CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	206-426
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Applicant's letter date:	12/22/2013
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Product:	Peramivir (RAPIVAB)
Indication:	Treatment of Acute Uncomplicated Influenza Infection in adults
Applicant:	BioCryst Pharmaceutical Inc, 4505 Emperor Blvd, Suite 200, Durham, NC 27703
Review Division:	Division of Antiviral Products
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1 EXECUTIVE SUMMARY

1.1 INTRODUCTION

Peramivir is an inhibitor of influenza viral neuraminidase of influenza A and B. This NDA proposes its market use for the treatment of acute uncomplicated influenza in patients 18 years and older. The drug product is formulated as a sterile solution for IV injection with a single recommended dose of 600 mg. The nonclinical pharmacology/toxicology studies of this NDA are discussed in this review.

1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS

The nonclinical safety profile of IV peramivir is primarily derived from in vivo and in vitro studies conducted using peramivir (or the code names of BCX-1812 and RWJ-270201 (b) (4) including:

- In vivo and in vitro animal pharmacokinetic studies
- Safety pharmacology studies
- Single-dose iv toxicity studies in rats, rabbits and mice
- Repeat-dose iv toxicity studies in rats and monkeys (duration up to 1-month)
- Reproductive iv toxicity studies in rats, and rabbits
- In vitro and in vivo mutagenicity assays
- Special juvenile studies (rats), nephrotoxicity (rabbits), & antigenicity studies (guinea pigs).

Bioavailability of peramivir was studied (b) (4) which led to the development of current IV formulation. The steady-state volumes of distribution of peramivir in mice, rats, rabbits, dogs, and monkeys were either close to or less than total body water (200 - 500 ml/kg). Plasma proteins binding of peramivir in animal species was less than 30%; peramivir primarily distributed to kidney, bladder and bile and did not partition into red blood cells. Peramivir was not significantly metabolized by the rat, dog or human hepatic system. It is neither a substrate nor an inhibitor of cytochromes P450 and p-glycoprotein. In vivo, the oral peramivir was not metabolized in the dog or monkey. In rats, < 5% was hydrolyzed to a cyclopentyl ring oxidized metabolite, and more than 90% was excreted in kidney unchanged after iv injection. In rabbits, peramivir can be glucuronidated into an acyl glucuronide metabolite: <10% following iv injection, or ≈10-30% after oral dosing. In a ^{14}C-peramivir study, radioactivity was detected in the milk of lactating rats, at levels below that in mother's plasma (AUC≈50%). Peramivir may be secreted in the rabbit kidney, but not in the rat or human kidney. The elimination half-life of IV peramivir was <2hrs in mice, ≈15 hrs in rats, or 15-20 hrs in monkeys.

No significant effects of peramivir were observed in a series of single-dose, in vivo and in vitro cardiovascular, respiratory, GI or CNS safety pharmacology studies. Peramivir did not show significant inhibitory activities on hERG potassium channels.

Key target organs of toxicity were explored through chronic toxicity studies. They are summarized as follows:

Kidney: Tubular dilatation and necrosis with protein casts in cortical areas, dilated tubules with mineralizations in corticomedullary junction areas, and multifocal tubular regeneration

were observed. Rabbits appeared to be the sensitive species for the nephrotoxic effects of peramivir, which occurred at exposure approximately 2-fold that of humans at the clinically recommended dose (AUC=102.7 ug.h/ml at 600mg). The sponsor speculated that the unique acyl glucuronide metabolite might be responsible for the toxicity (unproven). Renal toxicity did occur at high drug exposures in monkeys or with longer treatment duration in rats.

Liver: Abnormal liver function was observed concurrently with nephrotoxicity in rabbits at high iv doses (≥200 mg/kg, AUC=519-593 ug.h/ml). Significant liver toxicity was not reported in other animal species.

Peramivir tested negative in a battery of genotoxicity tests including the bacterial reverse-mutation, mammalian chromosomal aberration and clastogenicity assays.

An oral rat carcinogenicity study was completed during early drug development of oral peramivir. The study revealed no drug-related neoplasms (NOAEL=3000 mg/kg po, AUC=46.7 for males and 24.3ug.h/ml for females). Non-neoplastic lesions observed in this rat study included mineralization of the renal pelvis in both sexes, and tubular dilatation and vacuolation in females (NOAEL=150 mg/kg, AUC=2.5 for males, 4.5ug.h/ml for females).

In the reproductive toxicology studies, there were no findings in rats in an iv bolus study at the maximum feasible dose (NOAEL=600 mg/kg, exposure margin = 8), whereas continuous iv infusion did elicit dose-related increases in incidences of reduced renal papillae and dilated ureters without any maternal toxicity (NOAEL=50 mg/kg, exposure margin = 0.8). Both anomalies are neither a definitive malformation type nor a variation type, but listed under grey-zone category according to the literature. The findings suggested potential delays in the development of the urinary tract. However, because the effect was induced by continuous infusion and not by an iv bolus, its relevancy to humans is not known. No other remarkable findings in regard to malformations, skeletal anomalies or skeletal variants were observed. No maternal toxicity was observed in rat studies. For rabbits, no teratogenicity was detected, although there was maternal nephrotoxicity, which was dose-limiting (NOAEL=200 mg/kg, exposure margin=8).

In a 4-week iv juvenile rat study, peramivir did not affect physical development or behavior. No other toxicity effects were noted, except for body weight reductions at 240 mg/kg (NOAEL=120 mg/kg, AUC=158-177 ug.h/ml). Oral studies that were performed in juvenile rabbits and rats (2-week) showed dose-related increases in RBCs/neutrophils, and decreases in urinary pH/specific gravity (NOAEL=1500 mg/kg/day; AUC=22 ug.h/ml) in rats; and dose-related decreases in neutrophil (300 and 1200 mg/kg/day females) and renal cortical tubular changes (increased eosinophilic cytoplasmic material in the epithelial cells of primarily proximal cortical tubules, 1200 mg/kg/day, NOAEL= 300 mg/kg, AUC=50 ug.h/ml) in rabbits.

In summary, from nonclinical pharmacology/toxicology perspective, the safety profile of IV peramivir provides a manageable and acceptable risk for the single-dose indication for influenza infection.

1.3 RECOMMENDATIONS

1.3.1 Approvability

It is recommended that peramivir be approved for the proposed indication.

1.3.2 Additional Non-Clinical Recommendations

No additional nonclinical studies are recommended.

1.3.3 Labeling

The following texts are recommended by the reviewer:

8.1 Pregnancy
Pregnancy Category C. There are no adequate and well-controlled studies of RAPIVAB in pregnant women. Because animal reproduction studies are not always predictive of human response, and peramivir was shown to cross the placenta in animal studies, this drug should be used during pregnancy only if clearly needed.

Animal Data
Reproductive toxicity studies have been performed in rats and rabbits. In rats, no treatment-related maternal and fetal toxicities were observed when peramivir was given by iv bolus at the maximum feasible dose of 600 mg/kg, for which the exposure were 8-fold that in humans at the recommended dose. However, when peramivir was infused by iv continuously, fetal anomalies of reduced renal papilla and dilated ureters were observed. The exposure at the NOAEL was 0.8-fold the exposures in humans at the recommended dose. In rabbits, maternal toxicity (decreased food consumption and body weight, nephrotoxicity) and developmental toxicity (abortion or delivered early) were observed. The exposure at the NOAEL was 8-fold the exposure in humans at the recommended dose.

8.3 Nursing Mothers

RAPIVAB has not been studied in nursing mothers. Studies in rats demonstrated that RAPIVAB is excreted in milk at levels below the mother's plasma drug concentration. It is not known whether RAPIVAB is excreted in human milk. RAPIVAB was excreted into the milk of lactating rats with levels (AUC) approximately 0.5-fold those in the maternal blood. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for RAPIVAB and any potential adverse effects on the breastfed child from the drug or from the underlying maternal condition.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Carcinogenesis

Carcinogenicity studies by IV injection of peramivir were not performed. However, in an oral carcinogenicity study in Sprague-Dawley rats no drug-related neoplasms were observed at drug exposures 0.2 ~ 0.5-fold that of humans at the clinically recommended dose of 600 mg/day.

Mutagenesis
Peramivir was not mutagenic or clastogenic in a battery of in vitro and in vivo assays including the Ames bacterial reverse mutation assay, the Chinese hamster ovary chromosomal aberration test, and the in vivo mouse micronucleus test with IV administration.

Impairment of Fertility
Peramivir has no effects on mating or fertility in rats up to 600 mg/kg/day, at which exposures were approximately 8-fold that of humans at the clinically recommended dose.

13.2 Animal Toxicology and/or Pharmacology

Peramivir caused renal tubular necrosis and abnormal renal function parameters in rabbits. The toxicity profile included tubular dilatation and necrosis with protein casts in cortical areas, dilated tubules with mineralizations in corticomedullary junction areas, and multifocal tubular regeneration. Rabbit appeared to be a sensitive species for the nephrotoxic effects of peramivir, which were inducible at exposures approximately 2 ~ 4-fold that of humans at the clinically recommended dose.

2 DRUG INFORMATION

2.1 DRUG

I. CAS Registry Number
(b) (4)

II. Generic Name: Peramivir

III. Code Name: RWJ-270201 (b) (4); BCX1812

IV. Chemical Name:
(1S, 2S, 3R,4R)-3-[(1S)-1-(acetylamino)-2-ethylbutyl]-4-[(aminoiminomethyl) amino]-2-hydroxy-cyclopentane carboxylic acid, trihydrate

V. Molecular Formula/Molecular Weight:
(b) (4) $C_{15}H_{34}N_4O_7$ (trihydrate MW=382.4)

VI. Structure or Biochemical Description
VII. Pharmacologic Class: Antiviral- Inhibitor of influenza viral neuraminidase

2.2 RELEVANT INDS

INDs [redacted] 69,038, [redacted]

2.3 DRUG FORMULATION

Peramivir injection is a clear, colorless, sterile, isotonic solution for iv injection (20ml/vial, 10 mg/ml in 0.9% NaCl).

2.4 COMMENTS ON NOVEL EXCIPIENTS

None.

2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN

None.

2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN

Patient Population: Acute uncomplicated influenza in patients 18 years and older
Dose: Single 600 mg dose, administered via intravenous infusion over 15-30 minutes.
Regimen: Treatment with RAPIVAB should begin within 2 days of onset of symptoms of influenza

2.7 REGULATORY BACKGROUND

IND 69038 was received in the Division of Antiviral Products on 11/23/2005. It was based on an earlier oral formulation under IND [redacted] (original IND dated 8/15/1999), which was inactivated in 2002. The sponsor, BioCryst, has developed an IV formulation of this drug which led to the NDA stage of this product.

3 STUDIES SUBMITTED

3.1 STUDIES REVIEWED

Secondary Pharmacology
Secondary Pharmacological Evaluation of the HIV Integrase Inhibitor GSK1349572B in Radioligand Binding and Enzyme Assays and Isolated Tissue Assays
Safety Pharmacology
Effects of ERC-349572 Sodium on Central Nervous System in Rats
Binding activity of peramivir trihydrate on various receptors
Binding activity of peramivir trihydrate on various receptors
Cardiovascular (hemodynamic) evaluation of RWJ-270201 in dogs
Effect of [redacted] (neuraminidase inhibitors) and cisapride on the membrane K+ current I(Kr) in HERG-transfected HEK293 cells
Effects of BCX-1812 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
Effects of Peramivir trihydrate on Action Potential in Guinea Pig Papillary Muscles
Effects of Peramivir trihydrate on Cardiovascular System in Conscious Monkeys
Effects of Peramivir trihydrate on Cardiovascular System in Conscious Monkeys
Effects of Peramivir trihydrate on Central Nervous System in Rats
Effects of Peramivir trihydrate on Central Nervous System in Rats
Effects of Peramivir trihydrate on Respiratory System in Rats
Effects of Peramivir trihydrate on Respiratory System in Rats

Electrophysiological effects of the neuraminidase inhibitors [redacted] in cardiac tissue in vitro: concentration-dependent effects on isolated papillary muscles of the guinea-pig in conditions of normokalaemia
General behavior and other CNS-related effects of RWJ-270201 in CRL:CD-1 (ICR)BR, VAF/PLUS mice and Sprague-Dawley rats
Hemodynamic and electrocardiographic effects of RWJ-270201 in anesthetized rats
Influence of RWJ-270201 [redacted] on Pulmonary Function in the Anaesthetized Ventilated Guinea-Pig (Conducted by [redacted]
RWJ-270201 [redacted]: General Pharmacology Study (Effects on the central nervous system, on the digestive system, and on water and electrolyte metabolism)

Pharmacokinetics

Evaluation of bioavailability and pharmacokinetics of RWJ-270201 in female BALB/C mice following administration of single oral and intravenous bolus doses
Evaluation of bioavailability and pharmacokinetics of RWJ-270201 in male beagle dogs following administration of single oral and intravenous bolus doses (DM98361)
Evaluation of bioavailability and pharmacokinetics of RWJ-270201 in male Sprague-Dawley rats following administration of single oral and intravenous bolus doses
Evaluation of the bioavailability and pharmacokinetics of RWJ-270201 in male ferrets following administration of single oral and intravenous bolus doses
Intramuscular pharmacokinetic study of doses of Peramivir using the same volume per injection of Peramivir IM 75 mg/ml and Peramivir 150 mg/ml in male rats
Pharmacokinetics and disposition following single administration of [14C]-Peramivir trihydrate in monkeys
Pharmacokinetics and disposition following single administration of [14C]-Peramivir trihydrate in monkeys
Plasma Concentration Following Repeated Intravenous Administration of [14C]-Peramivir trihydrate in Rats
Plasma Concentration Following Repeated Intravenous Administration of [14C]-Peramivir trihydrate in Rats
Plasma Concentration Following Single Administration of [14C]-Peramivir trihydrate in Rats
Plasma Concentration Following Single Administration of [14C]-Peramivir trihydrate in Rats
Single Dose Intramuscular Pharmacokinetic Study with Peramivir 75 mg/ml and 150 mg/ml in Male Rats
Single Dose Intramuscular Pilot PK Study of Peramivir in Mice
Single Dose Intramuscular Pilot PK Study of Peramivir in Rats
Tissue distribution after single intravenous administration of [14C]-Peramivir trihydrate at high dose in juvenile and mature rats
Determination of protein binding and red blood cell partitioning of 14C-RWJ-270201 in mouse, rat, rabbit, dog, monkey and human
Quantitative whole-body autoradiography following single intravenous administration of [14C]-Peramivir trihydrate in pregnant rats
Quantitative whole-body autoradiography following single intravenous administration of [14C]-Peramivir trihydrate in pregnant rats
Quantitative whole-body autoradiography of [14C]RWJ-270201 in male and female Sprague Dawley rats and male long Evans rats following a single oral administration
Quantitative whole-body autoradiography of rats following single or multiple intravenous or single intramuscular administration of [14C]-Peramivir
The binding of RWJ-270201 [redacted] to the proteins of male and female human plasma (DM99305)
Tissue distribution after single intravenous administration of [14C]-Peramivir trihydrate at high dose in juvenile and mature rats
Tissue distribution following single intravenous administration of [14C]-Peramivir trihydrate in juvenile and mature rats
Tissue distribution following single intravenous administration of [14C]-Peramivir trihydrate in juvenile and mature rats
In vitro metabolism of the anti-infective compound, RWJ-270201 (DM98357)
In vivo metabolism of the anti-infective compound RWJ-270201 in the rat (DM98376)
In vivo metabolism of the anti-infective compound RWJ-270201 in the rat following a bolus intravenous dose (DM99326)
Study on the in vivo major metabolites of [14C]-Peramivir trihydrate in mice
Study on the in vivo major metabolites of [14C]-Peramivir trihydrate in mice
Study on the in vivo major metabolites of [14C]-Peramivir trihydrate in monkeys
Study on the in vivo major metabolites of [14C]-Peramivir trihydrate in monkeys
Study on the in vivo major metabolites of [14C]-Peramivir trihydrate in rats

Study on the in vivo major metabolites of [14C]-Peramivir trihydrate in rats
Study on the in vivo major metabolites of Peramivir in humans
An excretion study with 14C-RWJ-270201 administered by single nasogastric gavage to Cynomolgus monkeys
Excretion into milk following single intravenous administration of [14C]-Peramivir trihydrate in nursing rats
Excretion into milk following single intravenous administration of [14C]-Peramivir trihydrate in nursing rats
Excretion of 14C RWJ-270201 in male and female New Zealand white rabbits following a single oral administration
Mass balance of 14C-RWJ-270201 in male and female CD-1mice following a single oral administration
Pharmacokinetics and disposition following single administration of [14C]-Peramivir trihydrate in monkeys
Pharmacokinetics and disposition following single administration of [14C]-Peramivir trihydrate in monkeys
Urinary and fecal excretion following repeated intravenous administration of [14C]-Peramivir trihydrate in rats
Urinary and fecal excretion following repeated intravenous administration of [14C]-Peramivir trihydrate in rats
Urinary, fecal and biliary excretion following single intravenous administration of [14C]-Peramivir trihydrate in rats
Urinary, fecal and biliary excretion following single intravenous administration of [14C]-Peramivir trihydrate in rats
An investigation of the potential for RWJ-270201 to induce CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in cultured primary human hepatocytes
Effect of probenecid on plasma concentration after single intravenous administration of Peramivir trihydrate in rabbits
Effect of probenecid on plasma concentration after single intravenous administration of Peramivir trihydrate in rats
RWJ-270201 in vitro interaction with acetaminophen
Study on P-glycoprotein Mediated Drug Interaction of Peramivir trihydrate (S-021812)
Transport characteristics of RWJ-270201 in various cell lines
Dose-linearity of Plasma Concentration Following Single Administration of Peramivir trihydrate in Influenza A Virus-infected Mice
Drug metabolism support of a five-day oral toxicity study of RWJ-270201 (b) (4) in Sprague-Dawley rats
Drug metabolism support of a four-week oral toxicity study (DS99308) of RWJ-270201 (b) (4) in rats with a four-week recovery period
Drug metabolism support of a four-week oral toxicity study of RWJ-270201 in Cynomolgus monkeys with a four-week recovery period
Drug metabolism support of a four-week oral toxicity study of RWJ-270201 (b) (4) in rabbits with a four-week recovery period
Drug metabolism support of a seven-day intravenous toxicity study (DS99318) of RWJ-270201 (b) (4) in rats
Drug metabolism support of a seven-day intravenous toxicity study of RWJ-270201 (b) (4) in rabbits
Drug metabolism support of a three-day oral toxicity study of RWJ-270201 (b) (4) in Beagle dogs
Drug metabolism support of a two week oral toxicity study of RWJ-270201 (b) (4) in Cynomolgus monkeys
Drug metabolism support of a two-week oral toxicity study of RWJ-270201 (b) (4) in rats
Evaluation of bioavailability and pharmacokinetics of RWJ-270201 in male and female Cynomolgus monkeys following administration of single oral and intravenous bolus doses (10 mg/kg) (DM98404)
Evaluation of the bioavailability and pharmacokinetics of RWJ-270201 following multiple (14 days) oral or intravenous bolus (10 mg/kg) in male and female New Zealand white rabbits

