanopreputial gland from day 40 post partum (p.p.) and vaginal opening from day 30 p.p. was also checked for all live pups.

For all F1 breeders (one male and one female per litter where possible), behavior assessment included locomotor activity in an open field at the age of 6-7 weeks, learning ability in a water maze at the age of 9-10 weeks, and memorizing ability in a water maze at the age of 10-11 weeks. At approximately 11-13 week old, F1 breeders were paired in a period of 1-2 weeks. F1 male breeders were sacrificed for a detailed autopsy after littering of their corresponding F1 females, or after terminal sacrifice of apparently non-pregnant F1 females for verification of pregnancy. The complete male genital tract (testes, epididymides, prostate, seminal vesicles and deferent ducts), from F1 males which did not fertilize their corresponding females were collected for histopathologic evaluation. The ovaries and oviducts from F1 females, that were apparently not pregnant at terminal sacrifice, were collected for histopathologic evaluation. F1 females, which delivered a litter and showed no total litter loss, were sacrificed between LD3-5 for a detailed autopsy. At the same time, their F2 pups were weighed and examined for any gross external anomaly.

F1 males and females not selected for behavior testing and breeding were sacrificed for a detailed autopsy after achievement of sexual maturation of all littermates from the same sex in a litter.

## **Results**

### F0 Dams

Survival: No mortality was recorded among the F0 females.

Clinical Signs:

Increased salivation (mainly in lactation) and decreased activity in gestation were observed in mid- and high dosage F0 females and additional signs including ventral posture, irregular breathing, partial blepharoptosis, and piloerection during gestation were observed in the high dosage F0 females.

Body weight:

There was no treatment-related effect on body weight and body weight gain during gestation and lactation period.

Feed and water consumption:

There was no treatment-related effect on food consumption during gestation and lactation period

During gestation, mean water consumption was dose dependently increased in all treated groups when compared with that of the control group. During lactation, mean water consumption was similar between control and treated groups.

#### Uterine content:

There was no treatment-related adverse effect on the progress of gestation and parturition. Mean duration of gestation, gestation index (number of females with a live litter / number of pregnant females) and mean live birth index (number of live newborn pups / number of implantation sites) were unaffected. There was no treatment-related effect on mean body weight of the F1 pups at birth and the sex-ratio of F1 pups. Data is shown in the table below (excerpted from M2.6.7 page 134-135).

Daily dose (mg/kg) (Cont'd)	0 (control)	2.5	7	20
<b>F0 females:</b>				
No. pregnant	24	24	22	22
Mean No. implantations	12.4	12.8	11.4	12.4
Mean postimplantation loss (%)	0.09	0.06	0.10	0.10
Mean duration of gestation (days)	22.5	22.7	22.4	22.6
Abnormal parturition	0	0	1 <sup>a</sup>	0
Gestation index <sup>b</sup>	1.00	1.00	0.95	1.00
No. female with total litter loss	0	0	0	0
<b>F1 litters: (preweaning)</b>				
No. litters evaluated	24	24	21	22
Mean No. pups/litter	11.3	12.2	10.6	11.3
Mean No. live newborn pups/litter	11.2	12.1	10.6	11.2
Mean No. dead newborn pups/litter	0.0	0.0	0.0	0.0
Mean No. stillborn pups/litter	0.1	0.1	0.0	0.0
Pup sex ratios (% males) (at birth)	54	48	52	53
Live birth index	0.90	0.94	0.90	0.90

#### Necropsy observation:

An enlarged heart was observed in one female of each of the 2.5 and 7 mg/kg dose groups and in two females of the 20 mg/kg dose group. No histopathologic evaluation of the heart was performed as the finding was considered similar to that observed in the previous general toxicity studies and related to bradycardia.

#### Toxicokinetics:

The TK parameters are presented in the table below (excerpted from M2, section 2.6.7. page 133). Mean systemic exposure ( $AUC_{24h}$ ), increased slightly more than dose on GD17 and proportionally to dose on LD5.  $AUC_{24h}$  values of S 16257 were similar on GD17 and LD5, except for the high dose. Mean systemic exposures to S 18982 (N-demethylated metabolite) could not be accurately determined, because plasma concentrations were below the limit of quantitation (LOQ) for most of the samples.

Daily dose (mg/kg)		0 (control)	2.5	7	20
<b>F0 females:</b>					
Toxicokinetics of S 16257 (mean values) <sup>a</sup> :					
- $C_{max}$ (ng/ml)	G17		100	209	729
	L5		140	222	1096
- $AUC_{24}$ (ng.h/ml)	G17		622	1460	7719
	L5		608	1396	5037

F1 pups (data was summarized in the table below, excerpted from M2.6.7 page 135)

Survival: Mortality of F1 pups from birth to weaning was increased at 20 mg/kg when compared with controls. Consequently, the weaning index was slightly decreased in the high dose group.

Clinical signs: No significant treatment-related clinical signs were observed.

Body weight: Mean body weight of the F1 pups from birth to weaning was similar for treated and control groups.

Physical development: There were no treatment-related anomalies (incisor eruption on LD11, eye opening on LD15 and the auditory meatus opening on LD20).

Reflex test: There were not treatment-related anomalies of reflex test (surface righting on LD6, prehensile traction on LD13, pupillary reflex on LD19 and auditory startle on LD20).

Examination of found dead pups before LD9:

Skeletal examination did not reveal treatment-related findings.

Examination of the heart from 6 out of 20 found dead pups from the high dosage group was performed to verify cause of death. Four hearts showed an interventricular septum defect, which is considered to be treatment-related and the potential cause of death of the four corresponding pups. All six hearts showed an abnormal external shape.

Daily dose (mg/kg) (Cont'd)	0 (control)	2.5	7	20
F1 litters: (preweaning)				
Postnatal mortality before weaning (birth to L20) (%)	0.8	1.0	1.3	8.1**
Weaning index (L21)	0.99	0.99	0.99	0.92
Mean pup bodyweights (g) (at birth)	6.5	6.5	6.4	6.3
Mean pup bodyweights (g) (at weaning)	42.3	40.7	41.8	41.5
Pup clinical signs	-	-	-	-
Physical landmarks:				
Incisor eruption, eye opening, auditory meatus opening	-	-	-	-
Reflex tests:				
Surface righting, prehensile traction, pupillary reflex, auditory startle	-	-	-	-
Skeletal examination of dead F1 pups <sup>a</sup>	-	-	-	-
Pup necropsy observations <sup>b</sup>				
Heart:				
External abnormal shape	np	np	np	6/6
Septum defect	np	np	np	4/6

### F1 (post-weaning)

Data was summarized in the table below (excerpted from M2/2.6.7 page 136)

Mortality: none.

Body weight: No treatment-related effect on body weight gain was observed. Mean body weight gain of F1 male and female breeders, randomly selected at weaning (three weeks old), was similar between the 3rd and 10th week p.p. for treated and control groups.

Sexual maturation: Sexual maturation of F1 animals was not affected by treatment of the parental F0 generation. The onset and accomplishment of F1 male and female sexual maturation was similar in all groups.

Behavior assessment (F1 breeders): There were no treatment-related adverse effects on F1 males and females' locomotor activities in an open field as well as learning and memorizing abilities in a water maze.

#### Necropsy observation:

Necropsy of F1 animals at terminal sacrifice (including F1 breeders) or found dead after LD9 revealed an enlarged or misshapen heart in one mid-dosage male and three high dosage males; and abnormal shape of the heart was also detected in one high dosage female. Other findings with single cases in different groups (F1 breeders) were not considered treatment-related.

#### Histopathology evaluation:

Evaluation of ovaries and oviducts from non-pregnant F1 females, as well as that of the complete genital tract from the corresponding F1 males, did not reveal any change. Evaluation of mammary glands of two F1 females from the 2.5 mg/kg dose group, which lost their litter, did also not reveal any change.

Daily dose (mg/kg) (Cont'd)	0 (control)	2.5	7	20
<b>F1 males: (postweaning)</b>				
No. evaluated postweaning	24	24	21	22
No. found dead or killed <i>in extremis</i>	0	0	0	0
Clinical observations	-	-	-	-
Necropsy observations (terminal kill)				
Heart:				
. Enlarged or misshapen	-	-	1	3
. Depressed whitish area on apex of left ventricle	-	-	-	1
Bodyweight change (from 21pp to 70pp)	-	-	-	-
Balanopreputial gland cleavage	-	-	-	-
Motor activity (open field)	-	-	-	-
Learning and memory (water maze)	-	-	-	-
Mean No. days prior to mating	3.3	3.0	3.0	2.5
No. of males that mated	24	24	21	22
No. of fertile males	23	22	21	22
<b>F1 females: (postweaning)</b>				
No. evaluated postweaning	24	24	21	22
No. found dead or killed <i>in extremis</i>	0	0	0	0
Clinical observations	-	-	-	-
Necropsy observations (terminal kill)				
Heart: misshapen				
Premating bodyweight change	-	-	-	-
Bodyweight change (from 21pp to 70pp)	-	-	-	-
Vaginal opening	-	-	-	-
Motor activity (open field)	-	-	-	-
Learning and memory (water maze)	-	-	-	-
Mean No. days prior to mating	3.3	3.0	3.0	2.5
No. of females sperm positive	24	24	21	22
<b>F1 females: (postweaning) (Cont'd)</b>				
No. of pregnant females	23	22	21	22
Fertility index	0.96	0.92	1.00	1.00
Mean No. implantations	12.6	11.4	11.6	13.5
Mean No. postimplantation loss	0.08	0.14	0.10	0.04
Gestation index	1.00	1.00	0.95	1.00
No. female with total litter loss	0	1	0	0

#### Fertility, gestation and parturition for F1 breeders

There were not treatment-related effects on F1 breeders' fertility and progress from gestation to parturition. The results of the parameters were summarized in the tables above and below (excerpted from M2/2.6.7, pages 136-137).

#### F<sub>2</sub> Generation:

Survival: No treatment-related effect on mortality. No significant clinical signs observed.

Body weight: Mean body weight and mean body weight gain from birth to LD3 were similar for treated and control groups.

External evaluation: One live newborn pup of mid-dosage group with a shortened tail due to maternal cannibalism was not considered toxicologically significant. No other treatment-related findings.

Male/Female ratio: Not affected by the treatment of F0 females

External examination: No treatment-related findings were observed in pups found dead before terminal sacrifice or at terminal sacrifice between LD3-5.

Daily dose (mg/kg) (Cont'd)	0 (control)	2.5	7	20
<b>F2 Litters:</b>				
Number of F1 females with litter	23	22	20	22
Mean No. live conceptuses/litter	11.6	10.3	11.0	12.9
No. dead newborn <sup>3</sup> pups	1	2	0	0
No. stillborn pups	0	0	0	3
Mean % Postimplantation loss	0.08	0.14	0.10	0.04
Pup bodyweights (birth) (g)	6.3	6.3	6.3	6.1
Pup bodyweights (L3) (g)	9.2	9.1	9.2	8.7
Pup sex ratios (% males)	53.3	47.8	56.0	52.9
Live birth index	0.91	0.86	0.89	0.96
Viability index (L3)	1.00	0.95	1.00	0.99
External and skeletal examination of dead pups	-	-	-	-

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## **10 Special Toxicology Studies**

## **11 Integrated Summary and Safety Evaluation**

Reproductive and developmental toxicity was evaluated in the rat and rabbits and the relevant findings were summarized in the table below.

There were no treatment-related adverse effects on male and female fertility or early stage of embryonic development in the rats treated at up to 175 mg/kg/day.

In pregnant rats treated during organogenesis, external abnormal shape of the heart (dysplasia) with or without simple anomalies of the major proximal arteries was observed at 2.3 mg/kg/day and above. One fetal death with a delay in ossification and a spina bifida occulta situated between 5th and the 7th cervical vertebra as well as reduced mean fetal weight were observed at 19 mg/kg/day (approximately 14 times human AUC<sub>24h</sub> at MRHD). Teratogenic effects include interventricular septal defect and complex anomalies of the major proximal arteries observed at dosages  $\geq$  4.6 mg/kg/day (AUC<sub>24h</sub> of 1980 ng.h/mL, approximately 3 times human AUC<sub>24h</sub> at MRHD).

In pregnant rabbits treated during organogenesis, increased postimplantation loss was observed at lowest dosage of 7 mg/kg (AUC<sub>24h</sub> of 1664 ng.h/mL, approximately 5 times human AUC<sub>24h</sub> at MRHD) and above. Reduced fetal and placental weights and a small number of fetuses with ectrodactylia were observed at 28 mg/kg (AUC<sub>24h</sub> of 11603 ng.h/mL, approximately 34 times human AUC<sub>24h</sub> at MRHD).

Study	Route/Dose range mg/kg	Results <b>Reproductive and Development Toxicology</b>	Lowest dose with findings (mg/kg)	Multiples of human AUC	
				M	F
Rat fertility	Oral, daily 7,35, 175 14d pre-G6	Negative for male and female fertility NOAEL=175 mg/kg	175	NA	NA
Rat Embryo-Fetal, Pivotal + suppl.	Oral, daily 2.3, 4.6, 9.3, 19, GD6-15 0.5 1.5, 9.3 GD6-17	Reduced fetal weight, one fetal death with a delay in ossification and a spina bifida occulta (5th & 7th cervical vertebra )	19	-	14
		Teratogenic: interventricular septal defect and complex anomalies of primary arteries	4.6	-	3
		F1: external abnormal shape of the heart	2.3	-	2
		NOAEL= 1.5 mg/kg/day		-	1
Rabbit Embro-Fetal	Oral daily 7, 15, 28, GD6-18	Increased post-implantation loss	7	-	5
		Reduced fetal and placental weight ectrodactylia	28	-	34
		NOAEL for embryotoxicity <7		-	5
Rat, post-natal	Oral daily 2.5-20 GD6-LD20	F1:Increased postnatal mortality (dead pups: misshapen heart, interventricular septal defect); adult: abnormal shape of the heart	20	-	15
		F1 adult: enlarged heart	7	-	4

(b) (4)

NA: not available

In the rat pre-postnatal study, reduced postnatal survival associated with interventricular septum defect and abnormal shape of the heart was observed in the F1 pups delivered by dams treated at 20 mg/kg (AUC<sub>24h</sub> on LD5 = 5037 ng.h/mL, approximately 15 times

human  $AUC_{24h}$  at MRHD ). Enlargement of the heart in adult F1 rats was observed at dosages  $\geq 7$  mg/kg. No anomalies were observed for F2 generation from F0 dams treated at doses up to 20 mg/kg/day.

Taken together, as ivabradine was associated with lethal cardiac teratogenicity in rats, it should not be given to women during pregnancy, particularly during the embryogenesis of the heart and period of lactation.

(b) (4)



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11/25/2014

ALBERT F DEFELICE  
11/25/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW  
EVALUATION (Genetic Toxicology)

Application number: 206,143  
Supporting document/s: S016 (eCTD0014), S022 (eCTD0017)  
Applicant's letter date: April 30, 2014, June 27, 2014  
CDER stamp date: April 30, 2014, June 27, 2014  
Product: Ivabradine  
Indication: reduce the risk of [REDACTED] (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure [REDACTED] (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm), [REDACTED] (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated [REDACTED] (b) (4)  
Applicant: Amgen, Inc.  
Review Division: Division of Cardio-Renal Products  
Reviewer: Jean Q. Wu  
Supervisor/Team Leader: Albert DeFelice  
Division Director: Norman Stockbridge  
Project Manager: Alexis Childers

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# 1 Executive Summary

## 2 Drug Information

### 2.1 Drug

#### 2.1.1 CAS Registry Number (Optional)

Ivabradine: 155974-00-8

Ivabradine hydrochloride: 148849-67-6

#### 2.1.2 Generic Name

ivabradine

#### 2.1.3 Code Name

S 16257-2; AMG 998

#### 2.1.4 Chemical Name

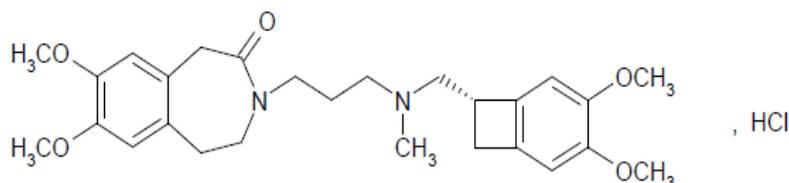
3-(3-(((7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl) methyl amino)propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride

#### 2.1.5 Molecular Formula/Molecular Weight

C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>, HCl/ 505.1 g/mol;

468.593 g/mol (base); Conversion factor from the salt to the base: 0.928

#### 2.1.6 Structure



#### 2.1.7 Pharmacologic class

hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel blocker

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND (b) (4)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

Ivabradine is provided as film-coated tablet in two strengths: 5 mg (oval) and 7.5 mg (triangular). The composition of the drug is listed in the table below (excerpted from Module 2.3. P, Table 1, Page 7)

Table 1. Composition of Ivabradine 5 mg and 7.5 mg Tablets

Component	5 mg		7.5 mg		Function	Reference to specifications
	Percentage (% w/w)	Quantity (mg/tablet)	Percentage (% w/w)	Quantity (mg/tablet)		
<b>Tablet</b>						
Ivabradine hydrochloride <sup>a</sup> (free base equivalent)	5.39 (5.00)	5.390 (5.000)	8.085 (7.50)	8.085 (7.500)	Drug substance	In-house
Lactose monohydrate	(b) (4)				(b) (4)	USP/NF, PhEur.
Maize starch	(b) (4)				(b) (4)	USP/NF
Maltodextrin	(b) (4)				(b) (4)	USP/NF, PhEur
Magnesium stearate	(b) (4)				(b) (4)	USP/NF, PhEur
Colloidal silicon dioxide	(b) (4)				(b) (4)	USP/NF, PhEur
(b) (4)	(b) (4)				(b) (4)	USP
<b>Core Tablet Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>		
<b>Film-Coating</b>						
(b) (4) salmon <sup>c</sup>	(b) (4)				(b) (4)	See 3.2.P.4.1
Polyethylene glycol 6000	(b) (4)				(b) (4)	USP/NF, PhEur
(b) (4)	(b) (4)				(b) (4)	USP
<b>Total</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>		

<sup>a</sup> The molecular weights of Ivabradine hydrochloride anhydrous and ivabradine free base are 505.1 g/mol and 468.6 g/mol, respectively. The free base accounts for 92.77% of the salt.

(b) (4)

## 2.4 Proposed Clinical Population and Dosing Regimen

Ivabradine is indicated to reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm) (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)

The proposed starting dose of ivabradine is 5 mg twice daily. After 2 weeks of treatment, if heart rate is between 50 and 60 bpm, the dose of 5 mg twice daily should be maintained. The dose should be increased to 7.5 mg twice daily if resting heart rate is persistently above 60 bpm.

As listed in the table below (excerpted from Module 2, Section 2.4, Page 7), the human plasma exposures of ivabradine and its N-desmethylated metabolite (S 18982) were estimated at steady state in patients receiving the maximum recommended human dose (MRHD), 7.5 mg bid. These values were derived from a population pharmacokinetic analysis using Phases II and III clinical data [Summary of Clinical Pharmacology Studies, in Section 2.7.2 (2.1.2.2)], and are accepted as references when preclinical doses are expressed as multiples of human exposures in current review, unless otherwise indicated.