Single-dose and Repeat-dose Studies

IV Studies

Seven-day intravenous toxicity study of RWJ-270201 (b) (4) in rabbits
Single intravenous infusion toxicity study of Peramivir trihydrate in monkeys
Single intravenous toxicity study of Peramivir trihydrate in monkeys
Single intravenous toxicity study of Peramivir trihydrate in rats
One month continuous intravenous infusion toxicity study of Peramivir trihydrate in monkeys
One month continuous intravenous infusion toxicity study of Peramivir trihydrate in rats
Seven-day intravenous toxicity study of RWJ-270201 (b) (4) in rabbits
Seven-day intravenous toxicity study of RWJ-270201 (b) (4) in rats
A 14-day continuous intravenous infusion toxicity study of RWJ-270201 (b) (4) in rats
Twenty-eight day intravenous GLP toxicity study of BCX-1812 in Cynomolgus monkeys
Twenty-eight day intravenous GLP toxicity study of BCX-1812 in rats
Two week oral toxicity study of RWJ-270201 (b) (4) in Cynomolgus monkeys
Two-week GLP toxicity study of peramivir in Cynomolgous monkeys with a two-week recovery period
Two-week intravenous toxicity study of peramivir in Cynomolgus monkeys with a 2-week recovery
Twenty-eight day intravenous GLP toxicity study of BCX-1812 in Cynomolgus monkeys
Twenty-eight day intravenous GLP toxicity study of BCX-1812 in rats

Four day range-finding intravenous nephrotoxicity study of RWJ-270201 (b) (4) in rabbits
Fourteen-day intravenous nephrotoxicity study of RWJ-270201 (b) (4) in rabbits with a two-week recovery period
One-week intravenous nephrotoxicity study of Peramivir trihydrate in rabbits
Seven-Day Intravenous Toxicity Study of RWJ-270201 (b) (4) in Rabbits
Single intravenous nephrotoxicity study of Peramivir trihydrate in rabbits

Non-IV Studies

A 52-week (once-weekly dosing) intramuscular toxicity study of BCX-1812 (peramivir) with a 4-week recovery period in Cynomolgus monkeys
A 14-day intramuscular toxicity study of Peramivir with a 14-day recovery period in rats
A 26-week (once every two weeks dosing) intramuscular toxicity study of BCX-1812 (peramivir) with a 4-week recovery period in Sprague Dawley rats
A thirteen-week oral toxicity study of RWJ-270201 (b) (4) in rabbits with a four-week recovery period
Five-day oral toxicity study of RWJ-270201 (b) (4) in Sprague-Dawley rats
Four-week oral toxicity study of RWJ-270201 in Cynomolgus monkeys with a four-week recovery period
Four-week oral toxicity study of RWJ-270201 (b) (4) in rabbits with a four-week recovery period
Four-week oral toxicity study of RWJ-270201 (b) (4) in rats with a four-week recovery period
Thirteen-week oral toxicity study of RWJ-270201 (b) (4) in Cynomolgus monkeys with a four-week recovery period
Thirteen-week oral toxicity study of RWJ-270201 (b) (4) in mice
Thirteen-week range-finding oral toxicity study of RWJ-270201 (b) (4) in rats
Three-day oral toxicity study of RWJ-270201 (b) (4) in Beagle dogs
Toxicokinetics of a thirteen-week oral toxicity study of RWJ-270201 (b) (4) in mice
Toxicokinetics of a thirteen-week range-finding oral toxicity study of RWJ-270201 (b) (4) in rats
Two week oral toxicity study of RWJ-270201 (b) (4) in Cynomolgus monkeys
Two-week oral toxicity study of RWJ-270201 (b) (4) in rats
14-day single dose intramuscular toxicity study with peramivir 75 mg/ml and 150 mg/ml in male rats
14-day single dose intramuscular toxicity study with peramivir 75 mg/ml and 150 mg/ml in rats

Genotoxicity

In vitro mutagenicity testing of RWJ-270201 (b) (4) in the bacterial/microsomal activation assay
In vitro chromosome aberration assay of RWJ-270201 (b) (4) using CHO-k1 cells
Bone marrow micronucleus assay in mice dosed orally with RWJ-270201 (b) (4)
Bone marrow micronucleus assay range finding study in mice dosed intravenously with RWJ-270201 (b) (4)
Mammalian erythrocyte micronucleus test
Micronucleus test of Peramivir trihydrate with mouse bone marrow cells
In vitro mutagenicity testing of RWJ-270201 (b) (4) in the bacterial/microsomal activation assay

Carcinogenicity

A 2-Year Oral Gavage Carcinogenicity Study of RWJ-270201 (b) (4) in the Albino Mouse
Two Year Oncogenicity Study of RWJ-270201 (b) (4) in Rats

Reproductive and Developmental Toxicity

Tissue distribution and excretion of [14C] RWJ-270201 in male, nonpregnant and pregnant female Sprague Dawley rats following a single oral administration
Biliary and milk excretion of [14C] RWJ-270201 in Sprague Dawley rats following single oral administration
Intravenous fertility and general reproductive toxicity study of RWJ-270201 (b) (4) in female rats
Intravenous fertility and general reproductive toxicity study of RWJ-270201 (b) (4) in male rats
A continuous intravenous infusion developmental toxicity study of RWJ-270201 (b) (4) in rat
Drug metabolism support of an intravenous developmental toxicity study (DS99316) of RWJ-270201 (b) (4) in rats
Intravenous development toxicity study of RWJ-270201 (b) (4) in rats
Intravenous developmental toxicity study of RWJ-270201 (b) (4) in rabbits
Intravenous dosage-range developmental toxicity study of RWJ-270201 (b) (4) in rabbits
Intravenous dosage-range developmental toxicity study of RWJ-270201 (b) (4) in rats
Intravenous developmental and perinatal/postnatal reproduction toxicity study of RWJ-270201 (b) (4) in rats, including a postnatal behavioral/functional evaluation

Juvenile Toxicology Studies

An oral (gavage) 2 week toxicity study of RWJ-270201 (b) (4) in neonatal/juvenile albino rabbits
An oral (gavage) 2 week toxicity study of RWJ-270201 (b) (4) in neonatal/juvenile albino rats
One-month intravenous toxicity study of Peramivir trihydrate in juvenile rats
Single intravenous toxicity study of Peramivir trihydrate in juvenile rats

Special Toxicology Studies
Preliminary muscle irritation study of peramivir acidic formulation in rabbits
Antigenicity study in guinea pigs: systemic anaphylaxis and passive cutaneous anaphylaxis reactions

3.2 STUDIES NOT REVIEWED

Acute oral toxicity study of RWJ-270201 (b) (4) in mice
Acute oral toxicity study of RWJ-270201 (b) (4) in New Zealand white rabbits
Acute oral toxicity study of RWJ-270201 (b) (4) in mice
Acute oral toxicity study of RWJ-270201 (b) (4) in New Zealand white rabbits
Validation of an analytical method for determination of Peramivir in monkey plasma by LC/MS/MS
Validation of an analytical method for determination of Peramivir in mouse plasma by LC/MS/MS
Validation of an Analytical Method for Determination of Peramivir in Rabbit Plasma by LC/MS/MS for PK Analysis
Validation of an analytical method for determination of Peramivir in rat plasma by LC/MS/MS
Collection of Sample for Determination of the PK of Peramivir after Single Intramuscular Dose to Ferrets
Collection of sample for determination of the PK of Peramivir after single intramuscular dose to monkeys
Dose-linearity of Plasma Concentration Following Single Administration of Peramivir trihydrate in Mice
Dose-linearity of Plasma Concentration Following Single Administration of Peramivir trihydrate in Mice
Dose-linearity of Plasma Concentration Following Single Administration of Peramivir trihydrate in Monkeys
Dose-linearity of Plasma Concentration Following Single Administration of Peramivir trihydrate in Rats
Dose-linearity of Plasma Concentration Following Single Administration of Peramivir trihydrate in Rats

3.3 PREVIOUS REVIEWS REFERENCED

Nonclinical studies, including safety pharmacology, ADME, repeat-dose toxicology, and genetic toxicology studies to support the NDA were reviewed previously under the various INDs. The reviews on key studies are attached to this document as Appendix 1 and are summarized in the appropriate sections of this review.

4 PHARMACOLOGY

Peramivir inhibits neuraminidase of influenza A and B viruses.

4.1 PRIMARY PHARMACOLOGY

Please see the Clinical Microbiology review for a complete review of the pharmacology of peramivir.

4.2 SECONDARY PHARMACOLOGY

Peramivir was evaluated for possible interactions in in vitro enzyme, receptors, ion channels and transporter binding site assays and also in isolated tissue assays. The inhibition ratio of peramivir for each receptor (e.g., adrenergic, muscarinic, opiate, histamine receptors), ion channel (e.g., potassium and calcium channels), or transporter (e.g., gamma amino butyric acid, serotonin transporters) tested in vitro was not significant, except that > 20% inhibition was found for the human alpha-1A-adrenergic (26%) and human serotonin $5HT_{2B}$ (40%) receptors.

4.3 SAFETY PHARMACOLOGY

Peramivir was tested for effects on the CNS in rats and mice; on the cardiovascular system both in vitro and vivo (in anesthetized rats, guinea pigs, and dogs; and in conscious monkeys); on the respiratory system in conscious rats and anesthetized guinea pigs; on the gastrointestinal system in mice; and on the renal system in rats. None of these single-dose safety pharmacology studies showed significant effects of peramivir. In vitro peramivir did not affect either the hERG channel

current at the maximum feasible concentration of 300 μM (≈115 ug/ml; ≈AUC=2760 ug.h/ml), nor did it affect the action potential of isolated guinea pig cardiac papillary muscles in 2 assays.

In vivo, peramivir did not affect general behavior and neurobehavioral function (≤ 100 mg/kg iv, in mice and rats); cardiovascular functions (≤ 10 mg/kg iv, in rats/guinea pigs; ≤ 60 mg/kg iv, in monkeys; 100 mg/kg intraduodenally in dogs); respiratory functions (≤ 10 mg/kg iv, in rats and guinea pigs); intestinal locomotion (≤ 300 mg/kg po, in mice); or urinary function (≤ 300 mg/kg po, in rats). In these whole animal studies, with the exception of CNS experiment (100 mg/kg iv), the estimated drug exposures (AUC) are predictably less than those achieved by the human recommended dose (102.7 ug.h/ml).

5 PHARMACOKINETICS/ADME/TOXICOKINETICS

5.1 PK/ADME

The absorption, distribution, excretion, and metabolism profile of PERAMIVIR has been studied in vitro and in vivo in animals.

ABSORPTION

IV peramivir would provide 100% systemic exposure. (b) (4)

DISTRIBUTION

The steady-state volumes of distribution of peramivir in mice, rats, rabbits, dogs and monkeys, were close to or less than total body water (200-500ml/kg). The plasma protein binding of peramivir was low (<30%). An IV ^{14}C-peramivir study in rats showed that it primarily distributed to kidney, bladder and bile. Peramivir does not partition into red blood cells.

METABOLISM

Peramivir was found not to be an inducer or inhibitor of liver microsomal enzymes (human). It was not extensively metabolized in the rat, dog or human hepatic S9 system (<4%, single metabolite). In vivo, the orally administered peramivir is not metabolized in the dog or monkey. In rats, it was slightly oxidatively hydrolyzed (5%) after oral administration (the cyclopentyl ring oxidized metabolite), and was excreted in unchanged form (>90%) after iv administration. In rabbits and humans, peramivir was moderately metabolized to a glucuronide (<10% after iv; 10-30% after oral dosing). An acyl glucuronide metabolite could be produced in vivo in rabbits (males, up to 33% of dose). Conjugation is the only major metabolic pathway in the rabbit and human. Peramivir is neither a substrate nor an inhibitor of p-glycoprotein.

ELIMINATION

IV peramivir was eliminated primarily in the urine, and nearly all urinary elimination occurred within the first 24 hours in rats and monkeys. However, oral peramivir was excreted primarily in the feces (rats, rabbits and monkeys), and <10% was in urine, and in rats, biliary excretion accounted for <1% of the dose. IV ^{14}C-peramivir study showed that radioactivity was present in the milk of lactating rats at early timepoints and rose to 7.4 at 4 hours after dosing (milk/plasma AUC≈50%). It is actively secreted in the kidney (rabbit, not in the rat). T½ after IV peramivir was < 2 hrs in mice, ≈15 hrs in rats, 10 hours in dogs and 15-20 hrs in monkeys.

6 GENERAL TOXICOLOGY

6.1 SINGLE-DOSE TOXICITY

Single-dose toxicity studies conducted in rats, mouse and monkeys revealed that (1) iv and im routes of drug administration caused local irritation and tissue damage at the drug injection sites, (2) white discolored feces were found in rats given a high oral dose (3000 mg/kg). No other target organ/system of toxicity was identified in these animal species.

A single-dose oral study in rabbits elicited significant renal toxicity: (1) pale, tan or red discolored foci/areas in the kidneys, without microscopic changes, were reported at 2400 mg/kg, (2) a single iv dose at 200 or 300 mg/kg produced renal tubular necrosis accompanied by increased BUN, creatinine, urine volume, and altered sodium and chloride excretions. More details on renal toxicity in rabbits are given in Section 10 below.

6.2 REPEAT-DOSE TOXICITY

Study Title: Seven-Day Intravenous Toxicity Study of RWJ 270201 (b) (4) in Rats

KEY FINDINGS:

No target organ of toxicity was identified in this 7-day iv study. The NOAEL was defined as 200 mg/kg (AUC=263 [female] -355 [male] ug.h/ml).

Study no.:	DS99318
Report Location:	EDR
Conducting laboratory:	R. W. Johnson Pharmaceutical Research Institute, Spring House PA
Date of study initiation:	6/22/1999
GLP compliance:	yes
QA report:	yes
Drug, lot #, and % purity:	S99-0071/99.4%

Methods

Doses:	Vehicle (NaCl 0.9%) or 20, 50, or 200 mg/kg/day
Species/strain:	Crl:CD®(SD) IGS BR, VAF/Plus® rats
Number/sex/group:	5
Route, formulation, volume:	IV, 1/day for 7 days (1.67-16.67ml/kg/day/12 mg/ml)
Satellite groups used for toxicokinetics or recovery:	2 (study# DM99363)
Age:	8 weeks
Weight:	(M) 232.8 - 295. 8 g; (F) 169.9 - 208.1 g

Results:

Mortality: Checked 1/day. No mortality.

Clinical signs: Checked 1/day. Unremarkable.

Body weights: Measured at predose and at study termination. Unremarkable.

Food consumption: Measured at predose and at study termination. Unremarkable.

Hematology: Measured at predose and at study termination. Lower (~0.89-0.96x) RBC, (~0.89-0.94x) Hb, and (~0.88-0.93x) Hct were observed (1 high dosage male, females at 50-200 mg/kg/day) (these effects were not reported in the following 14-day and 28-day studies.)

Clinical chemistry: Measured at predose and at study termination. Unremarkable.

Urinalysis: Measured at study termination. Unremarkable.

Gross pathology: Measured at study termination. Unremarkable.

Organ weights Increased pituitary weight (high dose males, $p \leq 0.05$), ovary and liver weight (trend in females only) were observed. Ovarian weight changes appeared to be dose-related. These effects were not reported in the following 14-day and 28-day studies.

Histopathology: Adequate Battery: yes
Peer review: yes
No significant gross or microscopic findings were reported.

Toxicokinetics:
AUC showed dose-related increases without gender differences or evidence of accumulation. T½ = 4.9-9.7 hr.

Table 1 – Toxicokinetics of 7-day IV study of peramivir in rats

Daily Dose (mg/kg)	0 (Control)		20		50		200	
Number of Animals	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Number of Animals (TK)[a]	M: 0	F: 0	M: 2	F: 2	M: 2	F: 2	M: 2	F: 2
Toxicokinetics: AUC $_{0\text{-}24}$[b] (ng•hr/mL)								
Day 1	SNC[c]	SNC	44,121	28,838	143,090	144,049	374,615	260,375
Day7	SNC	SNC	39,302	29,020[d]	180,395	135,064	354,664	262,567

The NOAEL was designated as 200 mg/kg by the sponsor.

Study Title: A 14-Day Continuous Intravenous Infusion Toxicity Study of RWJ 270201 (b) (4) in Rats

KEY FINDINGS:

- No target organ of toxicity was identified.
- The NOAEL was defined as 1152 mg/kg (AUC=1668 ug.h/ml).

Study no.:	DS99031
File Location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	16 August, 1999
GLP compliance:	yes
QA report:	yes
Drug, lot #, and % purity:	99P0071/99.4

Methods

Doses:	Vehicle (NaCl 0.9%) or 96, 384, 768, 1152 mg/kg/day
Species/strain:	Crl:CD®(SD) IGS BR, VAF/Plus® rats
Number/sex/group:	10
Route, formulation, volume:	IV, 96ml/kg/day; 1-12 mg/ml, 1/day for 14 days (via direct infusion into the femoral vein catheter continuously.)
Satellite groups used for toxicokinetics or recovery:	6 (DM99386)
Age:	(M) 260-305 g; (F) 195-229g
Weight:	11.5 weeks

Results:

Mortality: Checked 1/day. 4 dosing related deaths: 1 control male died on day 11, 1 male at 384mg/kg/day died on Day 6 (due to pulmonary edema), 1 male at same dose died on Day 13 (ulceration of the lower lip and teeth), 1 male at 1152mg/kg/day sacrificed on Day 13 (due to severed catheter).

Clinical signs: Checked 1/day. Unremarkable.

Body weights: Measured at predose and at study termination. Unremarkable.

Food consumption: Measured at predose and at study termination. Unremarkable.

Hematology: Measured at predose and at study termination. Unremarkable.

Clinical chemistry: Measured at predose and at study termination. Unremarkable.

Urinalysis: Measured at study termination. Unremarkable.

Gross pathology: Measured at study termination. Unremarkable.

Organ weights: Unremarkable. Increased kidney weight (9%, normalized to body weight) in females in middle and high dose groups and increased heart weight (10%) in the high dose group.

Histopathology: Adequate Battery: yes (examined in control and high dose groups)
Peer review: yes

No significant gross or microscopic findings were reported. The NOAEL was 1152 mg/kg/day.

Toxicokinetics:
Drug levels were dose-related without significant differences between Day 1 and Day15, and gender difference. No AUCs were available from sponsor's report but can be approximately estimated from the Css (steady-state plasma concentrations at Day 15, see below) by CssX24hr (i.e., 69.5[average Css]x24=1668 ug.h/ml for the 1152 mg/kg/day dose).

Table 2 – Toxicokinetics of 14-day IV study in rats

Daily Dose (mg/kg)	0 (Control)		96		384		768		1152	
Number of Animals Main Study	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Number of Animals (TK) [a]	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F 10
Toxicokinetics: Plasma Concentration, ng/mL										
Day 1 (Pre-infusion)	SNA[b]	SNA	0.18	0.00	0.00	0.00	0.00	0.00	0.00[c]	0.00[c]
Day 2 (24hr Postinfusion)	SNA	SNA	7,896	6,166[c]	27,406	23,758	48,022	53,831	93,777	70,830
Day 15 C_{ss}	SNA	SNA	6,889	7,092	17,546	28,903	49,260	61,784	69,809	68,745

Study Title: Twenty-Eight Day Intravenous GLP Toxicity Study of BCX-1812 in Rats

KEY FINDINGS:

- No target organ of toxicity was identified in this 28-day iv study. The NOAEL was defined as 120 mg/kg. AUCs at 120 mg/kg were 338 [male]-428 [female] ug·hr/ml, which were not as high as those achieved by continuous infusion (see previous 14-day study, AUC=1668 ug/ml for the dose of 1152 mg/kg/day). A separate continuous iv infusion study was attempted to escalate systemic drug exposures (see study below).