**Table 1. Ivabradine and S 18982 (Metabolite) Plasma C<sub>max</sub> and Estimated AUC<sub>24</sub> at Steady State in Patients at HTD**

	Ivabradine (n=492)	S 18982 (n=541)
Population C <sub>max</sub> (ng/ml)	31 ± 9.8	7.9 ± 2.3
Equivalent C <sub>max</sub> in µM	0.07	0.02 <sup>b</sup>
Population AUC <sub>24</sub> <sup>a</sup> (ng.h/ml)	346	128

Values are mean ± SD;

a: Calculated from AUC over 12 h period from [WS (2.7.2) 3.1/ Table 12; Table 34 and 35]

b: S 18982 MW = 454.6

## 2.5 Regulatory Background

A pre-NDA meeting was held between the sponsor and the Division on January 23, 2014. There was no IND opened in the US FDA (b) (4)

## 3 Studies Submitted

### 3.1 Studies Reviewed

#### In Vitro

NP03142: Genotoxicity Study of S 16257-02: In Vitro Test for Gene Mutation on Salmonella Typhimurium in The Histidine Reversion System on Escherichia Coli in The Tryptophan Reversion System (Ames Test)

NP05576: In Vitro Genetic Toxicology Study For The Detection Of Chromosome Aberrations On Cultured Human Lymphocytes

NP05144: Mutation Assay At The TK Locus In L5178Y Mouse Lymphoma Cells Using A Microtiter Cloning Technique (Trifluorothymidine Resistance) With S 16257-2

NP05489: S 16257-2: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells using the Microtitre® Fluctuation Technique

NP05912: Genotoxicity Study With S 16257-2: Measurement of Unscheduled DNA Synthesis in Isolated Rat Hepatocytes

In Vivo

NP03141: Mutagenicity Study Using the Micronucleus Test In Mice With S 16257-02

NP05409: S16257: Measurement of Unscheduled DNA Synthesis in Rat Liver using an In Vivo/In Vitro Procedure

NP05504: S 16257-2: Induction of Chromosome Aberrations in the Bone Marrow of Treated Rats

## 4 Pharmacology

## 5 Pharmacokinetics/ADME/Toxicokinetics

## 6 General Toxicology

## 7 Genetic Toxicology

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

**Study title: Genotoxicity Study of S 16257-02: In Vitro Test for Gene Mutation on Salmonella Typhimurium in The Histidine Reversion System on Escherichia Coli in The Tryptophan Reversion System (Ames Test)**

Study no.: NP03142

Study report location: M4/4.2.3.3.1

Conducting laboratory and location: (b) (4)

Date of study initiation: April 26, 1993

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: S 16257-02, Batch# EF558, purity=99.8%

### Key Study Findings

The results did not show mutagenic activity of S 16257-02 under the experimental conditions.

Methods

Strains: TA100, TA1535, TA1537, TA98, WP2 uvrA, WP2 uvrA(pKM101)

Concentrations in definitive study: See tables below for the first test and second test.

**First test**

Group identification code	D0	D1	D2	D3	D4	D5	D+
Compound administered	-	S 16257-02					①
Vehicle	Water for injection						
Dose ( $\mu\text{g}/\text{plate}$ )	0	50	150	500	1500	5000	
Volume per plate ( <i>ml</i> )	0.1						
Without metabolic activation	0.5 ml of S9 mix phosphate buffer/plate						
With metabolic activation	0.5 ml of S9 mix/plate						

**Second test**

Group identification code	D0	D1	D2	D3	D4	D5	D+
Compound administered	-	S 16257-02					①
Vehicle	Water for injection						
Dose ( $\mu\text{g}/\text{plate}$ )	0	500	1000	1500	2500	5000	
Volume per plate ( <i>ml</i> )	0.1						
Without metabolic activation	0.5 ml of S9 mix phosphate buffer/plate						
With metabolic activation	0.5 ml of S9 mix/plate						

## Basis of concentration selection:

Preliminary toxicity with or without metabolic activation was tested at 0, 5, 15, 50, 150, 500, 1500 and 5000  $\mu\text{g}/\text{plate}$ . Microscopic examination of the density and uniformity of this bacterial lawn indicates the degree of toxicity to bacterial growth. Toxicity may also be reflected by a reduction in the size or number of revertant colonies. The narrative of the report did not discuss the toxicity with the preliminary test result. The tabulated study report in the appendix stated no cytotoxic effects. The concentrations up to 5000  $\mu\text{g}/\text{plate}$  were selected in the definitive assays.

Negative control: vehicle, see the table of "acceptance limits for negative control"

Positive control: See the table below.

Strain	Without metabolic activation	With metabolic activation
TA100	sodium azide 8 µg/plate in physiological solution	2-anthramine 2.5 µg/plate in DMSO
TA1535		
TA1537		
TA98	4-nitroquinoline N-oxide 0.5 µg/plate in DMSO	2-anthramine 10 µg/plate in DMSO
WP2 <i>uvrA</i>	4-nitroquinoline N-oxide 2 µg/plate in DMSO	
WP2 <i>uvrA</i> ( <i>pKM101</i> )	4-nitroquinoline N-oxide 0.5 µg/plate in DMSO	2-anthramine 2.5 µg/plate in DMSO

Formulation/Vehicle: stock solution, 50 mg/mL/Water for injection

Incubation & sampling time:

The day before treatment, samples of each strain stored in liquid nitrogen were inoculated into Oxoid nutrient culture medium. Plates were analyzed after 3 days' (72 hours) incubation at 37°C. The number of revertant colonies was counted visually or using an electronic counter (*Artek 880*).

### Study Validity

The spontaneously revertant colony counts in the mutagenicity test negative controls were within the acceptance limits for each strain. The acceptance limits for negative control without metabolic activation was shown in the table below. After metabolic activation, a 15% deviation from these limits was tolerated.

Strain	Acceptance limits for negative control
TA100	150 - 250
TA1535	5 - 25
TA1537	3 - 15
TA98	20 - 55
WP2 <i>uvrA</i>	20 - 50
WP2 <i>uvrA</i> ( <i>pKM101</i> )	40 - 100

Each positive control group demonstrated the sensitivity of the test: the numbers of revertant colonies induced by the positive control agents were significantly higher than the spontaneous reversion values.

## Results

The summary of assay results with and without metabolic activation is listed in the tables below (excerpted from pages 41 and 42 of the report).

Small but statistically significant increase in the number of revertant colonies (highlighted) in the presence of S9 were observed at 500 µg/plate against WP2 *uvrA* (pKM101) and TA98 in the 1<sup>st</sup> assay, and at 5000 µg/plate against TA98 in the 2<sup>nd</sup> assay. A minimal but marginally statistical significant ( $p=0.049$ ) increase in the number of revertant colonies in the absence of S9 was observed at 2500 µg/plate against WP2 *uvrA* (pKM101) in the 2<sup>nd</sup> assay only.

The increase in the number of revertant colonies reached 1.7-fold of its control value at 500 µg/plate against WP2 *uvrA* (pKM101) in the presence of S9, whereas the other increases were about 1.2-fold of their respective control values. These increases were neither appreciable, nor exposure-dependent or reproducible, hence, were not considered biologically significant.

The results can be interpreted as negative for any unequivocal mutagenic activity of S 16257-02 under the experimental conditions.

## Genetic assays without metabolic activation

REVERTANT COLONIES COUNT		GROUP						
		D0	D1	D2	D3	D4	D5	D+
DOSE ( $\mu\text{g}/\text{plate}$ )	ASSAY							
	1	0.0	50.0	150.0	500.0	1500.0	5000.0	*
	2	0.0	500.0	1000.0	1500.0	2500.0	5000.0	*
STRAIN	ASSAY							
	1	150.7	153.3	214.7	202.3	163.3	177.3	719.0
	2	179.0	189.3	209.3	216.7	202.3	189.3	612.7
TA 1535	1	22.0	20.7	18.7	22.7	29.0	21.0	941.0
	2	17.0	15.0	18.0	16.3	18.0	22.0	1016.7
TA 1537	1	8.7	10.7	6.3	9.3	8.0	10.3	666.3
	2	8.7	8.3	8.0	6.3	10.7	6.3	725.0
TA 98	1	32.0	26.3	32.3	31.7	28.0	24.3	280.7
	2	48.7	46.7	46.3	45.0	50.0	57.7	397.3
WP2 uvr A	1	37.0	40.7	40.3	44.0	44.3	46.0	857.7
	2	32.7	39.7	39.7	33.7	38.0	37.7	864.0
WP2 uvr A (pKM 101)	1	99.3	100.0	94.0	81.7	103.3	83.3	878.3
	2	43.3	35.0	37.0	50.0	52.3	43.3	584.7

D0: negative control; D+: positive control; D1, D2, D3, D4 and D5: treated dose groups;  
 For WP2 uvrA (pKM101) in absence of S9, p value for assay 2 = 0.049 (the highest number at  
 2500  $\mu\text{g}/\text{plate}$  was approximately 1.2-fold of the control value)

## Genetic assays with metabolic activation

REVERTANT COLONIES COUNT		GROUP						
		D0	D1	D2	D3	D4	D5	D+
DOSE (µg/plate)	ASSAY							
	1	0.0	50.0	150.0	500.0	1500.0	5000.0	*
	2	0.0	500.0	1000.0	1500.0	2500.0	5000.0	*
STRAIN	ASSAY							
TA 100	1	253.3	238.7	255.7	242.7	254.7	268.7	1521.0
	2	231.7	234.3	242.7	247.0	229.3	258.3	2265.3
TA 1535	1	21.7	22.7	24.3	21.3	19.7	19.0	126.7
	2	20.3	23.7	28.3	32.7	28.3	27.3	158.0
TA 1537	1	18.0	17.3	14.7	17.3	17.7	20.7	287.3
	2	14.0	17.0	16.3	19.0	18.3	15.0	212.3
TA 98	1	50.3	42.7	57.0	60.0	56.7	57.3	1183.0
	2	58.0	63.3	66.3	69.3	60.3	71.3	1284.0
WP2 uvr A	1	46.7	50.0	52.7	50.0	40.7	40.3	643.0
	2	52.3	47.7	52.0	53.0	52.3	55.3	716.3
WP2 uvr A (pKM 101)	1	55.7	57.7	68.7	95.3	78.0	56.3	801.3
	2	114.7	96.3	99.0	87.7	108.3	82.0	854.0

D0: negative control; D+: positive control; D1, D2, D3, D4 and D5: treated dose groups;  
For TA98, p value for assay 1 = 0.007, p value for assay 2 = 0.039;  
For WP2 uvrA (pKM101), P value for assay 1 = 0.005.

## 7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

### Study title: *In Vitro* Genetic Toxicology Study For The Detection Of Chromosome Aberrations On Cultured Human Lymphocytes

Study no.: NP05576  
Study report location: M4/4.2.3.3.1  
Conducting laboratory and location: (b) (4)  
Date of study initiation: Sept. 6, 1994  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: S 16257-2, Batch # EG418, purity=101.3%

### Key Study Findings

Increase in aberration frequency and in number of breaks was observed at concentration of 50 µg/mL in the absence of S9 in both 1<sup>st</sup> and 2<sup>nd</sup> assays. However, the lack of exposure-related- activity made the results equivocal although the potential weak clastogenicity of ivabradine hydrochloride may not be excluded.

In the presence of S9, the 1<sup>st</sup> assay showed a small but significant increase in aberration frequency at 950 µg/mL (male donor) and pulverized chromosomes at 525 µg/mL (male donor). However, the finding was not exposure -dependent and reproducible (in the 2<sup>nd</sup> and 3<sup>rd</sup> assays), suggesting an overall negative result.

### Methods

Cell line: Human Lymphocytes (whole blood), replicate blood cultures were used (one derived from a male donor and one from a female donor).

Concentrations in definitive study:

- 1<sup>st</sup> assay: 50, 85, 125, 150 µg/mL without metabolic activation.  
280, 525, 950, 1800 µg/mL with metabolic activation.
- 2<sup>nd</sup> assay: 50, 85, 125 µg/mL without metabolic activation,  
525, 950, 1800, 2250 µg/mL with metabolic activation.
- 3<sup>rd</sup> assay: 525, 950, 1800 µg/mL with metabolic activation.

Basis of concentration selection:

The exposure levels were selected on the basis of the mitotic index reduction observed in the 1<sup>st</sup> assay.

Without metabolic activation (-S9):

The mitotic index reduction reached -52 % and -58% of the negative control in the 1<sup>st</sup> and the 2<sup>nd</sup> assay, respectively, at 125 µg/mL, which was the highest level chosen for metaphase analysis.

With metabolic activation (+S9):

At 1800 µg/mL, the highest level of the 1st assay, the mitotic index (MI) reduction was -37%. However, in the 2nd assay, the MI reduction was -52% at the same concentration and -66% at 2250 µg/mL. The highest exposure chosen for the metaphase analysis was 1800 µg/mL.

Negative control: vehicle

Positive control:

a) Bleomycin: 250 µg /mL (vehicle = saline)

b) Cyclophosphamide (vehicle = saline):

17.5 µg/mL (harvest at 52 hours); 12.5 µg/ml (harvest at 46 hours)

Formulation/Vehicle: S 16257-2 was dissolved in the treatment medium from 0.056 to 2.5 mg/mL, just prior to use. Vehicle=treatment medium

Incubation & sampling time:

a) 24 h treatment (-S9, 1<sup>st</sup> and 2<sup>nd</sup> assay)

Recovery Time: 4 h

Sampling time: 52 hours after stimulation

b) 2 h treatment (+S9, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> assay)

Recovery time: 24 h (1<sup>st</sup> assay) and 18 h (2<sup>nd</sup> assay)

Sampling time: 52 hours after stimulation (1<sup>st</sup> and 3<sup>rd</sup> assay); 46 hours after stimulation for the 2<sup>nd</sup> assay

For the 1<sup>st</sup> assay, the treatment is started 24 hours (26 hours with metabolic activation) after mitogenic stimulation of lymphocytes.

The second assay consists of the repetition of the first assay, at a sampling time of 46 hours with metabolic activation, with a readjusted range of concentrations selected on the basis of the results of the first assay.

The third assay was the reiteration of the 52 hours sampling time with metabolic activation at three exposures.

## Study Validity

The acceptance criteria:

- The mean number of abnormal metaphases should be less than 5%.
- The mean number of abnormal metaphases in the positive control groups must be statistically significantly increased over the negative control group.
- A minimum of three exploitable concentrations is required to analyze the results.

A combination of different criteria used to evaluate a positive response:

- a statistical increase in one group over the negative control group.
- a concentration-response effect.
- an unusual number of aberrations, especially exchanges, even in the absence of a statistically significant increase.
- the reproducibility of the above criteria.

All the acceptability criteria were met except that the cells from the female donor group of the 1<sup>st</sup> assay, when exposed to 17.5 µg/mL of cyclophosphamide, failed to show a statistically significant increase in the number of abnormal metaphases. However, the number of abnormal metaphases and the number of primary breaks were over the usual negative control range and clearly over the negative control of the assay. For these reasons, and with respect to the results obtained in the treated groups, the assay was considered acceptable.

## Results

The results are summarized in the table below excepted from pages 43-44 of the report. In the absence of S9, the 1<sup>st</sup> assay showed a total of 5 structural aberrations found in the male-donor cell culture at 50 µg/mL and the 2<sup>nd</sup> assay showed 6 structural aberrations found at the same exposure in the culture from the female donor. An increased number of breaks was observed in both cases. However, the increase in aberration frequency was not exposure-dependent (i.e. not observed at the two higher exposures).

Dose (µg/ml)	WITHOUT METABOLIC ACTIVATION									
	ASSAY 1 Harvest time : 52 hours					ASSAY 2 Harvest time : 52 hours				
	Group	Number of primary breaks		Number of abnormal metaphase		Group	Number of primary breaks		Number of abnormal metaphase	
		①		①②			①		①②	
	M	F	M	F		M	F	M	F	
0	D(-)	2	3	2	2	D(-)	2	0	2	0
50	D1	8	2	5	1	D1	2	9	1	6
85	D2	0	3	0	2	D2	3	4	3	4
125	D3	0	5	0	3	D3	2	2	2	2
250	D(+) <i>Bleomycin</i>	108	79	37	27	D(+) <i>Bleomycin</i>	46	48	25	21

① Number for 100 metaphase plates, unless otherwise indicated.

② Without achromatic lesions.

Dose (µg/ml)	WITH METABOLIC ACTIVATION									
	ASSAY 1 Harvest time : 52 hours					ASSAY 2 Harvest time : 46 hours				
	Group	Number of primary breaks ①		Number of abnormal metaphase ①②		Group	Number of primary breaks ①		Number of abnormal metaphase ①②	
		M	F	M	F		M	F	M	F
0	D(-)°	0	2	0	2	D(-)°	2	0	2	0
525	D2°	23/96	6/104	3/96	3/104	D1°	0	0	0	0
950	D3°	5	2	5	2	D2°	4	1	4	1
1800	D4°	1/142	4/58	1/142	3/58	D3°	2	2	2	1
17.5 12.5	D(+) Cyclophos- phamide	15	9	14	7	D(+) Cyclophos- phamide	12	12	11	12

① Number for 100 metaphase plates, unless otherwise indicated.

② Without achromatic lesions.

Dose (µg/ml)	WITH METABOLIC ACTIVATION					
	ASSAY 3 Harvest time : 52 hours					
	Group	Number of primary breaks ①		Number of abnormal metaphase ①②		
		M	F	M	F	
0	D(-)°	0	1	0	1	
525	D1°	4	2	3	2	
950	D2°	4	5	4	4	
1800	D3°	1	0	1	0	
17.5	D(+) Cyclophos- phamide	23	19	11	15	

In the presence of S9, the 1<sup>st</sup> assay showed a small but significant increase in aberration frequency at 950 µg/mL and pulverized chromosomes at 525 µg/mL for the culture from the male donor. However, the findings were not exposure-related and could not be reproduced in the 2<sup>nd</sup> and 3<sup>rd</sup> assays.

In conclusion, due to the reproducible positive finding at 50 µg/mL in the absence of S9, the potential weak clastogenicity of ivabradine hydrochloride may not be excluded though the lack of an exposure relationship could make the results equivocal.

### 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

**Study title: Mutagenicity Study Using the Micronucleus Test In Mice With S 16257-02**

Study no.: NP03141  
Study report location: M4/4.2.3.3.2  
Conducting laboratory and location:  (b) (4)  
Date of study initiation: May 17, 1993  
GLP compliance: Yes  
QA statement: Included  
Drug, lot #, and % purity: S 16257-02 Hydrochloride, EF 558  
Purity=99.8%

**Key Study Findings**

Single dose of S16257-02 up to 500 mg/kg (464 base mg/kg) did not induce micronuclei in the polychromatic erythrocytes (PCEs) of mouse bone marrow under study conditions.

**Methods**

Doses in definitive study: 0 (negative control), 125, 250, 500 mg/kg  
equivalent base dosages: 0, 116, 232, 464 mg/kg  
Frequency of dosing: Single dose  
Route of administration: Oral  
Dose volume: 25 mL/kg  
Formulation/Vehicle: Solution in distilled water/water  
Species/Strain: Mouse/ OF1 IFFA CREDO (male and female)  
Number/Sex/Group: 5/sex/group  
Satellite groups: n/a  
Basis of dose selection: Preliminary toxicity test (n=2 male/group) showed 100% mortality after a single oral dosage of 1250 mg/kg. Confirmatory toxicity test (n=5/sex/group) showed 30% mortality at 24 hours following a single dosage of 800 mg/kg by oral intubation in mice. The dosage of 500 mg/kg which provoked no death was considered as the maximum tolerated dose (MTD). Under these conditions, the following dosages were selected for the definitive micronucleus test: 500 mg/kg (MTD), 250 mg/kg (1/2MTD), 125 mg/kg (1/4MTD).  
Negative control: Vehicle  
Positive control: Cyclophosphamide 50 mg/kg, i.p.

## Study Validity

The mean number of micronuclei observed in the negative control animals must be close to that of the historical values for control animals.

The mean number of micronuclei observed in the positive control animals treated with cyclophosphamide must show a statistically significant difference from the negative control animals.

If deaths observed at the test dosage, the mortality rate must be less than 20 % per time. The dead animals are replaced by those in parallel treatment.

The sampling time: 24 and 48 hours after treatment

Micronuclei were scored in 2 x 1000 PCEs for each animal.

The PCE:NCE (normochromatic erythrocytes) ratio were determined in 1000 cells to assess bone-marrow toxicity.

The study criteria were met. Historical controls were provided. The study is considered valid.

## Results

The mean number of micronuclei for 1000 PCEs was summarized in the tables below (excerpted from pages 36 and 37 in the report). There were no statistically significant increases in the number of PCEs with micronuclei in the treated groups when compared to the control. There was no difference in the PCE:NCE ratio between groups.

In conclusion, single dose of S16257-02 up to 500 mg/kg (464 base mg/kg) did not induce micronuclei in the PCEs of mouse bone marrow under study conditions.

SAMPLING TIME 24 HOURS	GROUP	SEX	MICRONUCLEI MEAN FOR 1000 PCE	Standard error	MIN	MAX	Mann-Whitney U rank test		p
							UA	UB	
NEGATIVE CONTROL	SOLVENT	♂	0.9	0.22	0.5	1			
		♀	0.9	0.89	0	2			
		♂ + ♀	0.9	0.61	0	2			
POSITIVE CONTROL	CYCLOPHOS- PHAMIDE 50 mg/kg  ROUTE : I.P.	♂	28.3	8.11	18	39.5	0	25	=0.01
		♀	20.5	7.57	13	33	0	25	=0.01
		♂ + ♀	24.4	8.46	13	39.5	0	100	<0.01
COMPOUND STUDIED	HIGH DOSE 500 mg/kg	♂	0.9	0.96	0	2.5	9	16	N.S.
		♀	0.4	0.42	0	1	8.5	16.5	N.S.
		♂ + ♀	0.65	0.75	0	2.5	34	66	N.S.
	MIDDLE DOSE 250 mg/kg	M	0.5	0.35	0	1	4.5	20.5	N.S.
		♀	1.8	0.57	1	2.5	5	20	N.S.
		♂ + ♀	1.15	0.82	0	2.5	43	57	N.S.
	LOW DOSE 125 mg/kg	♂	1	0.35	0.5	1.5	10.5	14.5	N.S.
		♀	1.1	0.89	0	2	10.5	14.5	N.S.
		♂ + ♀	1.05	0.64	0	2	44.5	55.5	N.S.

N.S. = non-significant at the threshold of p = 0.05

SAMPLING TIME 48 HOURS	GROUP	SEX	MICRONUCLEI MEAN FOR 1000 PCE	Standard error	MIN	MAX	Mann-Whitney U rank test		p
							UA	UB	
NEGATIVE CONTROL	SOLVENT	♂	1.1	0.65	0.5	2			
		♀	0.9	0.55	0	1.5			
		♂ + ♀	1	0.58	0	2			
COMPOUND STUDIED	HIGH DOSE 500 mg/kg	♂	0.8	0.57	0	1.5	9.5	15.5	N.S.
		♀	0.5	0	0.5	0.5	5	20	N.S.
		♂ + ♀	0.65	0.41	0	1.5	30.5	69.5	N.S.
	MIDDLE DOSE 250 mg/kg	♂	0.5	0.5	0	1	6	19	N.S.
		♀	0.4	0.22	0	0.5	4.5	20.5	N.S.
		♂ + ♀	0.45	0.37	0	1	21.5	78.5	<0.05
	LOW DOSE 125 mg/kg	♂	0.4	0.42	0	1	4.5	20.5	N.S.
		♀	0.5	0.5	0	1	7	18	N.S.
		♂ + ♀	0.45	0.44	0	1	23	77	= 0.05

N.S. = non-significant at the threshold of  $p = 0.05$

#### 7.4 Other Genetic Toxicity Studies

##### Study title: Mutation Assay At The TK Locus In L5178Y Mouse Lymphoma Cells Using A Microtiter Cloning Technique (Trifluorothymidine Resistance) With S 16257-2

Study no.

NP05144

Study report location:

M4/4.2.3.3.1

Conducting laboratory and location:

(b) (4)

Date of study initiation:

Aug 3, 1994

GLP compliance:

Yes

QA statement:

Yes

Drug, lot #, and % purity:

S 16257-2, Batch # 46762, 100.4%

##### Key Study Findings

The result showed a weak mutagenicity of S16257-2 in the absence of S9 at concentration of 1000  $\mu\text{g/mL}$  and a negative mutagenic activity in the presence of S9 under study conditions.

##### Methods

Cell line: L5178Y (TK+/-) Mouse lymphoma cells

## Concentrations in definitive study:

Assay 1: 750, 375, 187.5 and 93.8 µg/mL without S9;  
1500, 750, 375, 187.5 and 93.8 µg/mL with S9

Assay 2: 1000, 750, 375, 187.5 and 93.8 µg/mL without S9;  
1500, 750, 375, 187.5 and 93.8 µg/mL with S9;

Assay 3: 1250, 1000, 800, 640 and 512 µg/mL without S9  
1750, 1500, 1250, 1000, and 750 µg/mL with S9;

## Basis of concentration selection:

The concentration selection was based on the cytotoxicity assay, in which concentrations of 0, 100, 300, 1000, 3000 and 5000 µg/mL were tested. The percent relative survival (%RS), and percent relative total growth (%RTG) were shown in the tables below.

Concentration (µg/mL)		0	100	300	1000	3000	5000
-S9	%RS	100	70.1	61.3	40.0	1.3	0.8
	%RTG	100	53.2	38.8	13.7	0	0
+S9	%RS	100	65.1	66.2	36.6	1.4	0
	%RTG	100	49.8	48.0	13.4	0	0

In the absence of S9, a high level of cytotoxicity (0.8 to 1.3% RS and 0% RTG) was observed at two highest concentrations (5000 and 3000 µg/mL). The intermediate concentration of 1000 µg/mL at which %RTG was 13.7% was chosen as the highest concentration for the definitive assay. However, due to a strong cytotoxicity in the definitive assay, the eventual concentrations retained for the first mutagenicity assay in the absence of S9 were 750, 375, 187.5 and 93.8 µg/mL.

In the presence of S9, a strong cytotoxicity (0 to 1.4% RS and 0% RTG) was observed at two highest concentrations. As the intermediate concentration of 1000 µg/mL provoked 36.6%RS and 13.4% RTG, the concentrations for the definitive assay with S9 were 1500 µg/mL (supposed having 10% RS), 750, 375, 187.5 and 93.8 µg/mL.

Negative control: vehicle

## Positive control:

In the absence of S9, Methyl Methanesulfonate (MMS), culture medium (Fischer) 0, 10 µg/mL;

In the presence of S9, Cyclophosphamide (CPA): culture medium 0, 2 µg/mL.

Formulation/Vehicle: S 16257-2 was dissolved in culture medium at 50 mg/mL.

Vehicle = culture medium.

## Exposure condition:

Cell cultures: duplicates except for positive control;

Treatment: 3 hours in the absence or the presence of S9

Expression period (recovery): 48 hours in the absence or the presence of S9

Plating for viability: 10-12 days.

### Study Validity

The sponsor's criteria for a valid assay:

1. the plating efficiency (PE) of the negative control is higher than 50% at T0 and T2 (Time 0 and 2 day after treatment).
2. the mutation frequency of the negative control is within a range of historical data of the Laboratory
3. the mutation frequency of the positive control is significantly increased compared with the solvent. The observed values must be close to those of historical positive controls.

The criteria were met and the study is considered valid. Historical data was provided in Table 1 of the report).

Criteria defined for a compound which was considered as mutagenic in the assays:

1. A multiplication by TWO of the number of spontaneous mutants has to be provoked by at least one dose.
2. A concentration/effect relationship that is an increase in the number of mutants with the exposure, must be observed, and does not necessarily imply a proportional increase (in the case of positive responses, a decrease in the number of mutants at the highest concentration, is frequently observed linked with cytotoxic effects).
3. The results have to be reproducible in an independent study, at least from a qualitative point of view.

### Results

The results from three assays are shown in the tables below (excerpted from Table 21, and Table 22 of study report, pages 36 and 37).

In the absence of S9, the 1st assay did not show any significant increase in the number of induced mutants. A slight increase (~1.6 fold) was observed at the highest concentration tested of 750 µg/mL. In the 2<sup>nd</sup> and 3<sup>rd</sup> assays, a significant increase (2.2 or 2.1 folds) in the number of mutants was observed at concentration of 1000 µg/mL (highest concentration tested in the 2<sup>nd</sup> assay) at which no strong cytotoxicity was observed. The highest concentration tested in the 3<sup>rd</sup> assay (1250 µg/mL) resulted in significant cytotoxicity (5% RS), hence, the marked increase in the number of mutants (3.8 folds) at this concentration should be taken into account with precaution for mutagenic activity. Under these conditions, the result showed that S16257-2 had a slight mutagenic activity at concentration of 1000 µg/mL.

In the presence of S9, no significant increase in the number of mutants was observed at all the concentrations tested in the 1<sup>st</sup> and the 3<sup>rd</sup> assays. A significant increase (2.6 folds) in the number of mutants noted at the highest concentration, 1500 µg/mL, in the

2<sup>nd</sup> assay was not reproducible in the 3<sup>rd</sup> assay at same and higher concentrations. Hence, there was no consistent evidence for mutagenicity of S16257-2 in the presence of S9 under study conditions.

In conclusion, the result showed weak mutagenicity of S16257-2 in the absence of S9 at concentration of 1000 µg/mL and a negative mutagenic activity in the presence of S9 under study conditions.

**RECAPITULATION OF THREE ASSAYS  
WITHOUT METABOLIC ACTIVATION**

ASSAY 1	TEST COMPOUND					MMS 10µg/ml
	DOSE µg/ml					
	0	93.8	187.5	375	750	
SURVIVAL RATE	100.0	91.5	84.4	82.5	61.2	102.8
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	91.8	64.2	86.9	106.2	144.0	718.2
<i>Ratio</i>		0.7	0.9	1.2	1.6	7.8

ASSAY 2	TEST COMPOUND						MMS 10µg/ml
	DOSE µg/ml						
	0	93.8	187.5	375	750	1000	
SURVIVAL RATE	100.0	108.8	98.7	86.7	73.0	70.6	96.7
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	68.7	86.6	81.7	96.0	75.1	152.4	678.4
<i>Ratio</i>		1.3	1.2	1.4	1.1	2.2	9.9

ASSAY 3	TEST COMPOUND						MMS 10µg/ml
	DOSE µg/ml						
	0	512	640	800	1000	1250	
SURVIVAL RATE	100.0	101.0	66.3	66.7	56.0	5.0	98.7
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	73.5	64.9	90.5	78.7	151.5	277.5	695.0
<i>Ratio</i>		0.9	1.2	1.1	2.1	3.8	9.5

**RECAPITULATION OF THREE ASSAYS  
WITH METABOLIC ACTIVATION**

ASSAY 1	TEST COMPOUND						CPA 2µg/ml
	DOSE µg/ml						
	0	93.8	187.5	375	750	1500	
SURVIVAL RATE	100.0	126.7	104.5	108.3	96.1	53.7	81.3
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	104.5	138.3	135.5	117.2	109.1	128.4	733.2
<i>Ratio</i>		1.3	1.3	1.1	1.0	1.2	7.0

ASSAY 2	TEST COMPOUND					CPA 2µg/ml
	DOSE µg/ml					
	0	187.5	375	750	1500	
SURVIVAL RATE	100.0	120.9	90.3	77.0	35.4	74.5
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	97.1	113.3	118.2	138.5	254.0	743.1
<i>Ratio</i>		1.2	1.2	1.4	2.6	7.7

ASSAY 3	TEST COMPOUND						CPA 2µg/ml
	DOSE µg/ml						
	0	750	1000	1250	1500	1750	
SURVIVAL RATE	100.0	96.2	91.4	79.6	64.9	40.1	79.0
MUTATION FREQUENCY x 10 <sup>-6</sup> CELLS (Mean of 2 cultures)	100.2	90.9	106.3	102.0	112.5	120.3	922.7
<i>Ratio</i>		0.9	1.1	1.0	1.1	1.2	9.2

**Study title: S 16257-2: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells using the Microtitre® Fluctuation Technique**

Study no.

NP05489

Study report location:

M4/4.2.3.3.1

Conducting laboratory and location:

(b) (4)

Date of study initiation:

January 30, 1995

GLP compliance:

Yes

QA statement:

Yes

Drug, lot #, and % purity:

S 16257-2, Batch # 46762, 100.4%

**Key Study Findings**

Overall, three experiments suggested a negative result in the absence of S9 and weak mutagenicity in the presence of S9. The result was different from another mouse

lymphoma assay (NP05144). The large variability in cytotoxicity among three assays may affect the reproducibility of the assay results, which made the study rather inconclusive.

### Methods

Cell line: L5178Y (tk) Mouse lymphoma cells

Concentrations in definitive study:

Experiment 1: 125 -1250 µg/mL without S9 and 250-1500 µg/mL with S9;

Experiment 2: 500 -2000 µg/mL without S9 and 500-1500 µg/mL with S9;

Experiment 3: 125 -2000 µg/mL without S9 and 62.5-1500 µg/mL with S9.

Basis of concentration selection:

The concentration selection was based on the cytotoxicity in the range-finder assay, in which 6 concentrations were tested. The raw plate counts and relative survival values are shown in the table below (excerpted from page 20 of the report). The highest survival concentrations were 625 µg/mL in the absence of S9 with 58.7% relative survival (%RS), and **1250** µg/mL in the presence of S9 with 10.1% relative survival. The definitive Experiment 1 was tested concentrations up to 1250 µg/mL in the absence of S9 and up to 1500 µg/mL in the presence of S9.

Based on the results of Experiment 1, the test concentrations in Experiment 2 were modified to ensure they were up to limits of toxicity. Concentrations up to 2000 µg/mL in the absence of S9 and 1500 µg/mL in the presence of S9 were tested. As shown in the tables of the result section, the top two concentrations in the absence and presence of S9 were later rejected from analysis due to excessive toxicity (%RS <10%). It was noted that more toxicity was observed in Experiment 2 than may have been expected from the results of Experiment 1.

In view of the results from Experiments 1 and 2, concentrations in Experiment 3 were extended and eight doses were tested ranging 125 - 2000 µg/mL in the absence of S9 and from 62.5 -1500 µg/mL in the presence of S9. The top concentrations selected yielded 31.8% and 37.8% RS in the absence and presence of S9, respectively.

Treatment µg/mL	In the absence of S-9		In the presence of S-9	
	Survival <sup>1</sup> at day 0*	% Relative survival	Survival <sup>1</sup> at day 0*	% Relative survival
0	20	100.0	26	100.0
156.25	23	129.3	22	69.5
312.5	18	84.3	22	69.5
625	14	58.7	16	41.4
1250	0	0.0	5	10.1
2500	0	0.0	0	0.0
5000	0	0.0	0	0.0

\*= 32 wells scored

<sup>1</sup>= 1.6 cells/well plated

Negative control: vehicle

Positive control: prepared in dimethyl sulphoxide (DMSO)

In the absence of S9, 4-nitroquinoline 1-oxide (4-NQO): 0.05 and 0.1 µg/mL

In the presence of S9, benzo(a)pyrene (BaP): 2 and 3 µg/mL

Formulation/Vehicle: S 16257-2 was dissolved in water at concentrations up to 2000 µg/mL. Vehicle = purified water.

Exposure condition:

Cell cultures: duplicates except for positive control;

Treatment: 3 hours in the absence or the presence of S9

Expression period: 2 days for Experiments 1 and 2; 3 days for Experiment 3.

Plating for viability: 10-12 days without 5-trifluorothymidine (TFT); 12-14 days with TFT.

### Study Validity

The sponsor's criteria for a valid assay:

1) the mutant frequencies in the negative (vehicle) control cultures fell within the normal range (not more than three times the historical mean value);

2) at least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value);

3) the % RS following treatment was higher than 10%. Dose levels yielding less than 10% relative survival were eliminated from further calculations.

The criteria for a valid study were met.

Criteria defined for a compound which was considered mutagenic:

1. the assay was valid;
2. the mutant frequency at one or more doses was significantly greater than that of the negative control;
3. there was a significant concentration-dependence as indicated by the linear trend analysis;
4. the effects described above were reproducible.

### Results

The results from three independent experiments are summarized below (excerpted from page 32-33 of the study report).

In the absence of S9, no statistically significant increase in mutant frequency was observed at concentrations up to 1250 µg/mL in Experiment 1, up to 750 µg/mL in Experiment 2 and up to 2000 µg/mL in Experiment 3. The relative survival at top concentrations of experiments 1 and 3 was 51% and 32%, respectively. Cytotoxicity

should be taken into consideration in evaluating the statistically significant increase in mutant frequency observed in Experiment 2 at concentration of 1000 µg/mL with a %RS <10%.

In the presence of S9, in Experiment 1, no statistically significant increase in mutant frequency was observed at concentrations up to 1500 µg/mL. In Experiment 2, a significant increase in mutant frequency was observed at concentrations 500-1000 µg/mL with %RS of 32.8-11.8%, and excessive cytotoxicity (<10% RS) was observed at higher concentrations of 1250 and 1500 µg/mL (top concentration). In Experiment 3, a slight but statistically significant increase in mutant frequency was observed at concentration of 1250 µg/mL but not at others including the higher concentration of 1500 µg/mL. The top concentrations of the 1<sup>st</sup> and 3<sup>rd</sup> assays resulted in %RS of 15% and 38%, respectively.

Experiment 1

Treatment (µg/mL)	%RS	-S-9 Mutant frequency#	Treatment (µg/mL)	%RS	+S-9 Mutant frequency#
0	100.0	138.07	0	100.0	130.75
125 \$	79.2	NE	250 \$	58.0	NE
250	89.5	130.76 NS	500	46.7	183.89 NS
500	86.8	90.53 NS	750	38.8	171.01 NS
750	59.1	84.81 NS	1000	33.8	164.01 NS
1000	50.8	126.08 NS	1250	22.4	166.49 NS
1250	50.8	108.24 NS	1500	15.7	152.40 NS
Linear trend		NS	Linear trend		NS
NQO			BP		
0.05	86.8	683.72	2	59.3	865.09
0.1	74.3	817.18	3	55.0	1380.19

Experiment 2

Treatment (µg/mL)	%RS	-S-9 Mutant frequency#	Treatment (µg/mL)	%RS	+S-9 Mutant frequency#
0	100.0	185.36	0	100.0	167.31
500	74.4	160.28 NS	500	32.8	294.44 *
750	60.9	178.48 NS	750	21.8	366.84 *
1000	6.7	276.54 *	1000	11.8	374.66 *
1250 X	3.0	NE	1250 \$\$, X	2.8	NE
1500 \$	0.3	NE	1500 X	2.5	NE
2000 \$	0.0	NE			
Linear trend		NS	Linear trend		***
NQO			BP		
0.05	78.4	1535.98	2	70.5	1586.02
0.1	38.0	1504.64	3	32.4	2347.08

Experiment 3

Treatment (µg/mL)	%RS	-S-9 Mutant frequency#	Treatment (µg/mL)	%RS	+S-9 Mutant frequency#
0	100.0	142.47	0	100.0	104.09
125 \$	84.8	NE	62.5 \$	101.5	NE
250 \$	96.4	NE	125 \$	91.0	NE
500 \$	84.2	NE	250 \$	93.7	NE
750	81.2	117.21 NS	500	78.4	122.43 NS
1000	77.6	128.85 NS	750	77.9	137.02 NS
1250	73.0	97.49 NS	1000	61.5	122.70 NS
1500	63.3	120.04 NS	1250	50.3	150.64 *
2000	31.8	99.27 NS	1500	37.8	109.73 NS
Linear trend		NS	Linear trend		NS
NQO			BP		
0.05	86.8	387.57	2	96.4	745.27
0.1	87.7	517.68	3	56.7	1391.29

# Per 10<sup>6</sup> viable cells  
 %RS Percent relative survival  
 \$ Not plated for viability / 5-TFT resistance  
 \$\$ Treatment excluded due to excessive heterogeneity  
 X Treatment excluded from test statistics due to excessive toxicity  
 NS Not significant  
 \*, \*\*, \*\*\* Significant at 5%, 1% and 0.1% level respectively  
 NE Not evaluated  
 NQO 4-nitroquinoline 1-oxide  
 BP Benzo(a)pyrene

Overall, three experiments suggested a negative result in the absence of S9 and a weak mutagenicity in the presence of S9. The result was different from another mouse lymphoma assay (NP05144). The large variability in cytotoxicity among three assays may affect the reproducibility of the assay results, rendering the study rather inconclusive.