Study no.: 0527-07232
File Location: EDR
Conducting laboratory: (b) (4)
Date of study initiation: 9 August 2007
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: 07P0677/102.8%

Methods

Doses:	Vehicle (NaCl 0.9%) or 15, 40, 120 mg/kg/day
Species/strain:	Hsd:Sprague Dawley SDR rats
Number/sex/group:	10 (15 for control)
Route, formulation, volume:	IV bolus (via femoral vein catheter, if nonpatent gave through lateral tail, 10ml/kg 1.5-12 mg/ml, 1/day for 30 days)
Satellite groups used for toxicokinetics or recovery:	4/group for TK 8 (14-days recovery period).
Age:	(M) 222.0-258.6 g (F) 200.0-236.1
Weight:	8-9 weeks

Results:

Mortality: Checked 1/day. 2 died (1 male each in control and low dose groups, not treatment related)

Clinical signs: Checked 1/day. Unremarkable.

Body weights: Measured at predose and at study termination. Unremarkable.

Food consumption: Measured at predose and at study termination. Unremarkable.

Hematology: Measured at predose and at study termination. Unremarkable.

Clinical chemistry: Measured at predose and at study termination. Unremarkable.

Urinalysis: Measured at study termination. Unremarkable.

Gross pathology: Measured at study termination. Unremarkable.

Organ weights Unremarkable.

Histopathology: Adequate Battery: yes
Peer review: no

No significant gross or microscopic findings were reported. The sponsor claimed that the no observed adverse effect level was 120 mg/kg/day under the conditions of this study.

Toxicokinetics: Cmax and AUC were dose-proportional. There was no significant accumulation and no gender differences in exposures. Exposures at 120 mg/kg/day were 338.24-428.23 ug·hr/ml with Cmax≥585.250 ug/ml at Day 27.

Table 3 – Toxicokinetics of 28-day IV study in rats

Daily Dose (mg/kg)	0 (Control)		15		40		120	
Number of Animals Main Study	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Number of Recovery Animals	M:4	F:4	M:0	F:0	M:0	F:0	M:4	F:4
Number of Toxicokinetic Animals	M:0	F:0	M:8	F:8	M:8	F:8	M:8	F:8
Toxicokinetics: Day 1 AUC $_{0\text{-}24\ hr}$ (ng·hr/mL)	NS[b]	NS	31,563	38,284	99,247	96,923	301,610	266,223
Toxicokinetics: Day 27 AUC $_{0\text{-}24\ hr}$ (ng·hr/mL)	NS	NS	39,946	45,933	112,245	122,990	338,240	428,228

Study Title: One-Month Continuous Infusion Toxicity Study of Peramivir Trihydrate in Rats

KEY FINDINGS:

- No target organ of toxicity was identified in this 30 day iv infusion study. The NOAEL was defined as 1440 mg/kg (AUC = 2330 (males) 1620 (females) ug.h/ml, Day 21).

Study no.: S-021812-TF-112-L
File Location: EDR
Conducting laboratory: (b) (4)
Date of study initiation: 12/30/2008
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: 07P0677/101.4%

Methods

Doses:	Vehicle (NaCl 0.9%) or 160, 480 and 1440 mg/kg/day
Species/strain:	(SPF) Crl:CD(SD) Sprague-Dawley rats
Number/sex/group:	10-15 (control:15)
Route, formulation, volume:	IV infusion 5 ml/kg/h for 24 hours/day for 30 days (via femoral vein catheter, when nonpatent the drug was given through lateral tail vein)
Satellite groups used for toxicokinetics or recovery:	4/group for TK 5/group (30 days recovery period, control and high dose)
Age:	(M) 173-218g; (F) 127-163g
Weight:	6wks

Results:

Mortality: Checked 1/day. One male died in low and middle dose groups each (not drug-related).

Clinical signs: Checked 1/day. Unremarkable.

Body weights: Measured at predose and at study termination. Unremarkable.

Food consumption: Measured at predose and at study termination. Unremarkable.

Hematology: Measured at predose and at study termination. Unremarkable.

Clinical chemistry: Measured at predose and at study termination. Unremarkable.

Urinalysis: Measured at study termination. Unremarkable.

Gross pathology: Measured at study termination. Unremarkable.

Organ weights Unremarkable.

Histopathology: Adequate Battery: yes
Peer review: no
Microscopic findings were unremarkable. The sponsor claimed that NOAEL was 1440 mg/kg for this study.

Toxicokinetics: Exposures reached steady-state at 15 minutes or 1 hour after infusion on Day 1. AUC=2330 (males) and 1620 (females) ug.h/ml at Day21 (AUC data on Day 31 were not derived from steady-state concentrations because infusion was terminated).

Table 4 – Toxicokinetics of one-month continuous IV study in rats

Daily Dose (mg/kg)	0 (Control)		160		480		1,440	
Number of Animals Main Study	M: 10	F: 10	M: 10	F 10:	M: 10	F: 10	M: 10	F: 10
Number of Animals (4-Week Recovery)	M: 5	F:5	M: 0	F: 0	M: 0	F: 0	M: 5	F: 5
Number of Animals (TK)[a]	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Toxicokinetics: AUC $_{0\text{-}24}$ (µg•hr/mL)								
Day 1[b]	BQL[c]	BQL	197	177	617	556	1,720	1,800
Day 7[d]	BQL	BQL	187	186	637	500	1,880	1,470
Day 14[d]	BQL	BQL	206	190	638	565	2,190	1,660
Day 21[d]	BQL	BQL	220	182	642	633	2,330	1,620
Day 31[e]	BQL	BQL	11.3	6.34	41.1	21.6	141	75.9

a 3 rats/sex/group; one additional (spare) rat/sex/groups per group was not used.
b Plasma samples were collected at 15 min, and 1, 6, and 24 hr after initiation of infusion; plasma concentrations at time zero (at initiation of dosing) were regarded as zero.
c Concentrations in control samples were below the lower limit of quantification at all intervals and time points.
d Plasma was collected immediately prior to replacement of the dosing solutions; plasma concentrations were assumed to be maintained at the levels immediately prior to replacement of the dosing formulation for 24 hours;.
e Plasma samples were collected immediately and at 15 min, and 1, 6, and 24 hr after termination of infusion (N = 2), the time immediately after termination of dosing was regarded as time zero.

Study Title: **2-Week IV Bolus Toxicity Study of Peramivir in Cynomolgus Monkeys With a Two-Week Recovery Period**

Key study findings:

- No target organ was identified in this study. The NOAEL is considered to be 45 mg/kg (AUC=249 μg.h/ml) for this study

Study no.:	806-023
Volume # and page #:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	6/2005
GLP compliance:	yes
QA report:	yes
Drug, lot #, and % purity:	S00-0150/100.2

Methods

Doses:	0, 5, 15, 45 mg/kg/day, 5 ml/kg/1-9 mg/ml
Species/strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/sex/group:	5 (Groups 1 and 4); 3 (Groups 2 and 3）
Route, formulation, volume:	IV bolus, 0.9% NaCl, 1/day for 14 days, 5ml/kg
Satellite groups used for toxicokinetics or recovery:	2/control & high dose (2-week recovery) 3-5/group for TK (pretest, day 1, 7 and 13)
Age:	1 yr 8 mos –2yrs 9 mos
Weight:	(M) 1.78-2.83kg; (F)1.86-2.77 kg

Results:

Mortality: Observations for moribund or dead animals were made 2 times daily. No mortality.

Clinical signs: Checked 1/day. No remarkable findings.

Body weights: Checked once/week. No remarkable findings.

Food consumption: Checked once/week. No remarkable findings.

Ophthalmoscopy: During pretest and at termination. No remarkable findings.

EKG: During pretest and at termination. No remarkable findings.

Hematology: During pretest and at termination. No remarkable findings.

Clinical chemistry: During pretest and at termination. No remarkable findings.

Urinalysis: During pretest and at termination. No remarkable findings.

Gross pathology: During pretest and at termination. No remarkable findings.

Organ weights: During pretest and at termination. No remarkable findings.

Histopathology: Adequate Battery: yes (see below)
Peer review: no

Toxicokinetics:

No gender difference in exposure levels and AUCs increased proportionally to dose (AUC=249 ug.h/ml on day 13 at 45 mg/kg). T½=12-16 hr on day 1 and 7-8 hr on day 13 for all doses (see below).

Table 5 – Toxicokinetics of 2-week IV study in monkeys

Day	Dose (mg/kg)	$AUC_{0-\infty}$ (ng•hr/mL)	$AUC_{0-\infty}$/Dose (hr•ng/mL•mg/kg)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL•mg/kg)	CL (mL/min/kg)	$t_{1/2}$ (hr)	V_{ss} (mL/kg)	T_{max} (hr)
1	5	27400±4170	5480±834	37300±2540	7450±509	3.10±0.505	16.2±4.12	337±29.7	0±0
	15	95200±20400	6340±1360	128000±21000	8500±1400	2.72±0.518	12.5±1.81	235±61.1[a]	0.042±0.102
	45	186000±24100	4130±536[a,b]	177000±32800	3940±730[a,b]	4.10±0.529[a,b]	11.5±1.77[a]	438±35.5[a,b]	0±0
7	5	25100±3790	5020±758	32800±3030	6550±606[c]	3.38±0.437	4.71±1.01[c]	200±23.2[c]	0±0
	15	87900±9690	5860±646[a]	152000±47900	10100±3190[a]	2.87±0.314	5.49±0.654[c]	126±23.1[a,c]	0.125±0.137
	45	232000±47900	5160±1060[c]	276000±52100	6120±1160[b,c]	3.35±0.676[c]	6.05±2.80[c]	241±20.8[a,b,c]	0±0
13	5	27800±4340	5560±869	24800±8260	4960±1650[c]	3.06±0.508	7.88±1.02[c]	238±41.4[c]	0.167±0.129
	15	118000±15300	7870±1020[a]	173000±46500	11500±3100[a,c]	2.15±0.260[a,c]	7.87±1.47[c]	138±30.4[a,c]	0.083±0.129
	45	249000±46200	5530±1030[b,c]	375000±91400	8330±2030[a,b,c]	3.10±0.548[b,c]	7.01±0.633[c]	230±43.7[b,c]	0.025±0.079

[a]Significantly different from 5 mg/kg/day on the same day; Student's t test p<0.05.
[b]Significantly different from 15 mg/kg/day on the same day; Student's t test p<0.05.
[c]Significantly different from the same dose on Day 1; Student's t test p<0.05.

Study Title: **Twenty-Eight Day Intravenous GLP Toxicity Study of BCX-1812 in Cynomolgus Monkeys (With a 14-Day Recovery Period)**

Key study findings:

- No target organ was identified in this 28-day study. The NOAEL = 90 mg/kg, with AUC= 473 (males) or 610 (females) µg.h/ml.

Study no.: 0527-07233
Volume # and page #: EDR
Conducting laboratory: (b) (4)

Date of study initiation: 7/17/2007
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: 07P0677/102.8
Methods

Doses:	0, 10, 30 or 90 mg/kg/day, 7.5 ml/kg
Species/strain:	Cynomolgus monkeys
Number/sex/group:	5(Groups 1 and 4); 3 (Groups 2 and 3）
Route, formulation, volume:	IV bolus, 0.9% NaCl, 1/day for 28 days
Satellite groups used for toxicokinetics or recovery:	2/control & high dose (2-week recovery) TK from main group (pretest, day 1 and 28)
Age:	⩾ 18mos
Weight:	(M/F) 1.73-2.40 kg

Results:

Mortality: Observations 2/day. One low dose male was sacrificed moribund on Day 21 (decreased activity, decreased appetite) due to misplaced catheter. Another control male in recovery group was found dead on Day 30 due to confirmed infections (Proteus mirabilis, Escherichia coli and Staphylococcus intermedius).

Clinical signs: Checked 1/day. No remarkable findings.

Body weights: Checked once/week. No remarkable findings.

Food consumption: Checked once/week. No remarkable findings.

Ophthalmoscopy: During pretest and at termination. No remarkable findings.

EKG: During pretest and at termination. No remarkable findings.

Hematology: During pretest and at termination. Unremarkable except that absolute large unstained cell count in females (≥30 mg/kg/day) was decreased.

Clinical chemistry: During pretest and at termination. Unremarkable except that triglycerides in males (≥10 mg/kg/day) was decreased.

Urinalysis: During pretest and at termination. No remarkable findings.

Gross pathology: During pretest and at termination. No remarkable findings.

Organ weights: During pretest and at termination. No remarkable findings.

Histopathology: Adequate Battery: yes
Peer review: no

No remarkable findings.

Toxicokinetics:

No gender difference in exposures was reported. AUCs and Cmax increased dose-proportionally (see below). Cmax = 48525-54467 ng/ml in the low dose, and 142833-154500 ng/ml in the mid-dose, and 515100 - 483200 ng/ml in the high dose group. AUC = 40121 hr·ng/ml (mid-dose), and 472773-610387 hr·ng/ml in the high dose group.

Table 6 - Toxicokinetics of 28-day IV study in monkeys

Dose	Gender	Day	C_{max} (ng/mL)	$AUC_{0\text{-}24hr}$ (hr-ng/mL)
0	Females	1	0	0
		28	7.44±10.6	6.47±8.90
	Males	1	0	0
		28	2.04±4.56	5.10±11.4
10	Females	1	48550±NA	41855±NA
		28	54467±3944	48950±11325
	Males	1	52767±8551	53933±10393
		28	48525±NA	40121±NA
30	Females	1	143833±2466	125713±8347
		28	154500±4444	140395±25080
	Males	1	148000±36149	152518±24988
		28	142833±16174	158838±13187
90	Females	1	487200±46160	596695±261796
		28	483200±69355	610387±295529
	Males	1	515100±45852	491039±73185
		28	486800±34917	472773±77615

Study Title: **One-Month Continuous Intravenous Infusion Toxicity Study of Peramivir trihydrate in Monkeys (With A 14-Day Recovery Period)**

Key study findings:

- This monkey study attempted to achieve highest drug exposures by using continuous iv infusion and escalating to a higher dose (720 mg) than the previous study used (see above). No definitive target organ was identified and NOAEL is considered by the sponsor to be 720 mg/kg for this study. (AUC=2945 µg.h/ml [both sexes] on day 14). Because there were emerging renal changes, the NOAEL should be 360 (AUC 1450-1510).

Study no.: S-021812 (b) (4)
Volume # and page #:
Conducting laboratory: (b) (4)
Date of study initiation: 4/28/2008
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: 05P0487/100.8
Methods

Doses:	0, 120, 360 or 720 mg/kg/day, 60 ml/kg/2-12 mg/ml
Species/strain:	Cynomolgus monkeys
Number/sex/group:	5(Groups 1 and 4); 3 (Groups 2 and 3）
Route, formulation, volume:	IV infusion, 0.9% NaCl, 2.5 ml/kg/hr for 30 days
Satellite groups used for toxicokinetics or recovery:	Main group (1 & 4 had a 1-month recovery period) TK performed in the main group animals.
Age:	(M) 2years and 8 months to 3 years and 3months; (F) 2years & 8-9 months
Weight:	(M) 2.05-3.95 kg; (F) 2.20-3.05 kg.

Results:

Mortality: None.

Clinical signs: Checked 1/day. No remarkable findings.

Body weights: Checked once/week. No remarkable findings.

Food consumption: Checked once/week. No remarkable findings.

Ophthalmoscopy: During pretest and at termination. No remarkable findings.

EKG: During pretest and at termination. No remarkable findings.

Hematology: During pretest and at termination. Unremarkable except: (1) decreases in prothrombin time (Day 14) and monocyte ratio/count (Day 30) in males (720 mg/kg), (2) decreases in the MCV in males (120 and 720 mg/kg, Days 14 and 30), (3) decrease in Hct in females (360 and 720 mg/kg, Day 30), (4) increases in the large unstained cell ratio in females (720 mg/kg, Day 14). These changes (Hct, MCV) were <10%.

Clinical chemistry: During pretest and at termination. Unremarkable, except that triglycerides were increased (LD: 29mg/dl & HD 31mg/dl vs. control 19mg/dl, females/Day 30).

Urinalysis: During pretest and at termination. No remarkable findings.

Gross pathology: During pretest and at termination. No remarkable findings.

Organ weights: During pretest and at termination. No remarkable findings. The relative kidney weights were increased in males (360 and 720 mg/kg), and absolute weight in females (right kidney, 720 mg/kg). Prostate weights in males (360 and 720 mg/kg, by 120~198%) and pituitary in females (720 mg/kg, by 44%) were also increased. All these organ weight increases (1) did not have correlated findings in histopathology exams (except slight renal changes, see below) and (2) were not seen in recovery group animals (control and HD).

Table 7 - Kidney weight changes in 1-month continuous IV infusion study in monkeys

Daily Dose (mg/kg)		0 (Control)		120		360		720	
Number of Animals		M: 5	F: 5	M: 3	F: 3	M: 3	F: 3	M: 5	F: 5
Organ Weights [a)]									
Kidney	Left	0.211 g%	0.214 g%	+15	-5	+30**	+21	+32**	+29
	Right	0.209 g%	0.200 g%	+14	+3	+27*	+18	+24*	+41

*a) For controls, group means are shown. For treated groups, percent differences from controls are shown (based on actual data, not on % differences). *: $p < 0.05$, **: $p < 0.01$.*

Histopathology: Adequate Battery: yes
Peer review: no

Slight vacuolar degeneration in tubular epithelium and regeneration of tubules were reported in 2/3 monkeys (males, 720 mg/kg). Bone marrow smears were unremarkable except that there was a decreased nucleated cell count (males 360 mg/kg, by 22%), which was not dose-related. The sponsor

indicated that the increased kidney weights (see above) were not significantly correlated in histological examinations, whereas renal findings did exist (see above). Findings in recovery group animals were unremarkable.

Table 8 - Renal histopathology in 1-month continuous IV infusion study in monkeys

Organ and tissue	Findings	Group	Control			120 mg/kg/day			360 mg/kg/day			720 mg/kg/day		
		\ Animal No.	01	02	03	11	12	13	21	22	23	31	32	33
Kidney	Cell infiltration, mononuclear cell, interstitium		-	±	±	±	-	±	±	-	±	-	±	-
	Hyaline cast, tubule, bilateral		-	-	-	-	-	-	-	-	-	-	-	-
	Hyaline cast, tubule, unilateral		-	-	-	-	-	-	-	-	-	-	-	-
	Mineralization, cortex		-	-	-	-	-	-	-	-	-	-	-	-
	Mineralization, papilla		-	-	-	-	-	-	-	-	-	-	-	-
	Regeneration, tubule, unilateral		-	-	±	-	-	-	-	-	-	±	-	±
	Vacuolar degeneration, epithelium, tubule, unilateral		-	-	-	-	-	-	-	-	-	-	-	±

±: Slight

Toxicokinetics:
No gender difference in exposure levels was reported. AUCs and Cmax increased proportionally to dose (see below). The pre-dosing levels on Day 14 and Day 31 were comparable to the levels measured at 6 hours post-dosing on Day 1. The AUC on Day 1 increased with dose. The AUC on Day 14 were comparable to those on Day 1. At end of dosing, drug levels decreased promptly after termination of infusion, indicating rapid elimination of the drug.