**Study title: Genotoxicity Study With S 16257-2: Measurement of Unscheduled DNA Synthesis in Isolated Rat Hepatocytes**

Study no. NP05912  
 Study report location: M4/4.2.3.3.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: Dec 23, 1994  
 GLP compliance: Compliant with (b) (4) guidelines  
 QA statement: Yes  
 Drug, lot #, and % purity: S 16257-2, Batch # EG418, 101.3%

**Key Study Findings**

No reproducible or dose-related increase in mean net nuclear grain counts was observed at up to 400 µg base/mL.

A slight increase in  $^3\text{H}$ -thymidine incorporation in the nuclei of rat hepatocytes in primary cultures was observed at 550  $\mu\text{g}/\text{mL}$ , suggesting a treatment induced unscheduled DNA synthesis.

### Methods

Cell: Rat hepatocytes in primary cultures

Induction: The unscheduled DNA synthesis was measured by autoradiography after the incorporation of  $^3\text{H}$ -thymidine.

Concentrations in definitive study:

1<sup>st</sup> Assay: 0 (culture medium), 55, 100, 180, 300, 550  $\mu\text{g}/\text{mL}$ ;

2<sup>nd</sup> Assay: 0 (culture medium), 100, 180, 300, 400, 550  $\mu\text{g}/\text{mL}$ ;

Basis of concentration selection:

The concentration selection was based on the preliminary cytotoxicity test, in which doses of 0 (culture medium), 3, 10, 30, 100, 300, 1000, 2000  $\mu\text{g}/\text{mL}$  were tested. As shown below, The relative survival (%) reached 11% at 1000  $\mu\text{g}/\text{mL}$ , indicating a cytotoxicity. The top dose for definitive assays was selected at 550  $\mu\text{g}/\text{mL}$ .

#### PRELIMINARY ASSAY

CYTOTOXICITY EFFECT ON RATS HEPATOCYTES	MEAN OF CELL NUMBERS	RELATIVE SURVIVAL (%)
GROUP AND DOSE		
D(-) (0 $\mu\text{g}/\text{ml}$ )	1144.3	100.0
D1 (3 $\mu\text{g}/\text{ml}$ )	1170.3	102.3
D2 (10 $\mu\text{g}/\text{ml}$ )	1082.7	94.6
D3 (30 $\mu\text{g}/\text{ml}$ )	930.7	81.3
D4 (100 $\mu\text{g}/\text{ml}$ )	910.0	79.5
D5 (300 $\mu\text{g}/\text{ml}$ )	942.0	82.3
D6 (1000 $\mu\text{g}/\text{ml}$ )	127.0	11.1
D7 (2000 $\mu\text{g}/\text{ml}$ )	11.0	1.0

Negative control: culture medium

Positive control: Solution of N-Acetylaminofluorene (2-AAF) in DMSO at 0.2  $\mu\text{g}/\text{mL}$  (2% v/v) in the medium

Formulation/Vehicle: Solution in water for injectable preparation (2% v/v).

Treatment/Recovery: 20 - 22 h treatment and recovery

Study Validity

The mean net nuclear grain counts of the medium negative controls were within the range of acceptability (1.0 and 0.2, in the first assay, and 1.9 and 2.6, in the second assay).

Positive controls induced at least a 4-fold increase in unscheduled DNA synthesis over the medium negative control, demonstrating the sensitivity of the test system.

Five usable concentrations of the tested compound were available.

The sponsor's criteria to evaluate a positive response:

- an increase above the solvent control value in the mean net nuclear grain count to at least 5 grains per nucleus, or in the number of cells with five or more net nuclear grains,
- a concentration-response effect,
- reproducibility of the above criteria.

The study was considered valid.

## Results

The results from three assays are shown in the tables below (summarized based on tables on page 36, 37, 39, and 40 of study report).

Assay 1, Dose ( $\mu\text{g}/\text{mL}$ )	0	55	100	180	300	550
Relative Survival (%)	100	98.9	85.4	89.3	85.7	41
Mean Net Nuclear Grain Counts culture 1/culture 2	1.0/ 0.2	0.8/ 0.3	0/ 0.9	0.4/ 1.1	0.3/ 2.0	7.8/ 1.9

Assay 2, Dose ( $\mu\text{g}/\text{mL}$ )	0	100	180	300	400	550
Relative Survival (%)	100	109.6	90.4	76.2	82.6	54.2
Mean Net Nuclear Grain Counts culture 1/culture 2	1.9/ 2.6	2.6/ 0.8	1.4/ 2.6	3.4/ 4.3	4.5/ 2.0	9.8/ 6.8

In conclusion, no reproducible or concentration-related increase in mean net nuclear grain counts was observed at up to 400  $\mu\text{g}$  base/mL. In both assays, a slight increase in  $^3\text{H}$ -thymidine incorporation in the nuclei of rat hepatocytes in primary cultures was observed at 550  $\mu\text{g}$  base/mL, at which relative survival was 41% to 54%, indicating a treatment induced unscheduled DNA synthesis. [note: The *in vivo* unscheduled DNA synthesis study in rat did not show a positive activity (see review of study NP05409 below).]

**Study title: S16257: Measurement of Unscheduled DNA Synthesis in Rat Liver using an In Vivo/In Vitro Procedure**

Study no.: NP05409  
 Study report location: M4/4.2.3.3.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: February 20, 1995  
 GLP compliance: (b) (4) compliance  
 QA statement: Included  
 Drug, lot #, and % purity: S 16257-2 Hydrochloride, 47734  
 Purity= 100.5%.

**Key Study Findings**

A single dose of S16257-02 up to 1000 mg/kg (males) or 650 mg/kg (females) did not induce unscheduled DNA synthesis in rat liver under study conditions.

At the high dosages, mean blood and liver, concentrations of S 16257-2 (expressed as the base) were between 48 and 17 µg/g for males and between 59 and 421 µg/g for females depending on the sampling time (2 or 12 hours after dosing).

**Methods**

Doses in definitive study: 0 (negative control), 300, 1000 mg/kg for males  
 0, 195, 650 mg/kg for females  
 Frequency of dosing: Single dose  
 Route of administration: Oral gavage  
 Dose volume: 20 mL/kg except 10 mL/kg for positive control  
 Formulation/Vehicle: Solution in purified water/purified water  
 Species/Strain: Rat/Wistar (male and female)  
 Number/Sex/Group: 5/sex/group  
 Satellite groups: See study design table below for blood samples  
 Basis of dose selection: A range-finding study was conducted in Wistar male and female rats treated with a single oral dose at dosage levels of 178.4, 274.6, 422.6, 650, 1000 mg/kg. One female animal died within 24 hours of dosing at 1000 mg/kg. Overt clinical signs of toxicity were observed at dosages of 650 mg/kg in females (eye closure, unsteady gait, tremors/shivers, and vocalization) and at 1000 mg/kg in males (eye closure, lethargy, unsteady gait, tremors, irregular breathing and uncoordinated movement). The maximum tolerated dosage (MTD) was determined at 650 mg/kg for females, and 1000 mg/kg for males,

which were selected as the maximum doses for main study.

In the main study, one male from the 1000 mg/kg and one female from the 650 mg/kg dosage groups died after dosing with S 16257-2, confirming the MTDs were achieved.

Negative control: Vehicle (purified water)

Positive control: For 2-4 hours sampling, dimethylnitrosamine (DMN), 10 mg/kg (in purified water), oral;  
For 12-14 hours sampling, 2-acetamidofluorene (2-AAF), 75 mg/kg (in corn oil), oral;

Sampling Time: 2-4 hours; 12-14 hours

Three rats/gender/group were euthanized 2 to 4 hours, or 12 to 14 hours, after dosing and livers were perfused with collagenase to provide primary hepatocytes for UDS analysis. After 4 h incubation in the presence of [<sup>3</sup>H]-thymidine, for 100 hepatocytes/rat, net nuclear grain counts were recorded by autoradiography. The net nuclear grain count (NNG), i.e. the number of grains present in the nucleus minus the mean number of grains in 3 equivalent areas of cytoplasm, was determined for each of 2 of the 3 slides, each animal and each dose.

Blood and liver samples were taken from satellite animals (3/sex/group/time point) at 2 hours and 12 hours post dosing for TK analysis.

### Study Validity

The criteria were set for a valid study:

1. The negative control animals had zero NNG counts or less (i.e a negative value).
2. The positive control treatments should have values of 5 or more NNG with 50% or more cells having NNG counts of 5 or greater.

The criteria for a positive response:

1. the test article yielded group mean NNG values greater than 0 and 20% or more of cells responding (mean NNG values  $\geq 5$ )
2. an increase was seen in both NNG and the percentage of cells in repair.

The study criteria were met.

As shown in the tables below in Results, male negative (vehicle) control animals gave a group mean NNG value of less than 0 with only 0.3 to 1.0% of cells in repair. Female negative (vehicle) control animals gave a group mean NNG value of less than 0 with only 1.3 to 1.7% of cells in repair. The vehicle control NNG value was consistent with historical control data.

Both positive control treatments produced NNG values greater than 5 with greater than 20% of cells in repair, indicating that the test system was sensitive to 2 known DNA damaging agents requiring metabolism for their action.

The study is considered valid.

## Results

As shown in the tables below (excerpted from pages 26 and 35 of the report), there were no increases in NNG counts or in the percentage of cells in repair in treated male and female groups when compared to their respective controls.

Group mean NNG values (Males)

### 12-14 hour experiment

Dose (mg/kg)	Net nuclear grain count (NNG)		Net grain count of cells in repair		Percent of cells in repair (NNG $\geq$ 5)	
	mean	SD	mean	SD	mean	SD
0 Water	-1.3	0.2	6.8	0.8	1.0	1.0
300	-1.6	0.4	5.7	0.9	1.3	1.5
1000	-1.6	0.4	5.0	0.0	0.3	0.6
75 2-AAF	13.0	1.6	14.1	1.5	89.0	3.5

### 2-4 hour experiment

Dose (mg/kg)	Net nuclear grain count (NNG)		Net grain count of cells in repair		Percent of cells in repair (NNG $\geq$ 5)	
	mean	SD	mean	SD	mean	SD
0 Water	-1.2	0.1	5.7	0.0	0.3	0.6
300	-1.7	0.0	6.7	0.0	0.3	0.6
1000	-2.0	0.5	5.0	0.0	0.3	0.6
10 DMN	11.2	0.6	12.4	0.7	87.0	1.7

Group Mean NNG Values (Females)

## 12-14 hour experiment

Dose (mg/kg)	Net nuclear grain count (NNG)		Net grain count of cells in repair		Percent of cells in repair (NNG $\geq$ 5)	
	mean	SD	mean	SD	mean	SD
0 Water	-1.3	0.2	5.4	0.1	1.7	1.5
195	-1.5	0.7	7.2	0.0	1.0	1.7
650	-1.6	0.3	5.5	0.7	0.7	0.6
75 2-AAF	9.2	0.1	10.5	0.3	81.3	4.0

## 2-4 hour experiment

Dose (mg/kg)	Net nuclear grain count (NNG)		Net grain count of cells in repair		Percent of cells in repair (NNG $\geq$ 5)	
	mean	SD	mean	SD	mean	SD
0 Water	-1.3	0.7	7.4	0.0	1.3	2.3
195	-1.4	0.8	8.1	3.0	1.7	2.1
650	-1.8	0.3	5.7	0.0	0.3	0.6
10 DMN	10.1	0.9	11.4	1.0	84.0	1.0

Mean plasma and liver exposure to ivabradine and its metabolite, S 18982, at 2 hr post-dose are shown in the table below (excerpted from table 34, Module 2, section 2.6.6. page 55). For the highest dosages, mean liver concentrations of S 16257-2 (expressed as the base) were between 48 and 17  $\mu\text{g/g}$  for males and between 59 and 421  $\mu\text{g/g}$  for females depending on the sampling time (2 or 12 hours after dosing).

**Table 34. Ivabradine and S 18982 Mean Plasma and Liver Concentrations in Wistar Rats 2 h After Single Oral Dosing**

Dose (mg/kg)	Plasma concentration (ng/ml)				Liver concentration (ng/g)	
	Males		Females		Males	Females
	2 h	Multiple of hC <sub>max</sub>	2 h	Multiple of hC <sub>max</sub>	2 h	2 h
<b>ivabradine</b>						
181			2100	68		28000
278	810	26			10000	
603			6300	203		59000
928	3400	110			48000	
<b>S 18982</b>						
181			34	4		750
278	42	5			640	
603			70	9		1500
928	190	24			2900	

n=3/gender/dose. hC<sub>max</sub> = Mean plasma maximum concentration at steady state in patients at the highest therapeutic dose.

In conclusion, a single dose of S16257-02 up to 1000 mg/kg (males) or 650 mg/kg (females) did not induce unscheduled DNA synthesis in rat liver under study conditions.

**Study title: S 16257-2: Induction of Chromosome Aberrations in the Bone Marrow of Treated Rats**

Study no.:

NP05504

Study report location:

M4/4.2.3.3.2

Conducting laboratory and location:

(b) (4)

Date of study initiation:

June 8, 1995

GLP compliance:

(b) (4) compliance

QA statement:

Included (reviewer scientist statement error in title, rats=mice

Drug, lot #, and % purity:

S 16257-2 Hydrochloride, 47734

Purity= 100.5%.

**Key Study Findings**

A single dose of S16257-02 up to 1000 mg/kg (males) or 650 mg/kg (females) did not induce chromosomal aberrations *in vivo* in rat bone marrow cells under study conditions.

**Methods**

Doses in definitive study:

See study design table below. Equivalent base dosages: 0, 232, 464, 928 for males; 0, 151, 302 or 603 mg/kg for females.

Frequency of dosing:

Single dose

Route of administration: Oral gavage  
 Dose volume: 20 mL/kg except 10 mL/kg for positive control  
 Formulation/Vehicle: Solution in purified water/purified water  
 Species/Strain: Rat/Wistar (male and female)  
 Number/Sex/Group: 5/sex/group  
 Satellite groups: See study design table below for TK samples  
 Basis of dose selection: The range-finding study 303/167 (i.e. NP05409, "Measurement of UDS in Rat Liver using an In Vivo/In Vitro Procedure") indicated that the maximum tolerated dose was determined at dosage of 1000 mg/kg in males, and 650 mg/kg in females. See "Basis of dose selection" in the review of study NP05409 in section 7.4.

In the main study, four females and three males died, and one male was sacrificed moribund in the high dosage groups and overt clinical signs of toxicity were observed in both male and female high dosage groups, confirming that MTDs were achieved.

Negative control: Vehicle  
 Positive control: Cyclophosphamide (CPA) 40 mg/kg, oral

Treatment (mg/kg)	Number of treated animals							
	18 hour sample		42 hour sample		Blood samples		Spares	
	♂	♀	♂	♀	♂	♀	♂	♀
Vehicle	5	5	5	5	2+2	2+2		
250	5				2+2			
500	5				2+2			
1000	5		5		2+2		5	
162.5		5				2+2		
325		5				2+2		
650		5		5		2+2		5
CPA, 40	5	5						

Study design including treatment groups and sampling times in main study was summarized in the table below (excerpted from page 15 of the report). Bone marrow from both femurs was collected. Bone marrow samples from rats treated at all three dosages were analyzed at the 18 hour post dosing and that from rats in the high dosage (1000 mg/kg for males, 650 mg/kg for females) and control groups were analyzed at the 42 hour post dosing. Slides were examined, uncoded, for mitotic index (MI) or percentage of cells in mitosis, based on 1000 cells scored per animal. Five hundred metaphases per dosage group (100 per rat) were analyzed. Blood samples for TK analysis were collected from satellite animals at 10 min and 2 hours post dosing.

**Study Validity**

The following criteria were set for an acceptable study:

1. the heterogeneity X2 test demonstrated acceptable variability between animals within groups, and
2. the proportion of cells with structural aberrations (excluding gaps) in negative control animals fell within the normal range, and
3. at least eight animals (males and females together) out of each group at each kill time and 400 cells out of an intended 500 were analyzable for analysis, and
4. the positive control chemical (CPA) induced a clear increase in the number of cells with structural aberrations.

The criteria for a positive response:

1. a statistically significant increase in the frequency of cells with structural aberrations occurred at one or more dosage and/or sampling time
2. the incidence of cells with aberrations at such data points exceeded the normal range;
3. corroborating evidence was obtained such as increased but insignificant increases in the incidence of structural aberrations at other doses or kill times, or dose response profiles.

Most study criteria were met. Less than 400 cells were available for analysis from the group of male rats treated with CPA, but as the cells analyzed contained a high frequency of aberrant cells, the slight lower number of cells was not considered to have significant impact on the validity of the assay. The study is considered acceptable.

**Results**

Mitotic indices from control and treated groups were comparable.

The frequencies of cells with structural chromosome aberrations from all treated groups were similar to and not significantly different from those observed in concurrent vehicle control groups at both sampling times. All group mean frequencies of aberrant cells were within the historical control range. The group data was summarized in the tables below (excerpted from pages 25 and 26 of the report).

**Table 1**

18 hour sample time: male animals

Treatment (mg/kg)	N <sup>o</sup> of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index % (group mean)
Vehicle	5	500	6	0		2.8
250	5	500	2	2	NS	4.7
500	5	500	3	1	NS	5.8
1000	5	500	4	2	NS	4.3
CPA, 40	5	319	147	131	p≤0.001	0.52

18 hour sample time: female animals

Treatment (mg/kg)	N <sup>o</sup> of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index % (group mean)
Vehicle	5	500	1	0		5.6
162.5	5	500	1	0	NS	5.2
325	5	500	3	1	NS	5.8
650	5	500	4	3	NS	3.7
CPA, 40	5	500	279	279	p≤0.001	0.98

**Table 2**

42 hour sample time: male animals

Treatment (mg/kg)	N <sup>o</sup> of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index % (group mean)
Vehicle	5	500	7	5		3.5
1000	5	483	1	0	p ≤ 0.05	5.6

Note: Statistical significance refers to a decrease in aberrant cell frequency

42 hour sample time: female animals

Treatment (mg/kg)	N <sup>o</sup> of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index % (group mean)
Vehicle	5	500	3	3		5.8
650	5	500	2	2	NS	5.4

Mean plasma exposure to ivabradine and its metabolite S 18982 were summarized in the table below (excerpted from Table 35 in Module 2, section 2.6.6, page 56).

*Note: The doses referred in the table below were equivalent base doses. The study report noted that the results for S 18982 are NOT validated because concentrations found in Quality Control samples were not within acceptability limits. The concentrations were given in the study report table just to indicate that S 18982 was present in plasma.*

**Table 35. Ivabradine and S 18982 Mean Plasma Concentrations in Wistar Rats 10 min and 2 h After Single Oral Dosing**

Dose (mg/kg)	Plasma concentration (ng/ml)							
	Males		Females		Males		Females	
	10 min	Multiple of hC <sub>max</sub>	10 min	Multiple of hC <sub>max</sub>	2 h	Multiple of hC <sub>max</sub>	2 h	Multiple of hC <sub>max</sub>
<b>ivabradine</b>								
151			4200	135			3000	97
232	1400	45			760	25		
302			3500	113			10000	323
464	4000	129			1200	39		
603			18000	581			4900	120
928	9100	294			6400	206		
<b>S 18982</b>								
151			64	8			37	5
232	58	7			17	2		
302			60	8			100	13
464	140	18			56	7		
603			270	34			nd	
928	390	49			230	29		

n=2/gender/dose. hC<sub>max</sub> = Mean plasma maximum concentration at steady state in patients at the highest therapeutic dose.

In conclusion, a single dose of S16257-02 up to 1000 mg/kg (males) or 650 mg/kg (females) did not induce chromosomal aberrations in rat bone marrow cells under study conditions.

## 8 Carcinogenicity

## 9 Reproductive and Developmental Toxicology

## 10 Special Toxicology Studies

## 11 Integrated Summary and Safety Evaluation

The results of genotoxicity assays are summarized in the table below.

**Table 1 Genotoxicity Summary**

Assay	+/- S9	Concentration/Dose Range <sup>c</sup> (µg/mL)	Results	<sup>a</sup> Multiples of $hC_{max}$
<b>In Vitro assays</b>				
Ames test	+/-	0, 46, 139, 464, 928, 1392, 2320, 4640 µg/plate	Negative	
Chromosomal Aberration in human lymphocytes	-	46*, 79, 116, 139	Equivocal	1,500*-4,500
	+	260, 487*, 882*, 1670, 2088	Negative	8,000-67,000
tk-gene mutation MLA (NP05144)	-	87 to 928*, 1160	Positive	2,800-30,000*
	+	87 to 1624	negative	2,800-52,000
tk-gene mutation MLA (NP05489)	-	116 to 1856	negative	5,200-60,000
	+	58 to 464*, 928*, 1160*, 1392	Positive (Inconclusive)	1,900-15,000*
UDS in rat hepatocytes	n/a	0, 55, 100, 180, 300, 400, 550*	Positive	1,800-18,000*
<b>In Vivo assays (single dose by oral gavage)</b>				
Micronucleus, mouse	n/a	0, 116, 232, 464 mg/kg	Negative	No TK data
Chromosomal Aberration, rat	n/a	M: 0, 232, 464, 928 mg/kg F: 0, 151, 302, 603 mg/kg	Negative	M: 294 <sup>b</sup> ; F: 581 <sup>b</sup>
UDS, rat	n/a	M: 0, 278, 928 mg/kg F: 0, 181, 603 mg/kg	Negative	M: 110 <sup>b</sup> ; F: 203 <sup>b</sup>

- $hC_{max}$  = mean plasma maximum concentration at steady state in patients at the highest therapeutic dose of 7.5 mg bid = 31 ng/mL.
  - Multiples of  $hC_{max}$  were based on the mean  $C_{max}$  from TK analysis in each rat study.
  - Ivabradine doses were expressed in terms of free base in this table, which could be calculated from dose of ivabradine hydrochloride  $\times$  0.928 (conversion factor).
- +/-: with/without exogenous metabolic activation (rat liver S9 mix);  
\* indicated that a significant finding was observed at that dose (exposure) level.

Ivabradine did not result in gene mutation in bacteria in vitro but was associated with a weak induction of unscheduled DNA synthesis in primary rat hepatocytes ex vivo and a weak induction of *tk* gene mutation in mouse lymphoma cells in vitro. The genotoxic responses were observed at dose concentrations > 15,000 fold of human  $C_{max}$  at maximum recommended human dose (MRHD), 7.5 mg bid, in these assays. The chromosomal aberration test in human lymphocytes produced an equivocal result for a possible weak clastogenic activity due to lack of dose-dependency.

In vivo, ivabradine did not show genotoxicity in three separate tests in mice and rats. The negative results were achieved at dosages up to 464 mg/kg (base) in mouse micronucleus test and at plasma exposures > 100 fold of human  $C_{max}$  at MRHD in rat chromosome aberrations test and rat liver UDS assay.

Given the uniformly negative in vivo results, the weak in vitro genotoxic responses observed at concentrations about 15,000 fold of human  $C_{max}$ , ivabradine is unlikely to pose a genotoxic risk in the proposed clinical use. The conclusion is substantiated by the results of 2-year carcinogenicity studies in rats and mice which showed no evidence

of tumorigenic potential after dietary administration of ivabradine at dosages up to 120/60 mg/kg/day (rats) and 405/180 mg/kg/day (mice), respectively.

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/s/  
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JEAN Q WU  
11/19/2014

ALBERT F DEFELICE  
11/19/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW  
EVALUATION (Carcinogenicity)

Application number: 206,143

Supporting document/s: S016 (eCTD0014), S022 (eCTD0017), S023 (eCTD0022)

Applicant's letter date: April 30, 2014, June 27, 2014, July 11, 2014

CDER stamp date: April 30, 2014, June 27, 2014, July 11, 2014

Product: Ivabradine

Indication: reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq$  70 beats per minute (bpm), (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)

Applicant: Amgen, Inc.

Review Division: Division of Cardio-Renal Products

Reviewer: Jean Q. Wu

Supervisor/Team Leader: Albert DeFelice

Division Director: Norman Stockbridge

Project Manager: Alexis Childers

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# 1 Executive Summary

## 2 Drug Information

### 2.1 Drug

#### 2.1.1 CAS Registry Number (Optional)

Ivabradine: 155974-00-8

Ivabradine hydrochloride: 148849-67-6

#### 2.1.2 Generic Name

ivabradine

#### 2.1.3 Code Name

S 16257-2; AMG 998

#### 2.1.4 Chemical Name

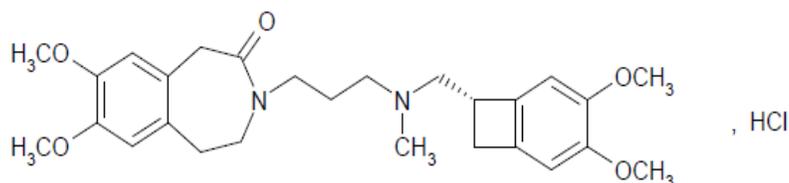
3-(3-(((7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl) methyl amino)propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride

#### 2.1.5 Molecular Formula/Molecular Weight

C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>, HCl/ 505.1 g/mol;

468.593 g/mol (base); Conversion factor from the salt to the base: 0.928

#### 2.1.6 Structure



#### 2.1.7 Pharmacologic class

hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND (b) (4)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

Ivabradine is provided as film-coated tablet in two strengths: 5 mg (oval) and 7.5 mg (triangular). The composition of the drug is listed in the table below (excerpted from Module 2.3. P, Table 1, Page 7)

Table 1. Composition of Ivabradine 5 mg and 7.5 mg Tablets

Component	5 mg		7.5 mg		Function	Reference to specifications
	Percentage (% w/w)	Quantity (mg/tablet)	Percentage (% w/w)	Quantity (mg/tablet)		
<b>Tablet</b>						
Ivabradine hydrochloride <sup>a</sup> (free base equivalent)	5.39 (5.00)	5.390 (5.000)	8.085 (7.50)	8.085 (7.500)	Drug substance	In-house
Lactose monohydrate	(b) (4)				(b) (4)	USP/NF, PhEur.
Maize starch	(b) (4)				(b) (4)	USP/NF
Maltodextrin	(b) (4)				(b) (4)	USP/NF, PhEur
Magnesium stearate	(b) (4)				(b) (4)	USP/NF, PhEur
Colloidal silicon dioxide (b) (4)	(b) (4)				(b) (4)	USP/NF, PhEur USP
<b>Core Tablet Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>		
<b>Film-Coating</b>						
(b) (4) salmon <sup>c</sup>	(b) (4)				(b) (4)	See 3.2.P.4.1
Polyethylene glycol 6000 (b) (4)	(b) (4)				(b) (4)	USP/NF, PhEur USP
<b>Total</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>	<b>102.0</b>		

<sup>a</sup> The molecular weights of Ivabradine hydrochloride anhydrous and ivabradine free base are 505.1 g/mol and 468.6 g/mol, respectively. The free base accounts for 92.73% of the salt.

(b) (4)

## 2.4 Proposed Clinical Population and Dosing Regimen

Ivabradine is indicated to reduce the risk of (b) (4) or hospitalizations for worsening heart failure in patients with chronic heart failure (b) (4) and in sinus rhythm with heart rate  $\geq 70$  beats per minute (bpm), (b) (4) maximally tolerated doses of beta blockers, or when beta blocker therapy is contraindicated (b) (4)

The proposed starting dose of ivabradine is 5 mg twice daily. After 2 weeks of treatment, if heart rate is between 50 and 60 bpm, the dose of 5 mg twice daily should be maintained. The dose should be increased to 7.5 mg twice daily if resting heart rate is persistently above 60 bpm.

As listed in the table below (excerpted from Module 2, Section 2.4, Page 7), the human plasma exposures of ivabradine and its N-desmethylated metabolite (S 18982) were estimated at steady state in patients receiving the maximum

recommended human dose (MRHD), 7.5 mg bid. These values were derived from a population pharmacokinetic analysis using Phases II and III clinical data [Summary of Clinical Pharmacology Studies, in Section 2.7.2 (2.1.2.2), and are accepted as references when preclinical doses are expressed as multiples of human exposures in current review, unless otherwise indicated.

**Table 1. Ivabradine and S 18982 (Metabolite) Plasma C<sub>max</sub> and Estimated AUC<sub>24</sub> at Steady State in Patients at HTD**

	Ivabradine (n=492)	S 18982 (n=541)
Population C <sub>max</sub> (ng/ml)	31 ± 9.8	7.9 ± 2.3
Equivalent C <sub>max</sub> in μM	0.07	0.02 <sup>b</sup>
Population AUC <sub>24</sub> <sup>a</sup> (ng.h/ml)	346	128

Values are mean ± SD;

a: Calculated from AUC over 12 h period from [WS (2.7.2) 3.1/ Table 12; Table 34 and 35]

b: S 18982 MW = [REDACTED]

## 2.5 Regulatory Background

A pre-NDA meeting was held between the sponsor and the Division on January 23, 2014. There was no IND opened in the US FDA [REDACTED] (b) (4). Hence, no carcinogenicity study protocols were submitted to the Division and ECAC for comments prior to study initiation or during the study.

## 3 Studies Submitted

### 3.1 Studies Reviewed

NP07944: S 16257-2: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice

NP07946: Toxicokinetics of S 16257 in male and female CD-1 mice after dietary administration of S 16257-2 for at least 104 weeks (TK report for study NP07944)

NP07943: S 16257-2: Potential Tumorigenic Effects in Prolonged Dietary Administration to Rats

NP07945: Toxicokinetics of S 16257 in male and female Wistar rats after dietary administration of S 16257-2 for at least 104 weeks (TK report for study NP07943)

*The following studies were briefly reviewed, and relevant findings are discussed in the reviews of the 2-year carcinogenicity studies:*

NP05454: Palatability Study in Mice by Dietary Administration for 4 Weeks

NP06393: S 16257-2: Subacute Toxicity Study to Mice by Dietary Administration for 13 Weeks.

NP05455: Palatability Study in Rats by Dietary Administration for 6 Weeks  
NP06450: S 16257-2: Subacute Toxicity Study to Rats by Dietary Administration for 13 Weeks.

## 4 Pharmacology

## 5 Pharmacokinetics/ADME/Toxicokinetics

## 6 General Toxicology

## 7 Genetic Toxicology

## 8 Carcinogenicity

### **Study title: S 16257-2: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice (Study No. NP07944)**

Conducting laboratory/location:

(b) (4)

Date of study initiation:

November 12, 1996

GLP compliance:

Yes

QA statement:

Included

Drug, lot #, and % purity:

S 16257-2, batch# 49 652, Purity: 100.4%  
(the conversion factor from free base to salt: 1.078)

CAC concurrence:

Study protocol was not discussed in ECAC.

### **Key Study Findings**

#### **Adequacy of Carcinogenicity Study**

S16257-2 was administered by diet to CD-1 mice at doses of 20, 90, and 405/180 mg/kg/day for up to 104 weeks (93 weeks for high dose male group). The study protocol was not submitted for discussion/concurrence by the ECAC (see regulatory history).

During the first 80 weeks, a significantly higher mortality was observed in the high dose (405 mg/kg/day) males and females when compared to the controls. During Weeks 81-93 and/or Weeks 84-104, the incidence of mortality in the high dose (reduced to 180

mg/kg/day) groups was comparable to the control groups. The high dose male group was terminated early at the end of Week 93 due to the low survival rate of 20%.

A body weight gain reduction in the 1<sup>st</sup> 80 weeks was observed in high dose males (-36%) and in all treated female groups in a dose-dependent manner (up to -33%). Body weight gain reduction was up to -38% over the treatment period (Week 1-93) for high dose males. The overall body weight gain for all treated females during Week 1-104 was reduced about -13% to -23% without dose-dependency.

The treatment-related non-neoplastic histopathology findings, observed in the high dose males and females, were primarily cardiac, which included atrial thrombus, dilated chambers, minimal to moderate myocardial degeneration, vacuolation and fibrosis, and minimal to slight myocardial cell hypertrophy, epicardial inflammation and fibroblast proliferation. A lesser degree of increased incidence of myocardial degeneration and vacuolation was also observed in the decedent mid-dose males. The pathology changes in the lungs, liver, spleen and thymus of the decedent mice were associated with cardiac toxicity.

Mortality and body weight gain reduction in the high dose males and females suggest that a MTD was achieved and possibly exceeded in this study. The duration of treatment, 93-week for high dosage males and 104-week for other groups, is considered acceptable.

The high dosage was associated with AUC<sub>24h</sub> of 7920 (23 X human AUC<sub>24h</sub>) and 14331 ng.h/mL (41 X human AUC<sub>24h</sub>) for male mice at Week 94 and female mice at the week of 104, respectively.

### **Appropriateness of Test Models**

A dietary administration of test article to CD-1 mice for up to 104 weeks is considered appropriate for tumorigenic potential evaluation.

### **Evaluation of Tumor Findings**

There were no treatment-related tumorigenic effects in ivabradine treated mice.

The statistically significant increase in incidence of lymphoid histiocytic sarcoma in the female low dose group was not dose-dependent, similar to the incidence in the male control group, and not observed in any other tissues/organs, hence, was not considered toxicologically significant.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 28, 2014 in Appendix 1).

## Methods

Doses:	20, 90, 405 (base) mg/kg/day <sup>a</sup> (week 1-80)/ 20, 90,180 (base) mg/kg/day <sup>a</sup> (week 81-104)
Frequency of dosing:	Daily in diet
Dose volume:	diet
Route of administration:	diet
Formulation/Vehicle:	Test article mixed with the diet <sup>b</sup> /Normal untreated diet for mice
Basis of dose selection:	The 13-week study (SVA 217/961267, NP06393) with dietary administration at dosages of 30, 100, 300 or 600 mg/kg/day showed a body weight gain decrease and an increase in heart weight at dosages $\geq 100$ mg/kg/day and $\geq 300$ mg/kg/day in males and females, respectively. Therefore, the high dosage of 405 mg/kg/day was chosen to elicit signs of minimal toxicity and to provide at least 30 times the human AUC <sub>24h</sub> estimated to be 346 ng/h.mL. The low dose of 20 mg/kg/day was chosen as no adverse effects were expected at this level and it provided at least 3 times the human therapeutic exposure.
Species/Strain:	CrI:CD-1(ICR)BR Mouse
Number/Sex/Group:	50
Age:	~5 weeks
Animal housing:	2 mice of the same sex/cage
Paradigm for dietary restriction:	All mice had free access to tap water and ground SDS Rat and Mouse No. 1 modified maintenance diet.
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	26/sex/group for TK profiling
Deviation from study protocol:	No deviations from the study protocol and amendments were considered to affect the integrity of the study
a.	<i>Food consumption and test article control data indicated that the actual doses received were 20, 91 and 403/179 mg/kg/day for males and 21, 91 and 408/184 mg/kg/day for females.</i>
b.	<i>Concentration of test article in the diet were changed as necessary to preserve the dosage levels (i.e. in line with body weight changes and food consumption)</i>

## Observations and Results

### Mortality and Clinical Signs

Animals were observed at least once weekly for mortality and moribundity. A detailed palpation of each mouse was performed once weekly for any palpable masses.

The distribution of unscheduled deaths during the study period was summarized below (excerpted from summary table of the report). During the first 80 weeks, a significantly higher mortality was observed in the high dose (405 mg/kg/day) males and females when compared to the control. Due to this high mortality, the high dose was reduced to 180 mg/kg/day from Week 81. During the week 81-93, the mortality incidence appeared similar between high dose and control groups. However, at the end of Week 93, the surviving number of high dose male reached 10, i.e. 20% survival point; thus, this group was terminated at Week 94 to ensure that there were enough animals to be examined to allow for a meaningful scientific interpretation of the terminal data.

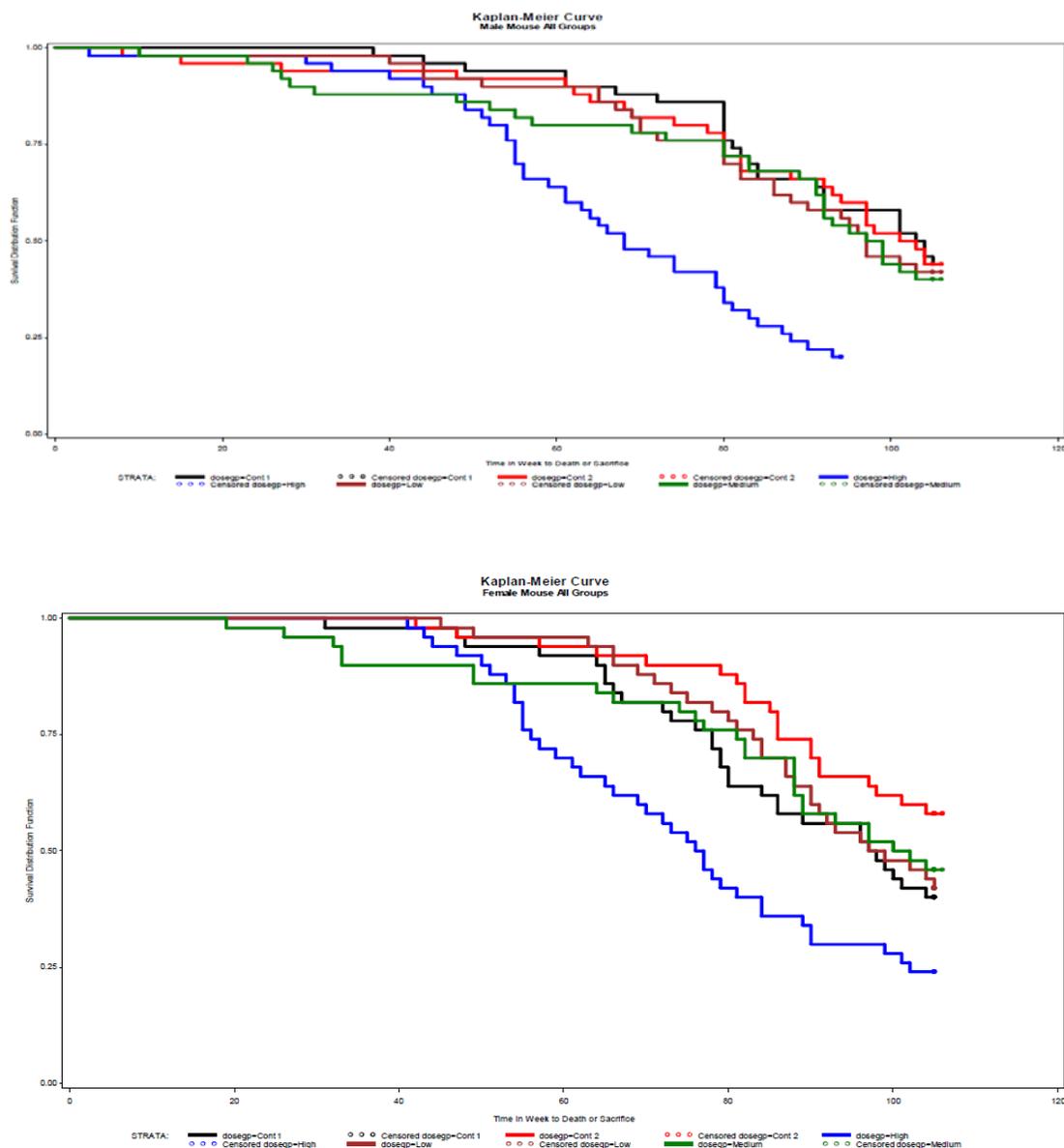
Number of decedents Weeks	Group/dosage (mg base/kg/day)									
	1M Control	2M Control	3M 20	4M 90	5M 405/180†	1F Control	2F Control	3F 20	4F 90	5F 405/180†
1 - 80	12	14	15(5)	14(6)	33(15)	18	6	11(8)	12(4)	29(16)
81 - 93	9	5	6(2)	9(3)	7(3)	4	11	12(3)	10(4)	6(1)
94 - 104	6	9	8(3)	7(3)	-	8	4	5(4)	5(5)	3(3)
1 - 104	27	28	29(10)	30(12)	40*(18)	30	21	28(15)	27(13)	38(20)
Main group mortality (%)	54	56	58	60	80	60	42	56	54	76
Main group survival (%)	46	44	42	40	20	40	58	44	46	24

( ) Satellite group animals

† Dosage was lowered from 405 mg base/kg/day to 180 mg base/kg/day from Week 81

\* Surviving Group 5 males were sacrificed in Week 94 due to high mortality

The survival test conducted by FDA statistical reviewer, Dr. A. Rahman, showed a statistically significant dose response relationship in mortality across the combined control and treated groups in both genders of mice. The pairwise comparison showed statistically significant increased mortality in the high dosage group in both genders when compared to their respective combined control groups (see the figures below, excerpted from Statistical Review and Evaluation for this NDA, by Dr. A. Rahman).