Table 9 - Toxicokinetics in 1-month continuous IV infusion study in monkeys

Daily Dose (mg/kg)		0 (Control)		120		360		720	
Number of Animals		M: 5	F: 5	M: 3	F: 3	M: 3	F: 3	M: 5	F: 5
Toxicokinetics: Peramivir									
Plasma concentration	Day 1, 6h	ND	ND	20.1	20.4	56.5	64.2	140	132
(μg/mL)	Day 14 [a)]	ND	ND	19.4	22.8	60.6	62.9	123	123
	Day 31 [b)]	ND	ND	23.1	22.7	56.5 [c)]	60.5	137	110
$AUC_{0\text{-}24h}$	Day 1 [d)]	NC	NC	470	472	1340	1450	3270	3090
(μg·h/mL)	Day 14 [e)]	NC	NC	466	547	1450	1510	2950	2940
	Day 31 [f)]	NC	NC	NC	NC	NC	NC	305	223

The first day of dosing is Day 1. ND: Not detected NC: Not calculated
a) Immediately prior to replacement of dosing formulations
b) Immediately prior to termination of infusion
c) n=2, the catheter of 1 male (Animal No. 21) fell out at blood sampling.
d) Trapezoidal rule: the plasma concentrations at time zero (at initiation of dosing) were regarded as zero (0).
e) Trapezoidal rule: the concentrations were assumed to be maintained at the levels immediately prior to replacement of the dosing formulations for 24 hours.
f) Trapezoidal rule: twenty-four hours after termination of infusion (n=2), the time immediately prior to termination of dosing was regarded as time zero.

7 GENETIC TOXICOLOGY

Peramivir was not mutagenic or genotoxic in the in vitro bacterial reverse mutation (Ames) assay, the in vitro mammalian Chinese hamster ovary chromosomal aberration test, and the in vivo

micronucleus test in mice. The following are studies conducted using intravenous administration (see Appendix for oral studies).

1. **Micronucleus Test of Peramivir Trihydrate with Mouse Bone Marrow Cells [By Intravenous Administration] (PMV-TF-040-L, 7/12/2007, Shionogi & Co., Ltd, Osaka, Japan).**

Method. This is a GLP study. Groups of 6 male mice (Crlj:CD1(ICR)) were given a single dose of peramivir iv at 0, 75, 150, or 300 mg/kg (highest soluble dose). Bone marrow smears were prepared 24 and 48 hours after dosing, and 2,000 polychromatic erythrocytes (PCE) were examined from each animal to calculate the incidence of micronucleated PCE. At least 1,000 total erythrocytes (PCEs + normochromatic erythrocytes [NCEs]) from each animal were also examined to calculate the percentage of PCEs as an index of the bone marrow cell proliferation suppressive effect.

Table 10 – Study design of iv micronucleus test of peramivir trihydrate (75 – 300 mg/kg iv) in mice

Group	Substance	Frequency	Dose level (mg/kg)	Concentration (mg/mL)	Dose volume (mL/kg)	No. of animals	Animal Nos.[a)]
Negative control	Saline	1	0	0	25	12	001 to 012
Low	Peramivir trihydrate	1	75	3	25	20	101 to 120
Mid	Peramivir trihydrate	1	150	6	25	20	201 to 220
High	Peramivir trihydrate	1	300	12	25	20	301 to 320
Positive control	MMC	1	2	0.2	10	6	401 to 406

a) Animals were used as follows: Animal Nos. ending in 01 to 06 for 24-hour treatment, Animal Nos. ending in 07 to 12 for 48-hour treatment and Animal Nos. ending in 13 to 20 for TK sampling.

Animals for TK sampling

Group	Substance	Dose level (mg/kg)	Concentration (mg/mL)	Dose volume (mL/kg)	No. of animals	Animal Nos.
Low	Peramivir trihydrate	75	3	25	8	113 to 120
Mid	Peramivir trihydrate	150	6	25	8	213 to 220
High	Peramivir trihydrate	300	12	25	8	313 to 320

TK samples were collected at 5 and 30 minutes after dosing from animals with Animal Nos. ending in 13 to 16 and those ending in 17 to 20, respectively.

Results. No mortality and no clinical signs were observed in the study. No statistically significant changes in % of PCEs were found in peramivir-treated group, whereas positive control (mitomycin C, i.p.) showed significant decreases. Peramivir did not cause a statistically significant increase in micronucleated PCEs (0.12%-0.15% vs. positive controls of 4.7-7%). Drug exposures were dose-related with the following mean drug levels reported: 230, 481 and 891µg/ml (for the 75, 150 and 300 mg/kg groups, respectively, at T5 minutes post-dosing) and 31.7, 62.0 and 110 µg/ml, respectively, at T30 minutes post-dosing. It was concluded that peramivir did not cause clastogenic or aneuploidy effects in this iv mouse micronucleus study.

2. Mammalian Erythrocyte Micronucleus Test [in Mouse Bone Marrow Cells with Intravenous Administration] (P01-002-037, (b) (4)

Method. This is a GLP study. Groups of 5 male and 5 female mice were given a single dose of peramivir iv at 0, 90, 160 or 360 mg/kg (highest soluble dose). Vehicle control (saline) and positive control (cyclophosphamide at 50 mg/kg) groups were similarly treated.

Five mice/group were sacrificed 24 hr post-dosing for bone marrow harvesting, with 5 more similarly sacrificed at 48 hours post-dosing from the vehicle and 360 mg/kg groups. An additional 5 mice per sex were intended to be used for TK at 30 minutes post-dose for the vehicle and peramivir groups, but these samples were not analyzed. The dosing solutions were not analyzed for concentration, and drug substance stability data was not provided. Femoral bone marrow preparations were evaluated for the incidence of micronuclei in PCEs and for the proportion of PCEs to total erythrocytes as an indicator of potential toxicity.

Table 11 - Study design of iv micronucleus test of peramivir (90 – 360 mg/kg iv) in mice

(b) (4)

Results. Peramivir did not produce an increase in micronucleated PCE in this study. It was concluded that peramivir tested negative in this iv mouse micronucleus assay.

3. Bone Marrow Micronucleus Assay Range-Finding Study In Mice Dosed Intravenously With RWJ-270201 (b) (4) (00450, R. W. Johnson Pharmaceutical Research Institute, Spring House PA, May 25, 2000)

Summary. This is a range-finding (non-GLP) study report on groups of 5 mice/sex mice that were given an iv dose of peramivir at 360 mg/kg (maximum soluble dose and volume). There were no significant changes in PCE/NCE ratio compared to vehicle control when erythrocytes were harvested at 24 or 48 hours after dosing. However, genotoxicity evaluation was not performed in this study.

8 CARCINOGENICITY

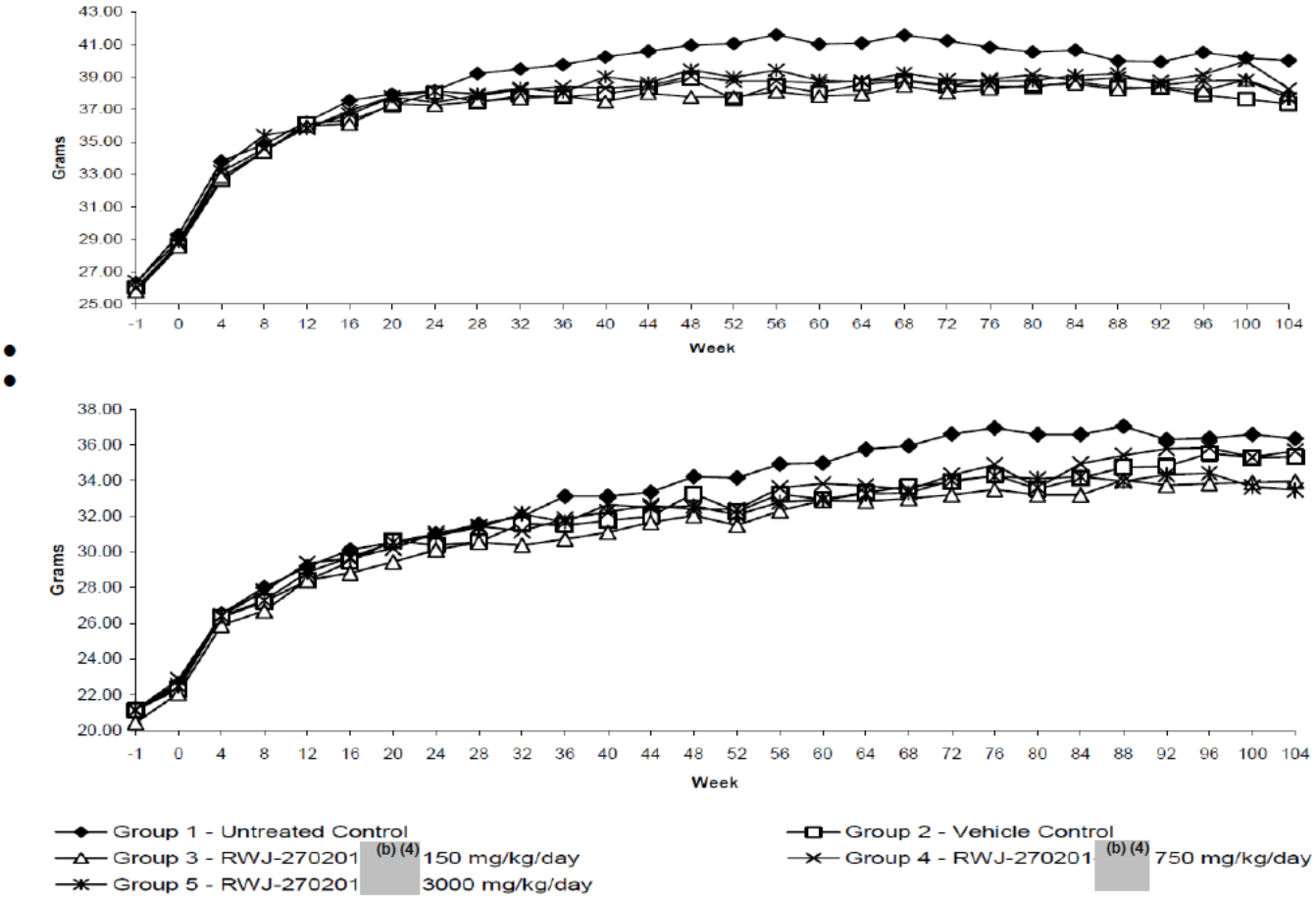
Study title: A 2-Year Oral Gavage Carcinogenicity Study of RWJ-270201 (b) (4) in the Albino Mouse

Study no.:	Sponsor Ref. No. DS00310 Testing Facility Study No. 89515
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12/4/2000 (dosing)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	99P0233, 99P0236, 00P0172 (86.66%, 83.8%, 85.18%, 86.5%, respectively)
CAC concurrence:	12/21/1999, concurred with sponsor proposed 0 (untreated), 0 (vehicle : Na carboxymethyl-cellulose, [% not known]), 150, 1000 and 3000 mg/kg

Key Study Findings

- This carcinogenicity study was terminated at month 17 without histopathology examinations because the original sponsor of RWJ-270201 (b) (4) Johnson and Johnson Inc, stopped the development of this drug.
- The sponsor reported no treatment related on survivability, food consumption, hematology ophthalmology, clinical signs, and incidences of palpable masses. No toxicokinetic information was available.
- A statistically significant reduction of body weight was recorded during the first 7 weeks for males and first 3 weeks for females (vehicle and drug-treated groups). For males from Week 28 a reduced group mean body weight occurred (vehicle and drug-treated groups), with statistical significance reached on a number of occasions. Similar observations were noted for females from Week 36 onwards.
- The study objective was not achieved for this study.

Figure 1. Body weight changes in 2-year oral mouse carcinogenicity study



Study title: Two Year Oral Oncogenicity Study of RWJ-270201 (b) (4) in Rats

Study no.:	(b) (4) 6336-143
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/7/2000 (Dosing)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	99P0233, 00p0110 (.90%) Control: 0.5% sodium carboxymethylcellulose; vehicle: distilled water

CAC concurrence:	ECAC met to discuss this protocol on 12/21/1999. ECAC's recommended doses: 0 (vehicle water), 0 (untreated), 150, 1000, 3000 mg/kg/day by oral gavage, based on MFD.

Key Study Findings

- RWJ-270201 (b) (4) administered by oral gavage to rats at doses of 0, 0, 150, 1000, 3000 mg/kg/day for up to 2 years did not significantly change survival rate or produce carcinogenic effects.
- The sponsor defined the NOAEL for non-neoplastic findings to be 3000 mg/kg/day because none of the tumors at the doses studied was proven statistically significant.
- The steady state (Day 182) $AUC_{0\text{-}24h}$ values for the high dose groups were 46.7 and 24.3 µg.h/ml for males and females, respectively, which were 0.5- and 0.2-fold the human exposures (102.7 ug.h/ml), respectively.

Adequacy of Carcinogenicity Study

The doses used for this study were based on MFD.

Appropriateness of Test Models

Crl:CD®(SD)IGS BR, VAF/Plus® rats are an appropriate animal model.

Evaluation of Tumor Findings

No tumor incidences satisfied the appropriate criteria to be described as statistically significant.

Methods

Doses:	0 (vehicle: distilled water), 0 (untreated), 150, 1000, 3000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	20 mL/kg/day
Route of administration:	Oral gavage
Formulation/Vehicle:	vehicle: distilled water
Basis of dose selection:	MFD, based on maximum dosage volume (20 ml/kg) and formulation (150 mg/ml).
Species/Strain:	Rats/ Crl:CD®(SD)IGS BR, VAF/Plus®
Number/Sex/Group:	65 (group 1-5)
Age:	Approximately 4 weeks
Animal housing:	Animals were pair housed in elevated, stainless steel, wire mesh cages during the first week of the stabilization period and individually housed
Paradigm for dietary restriction:	Not applicable
Dual control employed:	Yes (Group 1 was "vehicle control", Group 2 was untreated control; it was not clearly stated whether Group 1 was 5% carboxymethlycellulose or water)

Interim sacrifice:	No
Satellite groups:	Toxicokinetics (16/sex/group 6-8) For the AUC calculation the original human oral prophylactic dose was assumed to be 400 mg/day with a target daily AUC=1.3 ug·h/ml. For the current iv dose the exposure is around 102.7 ug.h/ml.
Deviation from study protocol:	Unremarkable

Observations and Results

Mortality

Survival to study termination was similar in vehicle control and drug-treated groups. Incidences of unscheduled deaths, including rats found dead or sacrificed in extremis, were similar among controls and drug-treated animals. The survival rates at termination were:

Table 12. The survival rates in 2-year oral rat carcinogenicity study

	Group1	Group 2	Group 3	Group 4	Group 5
Male:	20%	33%	23%	24%	19%
Females:	21%	23%	14%	23%	22%

No significant positive trend in mortality was observed in the males. A significant decrease in mortality for males was noted when the untreated group (Group 2) was compared with group 1 (water). No significant positive or negative trend in mortality was observed in the females.

Clinical Signs
White discolored feces were noted in all 1000 and 3000 mg/kg/day rats (drug was off-white in color).

Body Weights
There were no test article-related effects on body weights.

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

Gross Pathology
Macroscopic findings were sporadic and were not dose-dependent.

Histopathology

Peer Review
Adequate

Neoplastic
Male: Pheochromocytoma (benign and malignant combined) of the adrenal medulla were greater in 1000 and 3000 mg/kg/day males (See sponsor's table in the next page).

Female: Pheochromocytomas: 2 in 3000 mg/kg/day group.

Non-Neoplastic

Hyperplasia of the adrenal medulla (potential precursor lesion to pheochromocytoma) was greater in 3000 mg/kg/day males than other groups. This lesion occurred in 1 female in the 150 mg/kg group.

There were increases in incidence rate of (1) mineralization of the renal pelvis (42%-83% for MD and HD males; 37% for both MD and HD females) and tubules (50%-100% for MD and HD males; 150% for HD females), and (2) dilatation of renal tubule (females: 1-1-9-6 for ctrl-LD-MD-HD). In females, mineral deposits might be associated with minimal to slight tubular dilatation and minimal to moderate hyperplasia of the transitional epithelium of the renal pelvis (e.g., 1000 and 3000 mg/kg/day groups). The pelvic epithelial hyperplasia might be attributed to mechanical irritation by mineral deposits. Additionally, slight to moderate vacuolation of the tubular epithelium was present in several 3000 mg/kg/day females that died at an unscheduled interval. The sponsor stated that there were no distinct histomorphologic indicators of nephrotoxicity in terminal-sacrifice rats, however there is no sequential sacrifice to monitor renal toxicity and those died before end of dosing period might have died of renal toxicity.

In summary, no statistically significant increases in incidence rates for all hyperplastic and/or neoplastic findings among the vehicle and drug-treated groups (trend or group comparisons). Although increases in pheochromocytomas of the adrenal medulla were noted in treated males, these were not statistically significant.

Table 13 – Major histopathology findings in 2-year oral rat carcinogenicity study

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=T;FIND=ALL;SUBSET=ALL		SEX:	MALE				
		GROUP:	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION		NUMBER:	30	43	35	36	30
ADRENAL, MEDULLA (AM)	NUMBER EXAMINED:		29	0	35	36	30
	NOT REMARKABLE:		25	0	32	24	21
--B-PHEOCHROMOCYTOMA			3	0	2	8	6
--HYPERPLASIA			3	0	1	3	6
--M-MALIGNANT PHEOCHROMOCYTOMA			0	0	0	2	0
KIDNEY (KD)	NUMBER EXAMINED:		30	0	35	36	30
	NOT REMARKABLE:		2	0	0	1	0
--MINERALIZATION, TUBULE			14	0	25	28	21
--MINERALIZATION, PELVIS			12	0	14	17	22
--INFLAMMATION, SUPPURATIVE, PELVIS			6	0	5	7	5
--DILATATION, PELVIS			1	0	0	1	0
--DILATATION, TUBULE			1	0	0	0	0
--CYST			2	0	0	0	0
--HYPERPLASIA, TRANSITIONAL CELL, PELVIS			2	0	3	3	1
--INFLAMMATION, CHRONIC ACTIVE			3	0	0	1	0
--NEPHROPATHY, CHRONIC PROGRESSIVE			21	0	28	22	24
--CAST, PROTEINACEOUS			4	0	3	4	2
--FIBROSIS, CORTEX			2	0	2	0	1
--B-LIPOMA			0	0	0	1	0

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-106 DEATH=T;FIND=ALL;SUBSET=ALL	SEX:	FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	35	35	29	34	32

KIDNEY (KD) ..... NUMBER EXAMINED:	35	0	29	34	32
NOT REMARKABLE:	7	0	3	4	2
--INFILTRATE, LYMPHOPLASMACYTIC	0	0	0	0	1
--MINERALIZATION, PELVIS	19	0	23	26	26
--CYST, TUBULE	6	0	3	3	4
--NEPHROPATHY, CHRONIC PROGRESSIVE	2	0	1	0	0
--MINERALIZATION, TUBULE	2	0	1	2	5
--VACUOLATION, TUBULAR CELL	0	0	0	0	1
--DEGENERATION, TUBULAR	3	0	4	7	4
--INFLAMMATION, PELVIS	0	0	0	1	4
--HYPERPLASIA, TUBULAR	10	0	1	8	11
--DILATATION, PELVIS	1	0	0	0	2
--DILATATION, TUBULES	1	0	1	9	6
--PERIARTERITIS	1	0	0	0	0
--HYPERPLASIA, UROTHELIAL	2	0	3	10	6
--B-ADENOMA, TUBULAR CELL	0	0	0	0	1
--INFLAMMATION, ACUTE	0	0	1	0	0

Toxicokinetics

Systemic exposures increased with doses from 150 to 3000 mg/kg/day. The increases in Cmax and AUC were less than dose-proportional. Marked (> X2) sex differences were observed in AUC at 3000 mg/kg/day on Day 1. Cmax and AUC were similar for males and females. Drug accumulation occurred after multiple dosing (see below).

Table 14 - Toxicokinetics in 2-year oral rat carcinogenicity study

Group	Dose Level (mg/kg/day)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$t_{1/2}$ (hr)	CL/F (mL/hr/kg)
6	150	M	Mean	286	1.91	1.00	2527	16.8	NA	NA
			SD	56	0.38	0	720	4.8	NA	NA
			N	3	3	3	3	3	0	0
		F	Mean	418	2.78	5.67	4509	30.1	10.6	42095
			SD	137	0.91	5.69	1854	12.4	NA	NA
			N	3	3	3	3	3	1	1
7	1000	M	Mean	1151	1.15	1.00	13477	13.5	8.02	79183
			SD	129	0.13	0	4293	4.3	NA	NA
			N	3	3	3	3	3	2	2
		F	Mean	1317	1.32	2.00	13367	13.4	7.68	75645
			SD	416	0.42	1.73	4982	5.0	NA	NA
			N	3	3	3	3	3	2	2
8	3000	M	Mean	3158	1.05	6.67	50070	16.7	7.02	96288
			SD	2072	0.69	4.62	37782	12.6	NA	NA
			N	3	3	3	3	3	2	2
		F	Mean	2453	0.818	8.33	25484	8.49	NA	NA
			SD	223	0.074	6.35	11590	3.86	NA	NA
			N	3	3	3	3	3	0	0

Group	Dose Level (mg/kg/day)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$t_{1/2}$ (hr)	CL/F (mL/hr/kg)
6	150	M	Mean	494	3.29	1.00	2695	18.0	6.62	50926
			SD	159	1.06	0	618	4.1	NA	NA
			N	3	3	3	3	3	2	2
		F	Mean	492	3.28	1.00	3056	20.4	11.3	39803
			SD	122	0.81	0	525	3.5	NA	NA
			N	3	3	3	3	3	1	1
7	1000	M	Mean	1692	1.69	1.00	12463	12.5	6.81	54242
			SD	776	0.78	0	4288	4.3	NA	NA
			N	3	3	3	3	3	1	1
		F	Mean	1472	1.47	1.00	17256	17.3	7.35	70066
			SD	466	0.47	0	4249	4.2	NA	NA
			N	3	3	3	3	3	1	1
8	3000	M	Mean	3269	1.09	8.00	46670	15.6	10.7	136676
			SD	1492	0.50	4.00	26767	8.9	NA	NA
			N	3	3	3	3	3	1	1
		F	Mean	2514	0.838	1.00	24318	8.11	NA	NA
			SD	442	0.147	0	6331	2.11	NA	NA
			N	3	3	3	3	3	0	0

Dosing Solution Analysis
Unremarkable.

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Study title: **Intravenous Fertility and General Reproductive Toxicity Study of RWJ-270201-(b) (4) in Male Rats**

Key study findings

- The objective of study was to evaluate: (1) male reproductive functions, mating behavior and fertilization; (2) libido and epididymal sperm maturation that may not have been detected by histopathology of male rat reproductive organs in repeat-dose studies.
- The results of this study showed that no male reproductive toxicities were observed in this iv study (NOAEL designated at the high dose, 600 mg/kg). No TK was performed. Based on a separate 7-day iv study, in which the AUC for 200 mg/kg in males was 355 ug.h/ml, the estimated AUC for 600 mg/kg should be approximately 1065 ug.h/ml (355x3=1065 ug.h/ml).

Study no.: DS99307
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/30/1999
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: S99-0060/99.7

Methods

Doses: 0, 50, 200, 400, and 600 mg/kg/day; 10 weeks before cohabitation, through cohabitation (max 21 days) till day before sacrifice

Species/strain: Crl:CDBR VAF/Plus(SD) male rat
(M) 285-344/Age:67-73 days

Number/sex/group: 25 males/group (no TK group)

Route, formulation, volume, and infusion rate: IV (vehicle: NaCl 0.9%), 50 ml/kg, 2 ml/minute

Parameters and endpoints evaluated:

Clinical signs, BW/FC, necropsy: thoracic, abdominal and pelvic viscera; organ wt.: testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid) and prostate, plus sperm concentration and motility; histopathology: testes, left epididymis (corpus and caput), right epididymis, prostate and seminal vesicles (control and high dosage group); cohabitated females (untreated): sacrificed on DG 13; necropsy: thoracic/ abdominal/pelvic viscera, placentae, # of corpora lutea, implantation sites, viable/nonviable embryos). TK not evaluated.

Results

Mortality: 1 male each from the 200 and 400 mg/kg group died due to dosing/infusion procedures.

Clinical signs: Unremarkable.

Body weight: Unremarkable.

Feed Consumption: Unremarkable.

Necropsy: Unremarkable.

Fertility parameters (male reproductive tissues, mating/fertility index, corpora lutea, preimplantation loss, etc.):
No remarkable treatment related effects on sperm motility or counts, mating and histopathology of the testes, seminal vesicles, prostate and epididymides (neither for the Caesarean-sectioning parameters in cohabitated and untreated females, see table below):

Table 15 – Uterine findings in male rat fertility and reproductive IV toxicity study.

Daily Dose (mg/kg)	0 (Control)[a]	50[b]	200	400	600
No. of Pregnant Females	25	22	23	26	24
No. Aborted or with Total Resorption of Litter	0	0	0	0	0
Mean No. Corpora Lutea	18.2	18.4	17.7	18.2	17.4
Mean No. Implantations	15.5	15.6	15.6	15.5	14.7
Mean No. Live Conceptuses	15.0	15.1	15.2	14.9	13.9
Mean No. Resorptions	0.5	0.5	0.5	0.6	0.8

In conclusion: The male fertility NOAEL is 600 mg/kg/day.

Study title: **Intravenous Fertility and General Reproductive Toxicity Study of RWJ-270201-** (b) (4) **in Female Rats**

Key study findings

- No maternal and fetal toxicities were observed, up to the high dose of 600 mg/kg iv, in this rat fertility and general reproductive toxicity study. No TK was performed. The maternal and developmental NOAELs=600 mg/kg. Based on a separate 7-day iv study, in which the AUC for 200 mg/kg in females was 263 ug.h/ml, the estimated AUC for 600 mg/kg should be 789 ug.h/ml (263x3=789 ug.h/ml).

Study no.:	DS99309
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/11/1999
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	S99-0072/99.5

Methods

Doses:	0, 50, 200, 400, and 600 mg/kg/day; 15 days before cohabitation with undosed males through cohabitation (max of 21 days) until DG7
Species/strain:	Crl:CDBR VAF/Plus (SD) female rat (F) 145-235g/68 days
Number/sex/group:	25 pregnant females/group
Route, formulation, volume, and infusion rate:	IV in NaCl 0.9%, 50 ml/kg, 2 ml/min; GD 6-17
Parameters and endpoints evaluated:	Caesarean-sectioned and gross necropsy (GD 13): thoracic/abdominal/pelvic viscera, # of corpora lutea, #/position (*in utero)* of implantations (viable/nonviable embryos). No TK was performed.

Results

Mortality: 1 female each died from the control, 50, 200 mg/kg groups, and 2 females died in the 600 mg/kg group, due to dosing errors or non-drug related causes.

Clinical signs: Unremarkable.

Body weight: Unremarkable.

Feed Consumption: Unremarkable.

Necropsy: Unremarkable.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

No treatment related effects on estrous cycling, mating performance, fertility, reproductive and litter parameters were reported. Copulation rates were 100% and pregnancy rates 95.8%, 100.0%, 96.0%, 96.0% and 91.3% for the 0, 50, 200, 400 and 600 mg/kg/day, respectively. No maternal toxicity was observed at 600 mg/kg/day (MFD).

Table 16 - Uterine findings in female rat fertility and reproductive IV toxicity study.

Daily Dose (mg/kg)	0 (Control)	50	200	400	600
No. of Pregnant Females	23	24	24	24	21
No. with Total Resorption of Litter	0	0	0	0	0
Mean No. Corpora Lutea	16.2	15.9	16.1	15.5	15.6
Mean No. Implantations	15.4	15.2	15.2	15.0	15.4
Mean % Preimplantation Loss	5.4	4.8	7.7	4.0	1.5
Mean No. Live Conceptuses	14.6	14.1	14.3	14.4	14.5
Mean No. Resorptions	0.8	1.1	1.0	0.7	0.8
No. Dead Conceptuses	Not done, C-Sections performed on Day 13.				
Mean % Postimplantation Loss	5.4	8.1	6.5	4.2	5.1

In conclusion: The female fertility and reproductive toxicity NOAEL is 600 mg/kg/day. No teratogenic effects were observed in this study.

Study title: **Intravenous dosage-range developmental toxicity study of RWJ 270201** (b) (4) **in rats**

Key study findings

- No developmental toxicities were observed in this dose-range finding iv rat EFT study. No TK was performed. The developmental NOAEL was 600 mg/kg. No AUC was available. However, by using a separate 7-day iv study at 200 mg/kg, the AUC (263 ug.h/ml) could be extrapolated to 600 mg/kg dose as 789 (263x3) ug.h/ml, assuming a linear TK.

Study no.: DS99402
Conducting laboratory and location: (b) (4)
Date of study initiation: 2/21/1999
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: E/100.8
Methods

Doses:	0, 200, 300, 400, 500, 600 mg/kg/day; GD 6-17
Species/strain:	Rat/ Crl:CD®BR VAF/Plus® Rat (F, DG0) 217-247g/60 days
Number/sex/group:	8/group
Route, formulation, volume, and infusion rate:	IV in NaCl 0.9%, 50 ml/kg, 2 ml/min; GD 6-17
Parameters and endpoints evaluated:	Gross pathology; evidence of implantation; number of corpora lutea. No TK was performed.

Results

Mortality: 1 pregnant female each died in 400 and 500 mg/kg groups (due to unknown causes).

Clinical signs: Unremarkable.

Body weight: Unremarkable.

Feed Consumption: Unremarkable.

Necropsy: Unremarkable.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
Pregnancy rates were 8/8 for all groups except the 600 mg/kg group (7/8). The number of litters evaluated was 8, 8, 8, 7, 7, and 7 for the 0, 200, 300, 400, 500, and 600 mg/kg group, respectively. Reproductive parameters (numbers of corpora lutea, implantations, fetuses, resorptions or pre- and postimplantation loss), fetal weights or external alterations were not affected by treatment.

Table 17 - Uterine findings in dose-rangefinding developmental IV toxicity study in rats.

Daily Dose (mg/kg)	0 (Control)	200	300	400	500	600
Mean No. Corpora Lutea	18.5	15.5	16.5	18.3	17.1	16.8
No. Pregnant	8	8	8	7	7	7
Mean No. Implantations	14.8	14.6	14.8	14.4	15.3	15.0
Litters:						
No. Litters Evaluated	8	8	8	7	7	7
Mean No. Live Fetuses	14.5	14.4	14.6	14.0	14.7	14.1
Mean No. Resorptions (Early/Late)	0.1/0.1	0.2/0	0.1/0	0.4/0	0.6/0	0.7/0.1
No. of Litters with Dead Fetuses	0	0	0	0	0	0
Mean Fetal Body Weight (g)	5.22	5.28	5.24	5.3	5.3	5.37
Fetal Sex Ratios (Mean % live male fetuses)	43.6	51.1	49.9	55.5	49.1	56.0
Fetal Anomalies:						
Gross External (No. Litters/No. Fetuses Affected)						
Body – left hindpaw, extra digit	0/0	0/0	0/0	1/1	0/0	0/0

In summary, no developmental toxicities were detected up to 600 mg/kg in this dose-range finding iv study in the rat.

Study title: **Intravenous developmental toxicity study of RWJ 270201** (b) (4) **in rats**

Key study findings

- No remarkable toxicities were observed, up to 600 mg/kg, in this definitive developmental study performed in rats). The developmental NOAEL was designated at 600 mg/kg, AUC=789 ug.h/ml, as based on a separate 7-day iv study, in which the AUC for 200 mg/kg in females was 263 ug.h/ml.

Study no.: DS99316
Conducting laboratory and location: (b) (4) Pennsylvania
Date of study initiation: 6/13/1999
GLP compliance: Yes
QA report: Yes

Drug, lot #, and % purity: S99-0071/99.4

Methods

Doses: 0, 200, 400, and 600 mg/kg/day; GD 6-17

Species/strain: Crl:CDBR VAF/Plus Rat, (F) 166-218g/66-days old

Number/sex/group: 25(pregnant)/gp, TK (study# DM99307):6/gp.

Route, formulation, volume, and infusion rate: IV in NaCl 0.9%, 50 ml/kg, 2 ml/minute; GD 6-17

Parameters and endpoints evaluated: Gross pathology; implantation; # of corpora lutea.

Results

Mortality: 1 female died in control and 400 mg/kg groups (with normal necropsy).

Clinical signs: Unremarkable.

Body weight: Unremarkable.

Feed Consumption: Unremarkable.

Necropsy: Unremarkable.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
No remarkable findings were found in uterus, implantation and litter parameters among all groups.
No significant fetal abnormalities were observed.

Table 18 - Uterine findings in developmental IV toxicity study in rats.

Daily Dose (mg/kg)	0 (Control)	200	400	600
Mean No. Implantations	14.5	15.2	14.6	14.8
Mean % Preimplantation Loss	16.9	12.2	17.5	12.9 [a]
Litters:				
No. Litters Evaluated	24	24	22	24 [a]
Mean No. Live Fetuses	14.1	14.6	13.5	14.2
Mean No. Resorptions	0.4	0.7	1.1 [s]	0.5
No. of Litters with Dead Fetuses	0	0	0	0
Mean % Postimplantation Loss	2.7	4.8	7.6	3.5
Mean Fetal Body Weight (g)	5.13	5.18	5.15	5.16
Fetal Sex Ratios (Mean % live male fetuses)	50.0	45.9	49.0	48.2
% Fetuses with any Gross Anomaly	0.0	0.6	0.0	0.3
% Litters with any Gross Anomaly	0.0	8.3	0.0	4.2
% Litters with any Soft Tissue Anomaly	16.7	4.2	4.5	0.0
% Fetuses with any Soft Tissue Anomaly	2.4	0.6	0.7	0.0
% Litters with any Skeletal Anomaly	20.8	29.2	18.2	16.7
% Fetuses with any Skeletal Anomaly	3.5	5.5	2.6	3.4

Plasma drug concentrations showed dose-related increases, and there were no significant differences in levels between GD 6 and 17 (see below). No AUC was available.

Table 19 – Plasma concentration of peramivir (5-min post-dosing) in developmental IV toxicity study in rats

Summary of Mean (SD) Plasma Concentrations for RWJ-270201-000 in Pregnant Female Rats (N=6) on Gestation Days 6 and 17 Following Daily Intravenous Doses (0, 200, 400, or 600 mg/kg) of RWJ-270201 (b) (4) (DM99307)

Gestation Day	Dose (mg/kg/day)	RWJ-270201 Concentration (ng/mL)
6	0	0 (0)
	200	208153 (32001)
	400*	510613 (175944)
	600	592756 (174666)
17	0	0 (0)
	200	248297 (24584)
	400*	491066 (24903)
	600	755810 (258576)

* N=4

Study Title: **A Continuous Intravenous Infusion Developmental Toxicity Study of RWJ-270201 (b) (4) in Rats**

Key study findings

- In this continuous iv infusion study, there were treatment-related fetal anomalies occurred at 400 and 1000 mg/kg dose groups. The anomalies were reduced renal papilla(e) and dilated ureters, which could be considered as evidence of delayed development of the urogenital system, in the absence of maternal toxicity. The drug concentration at 400 and 1000 mg/kg doses were 29.8 and 61.5 ug/ml, respectively. The NOAEL for fetal anomalies was 50 mg/kg (AUC=84 ug.hr/ml).

- No other remarkable findings in regard to malformations, skeletal anomalies or skeletal variants were observed. Maternal NOAEL=1000 mg/kg (AUC=1475 ug.hr/ml).

Study no.: DS00312
Volume # and page #: 73.1-73.3
Conducting laboratory: (b) (4)
Date of study initiation: 8/28/2000
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: S99-0217/99.6
Methods
Doses: 0, 50, 400 and 1000 mg/kg/day
Species/strain: 100 Mated female Rat/Crl:CD®(SD) IGS BR,VAF/Plus®

Number/sex/group:	25/group (+TK satellite group: 6/group)
Route, formulation, volume, and infusion rate:	Continuous infusion (24 hr/day) from GD 6-17, inclusive; 0.5 ml/kg/hour; Vehicle:0.9% NaCl**.**
Parameters and endpoints evaluated:	# implantation/corpora lutea, fetal abnormalities (external, visceral and skeletal examinations, GD21).

Results

Mortality: None.

Clinical signs: Unremarkable.

Body weight: Unremarkable.

Feed Consumption: Unremarkable.

Necropsy: Unremarkable.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
The numbers of corpora lutea, implantation sites, live fetuses, dead fetuses, resorptions, the sex ratio and the pre- and postimplantation losses were unremarkable. Male, female and total (combined) fetal weights were comparable to controls.

There were significant dose-related increases in incidences of reduction of the renal papilla(e) and dilatation of the ureters. The sponsor stated that these were indications of dose-related delays in the development of the urinary tract, and that the teratological importance of this finding is unclear. No other remarkable findings in regard to malformations, skeletal anomalies or skeletal variants were observed.