Histopathology examination of the main group decedents during the 1st 80 weeks revealed an increased incidence and severity of heart lesions that included myocardial degeneration, myocardial vacuolation, myocardial fibrosis, dilated chambers, myocardial cell hypertrophy, atrial thrombi and epicardial inflammation with edema or fibroblast proliferation, predominantly in the high dosage (405 mg/kg/day) males and females when compared to the controls.

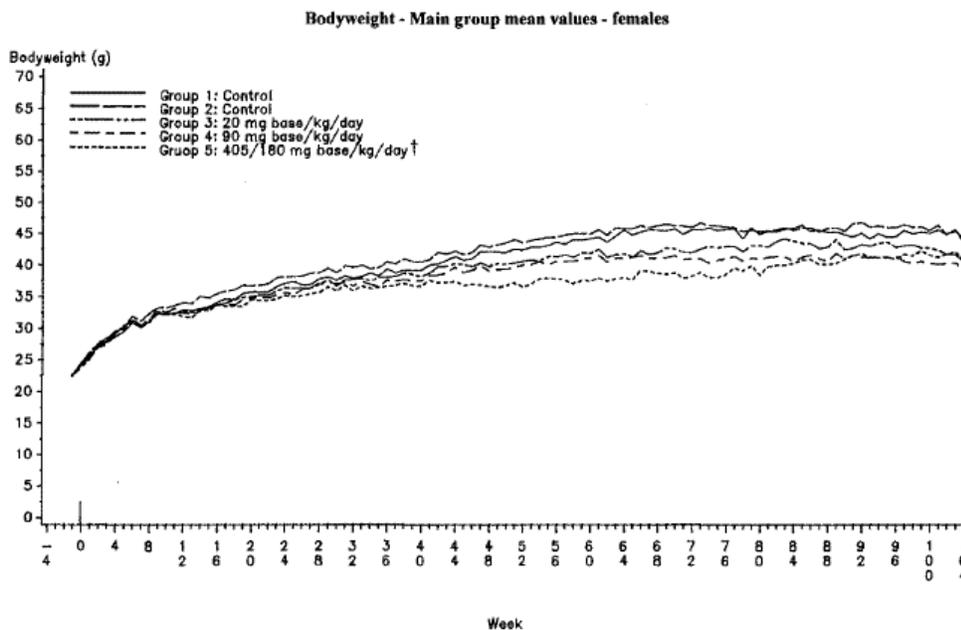
There were no other test article-related adverse clinical observations.

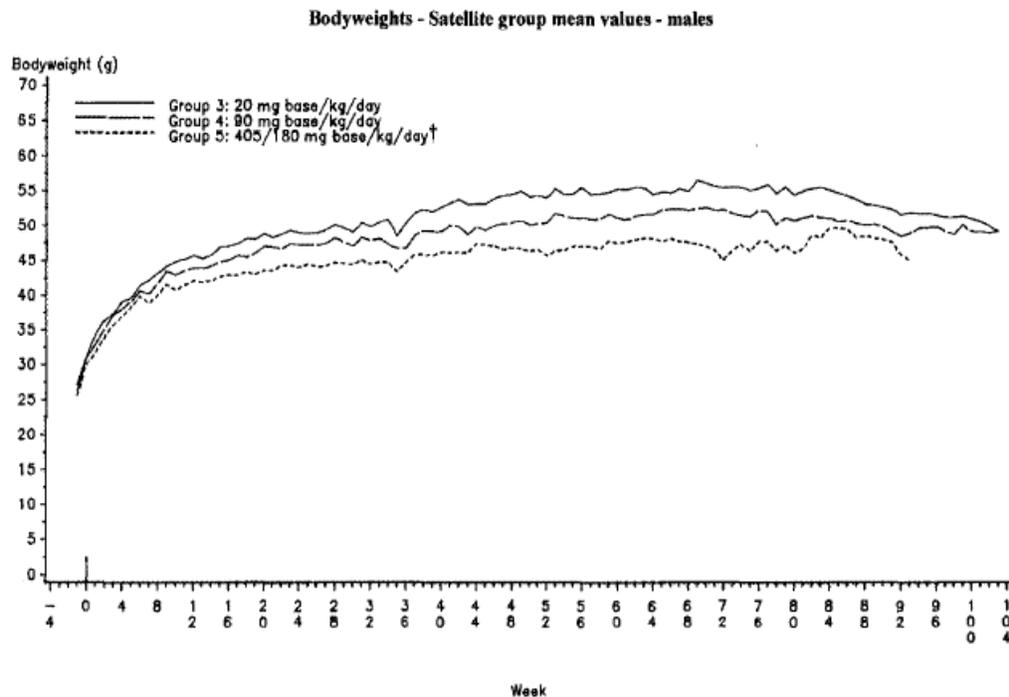
## Body Weights

Body weight was collected at randomization, one week prior to and on the day of initial treatment, and once a week thereafter.

Body weight gain g (% of combined control)	Dose Mg/kg/day	Male					Female				
		0	0	20	90	405/180	0	0	20	90	405/180
Week 0-80		22.7	24.0	21.9 (94)	24.3 (104)	14.9 (64)	21.1	20.7	19.3 (92)	16.5 (79)	13.9 (67)
Week 0-93		23.0	22.3	21.5 (95)	21.7 (96)	13.5 (62)	20.4	21.3	19.1 (92)	16.5 (79)	17.4 (83)
Week 0-104		21	17	19 (0)	19 (0)	N/A	20.8	18.6	16.7 (86)	14.8 (77)	17.0 (87)

As shown in the table above and figures below (excerpted from page 51-52 of the report), a dose-related reduction in group mean bodyweight gain was noted in the first 80 weeks among all treated female groups up to -33% and in high dose males (-36%) when compared with concurrent controls. Following the reduction of the high dosage from 405 to 180 mg/kg/day, female bodyweight gain exhibited a slight improvement between Weeks 81 to 104. This resulted in an overall weight gain for high dose females that was comparable to that for low dose females while the reduction for the mid-dose females reached -23% over the period of 104 weeks. The overall bodyweight gain for high dose males was reduced about -38% during the Week 1-93 treatment period while that for low and mid-dose males was similar to the concurrent controls during the 104-week treatment period.





## Food Consumption

The quantity of food consumed by each cage of mice was recorded weekly. Individual mouse food intake per week was calculated as  $[(\text{total food given} - \text{total food left}) / \text{number of animal days}] \times 7$ .

Food conversion ratios were calculated, where possible, over the period Weeks 1 to 24, from the bodyweight and food consumption data as weight of food consumed per unit gain in bodyweight.

Daily monitoring by visual appraisal of the water bottles was maintained throughout the study. Weekly water consumption was monitored and measured during selected intervals.

There were no significant test article-related effects on food consumption through the 93 week (high dosage males) or 104 week treatment period. Marginally lower food consumption was observed in the mid-dosage females.

The overall efficiency of food utilization during the 1<sup>st</sup> 24 weeks was inferior for all treated animals when compared to that of control. It was not in a dose-related trend in male groups and was severe in only high dosage males.

The overall water intake for high dosage males was higher than that of controls through the first half of the study up to Week 47 whereas the water intake for high dosage females was lower than that of concurrent controls between Weeks 27 to 55. The overall water intake at low and mid- dosages was generally unaffected.

## Hematology

Blood samples were taken from all animals, where possible, at sacrifice or at scheduled termination.

A marginal but statistically significant increase in total RBC was observed in the males and females treated at 405 mg/kg/day in Week 80. Such a change was not observed at termination. There was no significant test article-related effect on total WBC.

## Gross Pathology

The surviving high dosage males were sacrificed at the end of 93 weeks of treatment due to high mortality in this group. All other survivors were sacrificed on completion of at least 104 weeks of treatment. A complete gross pathology examination was conducted for all animals that were subject to unscheduled death or scheduled termination.

Daily Dose (mg base/kg/day)	0 (Control)		0 (Control)		20		90		405/180*	
	M:50	F:50	M:50	F:50	M:50	F:50	M:50	F:50	M:50	F:50
<b>Macroscopic pathology</b>										
<b>Lesions (all animals examined)</b>	50	50	50	50	50	50	50	50	50	50
Heart - Enlarged	2	0	4	0	2	0	7	1	22	18
Thrombus – left atrium	2	0	6	0	1	1	3	0	23	10
Pale areas – ventricle/s	0	0	3	0	0	0	2	0	1	5
Mediastinal Lymph Nodes – Enlarged	3	3	0	1	1	3	1	2	5	7
<b>Lesions (Decedent animals only) number examined</b>	28	30	28	21	29	29	30	27	40	38
Heart - Pale area/s – atrium/a	1	0	0	0	0	0	2	0	5	0
Lungs - Congested	7	5	4	1	6	9	5	7	17	18
Oedematous	0	0	1	1	2	0	0	0	4	6
Thoracic Cavity – Contained fluid	2	2	2	0	2	6	2	2	10	15
Adipose Tissue – Minimal	7	8	7	6	8	11	16	9	17	17

The macroscopic findings were summarized above (excerpted from summary table of the report). Most treatment related findings were observed in the high dosage male and female groups, which included an increased incidence of the heart enlargement, thrombus in the left atrium and pale areas on the atrium (males) and ventricles (females); an increased incidence of congestion and edema in the lungs; excess fluid present in the thoracic cavity; and enlargement of the mediastinal lymph nodes, and a reduction of the adipose tissue. The histopathology changes associated with these findings are discussed below.

A slightly increased incidence of the heart enlargement and a reduction of the adipose tissue were also noted in the mid-dose males.

## Histopathology

Samples of any lesions, and all the tissues listed below from all animals were preserved in buffered 10% formalin (except eyes, which were preserved in Davidson's fixative and testes/ epididymides which were fixed in Bouin's solution and then transferred to 70% alcohol). Tissues for microscopic examination were as specified in the list below. All

specified tissues from all mice in the control and high dosage groups, all specified tissues from unscheduled death or moribund sacrificed mice in the low and mid-dosage groups, and all tissues with lesions from mice in the low and mid-dosage groups at the termination were examined microscopically.

Peer Review: Yes

adrenal glands*	heart*	sciatic nerve
alimentary tract*	kidneys*	seminal vesicles*
(oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum)	larynx and pharynx	skeletal muscle
aorta*	liver*	skin
brain* (medullary, cerebellar and cerebral sections)	lungs* (all lobes and mainstem bronchi)	spinal cord* (cervical region)
clitoral gland	lymph nodes* (cervical and mesenteric)	spleen*
eyes*	mammary gland*	sternum* (for bone and marrow)
femur (with joint)*	other macroscopic abnormalities*	testes* (with epididymides)
gall bladder*	ovaries*	thymus* (where present)
Harderian gland*	pancreas*	thyroids* (with parathyroid glands)
head (to preserve nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lachrymal gland and Zymbal's gland)	pituitary gland*	tongue*
	preputial gland*	trachea*
	prostate*	urinary bladder*
	salivary glands* (submandibular)	uterus* (corpus and cervix)
		vagina*

\* Tissues required for histopathological examination

Neoplastic

There were no treatment-related neoplastic findings.

Based on Statistical Review and Evaluation by Dr. A. Rahman, there was no statistically significant dose response relationship in any of the observed tumor types in either gender. The pairwise comparison showed statistically significant increased incidence of lymphoid histiocytic sarcoma in the female low dosage group when compared to the combined control. The incidence of histiocytic sarcoma is shown below. Apparently, it was not dose-dependent and the incidence of histiocytic sarcoma observed in the male control group approached that in the low dosage female cohort. There was no histiocytic sarcoma observed in any other organs/tissues. The NTP carcinogenicity studies in rats and mice (TR-551, CAS No. 97-54-1) indicated that a historical control incidence range for histiocytic sarcoma (all tissues) in B6C3F1 female mice by all routes exposure is 0%-8% or 2.5%±2.5%. Taken together, the increased incidence of histiocytic sarcoma in the female low dose group was considered random, and not toxicologically significant.

Dose (mg/kg/day)	Male					Female				
	0	0	20	90	405/180	0	0	20	90	405/180
Incidence of histiocytic sarcoma in lymphoid	5	1	0	0	2	1	2	8	2	3

## Non Neoplastic

The noteworthy non-neoplastic histopathology changes were summarized below (excerpted from summary table of the report).

Daily Dose (mg base/kg/day)	0 (Control) <sup>1</sup>		0 (Control) <sup>2</sup>		20		90		405/180 <sup>a</sup>	
	M:50	F:50	M:50	F:50	M:50	F:50	M:50	F:50	M:50	F:50
<b>Histopathology – Non-Neoplastic Lesions</b>										
<b>Heart</b>										
Number hearts examined:	28	30	28	21	29	29	30	27	40	38
Atrial thrombus	1	0	3	0	2	1	7*	0	31***+	28***+
Dilated chambers	1	0	4	1	5	3	5	0	24***+	24***+
Myocardial degeneration	5	7	13*	2	11	12+	13*	11+	37***+	36***+
Myocardial vacuolation	18	13	18	6	18	11	21	10	37***+	36***+
Myocardial fibrosis	23	13	22	7	19	16	26	8	33	35***+
Myocardial cell hypertrophy	0	0	2	0	3	0	5*	0	24***+	19***+
Epicardial inflammation and fibroblast proliferation	2	1	4	0	2	2	1	1	11*	23***+
<b>Lungs</b>										
Number lungs examined:	28	30	28	21	29	29	30	27	40	38
Alveolar septal fibrosis	0	3	3	0	4	1	7**	0	17***+	17***+
Alveolar septal thickening and/or degeneration	0	2	3	1	2	3	5*	1	24***+	25***+
Alveolar eosinophilic material	1	1	2	0	3	2	4	1	20***+	16***+
Foamy alveolar macrophages	5	6	5	3	5	8	8	5	28***+	26***+
<b>Thymus</b>										
Number thymus examined	20	28	20	16	21	25	22	25	28	29
Involution/atrophy	10	11	11	4	12	9	16	7	24***+	19*+
<b>Spleen</b>										
Number spleens examined	28	30	28	21	29	29	30	27	40	38
Haemosiderosis	8	9+	3	1	6	6	4	2	15+	21*+
<b>Liver</b>										
Number livers examined	28	30	28	21	29	29	30	27	40	38
Centrilobular hepatocyte vacuolation	12	11	9	6	12	12	7	8	18	31***+
Sinusoidal dilatation/congestion	2	1	4	0	3	2	2	0	13*	12***+

\*  $p < 0.05$ , \*\*  $p < 0.01$ , Fisher's Exact test compared to control<sup>1</sup>  
+  $p < 0.05$ , ++  $p < 0.01$ , Fisher's Exact test compared to control<sup>2</sup>  
<sup>a</sup> Dosage level was lowered from 405 mg base/kg/day to 180 mg base/kg/day from Week 81

Treatment related non-neoplastic microscopic findings, noted principally among decedent high dosage male and female mice, consisted of atrial thrombus, dilated chambers, minimal to moderate myocardial degeneration, minimal to moderate myocardial vacuolation, minimal to moderate myocardial fibrosis, minimal to slight myocardial cell hypertrophy, and minimal to slight epicardial inflammation and fibroblast proliferation. A lesser degree of increased incidence of myocardial degeneration and vacuolation was also observed in the decedent males treated at dose of 90 mg/kg/day. The significant changes observed in the lungs, liver, spleen and thymus of the decedent mice were associated with the myocardial toxicity and could be a consequence of heart failure. No other treatment related microscopic findings were observed.

## Toxicokinetics

Blood samples were collected from animals in the satellite groups on one day during Weeks 13, 26, 52, 80 and at termination. Samples were withdrawn at approximately 7, 10, 16 and 21 hours with different animals used at each time point.

The TK analysis was reported in a separate report (NP07946). The plasma exposure data of ivabradine (S16257) and its major metabolite (S18982) are summarized in the

table below (excerpted from report NP07946, pages 25-26).

20 mg base form/kg/day															
	Week 13			Week 26			Week 52			Week 80			Week 104		
	M	F	F/M	M	F	F/M	M	F	F/M	M <sup>(1)</sup>	F <sup>(2)</sup>	F/M	M <sup>(3)</sup>	F <sup>(4)</sup>	F/M
<b>C<sub>min,ss</sub></b> (ng/ml)	35.2	39.2	-	46.0	49.5	-	45.8	68.4	-	31.0	71.5	-	12.3	13.4	-
<b>C<sub>max,ss</sub></b> (ng/ml)	120	99.4	-	113	91.7	-	118	103	-	71.5	83.4	-	47.3	78.4	-
<b>t<sub>max</sub></b> (h)	21:00	07:00	-	21:00	21:00	-	21:00	21:00	-	21:00	10:00	-	21:00	21:00	-
<b>AUC<sub>24,ss</sub></b> (ng.h/ml)	2112	1789	0.847	1940	1641	0.846	1782	2037	1.14	1182	1845	1.56	730	1189	1.63
<b>% S 18982 (AUC<sub>24,ss</sub>)</b>	18.3	13.7	-	21.5	13.5	-	20.9	11.1	-	21.8	9.81	-	23.5	11.7	-

- (1) depending on the sampling time only 5 or 6 animals were sampled instead of 6
- (2) depending on the sampling time only 4 or 5 animals were sampled instead of 6
- (3) depending on the sampling time only 3 to 5 animals were sampled instead of 6
- (4) at each sampling time only 3 animals were sampled instead of 6

90 mg base form/kg/day															
	Week 13			Week 26			Week 52			Week 80			Week 104		
	M	F	F/M	M	F	F/M	M	F	F/M	M <sup>(1)</sup>	F <sup>(2)</sup>	F/M	M <sup>(3)</sup>	F <sup>(3)</sup>	F/M
<b>C<sub>min,ss</sub></b> (ng/ml)	185	146	-	278	153	-	176	209	-	232	213	-	107	93.3	-
<b>C<sub>max,ss</sub></b> (ng/ml)	806	380	-	466	375	-	478	493	-	359	421	-	380	526	-
<b>t<sub>max</sub></b> (h)	10:00	21:00	-	10:00	10:00	-	21:00	21:00	-	21:00	21:00	-	21:00	21:00	-
<b>AUC<sub>24,ss</sub></b> (ng.h/ml)	8588	7277	0.759	9208	6689	0.724	7827	8109	1.04	6802	7348	1.08	6010	8670	1.44
<b>% S 18982 (AUC<sub>24,ss</sub>)</b>	24.2	18.1	-	25.3	16.6	-	22.8	14.9	-	26.7	16.5	-	23.4	13.6	-

- (1) at each sampling time only 5 animals were sampled instead of 6
- (2) depending on the sampling time only 5 or 6 animals were sampled instead of 6
- (3) depending on the sampling time only 3 or 4 animals were sampled instead of 6

405 mg base form/kg/day												180 mg/kg/day			
	Week 13			Week 26			Week 52			Week 80			Week 104		
	M	F	F/M	M	F	F/M	M	F	F/M	M <sup>(1)</sup>	F <sup>(1)</sup>	F/M	M <sup>(1)</sup>	F <sup>(2)</sup>	F/M
<b>C<sub>min,ss</sub></b> (ng/ml)	908	737	-	759	1022	-	747	1041	-	820	1094	-	172	254	-
<b>C<sub>max,ss</sub></b> (ng/ml)	1534	1544	-	2884	2444	-	1482	2062	-	1290	2232	-	425	1085	-
<b>t<sub>max</sub></b> (h)	21:00	21:00	-	10:00	10:00	-	21:00	21:00	-	21:00	10:00	-	21:00	10:00	-
<b>AUC<sub>24,ss</sub></b> (ng.h/ml)	29195	27410	0.939	38698	40762	1.05	25489	36980	1.45	25022	43160	1.72	7920	14331	1.81
<b>% S 18982 (AUC<sub>24,ss</sub>)</b>	37.1	24.5	-	35.5	20.8	-	32.9	14.6	-	36.6	16.3	-	-	-	-

- (1) depending on the sampling time only 2 or 3 animals were sampled instead of 6 ; last week of treatment = week 93 instead of 104
- (2) at each sampling time only 3 animals were sampled instead of 6
- M : Male F : Female
- % S 18982 (AUC<sub>24,ss</sub>) : ratio of AUC<sub>24,ss</sub> S 18982/S 18257 expressed as percentage
- : not determined

Mean plasma exposure to ivabradine increased dose-proportionally in females, and slightly less than dose-proportionally in males. In females, AUC<sub>24h</sub> for ivabradine did not change over time. In males, mean plasma exposure decreased progressively over time, by up to 18% and 65% in Weeks 52 and 104, respectively, at low and mid-dose levels,

and to a lesser extent at the high dose level. Up to Week 26, there was no gender difference. At the end of the dosing period, mean plasma exposure was lower in males than in females. Exposure to metabolite S 18982 was about 15% and 25% that to ivabradine in female and male mice, respectively.