Table 20 – Uterine findings in developmental toxicity study on peramivir in rats (continuous IV infusion)

Daily Dose (mg/kg)	0 (Control)	50	400	1000
Mean No. Corpora Lutea	17.6	16.5	17.5	17.9
Mean No. Implantations	16.1	15.0	15.8	16.4
Mean % Preimplantation Loss	8.4	9.1	9.4	8.1
Litters:				
No. Litters Evaluated	25	24	24	24
Mean No. Live Fetuses	14.9	14.3	15.0	15.5
No. Litters with Total Resorption	1	0	0	0
Mean No. Resorptions	1.2	0.8	0.8	0.9
No. of Litters with Dead Fetuses	0	0	0	0
Mean % Postimplantation Loss	7.6	5.1	5.2	5.5
Mean Fetal Body Weight (g)	5.06	5.01	5.04	5.05
Fetal Sex Ratios (mean % live male fetuses)	50.3	42.5 [d]	52.2	54.0
Kidney – Reduction of renal papilla(e) [g]	0/0	1/1	4/6	5/5
Ureters – Dilated [h]	6/8	10/16	10/24 [i]	13/31 [j]
Megaureter [g]	2/2	2/3	5/5	8/10 [i]

g Significant Linear Trend Test (One-Sided Cochran-Armitage Test, Litter Data) p <0.01
h Significant Linear Trend Test (One-Sided Cochran-Armitage Test, Litter Data) p <0.05
i Significantly different from controls (Logistic Regression, Bonferroni Adjusted) p<0.05
j Significantly different from controls (Logistic Regression, Bonferroni Adjusted) p<0.01

Systemic exposures were evident at 24 hours postdose on GD6 and prior to the end of infusion on GD17 in pregnant rats at all dose levels. The increases in exposure levels were dose-related, for both GD6 and GD17, and there were slight increases in drug levels from GD6 to GD17. For the 50 and 1000 mg/kg dose groups, drug concentration prior to end of infusion was 3.5 and 62 ug/ml, respectively, which provided estimated AUC values of 84 ug.hr/ml and 1475 ug.hr/ml.

Table 21 - Plasma concentration of peramivir in developmental toxicity study in rats (continuous IV infusion)

Gestation Day	Dose (mg/kg/day)	RWJ-270201 Conc (ng/mL)
6[a]	0	0.00 (0.00)
	50	2487 (863)
	400	20480 (4396)
	1000	46866 (4417)
17[b]	0	0.00[c] (0.00)
	50	3490 (462)
	400	29824 (4820)
	1000	61471 (3639)

[a] Collected 24 hours following onset of infusion
[b] Collected just prior to the end of infusion
[c] N = 5

The study report concluded that the fetal NOAEL was 1000 mg/kg/day but the sponsor assigned it as 50 mg/kg based on delayed development of the urogenital system observed at 400 and 1000 mg/kg/day.

Study title: **Intravenous dosage-range developmental toxicity study of RWJ 270201 (b) (4) in rabbits**

Key study findings

- Severe maternal toxicity (nephrotoxicity; lethal dose=300 mg/kg, sublethal 200 mg/kg) occurred in this rabbit dose-ranging developmental toxicity study at all doses tested (200-600 mg/kg). Fetal toxicity, as reflected by body weight loss, also occurred at all doses.
- No teratogenicity findings were observed. There was no NOAEL for either maternal or fetal toxicity.

Study no.:	DS99403
Conducting laboratory and location:	(b) (4) PA
Date of study initiation:	2/17/1999
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	E/100.8

Methods

Doses:	0, 200, 300, 400, 500, 600 mg/kg/day; GD 7-19
Species/strain:	[Hra:NZW)SPF] Rabbits, 2.5-5.5kg/5-7mos
Number/sex/group:	5 females/group (No TK)
Route, formulation, volume, and infusion rate:	IV in NaCl 0.9%, 50 ml/kg, 2 ml/minute
Parameters and endpoints evaluated:	Gross pathology, evidence of implantation, number of corpora lutea, fetal abnormalities (external, visceral and skeletal examinations, DG29).

Results

Mortality: Drug-related deaths or moribundities occurred, with the following frequencies: 0, 0, 3, 3, 5, and 3 for the 0, 200, 300, 400, 500, and 600 mg/kg groups, respectively (lethal dose was ≥300 mg/kg). For the 600 mg/kg group, the remaining two animals were aborted and sacrificed (one moribund, the other sacrificed due to aborting status). For the 300 mg/kg group, 1 more animal was sacrificed moribund, in addition to 3 that were already dead. No animals left in the 500 and 600 mg/kg groups at scheduled sacrifice. Animals left for scheduled sacrifice were 5, 5, 2, 2, 0, 0 for the 0, 200, 300, 400, 500, 600 mg/kg/day dosage, respectively.

Clinical signs: Increased incidences of decreased feces were observed in all drug-treated groups. There were also absence of urine and labored breathing observed in animals of drug- treated groups.

Body weight: Body weight loss was observed for all drug treated groups during treatment (no dose response relationship). There were weight gains after dosing ended in all drug-treated groups (which were still alive).

Feed Consumption: It was reduced in a dose-related manner during treatment (post-treatment was comparable to control for all groups except it was lower for the 200 mg/kg/day).

Necropsy: Postmortem findings included pale and/or white, mottled kidneys in animals that died, were sacrificed moribund, or survived to scheduled sacrifice. Histopathology showed dose-related increases in severity of renal toxicity (see table below). The profile of toxicities included tubular nephrosis (correlated with pale, mottled kidneys), cortical tubular epithelial necrosis, and glomerulopathy (associated with the tubular nephrosis, observed more frequently in 200 and 300 mg/kg/day groups). In addition, increases in BUN (≈2X-3X), creatinine, and GGT were observed for all surviving drug-treated animals. Other postmortem findings included fluid in the thoracic and/or abdominal cavity and discolored lungs.

Table 22 - Necropsy findings in dose-rangefinding developmental IV toxicity study in rabbits

Daily Dose (mg/kg)	0 (Control)	200	300	400	500	600
Kidney						
Bilateral, Pale or Pale White	0	3	4	4	5	3
Slightly Mottled or Mottled	0	0	0	2	1	0
Stomach, Mucosal Surface, Red Area	0	0	0	0	0	1
Liver, Brown or Discolored Area	0	0	1	0	0	1
Abdominal Cavity, Clear to Red Fluid	0	0	0	3	5	0
Thoracic Cavity, Clear to Yellow Fluid	0	0	0	3	5	0
Lungs,						
Numerous White Areas, Red Foci or Red Areas	0	0	0	2	3	0

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
Pregnancy rates were 4/5 for the control group and 5/5 for all other groups. No animal resorbed their entire litter or had dead fetuses. Fetal weights were reduced in a dosage-dependent manner. No other significant differences were observed for reproductive parameters measured (i.e., number of corpora lutea, implantation sites, live fetuses, pre and post-implantation loss, and early and late resorptions) or fetal alterations.

Table 23 - Uterine findings in dose-rangefinding developmental IV toxicity study in rabbits

Daily Dose (mg/kg)	0 (Control)	200	300	400	500	600
Maternal Body Weight[b], kg (%)						
DG 29[f]	4.31	-11.6	-10.4	-6.4	Not Done	Not Done
DG 29 wt. minus uterine wt.[f]	3.86	-11.4	-8.0	-4.1		
Food Consumption[d], g/day (%)						
DG 7-29[f]	178.4	-37.2	-38.3	-44.8	Not Done	Not Done
Caesarean-Section Observations[e]					Not Done	Not Done
Mean No. Corpora Lutea	9.5	9.2	11.0	13.5		
Mean No. Implantations	8.2	7.6	10.5	12.0		
Mean No. with Total Resorptions	0	0	0	0		
Litters:						
No. Litters Evaluated	4	5	2	2	0	0
Mean No. Live Fetuses	8.0	7.6	6.5	11.5		
No. of Litters with Dead Fetuses	0	0	0	0		
Mean Fetal Body Weight (g)	44.0	41.7	40.2	39.4		
Fetal Anomalies:						
Gross External						
Tail, constricted						
% Litters	0.0	0.0	0.0	50.0		
% Fetuses	0.0	0.0	0.0	4.3		

TK analysis on a separate 14-day oral/iv PK study in rabbits showed that on day 14, the drug exposures after 10 mg/kg had systemic drug exposures (AUC0-24hr) of 0.2 (po) or 34/40 (iv) ug.h/ml (male/female). Oral bioavailability of the drug in rabbits in day 1 and 14 were estimated at 2.6/11.8% and 4.6/5.4% respectively (male/female).

In conclusion, the drug caused dose-related maternal (i.e., renal) and fetal (BW reductions) toxicity at all doses studied while no significant adverse teratogenicity findings had been found.

Study title: **Intravenous developmental toxicity study of RWJ 270201** (b) (4) **in rabbits**

Key study findings

- Maternal nephrotoxicity may have contributed to the abortions and other clinical effects (body weight losses, and decreased feed consumption) seen in the dams.
- NOAEL for developmental toxicities was 200 mg/kg (no AUC, 5-min post-dosing mean drug level = 64.8 ug/ml) while there was ongoing nephrotoxicity in dams.
- Maternal NOAEL=50 mg/kg/day (1 maternal death, 3 aborted or delivered early at 100 mg/kg/day), with 5-min post-dosing mean drug level of 64.8 ug/ml.
- No teratogenic effects were observed. No AUC was available because drug concentrations were measured at one time-point, at which it was not steady-state (5-min post-dosing).

Study no.: DS99317
Conducting laboratory and location: (b) (4)

(b) (4)

Date of study initiation: 6/14/1999
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: S99-0071/99.4

Methods

Doses:	0, 25, 50, 100 and 200 mg/kg/day on Gestation Day (GD) 7-19
Species/strain:	Hra:NZW SPF Rabbits (F)3.0-4.6kg/6 mos
Number/sex/group:	25 pregnant females/group (no TK group)
Route, formulation, volume, and infusion rate:	IV in NaCl 0.9%, 50 ml/kg, 2 ml/minute
Parameters and endpoints evaluated:	Gross pathology (GD 29): evidence of implantation, number of corpora lutea, fetal abnormalities (external, visceral and skeletal examinations)(TK reported under DM99308).

Results

Mortality: Two died in the 200 mg/kg group (1 on Day 1 of dosing and the other on GD 27 [8 days after dosing]) and one died in the 100 mg/kg/day group (GD 9). Cause of death was unknown.

Clinical signs: Abnormal feces (scant or no feces, dried, soft or liquid) and emaciation in the 200 mg/kg were considered drug related.

Body weight: Significant body weight losses (200 mg/kg, during dosing period) were observed. During post-dosage period, there were BW gains.

Feed Consumption: Absolute and relative feed consumption (200 mg/kg, during dosing period) were reduced whereas during post-dosage period, feed consumption was increased.

Necropsy: Pale cortex of the kidneys was found in 2 rabbits (200 mg/kg)(similar finding was observed at this dosage level in the dosage range-finding study [see above]).

Table 24 - Necropsy findings on kidney in developmental IV toxicity study in rabbits

Daily Dose (mg/kg)	0 (Control)	25	50	100	200
Kidney					
Left pelvis, calculi	0	0	1	0	0
Cortex pale, bilateral	0	0	0	0	2

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
Increased incidences of abortions occurred at 100 and 200 mg/kg.

Table 25 – Survival and TK in developmental IV toxicity study in rabbits

Daily Dose (mg/kg)	0 (Control)	25	50	100	200
Dams/Does:					
Toxicokinetics: Plasma Concentration (ng/mL) at 5 min [b c]					
DG 7	0.44 [d e]	31,962 [e]	86,021	119,965	321,633
DG 19	1.44 [d e]	27,096 [e]	64,833	137,985	476,255 [e]
No. Mated Females	25	25	25	25	25
No. Pregnant [f]	23 [g]	23 [h]	22 [i]	22	20 [j]
No. Died or Sacrificed Moribund	0	0	0	1 [k]	2 [l]
No. Aborted or Delivered Early	0	0	0	3 [m]	2 [n]

Reproductive and litter parameters were significantly affected. There were increases in % fetal skeletal anomalies (e.g., skull irregular ossifications) at 50 mg/kg, but the sponsor indicated the findings were not dose-related (the number of fetus and litters examined were sufficient for the mid-dose and high-dose groups considering they were lethal doses and thus supportive of sponsor's non-dose-related claim). No other significant gross external, soft tissue or skeletal malformations or variations in the fetuses that were considered to be drug-related.

Table 26 - Uterine findings in developmental IV toxicity study in rabbits

Daily Dose (mg/kg)	0 (Control)	25	50	100	200
Mean No. Corpora Lutea	10.1	9.7	9.9	10.6	9.6
Mean No. Implantations	9.5	8.7	8.8	9.7	8.1
Mean No. with Total Resorptions	0	0	0	0	0
Mean % Preimplantation Loss	6.4	9.4	10.7	8.9	17.4 [o]
Litters:					
No. Litters Evaluated	23	23	22	18	16
Mean No. Live Fetuses	9.3	8.6	8.5	9.5	7.8
Mean No. Resorptions	0.2	0.1	0.3	0.2	0.3
No. of Litters with Dead Fetuses	0	0	1	0	0
Mean % Postimplantation Loss	1.5	1.4	3.5	1.7	2.5
Mean Fetal Body Weight (g)	42.76	44.04	44.15	42.66	44.18
Fetal Sex Ratios (% Live Male Fetuses)	46.1	44.2	51.8	45.4	54.4

(Table 26 - Cont'd)

Daily Dose (mg/kg)	0 (Control)	25	50	100	200
% Fetuses with any gross external alteration	0.5	0	0	0	0
Visceral Anomalies	No compound related Anomalies				
% Litters with any soft tissue alteration	8.7	4.3	13.6	5.6	0
% Fetuses with any soft tissue alteration	0.9	0.5	1.6	0.5	0
Skeletal Anomalies (No. Litters/No. Fetuses with finding)					
Thoracic vertebrae - Hemivertabra	0/0	0/0	1/1	1/1	0/0
Lumbar vertebrae - Hemivertabra	0/0	0/0	0/0	0/0	1/1
Ribs –					
Fused	0/0	0/0	0/0	0/0	1/1
Split	0/0	0/0	0/0	1/1	0/0
Thickened	0/0	0/0	0/0	0/0	1/1
% Litters with any skeletal anomaly	30.4	39.1	59.1	38.9	25.0
% Fetuses with any skeletal anomaly	5.6	6.6	11.8 [$]	4.7	5.6
Total Affected Fetuses (Litters) – Any anomaly	14(8)	14(9)	25(15)	9(7)	7(4)
% Litters with Any Anomaly	34.8	39.1	68.2	38.9	25.0
% Fetuses with Any Anomaly	6.5	7.1	13.4 [$]	5.3	5.6

There were dose-related increases in plasma concentration for both GD7 and GD19, and there were no apparent differences in plasma concentrations between GD7 and GD19. No AUCs could be derived because only single post-infusion (5-minutes) levels were measured, at which it was not at steady-state.

Table 27 - Plasma concentrations of peramivir in developmental IV toxicity study in rabbits

Summary of Mean (SD) Plasma Concentrations for RWJ-270201-000 in Pregnant Female New Zealand White Rabbits (N=6) on Gestation Days 7 and 19 Following Daily Intravenous Doses (0, 25, 50, 100, or 200 mg/kg) of RWJ-270201 (b) (4) (DM99308)

Gestation Day	Dose (mg/kg/day)	RWJ-270201 Concentration (ng/mL)
7	0[a]	0.44[c] (0.89)
	25[a]	31962 (14117)
	50[b]	86021 (25147)
	100[b]	119965 (33468)
	200[b]	321633 (60204)
19	0[a]	1.44[c] (1.07)
	25[a]	27096 (10343)
	50[b]	64833 (6332)
	100[b]	137985 (34855)
	200[a]	476255 (101202)

[a] N=4
[b] N=5
[c] Although values were measured, they were determined to be endogenous interference peaks of such small magnitude that they do not impact the values for the remaining dose groups

Concentrations measured at 5 minutes post-infusion.

In conclusion, maternal NOAEL=50 mg/kg/day (1 maternal death, 3 aborted or delivered early at 100 mg/kg/day) with post-infusion (5-minutes) drug level of 64.8 ug/ml. Maternal toxicity at 200 mg/kg/day included death, abortions, increased incidences of clinical signs, body weight losses or reduced body weight gain, and decreased feed consumption. No teratogenic effects were observed (Fetal NOAEL=200 mg/kg). The original study report concluded that the maternal NOAEL=100 mg/kg/day, for which the post-infusion (5-minutes) level was 38 ug/ml. This NOAEL was corrected in sponsor's integrated summary as 50 mg/kg.

Study title: Reproduction Toxicity Study of RWJ-270201 (b) (4) in Rats, Including a Postnatal Behavioral/Functional Evaluation

Key study findings

- Maternal and developmental NOAELs were 600 mg/kg/day for this postnatal reproductive toxicity in rats. Same NOAELs were applicable for the F1 generation males and females. No TK was performed. The AUC could be approximated at 789 ug.h/ml, as based on a separate 7-day iv study, in which the AUC for 200 mg/kg in females was 263 ug.h/ml.

Study no.: DS99310
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: 5/2/1999
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: S99-0072/99.5

Methods

Doses:	0, 50, 200, 400, 600 mg/kg/day; GD 6 to DL 20 (day 20 of lactation) or to GD24 (if dam did not deliver a litter)(DL 1=day of delivery)
Species/strain:	Crl:CD IGS BR VAF/Plus Rat 236-277g/83days
Number/sex/group:	25 pregnant rats/group (No TK group)
Route, formulation, volume, and infusion rate:	IV in NaCl 0.9%, 50 ml/kg, 2 ml/minute; GD 6-17

Parameters and endpoints evaluated:
F0: clinical signs, BW/FC, mating, litter size/pup viability, fertility/gestation indices, lactation/ viability indices; F1 pups: viability, clinical signs, BW; F1 female pups necropsied (DL 21): thoracic/abdominal/pelvic viscera exams, #/distribution of implantation sites; F1 pups not selected for further study on DL 21: necropsied, single x-section at frontal-parietal suture level (exams for hydrocephaly); F1 rats: viability, clinical signs, BW/FC and reproductive capacity (age of vaginal patency or preputial separation), passive avoidance testing for learning, short-term retention and long-term retention, water maze testing for coordination, swimming ability, learning and memory; F1 males (post 21-day cohabitation period): thoracic/abdominal/pelvic viscera exams, testes/epididymides (weights); F1 females (GD21): thoracic/abdominal/pelvic viscera exams, corpora lutea number/distribution, implantation sites, live/dead fetuses, early/late resorptions, fetal sex and gross external alterations.

Results
F_0 Dams
No deaths. Clinical signs and changes in body weights, body weight gains, feed consumption, natural delivery and litter parameters were unremarkable.

F1 (preweaning) No deaths were reported. Observations in clinical or necropsy, and changes in body weights, or body weight gains, or absolute or relative feed consumption in the F1 generation rats were unremarkable. No treatment related effects on implantation, viability/lactation indices (see below).

Table 28 – Effects of Peramivir in Postnatal Reproduction Toxicity Study in Rats

Daily Dose (mg/kg)		0 (Control)	50	200	400	600
F_1 Litters: (Preweaning)						
	No. Litters Evaluated	21	25	24	24	24
	Mean No. of Implantations	17.0	17.0	17.1	16.4	16.5
	Mean No. Pups/Litter	15.8	15.9	16.0	15.4	14.8
	Mean No. Liveborn Pups/Litter	15.8	15.6	15.8	15.2	14.8
	No. of Litters with Stillborn Pups	2	1	2	3	0
	No. of Dams with No Liveborn Pups	0	0	0	0	0
	Postnatal Survival to Day 7 % (Viability Index)	91.2	96.4 [g]	93.4	94.0	99.2 [h]
	Postnatal Survival to Weaning % (Lactation Index)	98.3	98.1	98.3	98.2	100 [c]
	Pup Sex Ratios					
	% Male pups/Litter, Day 1	49.7	51.0	49.0	54.7	49.1
	Pup Necropsy Observations					
	Litters Evaluated [i]	20	25	23	24	24
	Pups Evaluated	249	320	302	301	301
	Appeared Normal [j]	249/20	319/24	302/23	300/23	300/23
	Thoracic and Abdominal Viscera, Pale	0/0	1/1	0/0	0/0	1/1
	Kidney – right pelvis, Slight dilation	0/0	0/0	0/0	1/1	0/0

F1 males behavioral (postweaning): Unremarkable (see below).

(Table 28 – Continued)

Daily Dose (mg/kg)		0 (Control)	50	200	400	600
F_1 Males: (Postweaning)						
	No. Evaluated Postweaning	25	25	25	25	25
	No. Died or Sacrificed Moribund	1	1	2	0	2
	Epididymides, small	0	1	0	1	0
	Kidneys, dilated pelvis, moderate	0	0	1	0	0
	Stomach – small amount of food or no food [1]	1	0	1	0	2
	Testes, small	0	1	0	1	0
	Sensory Function	Not evaluated				
	Motor Activity	Subjectively evaluated in water maze test.[m]				
	Learning and Memory	No remarkable findings in passive avoidance or water maze performance.				
	No. of Fertile Males	23	21	21	22	22

F1 females behavioral (postweaning): Unremarkable (see below). Mating and fertility indices, litter parameters were unremarkable. Litter averages for corpora lutea and implantations were unremarkable (see below).

(Table 28 – Continued)

Daily Dose (mg/kg)		0 (Control)	50	200	400	600
F_1 Females: (Postweaning)						
	No. Evaluated Postweaning	25	25	25	25	25
	No. Died or Sacrificed Moribund	1	1	1	0	0
	No. Delivered and Sacrificed	0	0	0	1[n]	0

(Table 28 – Continued)

	Necropsy Observations					
	Appeared Normal	24	25	24	24	25
	Kidneys, dilated pelvis, moderate	0	0	0	1	0
	Spleen, surface, numerous constricted areas	0	0	0	0	1

	Mean Age of Vaginal Patency (days)	32.2	31.9	32.5	32.7	32.3
	Sensory Function	Not evaluated				
	Motor Activity	Subjectively evaluated in water maze test.[In]				
	Learning and Memory	No remarkable findings in passive avoidance or water maze performance				
	Mean No. Days Prior to Mating	2.4	2.6	2.8	2.5	3.5
	No. of Females Sperm-Positive	24	24	24	25	25
	No. of Pregnant Females	23	21	22	23	24
	Mean No. Corpora Lutea	19.1	19.8	20.1	19.4	19.0
	Mean No. Implantations	16.0	17.1	15.9	16.7	16.1
	Mean % Preimplantation Loss	Not reported				

F_2 Generation
F2 litter parameters and fetal measurements were unremarkable.

(Table 28 – Continued)

Daily Dose (mg/kg)		**0 (Control)**	**50**	**200**	**400**	**600**
F_2 Litters:						
	Mean No. Live Conceptuses/Litter	15.6	16.4	15.4	16.4	15.8
	Mean No. Resorptions	0.4	0.7	0.5	0.4	0.3
	No. of Litter with Dead Conceptuses	0	0	0	0	0
	No. Dead Conceptuses	0	0	0	0	0
	Mean % Postimplantation Loss	2.3	5.0	3.3	2.1	2.1
	Fetal Body Weights (g)	5.18	5.09	5.20	5.13	5.18
	Fetal Sex Ratios (% males/ litter)	48.7	46.2	50.0	49.8	47.1
	Fetal Anomalies					
	No. Fetuses/No. Litter Examined	359/23	344/21	338/22	360/22	379/24

Conclusions: Maternal and developmental NOAELs were 600 mg/kg/day (MFD, based on formulation solubility) for this postnatal reproductive toxicity in rats. Same NOAELs were applicable for the F1 generation males and females. No TK was performed.

10 SPECIAL TOXICOLOGY STUDIES

Study Title: One-Month Intravenous Toxicity Study of Peramivir Trihydrate in Juvenile Rats

KEY FINDINGS:

- No target organ of toxicity was identified in this 4-week juvenile rat iv study, except that a moderate reduction of body weight was observed in the high dose group. The NOAEL was designated at 120 mg/kg (AUC=177 [male] or 158 [female] ug.h/ml).

Study no.:	S-021812-TF-110-L
File Location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	1/20/2009
GLP compliance:	yes
QA report:	yes
Drug, lot #, and % purity:	05P0487/100.8%

Methods

Doses:	Vehicle (NaCl 0.9%), 60, 120, or 240 mg/kg, 4 weeks
Species/strain:	Crl:CD(SD) rats
Number/sex/group:	16
Route, formulation, volume:	IV bolus, 10-50 ml/kg/3-12 mg/ml
Satellite groups used for toxicokinetics or recovery:	8/group (12/sex in control)
Age:	9days
Weight:	(M) 20.7-28.1; (F)19.2-25.8 g

Results:

Mortality: Checked 1/day. One middle dose female died (day 22) without significant findings and the event was not considered to be drug-related.

Clinical signs: Checked 1/day. Unremarkable in physical development (appearance of growth of hair, eruption of incisors or eyelid opening), early behavior (back righting) and genital development (preputial separation or vaginal opening).

Body weights: Measured at predose and at study termination. BW gain reduced by 6-7% in females at 240 mg/kg (Day 16-25).

Food consumption: Measured at predose and at study termination. Unremarkable.

Hematology: Measured at predose and at study termination. Unremarkable.

Clinical chemistry: Measured at predose and at study termination. Unremarkable.

Urinalysis: Measured at study termination. Unremarkable.

Gross pathology: Measured at study termination. Unremarkable.

Organ weights Unremarkable.

Histopathology: Adequate Battery: yes
Peer review: no
Histopathology exams were performed in control and high dose groups only (with the lung and kidneys that were also examined in either low or middle dose groups). The findings were unremarkable.

Toxicokinetics:
AUC increased dose-proportionally. AUCs on Days 14 and 28 were lower than those on Day 1, without gender differences. The T½ (Day 1)=2.3-2.6 hours (Days 14 & 28 =0.5-0.6 hrs at 60 and 120 mg/kg and 2.1-2.6 at 240 mg/kg). On Day 28, AUCs were 90, 177, and 323 µg.hr/ml for males and 79, 158, and 320 µg.hr/ml for females, respectively (see below).

Table 29 – Toxicokinetics of one-month IV Toxicity Study of peramivir in juvenile rats

	MALE			FEMALE		
Group	C_0 (µg/mL)	$T_{1/2}$ (h)	AUC_{0-24h} (µg·h/mL)	C_0 (µg/mL)	$T_{1/2}$ (h)	AUC_{0-24h} (µg·h/mL)
Low 60 mg/kg/day	206	0.5	90.1	229	0.6	78.9
Mid 120 mg/kg/day	389	0.5	177	427	0.5	158
High 240 mg/kg/day	899	2.6	323	912	2.1	320

Mean ± S.D. (n=4)
N.E.: Not estimated

Study Title: Single [Dose] Intravenous Toxicity Study of Peramivir Trihydrate in Juvenile Rats

KEY FINDINGS:
No target organ of toxicity was identified in this single-dose study. The NOAEL was defined as 240 mg/kg (AUC=456-482 ug.h/ml).

Study no.: S-021812-TF-109-L
File Location: EDR
Conducting laboratory: (b) (4)
Date of study initiation: 10/10/2008
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: 05P0487/100.8%

Methods
Doses: 0 (NaCl 0.9%), 10, 120, 240 mg/kg (14-day observ.)

Species/strain:	SPF Crl:CD(SD) rats
Number/sex/group:	8
Route, formulation, volume:	IV bolus, 20 ml/kg, 0.5-12 mg/ml
Satellite groups used for toxicokinetics or recovery:	8/group 12
Age:	9 & 21days old (4/group/9day old, 4/group/21day old)
Weight:	9 days (M)19.9-28.6 g; (F) 20.7-26.6g;21 days (M) 60.1-69.9 g

Results: No deaths occurred in this single dose study. Clinical signs were unremarkable, except that 1 male rat in the high dose group showed irregular respiration and incomplete eyelid opening (at 5 minutes post-injection) at 21 days of age. AUC increased dose-proportionally without gender differences.

Table 30 – Toxicokinetics of single-dose IV study in juvenile rats.

Dose (mg/kg)	AUC $_{0\text{-}24}$ µg·hr/mL			
Age:	9 days old		21 days old	
	Male	Female	Male	Female
10	59.4	58.9	21.4	20.6
120	696	629	298	285
240	1430	1290	482	456

Study title: **Four-Day Range-Finding Intravenous Nephrotoxicity Non-GLP Study of RWJ-270201** (b) (4) **in Rabbits**

Key study findings: This 4-day repeat dose special nephrotoxicity study on peramivir focused on defining an NOAEL and characterizing a more detailed renal toxicity profile in rabbits. Both doses tested (200 and 300 mg/kg) caused acute tubular necrosis with only 4-day dosing. Tubular necrosis was multifocal and that occurred in cortical area only. Intra-luminal tubular proteinaceous casts and multifocal tubular dilatation could also be found. Additionally, there were also tubular mineralizations (multifocal) in dilated tubules near corticomedullary junction (not associated with areas of acute necrosis). Tubular regeneration (multifocal) did occurred, which was characterized by increased cytoplasmic basophilia, mitoses, and piling up of lining epithelia. No NOAEL was available for this range-finding study.

Study no.:	**DS00417**
File Location:	EDR
Conducting laboratory and location:	The RWJPRI, Spring House, PA
Date of study initiation:	13 March 2000
GLP compliance:	No
QA report:	No
Drug, lot #, and % purity:	S99-0072/99.5
Methods	
Doses:	0, 200 or 300 mg/kg/day for 4 days
Species/strain:	Rabbit, NZW (M) 2.6-3.1 kg; (F) 2.8-3.2 kg; age: 3-5 month
Number/sex/group or time point (main study):	2/sex/group

Route, formulation, volume, and infusion rate: IV (in 0.9% NaCl), 16.7-25ml/kg/day /12 mg/ml

Satellite groups used for toxicokinetics or recovery: None

Results:

Mortality: None.

Clinical signs: Both high-dose males exhibited decreased food consumption on Day 3, one male having decreased feces on Day 4.

Body weights: Unremarkable.

Food consumption: Unremarkable.

Hematology: Decreases in RBC parameters (males, 300 mg/kg).

Clinical chemistry: Increased BUN, creatinine, sodium and phosphorus (correlated with renal pathology findings (see below). Higher serum ALT, AST, GGT, bilirubin, cholesterol, and triglycerides were also observed (suggestive of hepatic target organ effects).

Urinalysis: Lower urine volumes, higher urinary creatinine and ratios for excreted protein/Cr, ALP/Cr, GGT/Cr and NAG/Cr, and lower K/Cr ratios were observed (correlated with nephrotoxicity, see below).

Gross pathology: Pale, diffuse discoloration of the kidneys occurred in all treated rabbits.

Histopathology: Adequate Battery: No (kidneys only)

Peer review: No

Acute tubular necrosis (mild to marked) was seen in kidneys of all treated animals. Nephrosis was moderate (1 of 4 showed marked). Necrosis was multifocal and limited to cortical tubules. Tubular regeneration was also multifocal (also seen in 1 of 4 controls [2/sex]). Regenerative tubules showed increased cytoplasmic basophilia, mitoses, and piling up of lining epithelia. Intra-luminal tubular proteinaceous casts and multifocal tubular dilatation were seen in all drug-treated rabbits. Tubular mineralization was multifocal in 2 of 4, 1 of 4, and 4 of 4 rabbits in control, 200 mg/kg and 300 mg/kg groups, respectively. The mineralization was limited to dilated tubules near corticomedullary junction and not associated within areas of acute necrosis. No NOAEL was available for this range-finding study.

Study title: Fourteen-Day Intravenous Nephrotoxicity Study of RWJ-270201 (b) (4) in Rabbits with a Two-Week Recovery Period (See actual dosing periods and variance due to mortalities in Methodology)

Key study findings:

- This special toxicity study (using one dosage at 200 mg/kg) focused on time-dependent development of nephrotoxicity in rabbits with additional electron microscopy (EM) investigation. Acute tubular necrosis in both male and female rabbits could be elicited after just one day dosing of peramivir (at 200 mg/kg). The lesion (i.e., acute tubular necrosis) was more pronounced in males than females because greater drug exposures were observed in males as renal elimination of the drug has been compromised by nephrotoxicity. The renal toxicity was not reversible in males but showed reversibility in the females following the 2- and 4-week of recovery (study title reflected only 2-week recovery).
- In addition to nephrotoxicity, drug-induced hepatocellular vacuolation (hepatic fatty change in males only) and increases in AST, ALT, GGT and/or bilirubin were also observed in males after day 1 of dosing. Liver toxicity in females was reflected in clinical chemistry alterations without structural damage at the 200 mg/kg dose studied.

- Mean drug concentrations showed 519916 and 593783 ng/ml for males and females, respectively, after Day 1 dosing. However, on Day 7 the mean pre-infusion drug concentrations in males were 644065 ng/ml as compared to 4315 ng/ml in the females. No AUC was available because there was only one blood sampling time point.

Study no.: DS00432
File Location: EDR
Conducting laboratory: (b) (4)
Date of study initiation: 1/8/2000
GLP compliance: No
QA report: No
Drug, lot #, and % purity: S99-0217/99.6
Methods

Doses:	Vehicle (NaCl 0.9%) or 200 mg/kg for 14 days (original plan, actual dosing 9 days for males and 7 days for females due to mortalities)
Species/strain:	Rabbit/New Zealand White[Hra:(NZW)SPF]
Number/sex/group:	10 (control), 20 (200 mg/kg)
Route, formulation, volume:	IV (50 ml/kg, 2ml/min)
Satellite groups used for toxicokinetics or recovery:	No separate TK and no separate recovery group. TK done in Day 1 and 7
Age:	6 months
Weight:	(M) 2.5-3.9;(F) 3.0-4.0kg

Results:
Scheduled sacrifice was followed to evaluate rabbit-specific nephrotoxicity in this non-GLP study (one dosage at 200 mg/kg only). Two males from controls and four males from the 200 mg/kg/day group were randomly selected and sacrificed on days 2 and 4 (DS [day of sacrifice] 2 and 4). Two males per group were randomly selected and sacrificed on DS 8. All surviving males (4 from the control and 5 from the dosed group) were sacrificed on DS 10. Two females from control and four females from 200 mg/kg/day group were sacrificed on DS 2, 4, and 8. Two females from control and four females from 200 mg/kg/day group were sacrificed two weeks post-treatment. Two females from control and four females from the 200 mg/kg/day group were sacrificed four weeks post-treatment.

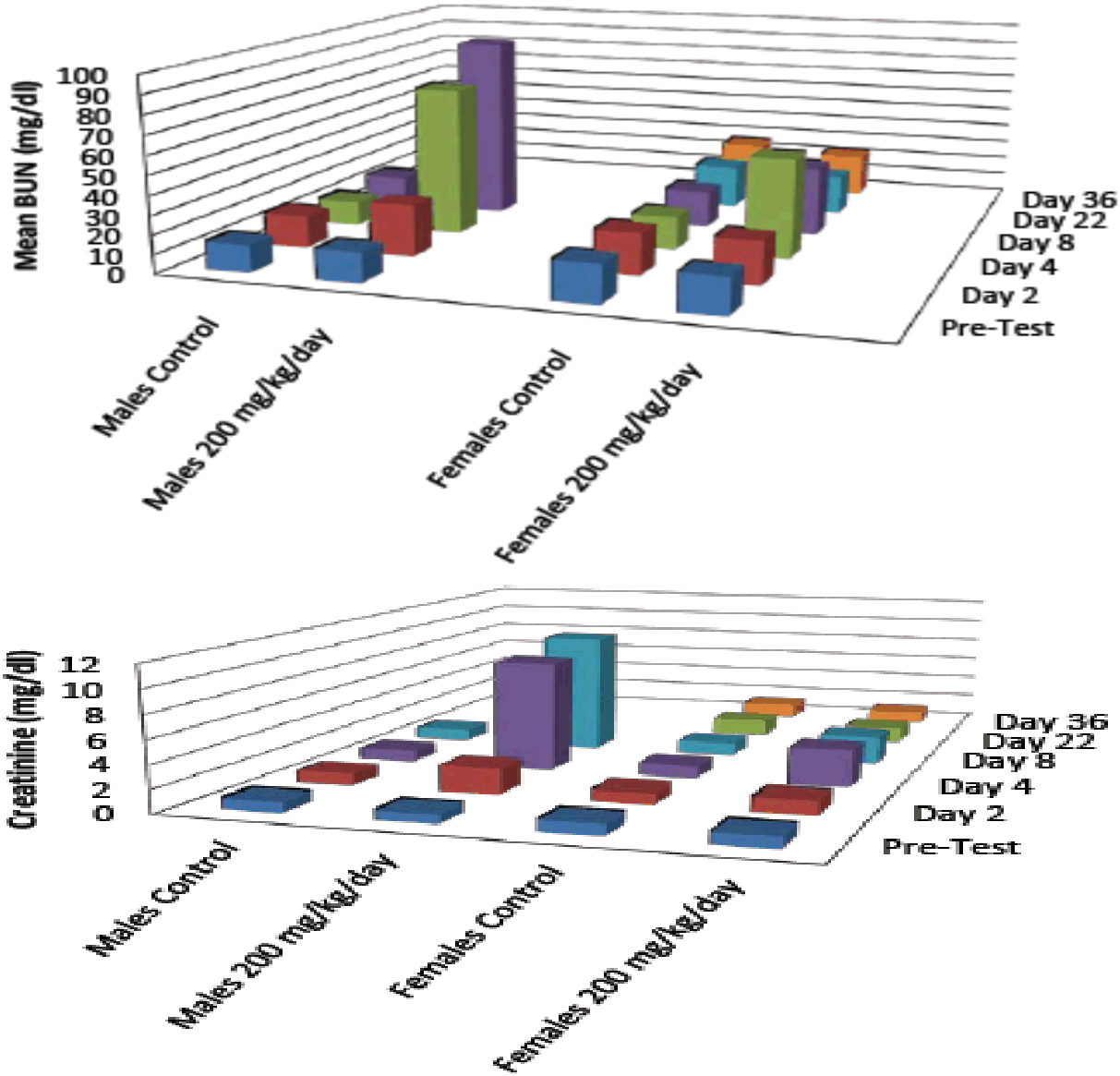
Kidney necropsy: All male and female rabbits sacrificed on DS 2 appeared normal. On DS4, all treated males sacrificed showed pale kidneys; one of these also had a small, firm and tan right lateral lobe of the liver. On DS 8, 2/3 males had fluid in the thoracic cavity, pale kidneys, and friable perirenal adipose tissue. On DS10, 4 of the treated males had pale and/or mottled kidneys; one appeared normal. All treated females sacrificed on DS 4 and 8 had pale kidneys. On DSs 22 and 36, all animals' kidneys appeared normal. Terminal body weights for males were reduced in the treated group. Body weight loss was increased for males in the treated group from Days 4-8, 8-10, and 1-10.