### Stability and Homogeneity

Analysis of samples taken at 3 monthly intervals from the diets administered to the animals indicated that the achieved concentrations were within -10%/+7.3% of nominal.

### Study title: S 16257-2: Potential Tumorigenic Effects in Prolonged Dietary Administration to Rats (Study No. NP07943)

Conducting laboratory/location: (b) (4)

Date of study initiation: November 12, 1996

GLP compliance: Yes

QA statement: Included

Drug, lot #, and % purity: S 16257-2, batch# 49 652, Purity: 100.4%  
(the conversion factor from free base to salt: 1.078)

CAC concurrence: Study protocol was not discussed in ECAC.

### Key Study Findings

#### Adequacy of Carcinogenicity Study

S16257-2 was administered by diet to Wistar Rats at dosages of 7.5, 30, and 120/60 mg/kg/day for up to 104 weeks. The study protocol was not submitted for a discussion/concurrence of the ECAC (see regulatory history).

There was no treatment-related effect on survival.

A body weight gain reduction in the 1<sup>st</sup> 52 weeks was observed in high dosage males up to -26% and in all treated female groups in a dose dependent manner up to -36% when compared with the combined controls. Overall for the period of 104 weeks, the group mean bodyweight gain was reduced up to -29% for treated males and -42% for treated females (in a dose-dependent manner) when compared with the combined controls.

Treatment-related non-neoplastic findings were observed in the heart and the lungs. There were increased incidence of heart enlargement in both mid-dose males and females, thrombus in the left atrium and fenestration of the ventricles in high dose males, and the ventricle fenestration in the high dose females. The associated histopathological changes included increased incidence/severity of myocardial fibrosis and systrophic mineralization/chondroid metaplasia in the chordae tendineae at doses  $\geq$  30 mg/kg/day. There were increased incidences of pale focus/i or areas in the lungs of

all treated male and female rats, most evident in the mid- and high dosage groups. The associated histopathological changes in the lung included increased incidence of aggregation of alveolar macrophages with focal septal thickening in the high dose males.

Significant body weight gain reduction and cardiac toxicity findings in the high dosage males and females indicate that a MTD was achieved..

The high dosage was associated with  $AUC_{24h}$  of 7950 (23xhuman  $AUC_{24h}$ ) and 8436 ng.h/mL (24xhuman  $AUC_{24h}$ ) for male and female rats, respectively, at the week of 104.

### **Appropriateness of Test Models**

Dietary administration of test article to Wistar rats for up to 104 weeks is considered appropriate for tumorigenic potential evaluation.

### **Evaluation of Tumor Findings**

There were no treatment-related tumorigenic effects in ivabradine treated rats.

The statistically significant increased incidence of tubulostromal adenoma in ovaries of mid-dosage female rats was not dose-dependent pattern and within the published historical control incidence range in Wistar rats, hence, was not considered toxicologically significant.

The marginally increased incidence of uterine epithelial tumors, mainly uterine adenocarcinoma, in the treated female rats, associated with increased incidence of uterine masses, was not in a dose-dependent pattern and did not reach statistical significance whether incidence of individual tumor types or combined uterine epithelial tumors was analyzed.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 28, 2014 in Appendix 1).

## Methods

Doses: 7.5, 30, 120 (base) mg/kg/day<sup>a</sup> (week 1-52)/  
 7.5, 30, 60 (base) mg/kg/day<sup>a</sup> (week 53-104)

Frequency of dosing: Daily in diet  
 Dose volume: diet  
 Route of administration: diet  
 Formulation/Vehicle: Test article mixed with the diet<sup>b</sup>  
 /Normal untreated diet for rats

Basis of dose selection: The 13-week study (NP06450) with dietary administration at dosages of 20, 50, 100, 150 or 200 mg/kg/day showed a body weight gain decrease at doses  $\geq 100$  mg/kg/day and increased heart weight as well as provoking myocardial lesions at dosages  $\geq 20$  mg/kg/day. Therefore, a dosage of 120 mg/kg/day was chosen to elicit signs of toxicity and provide approximately 14-25 fold rat/human AUC ratio (estimated human AUC<sub>24h</sub> of 346 ng/h.mL) in males and >25-fold in females. The low dose of 7.5 mg/kg/day was chosen to achieve an AUC approximately equal to the AUC at MRHD. The high dosage was reduced to 60 mg/kg/day after 1 year of treatment due to the decrease in overall body weight gain in males (-26%) and females (-36%) when compared to the combined controls. In addition, a separate 52-week oral gavage rat study (NP07026) showed increased heart weight and myocardial lesions at dosage  $\geq 3$  (females) and  $\geq 16$  mg/kg bid (males). Mortalities were observed after Day 188 and all deaths in high dosage groups and 1 out of 2 deaths in the mid-dosage males were attributed to severe cardiac toxicity. The AUC<sub>24h</sub> at dose of 16 mg/kg bid in Week 52 was 4384 ng.h/mL (male) and 12426 ng.h/mL (female) which was lower than or similar to the AUC of high dosage male or female rats, respectively, in Week 52 of this 2-year carcinogenicity study.

Species/Strain: HanIbm Wistar Rat  
 Number/Sex/Group: 50  
 Age/Weight: ~8 weeks/male:134-179 g; female:101-140 g  
 Animal housing: 5 rats of the same sex/cage for main group  
 3 rats of the same sex/cage for satellite group  
 Paradigm for dietary restriction: All rats had free access to tap water and ground SDS Rat and Mouse No. 1 modified maintenance diet.

Dual control employed: Yes  
 Interim sacrifice: No  
 Satellite groups: 6/sex/group for TK profiling  
 Deviation from study: No deviations from the study protocol and

protocol: amendments were considered to affect the integrity of the study

- a. Food consumption and test article control data indicated that the actual doses received were 7.4, 30 and 119/59 mg/kg/day for males and 7.5, 30 and 119/60 mg/kg/day for females.
- b. Concentration of test article in the diet were changed as necessary to preserve the dosage levels (i.e. in line with body weight changes and food consumption)

## Observations and Results

### Mortality and Clinical Signs

Animals were observed at least once weekly for mortality and morbidity. A detailed palpation of each rat was performed once weekly for any palpable masses.

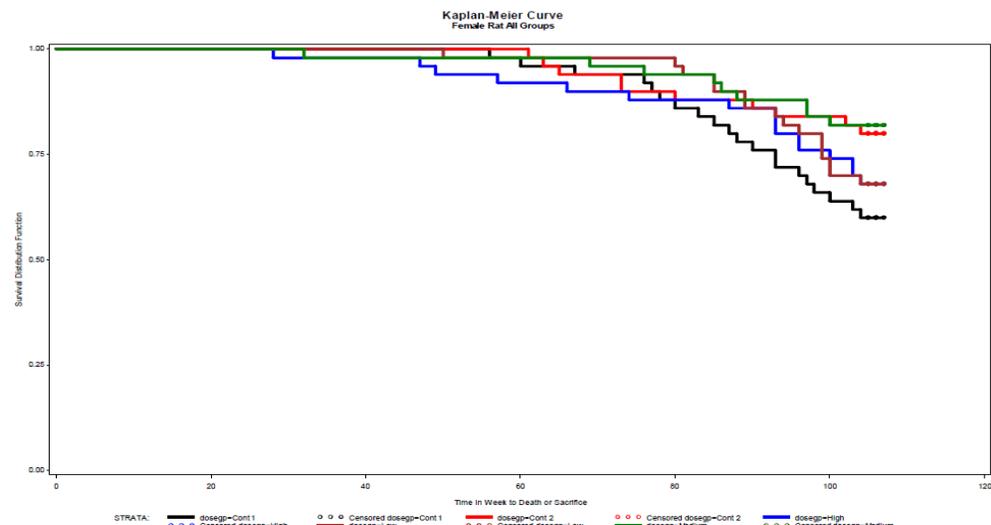
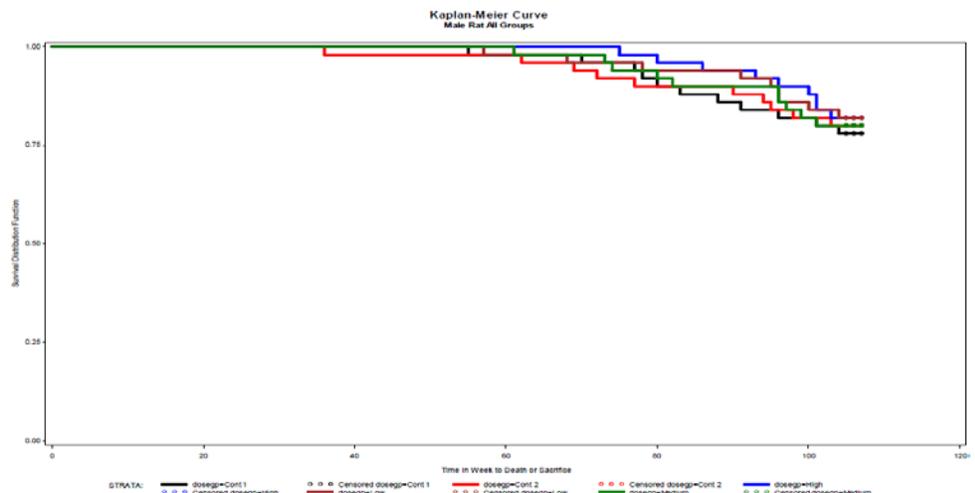
The distribution of unscheduled deaths during the study period was summarized below (excerpted from page 32 of the report). There were no treatment-related effects on the incidence or distribution of unscheduled deaths between control and treated groups over the 104 week treatment period.

Number decedents weeks	Group/dosage (mg base/kg/day)									
	1M Control	2M Control	3M 7.5	4M 30	5M 120/60†	1F Control	2F Control	3F 7.5	4F 30	5F 120/60†
1 - 52	0	1	0	0	0	0	0	1	1	3
53 - 104	11	9	9	10	9	20	10	15	8	13
1 - 104	11	10	9 (2)	10 (2)	9 (3)	20	10	16 (6)	9 (4)	16 (4)
Main group mortality (%)	22	20	18	20	18	40	20	32	18	32
Main group survival (%)	78	80	82	80	82	60	80	68	82	68

( ) Satellite group animals

† Dosage was lowered from 120 mg base/kg/day to 60 mg base/kg/day from Week 53

The survival analysis, as shown in the figure below (excerpted from Statistical Review by Dr. Rahman), did not reveal statistically significant dose response relationship in mortality across the treated and the combined control groups and the pairwise comparison did not show statistically significant increased mortality in treated groups when compared to the combined control.



There were no test article-related adverse clinical observations.

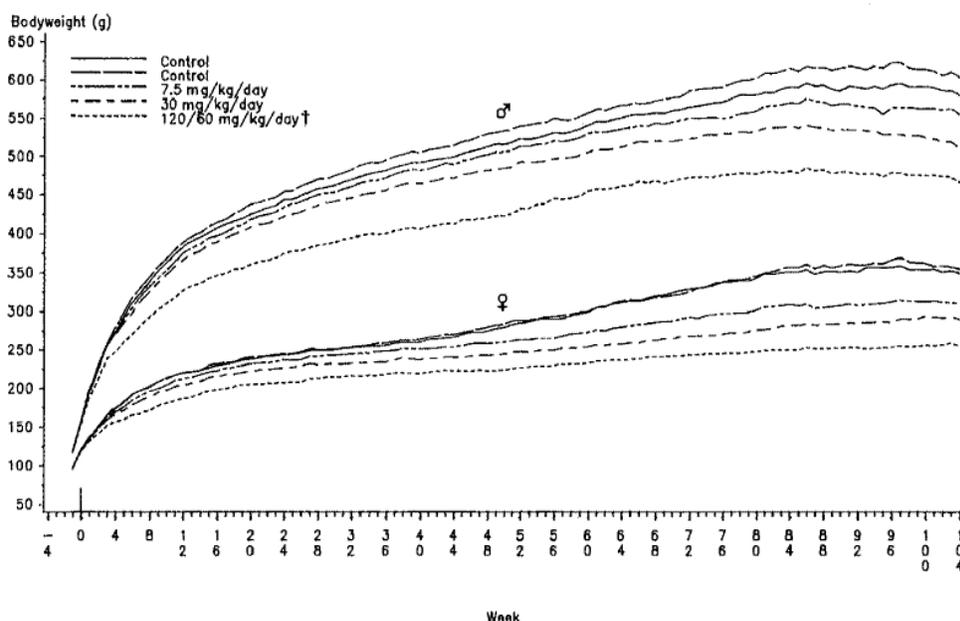
### Body Weights

Body weight was collected at randomization, one week prior to and on the day of initial treatment, and once a week thereafter.

*Body weight gain (g) (% of combined control)	Dose Mg/kg/day	Male					Female				
		0	0	7.5	30	120/60	0	0	7.5	30	120/60
Week 0-52		368	385	362 (96)	338 (90)	277 (74)	166	168	145 (87)	124 (74)	107 (64)
Week 53-104		56	63	42 (95)	17 (31)	34 (66)	64	67	45 (70)	49 (59)	28 (39)
<b>Week 0-104</b>		424	448	404 (92)	355 (81)	311 (71)	230	235	190 (82)	173 (74)	135 (58)

\*The body weight gain was calculated based on the mean body weight at Week 0, Week 52 and Week 104.

As shown in the table above and figures (excerpted from page 43) below, during the first 52 weeks of treatment, a reduction in group mean bodyweight gain was noted in all treated female groups in a dose-related pattern up to -36% and in mid- and high dose males up to -26% when compared to that of combined controls. From Week 53, the high dose level was decreased from 120 to 60 mg/kg/day. Overall (Weeks 52 to 104) body weight gains in the treated groups remained lower than that of the combined controls. Group mean bodyweight gain for the treated female groups, over this period, also remained lower than that of concurrent combined controls, with the values following a dose-related trend. Overall in the period of 104 weeks, the group mean bodyweight gain among all male and female treated groups was reduced up to -29% for males and -42% for females in a dose-dependent manner when compared with the combined controls.



## Feed Consumption

The quantity of food consumed by each cage of rats was recorded weekly. Food intake per rat (g/rat/week) was calculated as [(total food given-total food left)/number of animal days] x 7. Food conversion ratios were calculated, where possible, over the period Weeks 1 to 26, from the bodyweight and food consumption data as weight of food consumed per unit gain in bodyweight. Daily monitoring by visual appraisal of the water bottles was maintained throughout the study. Weekly water consumption was monitored in the selected intervals.

During the first 52 weeks of treatment a dosage related reduction in food consumption was noted among male and female treated groups when compared to that of combined controls. From Week 53, when the high dosage was reduced to 60 mg/kg/day, there was a slight improvement in food consumption for both sexes at this dosage (Weeks 53 to 60), although the mean food intakes remained significantly lower than that of the

concurrent combined controls. Food consumption for both sexes remained significantly lower among rats receiving mid and low dosages during this period.

Over the 104 week treatment, mean food consumption for male and female treated groups was lower than that of their respective combined controls in a dose-dependent pattern and with all the differences attaining statistical significance.

The overall efficiency of food utilization during the 1<sup>st</sup> 26 weeks was marginally inferior for males and females treated at 120 mg/kg/day, which reflected the lower body weight gain for these animals without a commensurately lower food consumption.

The mean water intake in the high dose males and females as well as that in the mid-dose females was generally lower than their respective controls. The water intake in the other treated groups was comparable to the respective controls over the study period.

## **Hematology**

Blood samples were taken from all animals, where possible, at sacrifice or at scheduled termination.

A slight but statistically significant decrease in total WBC was observed in the mid- and high dosage males in Week 104 (5.33, and 4.77 x10<sup>9</sup>/L, respectively), when compared to the control (5.70-6.30x10<sup>9</sup>/L). There were no significant changes of the measured parameters in other treated groups.

## **Gross Pathology**

All surviving main group animals were sacrificed on completion of at least 104 weeks of treatment. A complete gross pathology examination was conducted for all animals with unscheduled death or scheduled termination. The major organ weights of the rats subject to unscheduled termination were recorded at discretion of the pathologist. Organ weights were reported when necessary to clarify macroscopic findings.

The macroscopic findings are summarized below. There were treatment related findings observed in the heart, lung and uterus. The histopathology changes associated with these findings are discussed in the next section.

In the heart, there were increased incidences of the heart enlargement observed in the mid- and high dosage males and females, thrombus in the left atrium in the high dose males, and fenestration of the ventricles observed in both genders at mid- and high dosages as well as in the low dosage males.

In the lung, increased incidences of pale focus/i or areas were observed in all treated groups, especially in the mid- and high dose groups.

In the uterus, excess mass/es were observed in the high dosage rats.

In the spleen, the marginal increased incidence of the enlargement was observed in mid- and high dosages but without associated histopathologic changes.

Males on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	50		50		50		50		50	
Animals completed	Decedent	Terminal								
	12	38	10	40	9	41	10	40	9	41
<b>Lungs</b>										
Mass/es	0	0	0	1	0	0	0	1	0	0
Raised focus/i	0	0	0	1	0	0	0	0	0	1
Pale area/s or focus/i	4	6	2	6	5	7	6	11	4	21
Petechiae	0	19	1	23	0	17	1	5	0	5
Congested	4	1	3	2	4	2	3	8	5	3
Dark focus/i	1	0	0	2	0	0	0	0	1	0
<b>Heart</b>										
Enlarged	0	0	1	0	0	0	1	5	5	14
Thrombus - left atrium	0	0	0	0	0	0	0	0	1	1
Fenestrated - ventricle/s	0	0	0	1	0	2	0	3	0	12
Pale	1	0	0	1	0	0	0	2	1	3
Pale area/s - atrium	0	0	0	0	0	0	0	0	0	1
<b>Spleen</b>										
Enlarged	2	8	2	6	2	9	3	8	3	11
Pale area/s	1	0	0	0	0	0	0	0	0	0

Females on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	50		50		50		50		50	
Animals completed	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal
	20	30	10	40	16	34	9	41	16	34
<b>Lungs</b>										
Mass/es	0	0	0	0	0	0	0	0	1	0
Nodule/s	0	1	0	1	0	0	0	0	0	0
Raised focus/i	0	0	0	0	0	0	0	0	1	0
Pale area/s or focus/i	6	12	3	12	8	13	2	24	8	17
Petechiae	0	18	0	18	0	20	0	13	0	2
<b>Lungs</b>	(continued)									
Congested	5	3	3	4	6	4	3	12	6	5
Dark focus/i	1	0	0	0	3	0	0	0	0	0
<b>Heart</b>										
Enlarged	1	2	0	2	1	1	0	7	4	10
Fenestrated - ventricle/s	0	0	0	0	0	0	0	0	0	3
Pale	0	1	0	2	0	1	0	1	1	1
<b>Spleen</b>										
Enlarged	4	6	1	7	2	4	5	11	8	3
Pale	1	0	0	0	0	1	1	0	0	0
<b>Uterus</b>										
Mass/es	3	5	3	5	4	6	1	6	3	9
Pale raised area/s	1	0	0	0	1	0	0	0	0	0
Swelling/s	1	4	0	2	0	3	0	1	2	4
Fluid swelling/s	2	4	0	5	3	8	1	7	2	7
Fluid distension	1	1	0	1	1	1	0	2	1	2
Thickened	1	0	0	1	0	0	0	0	0	0

### Histopathology

Samples of any lesions, and all the tissues listed below (excerpted from page 29) from all animals were preserved in buffered 10% formalin (except eyes, which were preserved in Davidson's fixative and testes/ epididymides which were fixed in Bouin's solution and then transferred to 70% alcohol). All specified tissues (marked as\* below) from all rats in the control and high dose groups, all specified tissues from unscheduled death or moribund sacrificed rats in the low and mid-dose groups, and all tissues with lesions from rats in the low and mid-dose groups at the termination, were examined microscopically.

Peer Review: Yes

adrenal glands*	heart*	sciatic nerve
alimentary tract*	kidneys*	seminal vesicles*
(oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum)	larynx and pharynx	skeletal muscle
aorta*	liver*	skin
brain* (medullary, cerebellar and cerebral sections)	lungs* (all lobes and mainstem bronchi)	spinal cord* (cervical region)
clitoral gland	lymph nodes* (cervical and mesenteric)	spleen*
eyes*	mammary gland*	sternum* (for bone and marrow)
femur (with joint)*	other macroscopic abnormalities*	testes* (with epididymides)
gall bladder*	ovaries*	thymus* (where present)
Harderian gland*	pancreas*	thyroids* (with parathyroid glands)
head (to preserve nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lachrymal gland and Zymbal's gland)	pituitary gland*	tongue*
	preputial gland*	trachea*
	prostate*	urinary bladder*
	salivary glands* (submandibular)	uterus* (corpus and cervix)
		vagina*

\* Tissues required for histopathological examination

Neoplastic

There were no treatment-related neoplastic findings.

Based on the statistical review by Dr. A. Rahman, there was no statistically significant dose response relationship in any observed tumor types in either gender. However, the pairwise comparison showed statistically significant increased incidence of tubulostromal adenoma in ovaries of mid-dose female rats when compared to the combined control (see the table below). According to the literature report (<sup>ii</sup>Ref. *Carlus M et al 2013*) with eight 2- year carcinogenicity studies in Wistar rats, the ovary tubulostromal adenoma could be observed in control rats at incidence of 3% with a range of 0-6.7%. Given that the incidence of this finding was not dose-dependent and was within the range of 0-6.7%, it was not considered toxicologically significant.

<b>Female rats, Dose (mg/kg/day)</b>	<b>0</b>	<b>0</b>	<b>7.5</b>	<b>30</b>	<b>120/60</b>
<b>Ovaries:</b> tubulostromal adenoma	0	0	1	3	0
<b>Uterine:</b>					
Endometrial adenoma	0	0	1	0	1
Endometrial adenocarcinoma	3	5	6	4	8
Uterine carcinoma (anaplastic)	1	0	0	0	0
<i>Combined uterine epithelial tumor</i>	4	5	6	4	9

As listed in the table above, uterine epithelial tumors, mainly adenocarcinomas, were observed in control and treated females, and associated with the uterine masses reported in the gross pathology evaluation. The historical control data from the CRO is

listed below (excerpted from page 35 of the report). The incidence of uterine epithelial tumors in both control and treated groups fell at the upper limit or outside of the historical range. Based on FDA statistical analysis of uterine tumors, pairwise comparison showed that incidence of endometrial adenocarcinoma alone or combined epithelial tumors including endometrial adenoma, endometrial adenocarcinoma and uterine carcinoma (anaplastic) of the high dose females was not statistically significantly different from that of the combined control.

**Uterine epithelial tumours in HAN Wistar control group rats**  
at [redacted] (b)(4)

Study code	9220	9221	93A	93B	9402
Endometrial adenoma	0	0	0	0	1
Endometrial adenocarcinoma	4	1	2	2	1
Uterine carcinoma (anaplastic)	0	0	0	0	0
Number of uteri examined	50	50	55	55	55

**Uterine epithelial tumours in HAN Wistar control group rats**  
at [redacted] (b)(4)

Study code	017	019a	019b	20a	20b	20c	22a	22b	23a	23b
Endometrial adenoma	1	0	2	0	0	0	0	1	0	1
Endometrial adenocarcinoma	1	1	0	3	0	0	2	3	1	4
Uterine carcinoma (anaplastic)	0	0	0	0	0	0	0	0	0	0
Number of uteri examined	50	50	50	55	55	55	50	50	60	60

### Non-Neoplastic

The noteworthy non-neoplastic histopathology changes are summarized below.

An increased incidence of minimal to moderate myocardial fibrosis was observed at high dosage in both genders as well as at mid-dosage in females. Excess dystrophic mineralization/chondroid metaplasia in chordae tendineae was observed in all treated males and at mid- and high dosage in females. These findings were consistent with gross pathology lesions in the heart.

Group	Male					Female				
	1	2	3	4	5	1	2	3	4	5
Dose level (mg base/kg/day)	0	0	7.5	30	120/60A	10	0	7.5	30	120/60A
Myocardial fibrosis										
Total	35	39	31	37	45*	20	10	19	32*	35**
Minimal	21	28	22	28	22	18	10	18	24	16
Slight	10	10	8	8	19*	2	0	1	8*	15**
Moderate	4	1	1	0	4	0	0	0	0	4
Marked	0	0	0	1	0	0	0	0	0	0
Dystrophic mineralisation/chondroid metaplasia in chordae tendineae	1	0	2	4	16**	0	0	0	6*	15**
Number of hearts examined	50	50	50	50	50	50	50	50	50	50

\*  $p < 0.05$  \*\*  $p < 0.01$  with Fisher's Exact test when compared to Group 1  
A- dose level reduced to 60 mg base/kg/day at Week 53

An increased incidence of aggregations of alveolar macrophages with focal septal thickening was observed in the lungs of males at mid- and high dosages, consistent

with the gross pathology findings in the lungs. There were no significant findings in the lungs of the treated females.

Group	1	2	Male 3	4	5
Dose level (mg base/kg/day)	0	0	7.5	30	120/60A
Aggregations of alveolar macrophages with focal septal thickening	14	13	11	17	25*
Number of lungs examined	50	50	50	50	50

\*  $p < 0.05$  with Fisher's Exact test  
 A- dose level reduced to 60 mg base/kg/day at week 53

There were no other significant gross or microscopic non-neoplastic lesions..

### Toxicokinetics

Blood samples were collected from animals in the satellite groups on one day during Weeks 13, 26, 52, 78 and 105 (satellite group animals continued to receive the test article I diet until the day of termination). Samples were withdrawn at approximately 7, 16 and 22 hours.

The plasma exposure data of ivabradine (S16257) is summarized in the table below (excerpted from study report NP07945, page 28). Mean plasma exposure increased dose-proportionally in both genders, and was higher in females than in males throughout the study. No time-effect was observed in Week 13 to Week 105 interval for males. Exposure in low-dosage females was gradually decreased by approximately 42% in this interval. Most of S18982 concentrations were below the limit of quantitation (i.e. 2.5 ng/ml).

7.5 mg/kg/day (expressed as base form)																		
Week 13			Week 25			Week 39			Week 52			Week 78			Week 104			
M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	
$C_{min,ss}$ (ng/ml)	18.8	40.7	-	18.1	47.6	-	12.3	24.3	-	11.2	41.3	-	15.4	23.2	-	27.4	20.86	-
$C_{max,ss}$ (ng/ml)	33.7	88.0	-	29.7	85.5	-	23.4	73.8	-	20.4	58.5	-	29.9	48.0	-	47.5	48.1	-
$t_{max}^*$ (h)	7:00	22:00	-	7:00	22:00	-	16:00	14:30	-	7:00	11:30	-	7:00	16:00	-	7:00	7:00	-
$AUC_{24,ss}$ (ng.h/ml)	628	1470	2.34	581	1595	2.75	452	1282	2.84	380	1249	3.28	544	816	1.50	888	859	0.968

30 mg/kg/day (expressed as base form)																		
Week 13			Week 26			Week 39			Week 52			Week 78			Week 104			
M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	
$C_{min,ss}$ (ng/ml)	42.9	102	-	30.9	157	-	35.4	95.8	-	26.5	160	-	39.5	84.0	-	196	86.0	-
$C_{max,ss}$ (ng/ml)	98.5	345	-	116	251	-	114	371	-	102	226	-	212	259	-	342	246	-
$t_{max}^*$ (h)	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-
$AUC_{24,ss}$ (ng.h/ml)	1733	5537	3.20	1781	4776	2.71	1711	5321	3.11	1505	4545	3.02	2766	4026	1.46	6376	3984	0.625

	120 mg/kg/day (expressed as base form)												60 mg base form/kg/day					
	Week 13			Week 26			Week 39			Week 52			Week 78			Week 104		
	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M	M	F	F/M
$C_{min,ss}$ (ng/ml)	247	329	-	231	671	-	125	286	-	119	316	-	108	79.4	-	201	176	-
$C_{max,ss}$ (ng/ml)	470	854	-	515	1153	-	432	1279	-	424	744	-	338	426	-	480	594	-
$t_{max}^*$ (h)	7:00	7:00	-	7:00	18:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-	7:00	7:00	-
$AUC_{24,ss}$ (ng.h/ml)	8680	14870	1.71	8860	22181	2.50	6466	16616	2.57	6329	12823	2.03	5151	5987	1.16	7950	8435	1.06

## Stability and Homogeneity

Analysis of samples taken at 3 monthly intervals from the diets administered to the animals indicated that the achieved concentrations were within -18%/+3% of nominal.

## 10 Special Toxicology Studies

### 11 Integrated Summary and Safety Evaluation

The carcinogenicity potential of ivabradine (S16257-2) was assessed in 104-Week dietary studies in CD-1 mice and Wistar rats.

#### Mouse

S16257-2 was administered by diet to CD-1 mice at dosages of 20, 90, and 405/180 mg/kg/day for up to 104 weeks. The high dose was reduced to 180 mg/kg/day due to the high mortality observed in the high dose males and females during the first 80 weeks of treatment. The high dose male group was terminated early at the end of Week 93 when survival reached 20%.

Significantly reduced survival rate was observed in the high dose males and females.

A body weight gain reduction in the 1<sup>st</sup> 80 weeks was observed in high dose males (-36%) and in all treated female groups with a dose-dependent manner (up to -33%). Body weight gain reduction was up to -38% over the treatment period (Week 1-93) for high dose males. The overall body weight gain for all treated females during Week 1-104 was reduced about -13% to -23% without dose-dependence.

Higher mortality and body weight gain reduction in the high dose males and females indicated that at least an MTD was achieved. The route (diet) and the duration of 104 weeks (93 weeks for high dose male group) are considered acceptable.

Treatment-related non-neoplastic findings, primarily cardiac, included atrial thrombus, dilated chambers, minimal to moderate myocardial degeneration, vacuolation and fibrosis, and minimal to slight myocardial cell hypertrophy, epicardial inflammation and fibroblast proliferation observed in the high dose males and females. A lesser degree of

increased incidence of myocardial degeneration and vacuolation was also observed in the decedent mid-dosage males. The pathology changes in the lungs, liver, spleen and thymus of the decedent mice were associated with cardiac toxicity. The increased mortality was attributed to the cardiac toxicity.

There were no treatment-related tumorigenic effects in ivabradine treated mice.

The statistically significant increased incidence of lymphoid histiocytic sarcoma in the female low dosage group only was not dose-dependent, similar to the incidence in the male control group, and not observed in any other tissues/organs, hence, was not considered toxicologically significant.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 28, 2014).

The plasma exposures of ivabradine and the human exposure multiples in the 104-week mouse study are summarized in the tables below (excerpted from Section 2.6.6. page 59, Table 38).

**Table 38. Ivabradine and S 18982 Mean Plasma AUC<sub>24</sub> in CD-1 Mice After 104-week Oral Dosing**

Dose (mg/kg/d)	AUC <sub>24</sub> (ng.h/ml)							
	ivabradine				S 18982			
	Males		Females		Males		Females	
	Week 104	Multiple of hAUC <sub>24</sub>	Week 104	Multiple of hAUC <sub>24</sub>	Week 104	Multiple of hAUC <sub>24</sub>	Week 104	Multiple of hAUC <sub>24</sub>
20	730	2	1189	3	172	1	140	1
90	6010	17	8670	25	1406	11	1179	9
405 <sup>a</sup>	2502 2	72	43160	125	9164	72	7033	55
180 <sup>b</sup>	7920	23	14331	41	nd		nd	

n=6/gender/dose/sampling time <sup>a</sup> week 80 <sup>b</sup> week 93. AUC<sub>24</sub> = Area under the concentration curve over 24 hours; hAUC<sub>24</sub> = mean plasma exposure at steady state over 24 hours in patients at the highest therapeutic dose.

### Rat

Ivabradine (S16257-2) was administered by diet to Wistar Rats at doses of 7.5, 30, and 120/60 mg/kg/day for up to 104 weeks. The high dosage was reduced to 60 mg/kg/day from Week 53 due to significant body weight gain reduction in the first 52 weeks and mortality with severe cardiac toxicity observed in a separate 52-week rat oral gavage study in which the exposures at a dosage of 16 mg/kg bid were lower than or similar to the AUC of the high dose male or female rats, respectively, by Week 52 in this carcinogenicity study.

There were no treatment-related effects on survival.

In the first 52 weeks, significant body weight gain reduction was observed in the high dosage males (-26%) and in all treated female groups in a dose-dependent manner up to -36%. Overall (Weeks 0 to 104), a dose-dependent mean bodyweight gain reduction was up to -29% for treated males and -42% for treated females when compared to the respective combined controls.

Treatment-related non-neoplastic findings were observed in the heart and the lungs. There were increased incidences of heart enlargement in both mid-dose males and females, thrombus in the left atrium and fenestration of the ventricles in high dose males, and ventricle fenestration in high dose females. The associated histopathological changes of the heart included increased incidence/severity of myocardial fibrosis and systrophic mineralization/chondroid metaplasia in the chordae tendineae in the rats treated at dosages  $\geq 30$  mg/kg/day. There were increased incidences of pale focus/i or areas in the lungs of all treated cohorts, especially in the mid- and high dose groups. The associated histopathological changes included increased incidence of aggregation of alveolar macrophages with focal septal thickening in the high dose males.

Significant body weight gain reduction and cardiac toxicity findings in the high dose rats suggest that a MTD was achieved in this study. The route (diet) and the duration of 104 weeks are considered acceptable.

There were no treatment-related tumorigenic effects in ivabradine treated rats.

The statistically significant increased incidence of tubulostromal adenoma in ovaries of mid-dose female rats was not dose-dependent, and within the published control incidence range in Wistar rats, hence was not considered toxicologically significant.

The marginally increased incidence of uterine epithelial tumors, mainly uterine adenocarcinoma, in the treated female rats, associated with increased incidence of uterine masses, was not dose-dependent and did not reach statistical significance whether incidence of individual tumor types or combined uterine epithelial tumors was analyzed.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 28, 2014).

The plasma exposures of ivabradine and the human exposure multiples in the 104-week rat study are summarized in the tables below (excerpted from Section 2.6.6. page 61, Table 40)

**Table 40. Ivabradine Mean Plasma AUC<sub>24</sub> in Wistar Rats After 104-week Oral Dosing**

Dose (mg/kg/d)	AUC <sub>24</sub> (ng.h/ml)			
	Males		Females	
	Week 104	Multiple of hAUC <sub>24</sub>	Week 104	Multiple of hAUC <sub>24</sub>
7.5	888	3	859	2
30	6376	18	3984	12
120 <sup>a</sup>	6329	18	12823	37
60	7950	23	8436	24

n=5/gender/dose/sampling time <sup>a</sup> week 52. AUC<sub>24</sub> = Area under the concentration curve over 24 hours;  
hAUC<sub>24</sub> = mean plasma exposure at steady state over 24 hours in patients at the highest therapeutic dose.

### Conclusion

Ivabradine was not tumorigenic in the 104-Week carcinogenicity studies with dietary administration in CD-1 mice and Wistar rats. The statistically significant increase in the incidences of lymphoid histiocytic sarcoma in the low dose female mice and tubulostromal adenoma in ovaries of mid-dose female rats are not considered toxicologically significant.

## 12 Appendix/Attachments

### Appendix 1: EXEC CAC MINUTES

<sup>i</sup> TR-551 CAS No. 97-54-1: Toxicology and Carcinogenesis Studies of Isoeugenol in F344/N Rats and B6C3F1 Mice (Gavage Studies)

<sup>ii</sup> *Marin Carlus et al: Historical Control Data of Neoplastic Lesions in the Wistar Hannover Rat among Eight 2-year Carcinogenicity Studies. Experimental and Toxicologic Pathology 65, 2013: 243-253*

Appendix 1 is 3 pages of the Duplicate Executive CAC Meeting Minutes dated 8/28/14 that can be found in the Administrative Correspondence Section of this Approved NDA. Please refer to this section for these Meeting Minutes.

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/s/  
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ADELE S SEIFRIED  
08/28/2014

PAUL C BROWN  
08/28/2014

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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JEAN Q WU  
11/18/2014

ALBERT F DEFELICE  
11/18/2014

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 206,143    Applicant: Amgen, Inc.

Stamp Date: 6/27/2014

Drug Name: Ivabradine                      NDA/BLA Type: NDA

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

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

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?	x		In the email response dated 7/30/2014, the sponsor clarified that human dose multiples are expressed in AUC24hr at highest human dose of 7.5 mg bid in the proposed labeling sections relative to pharmacology/toxicology.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		In the email response dated 7/30/14, the sponsor clarified that the proposed specifications for impurities in Section 2.6.7 Toxicology Tabulated Summary, Page 22-23 were <b>not</b> the final impurity specifications for commercial drug substance. As defined in 3.2.S.4.1, the proposed commercial drug substance specifications were set below the ICH limit and no control impurities above the ICH qualification limit are proposed.
11	Has the applicant addressed any abuse potential issues in the submission?	n/a		Pending review, there is no significant concern for a potential abuse of ivabradine from pharm/tox perspective.
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?   x  yes\_\_\_\_\_**

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

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

None.

Jean Q. Wu

\_\_\_\_\_  
Reviewing Pharmacologist

\_\_\_\_\_  
Date

Albert DeFelice

\_\_\_\_\_  
Team Leader/Supervisor

\_\_\_\_\_  
Date

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement  
010908

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/s/  
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JEAN Q WU  
08/04/2014

ALBERT F DEFELICE  
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