Kidney organ weight: For males, absolute kidney weights (left and right) and the ratio of these weights to terminal body weights were increased in the treated group at each scheduled necropsy (DS 2, 4, 8, and 10). For females, absolute right and left kidney weights on DS 2 were comparable to the control while the ratios of these weights to terminal body weights were reduced. Absolute kidney weights (left and right) and the ratio of these weights to terminal body weights were

increased in the treated group at all other scheduled necropsies (DS 4, 8, 22, and 36). Body weights and body weight changes for the females were unremarkable.

Clinical Chemistry: Indicators of nephrotoxicity included moderate-marked increases (2x - 13x) in BUN (see chart 1 below) and creatinine (see chart 2 below). Overall, renal dysfunction was more severe and occurred earlier for male (DS 2) than for females, progressing through DS 8 and seemed to be reversible following both recovery periods in females (males were not done for recovery study).

Figure 2 - Clinical chemistry (BUN and creatinine) in IV nephrotoxicity study in rabbits



Histology: Acute tubular necrosis was apparent after 1 day of dosing (DS 2) in males and females and persisted up to DS 8 (7 days of dosing). Between DSs 4 and 8 (3 and 7 days of dosing, respectively), tubular epithelial cell regeneration appeared with complete tubular restoration in some areas. At and after DS 8, tubular regeneration was the predominant microscopic change. Other

associated findings included multifocal areas of mineralization (dystrophic calcification) within and surrounding affected tubules, mild multifocal glomerular atrophy secondary to tubular dilation (tubular blockage) and proteinaceous cast formation. These effects seemed reversible in females after both 2- and 4-week recovery periods, whereas residual mineralization and small foci of cortical fibrosis still existed.

Electron microscopy: EM performed in males and females on DS 2 confirmed the onset of tubular necrosis by LM after only 1 day dosing. The earliest discernible lesion was apical cell swelling of the proximal convoluted tubule, which progressed to marked generalized cell swelling, irreversible cellular degradation and eventual necrosis with preserved underlying tubular basement membranes. No glomerular abnormalities were observed by EM.

Liver Toxicity: Drug-induced hepatocellular vacuolation (hepatic fatty change) was also observed in males (correlated with increases (1.5-7X the upper limit of controls) in AST, ALT, GGT and/or bilirubin from DSs 2 through 8, with peak levels at DS 4). Higher cholesterol and triglycerides also occurred in males (2X- 7X) with increasing occurrence and magnitude from DSs 2 to 8. In females, mild increases (1-3X) in AST and bilirubin occurred at DS 4. Mild increases in cholesterol and triglyceride also occurred (similar to male pattern, see above). AST, bilirubin and cholesterol levels in treated females recovered following both recovery periods, but triglyceride levels were not. In summary, the hepatic fatty lesion was seen in males only whereas females showed clinical chemistry alterations without structural damage at the 200 mg/kg dose studied. The sponsor indicated that weight loss (seen in males only) and more severe renal lesions might play a role in the hepatic fatty changes observed in males.

TK: No significant gender differences in exposures (at end of infusion on Day 1: males 519,916; females 593,783 ng/ml). Gender differences occurred on Day 7 (pre-dosing: males 644,065; females 4,315 ng/ml). Post-infusion concentrations were also increased by a similar amount in the males. The sponsor indicated that males might fail to eliminate the drug (due to nephrotoxicity), resulting in higher plasma drug concentrations than females (see table below). Similar profile was also observed in another nephrotoxicity study (S-021812-TF-094-L, see next). Note that day 7 pre-dosing levels $\geqslant$ 1,350,000 ng/ml in 3/5 moribund animals which subsequently died on Days 7-8.

Table 31 - Percent of animals with renal histology findings in IV nephrotoxicity study in Rabbits

Acute Tubular Necrosis (Percent of animals)				
Necropsy Day	**Males**		**Females**	
	Control	**200 mg/kg**	**Control**	**200 mg/kg**
Day 2 (SS)	0	0	0	75
Day 4 (SS)	0	25	0	100
Day 6-7 (US)	NA	100	NA	NA
Day 8 (SS)	0	100	0	75
Day 10 (US)	0	100	NA	NA
Day 22 (SS)	NA	NA	0	75
Day 36 (SS)	NA	NA	0	50
Tubular Regeneration (Percent of animals)				
Necropsy Day	**Males**		**Females**	
	Control	**200 mg/kg**	**Control**	**200 mg/kg**
Day 2 (SS)	0	0	0	75
Day 4 (SS)	0	25	100	100
Day 6-7 (US)	0	100	NA	NA
Day 8 (SS)	0	100	0	100
Day 10 (US)	0	100	NA	NA
Day 22 (SS)	NA	NA	0	75
Day 36 (SS)	NA	NA	0	50
NA= No animals necropsied at this interval SS = Scheduled Sacrifice US= Unscheduled Sacrifice or Death				

Table 32 – Toxicokinetics in IV nephrotoxicity study in Rabbits

Animals That Died			
Toxicokinetic Sampling Interval:	**Day 1 Plasma Conc. (ng/mL) End of infusion**	**Day 7 Plasma Conc. (ng/mL) Pre infusion**	**Day 7 Plasma Conc. (ng/mL) Post infusion**
Males Range of Plasma Conc.(ng/mL)	399,610-494,580 (5)	1,353,530–1,466,488 (3)	1,859,015 -1,873,538 (2)
Animals That Survived			
Males Range of Plasma Conc.(ng/mL) (n)	397,538 – 668,730 (15)	50,215 – 835,743 (7)	820,592 -1,462,234 (7)
Females Range of Plasma Conc.(ng/mL) (n)	387,221 – 750,228 (20)	814 – 10,771 (12)	415,438 -1,036,880 (12)

In summary, repeat dosing of 200 mg/kg iv (up to 9 days) resulted in mortality due to severe renal pathology. Ultrastructural lesions in kidneys in male and female rabbits could be elicited after first day dosing at 200 mg/kg (found at DS 2, day of sacrifice). Profile of lesions seen with EM were similar to those by LM, i.e., acute tubular necrosis, which was more pronounced in males than females, partly due to greater drug exposures in males as renal elimination of the drug has been compromised by nephrotoxicity. The renal lesions seemed to be reversible in the females following the 2- and 4-week recovery periods.

Study title: One-Week Intravenous Toxicity Study of Peramivir Trihydrate in [Male] Rabbits

Key study findings:

- This 7-day iv special nephrotoxicity study in male rabbits showed an NOAEL of 100 mg/kg (for renal toxicity) with AUC=219 ug.h/ml.

Study no.: S-021812-TF-094-L
File Location: EDR
Conducting laboratory: (b) (4)
Date of study initiation: 5/21/2008
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: 05P0510/100.4
Methods

Doses:	0 (NaCl 0.9%) or 50, 100 and 200 mg/kg for 7 days
Species/strain:	Rabbit/Kbl:JW (male)
Number/sex/group:	4 (male)
Route, formulation, volume:	IV (2ml/min)
Satellite groups used for toxicokinetics or recovery:	4 males Day 1 and 7 (no recovery phase)
Age:	11-12 Week
Weight:	2.13-2.64 kg (male)

Results:
Mortality: Checked 1/day. No mortality.

Clinical signs: Checked 1/day. Unremarkable.

Body weights: Measured at predose and at study termination. One of 4 high dose males exhibited scant feces and suppressed body weight gain or decreased body weights associated with decreased food consumption (≈10%) from initiation of dosing.

Food consumption: Measured at predose and at study termination. Unremarkable.

Hematology: Not performed.

Clinical chemistry: Measured at predose and at study termination. There were no findings attributed to treatment with test article.

Urinalysis: Measured at study termination. In 200 mg/kg group, one male showed increases in BUN (x3) and creatinine on Days 3 and 8 (x90); presence of urinary protein, glucose and occult blood on Day 3; and tendencies to decrease in the specific gravity and urinary excretion levels of electrolytes (sodium, potassium and chloride) on Days 3 and 7; light brownish discoloration of the cortex of the kidneys; and microscopic renal lesions including dilatation of the tubules, hyaline casts, regeneration of the tubules and necrosis of the tubular epithelium. In addition, this male animal exhibited scant feces and suppressed body weight gain or decreased.

Gross pathology: Measured at study termination. Unremarkable.
Organ weights Unremarkable.
Histopathology: No Peer review was performed. Histology was done for kidney only (see findings described for 1 high dose male under Urinalysis section).

Toxicokinetics:
AUC0-24 (systemic exposures) were dose-proportional and no dose-related effects on the mean elimination T½ (Day 1). Exposures immediately after dosing on Day 7 were 80.1, 159 and 427 µg/ml and the mean T½ values were 3.28, 3.55 and 2.72 hours at 50, 100 and 200 mg/kg, respectively, similar to those seen on Day 1. The AUC0-24 were 113, 219 and 962 µg·hr/ml at 50, 100 and 200 mg/kg, respectively, and the levels at 200 mg/kg tended to be higher than that on Day 1. However, this was due to Animal No. 4M01(AUC0–24 of 2,170 µg.hr/ml) that exhibited effects on the kidneys.

Table 33 – Toxicokinetics of one-week IV study in male rabbits

Daily Dose (mg/kg)	0 (Control)	50	100	200
Number of Animals	M: 4	M: 4	M: 4	M: 4
Toxicokinetics: AUC_{0-24} (µg•hr/mL)				
Day 1	BQL[a]	92.2	194	540
Day 7	BQL	113	219	962 [b]

BQL- below the lower limit of quantification for all time points.
b Day 7 AUC for #2M01 was approximately four fold higher than other values in this group.

In conclusion, the NOAEL of peramivir on the kidneys in rabbits was considered to be 100 mg/kg/day under the conditions of this study.

Study Title: Seven-Day Intravenous Toxicity Study of Peramivir in New Zealand White Rabbits with Supporting Toxicokinetics

Key study findings:

- The NOAEL for nephrotoxicity in this 7-day iv study was defined as 100 mg/kg (AUC= 309 and 366 μg.h/ml in males and females, respectively).

Study no.:	DS99022 (TK DM99079)
File Location:	EDR
Conducting laboratory:	R. W. Johnson Pharmaceutical Research Institute, Spring House PA
Date of study initiation:	5/20/1999
GLP compliance:	yes
QA report:	yes
Drug, lot #, and % purity:	S99-0072/99.5

Methods

Doses:	0 (NaCl 0.9%), 10, 25, 50, 100 mg/kg for 7 days
Species/strain:	Rabbit/Kbl:JW
Number/sex/group:	3
Route, formulation, volume:	IV (0.8- 8.3 ml/kg/day/2 mg/ml)
Satellite groups used for toxicokinetics or recovery:	2 Day 1 and 7
Age:	19 Week
Weight:	(M) 2.6-2.9 kg; (F) 2.7-3.0 kg

Results:

Mortality: Checked 1/day. No mortality.

Clinical signs: Checked 1/day. Unremarkable.

Body weights: Measured at predose and at study termination. Unremarkable.

Food consumption: Measured at predose and at study termination. Unremarkable.

Hematology: Measured at predose and at study termination. Decreased RBC, HB, and HCT (~0.98 to ~0.95×) and increased MCV (~1.04×)/reticulocyte % were observed in 1 male (100 mg/kg/day). Decreased RBC, HB, and HCT (~0.95 to ~0.85×) were observed in 3 low dose females (25 mg/kg) and 1 high dose female. In addition, 2 low dose females had high (~1.6 to ~2.3×) reticulocyte counts, one of them had cage injury to a hind limb on Day 5 that resulted blood loss. The sponsor considered that effects observed at 100mg/kg were considered drug-related.

Clinical chemistry: Measured at predose and at study termination. Increased fibrinogen (~2 to 3×) levels were observed in males (50, 100 mg/kg) and a female (25 mg/kg), which had a cage injury to a hind limb on Day 5 that probably reflected the increased fibrinogen at 25 mg/kg. No dose-response in fibrinogen levels was evident.

Urinalysis: Measured at study termination. Lower urine volumes (~0.5×) were observed for treated males and a female at 100 mg/kg/day. Two females at 50 mg/kg had higher urinary output volumes (~3×). Specific gravity was inversely related to urine volume. Increased urine sodium, potassium, chloride, creatinine, and/or protein concentrations (~1.5 to ~2.0×) were observed in males at 100 mg/kg. Increased urine sodium, potassium, and chloride (~1.1 to ~1.3×) were also observed in females at 100 mg/kg. The sponsor stated that these changes were variable and not dose-related and thus not treatment related. GFR (calculated from creatinine clearance) were variable and comparable with controls. When urine chemistry are normalized to creatinine excretion, a trend toward increased ratios of sodium and chloride to creatinine reflected mild increases in urinary output of these parameters for the middle and high dose groups. These changes indicated mild shifts in sodium and chloride excretion and were treatment-related. In the absence of kidney histopathology and unchanged GFR, the nephrotoxic effects were marginal and could be emerging, which became definitive at the 200 mg/kg dose (see previous studies, Study#S-021812-TF-094-L and DS00417).

Gross pathology: Measured at study termination. Unremarkable.

Organ weights ⩾50 mg/kg/day. Unremarkable. Increased adrenal weights were observed in female rabbits at

Histopathology: No Peer review was performed.

The sponsor claimed no significant microscopic damages in kidney occurred. Note that females at 50 and 100 mg/kg, and males at 100 mg/kg showed signs of renal toxicity (nephrosis, see below).

Table 34 – Renal histopathology of one-week IV study in rabbits

	INCIDENCE OF FINDINGS (NUMERIC)									
	Males					Females				
FINDINGS / DOSAGE	0 mg/kg /day	10 mg/kg /day	25 mg/kg /day	50 mg/kg /day	100 mg/kg /day	0 mg/kg /day	10 mg/kg /day	25 mg/kg /day	50 mg/kg /day	100 mg/kg /day
KIDNEY	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Not remarkable	2	1	2	1	1	2	1	2	1	
Nephrosis	1	1	1	1	2	1	2		2	2
Mineralization	1	2	1	1	2	1		1	1	2
Inflammation, lymphocytic, pelvic.										1
Concretion, pelvic					1					

Toxicokinetics (Study DM99079):
AUC0-24 were dose-proportional in both sexes for Days 1 and 7, with T½ =4-11 hr (Day 1). AUC0–24 (Day 7) at 100 mg/kg/day = 337.72 ug•hr/ml. Clearance =244-322 (mL/h·kg) and Vd (Days 1 and 7)= 368-1653 mL/kg. Vd approximated total body water, suggesting that the drug distributed evenly throughout the body in rabbits. No gender differences were evident for the PK parameters evaluated.

Table 35 - Toxicokinetics of one-week IV study in rabbits

Interval - Sex	Dose (mg/kg)	C_0 (ng/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)	CL (mL/h·kg)	Vd_{ss} (mL/kg)
Day 1 – Male[a]	10	57030	40721	11.39	249	1653
	25	127549	83674	4.81	299	409
	50	271363	205014	4.31	244	368
	100	372637	345187	4.32	290	439
Day 1 – Female[a]	10	33158	32781	5.65	307	441
	25	147800	106950	9.04	252	916
	50	248512	183473	5.48	273	438
	100	459506	389324	5.30	268	454
Day 7 – Male[b]	10	50013	33773	9.24	288	740
	25	116513	81355	4.53	309	443
	50	274727	184090	5.26	270	477
	100	430406	309347	4.47	322	483
Day 7 – Female[b]	10	49894	31658	6.36	318	648
	25	142443	96195	6.47	263	488
	50	171417	162236	5.19	309	512
	100	477912	366088	5.31	279	441

[a] AUC (0-∞)
[b] AUC (0-24)

In conclusion, the NOAEL for nephrotoxicity in rabbits was considered to be 100 mg/kg/day (337.72 ug•hr/ml) under the conditions of this 7-day study.

Study title: Antigenicity Study in Guinea Pigs: Systemic Anaphylaxis and Passive Cutaneous Anaphylaxis Reactions

Key study findings: Peramivir is not antigenic as demonstrated in guinea pig anaphylaxis models.

Study no.: DS00320
File Location: EDR
Conducting laboratory and location: (b) (4)
PA
Date of study initiation: 10/9/2000
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: S99-0217/99.6%

Methods

Doses: IV (1 & 10 mg/kg), SC (0.5 & 5 mg/kg) sensitized 1/week for 4 weeks.
Species/strain: Hartley guinea pig Crl:(HA)BR
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: 1 ml/kg/1.0-10 mg/ml(iv)/0.5-5.0 mg/ml

	(sc)80; 10 mL/kg via oral gavage
Age:	5-6 weeks

Results:

No signs of anaphylaxis were elicited by iv (1 and 10 mg/kg) and sc (0.5 and 5 mg/kg) administration of peramivir in the guinea pigs used. Animals in the positive control groups (ovalbumin) showed moderate to severe signs of anaphylaxis after challenge dosing (labored breathing or deaths). None of the peramivir or vehicle sensitized animals showed signs of anaphylaxis when challenged with peramivir. In a passive cutaneous anaphylaxis assay (i.e., using sensitized animal's sera injected to untreated animals, which were then challenged by IV peramivir, saline or ovalbumin), no significant effects were reported for the vehicle or peramivir group (reactions were seen with positive controls). The sponsor concluded that peramivir was not antigenic as demonstrated in this study.

Local Toxicity Studies on Peramivir

Local toxicity elicited at the iv injection sites were evident in some of the iv toxicity studies performed in rats, rabbit and monkeys. In the rat, the one-month continuous iv infusion study caused perivascular inflammatory cell infiltration, proliferation of the venous intima, thickening/necrosis of the vascular wall and/or thrombus formation at indwelling catheter site (posterior vena cava). In rabbits, in the single-dose and 7-day studies, the injection sites showed thrombus formation, perivascular hemorrhage, inflammatory cell infiltration, edema and/or necrosis. In monkeys, inflammation, hemorrhage/fibrous thickening of the vascular wall, thrombus, granuloma and mineralization in the infusion area also occurred at the injection sites. These findings were not dose-related and could occur in controls, and were apparently resulted from the injection or infusion procedures.

11 INTEGRATED SUMMARY AND SAFETY EVALUATION

In support of the indication of a single iv dose of peramivir for acute uncomplicated influenza, the sponsor has conducted (1) repeat dose iv toxicity studies on peramivir in rats (up to 1-month) and monkeys (up to 1-month), (2) i*n vitro and in vivo* genotoxicity studies, (3) reproductive and developmental toxicity studies including fertility studies in male and female rats, embryo-fetal developmental studies in rats and rabbits, and a peri- and post-natal developmental study in rats, all administered via the iv route, and (4) juvenile studies in rats.

In animal species assessed in the nonclinical toxicology program, pharmacokinetics/ toxicokinetics were concurrently assessed. In general, systemic exposures were linear and proportionate to dose (0.4-10 mg/kg in mice, 3-30 mg/kg in rats and monkeys), and no gender differences in pharmacokinetic parameters were observed. T½ of IV peramivir was < 2 hr in mice, ≈15 hrs in rats, 15-20 hr in monkeys. The drug is excreted primarily in the urine after iv administration, and not extensively metabolized (<4%), with a cyclopentyl ring oxidized metabolite found in rats or an acyl glucuronide metabolite in rabbit (no metabolite in monkeys). Plasma protein binding in animal species were ≤18%-30%, and it does not partition into red blood cells. Volume of distribution was about 1/3-2/3 of total body water. Peramivir is neither a substrate nor an inhibitor of p-glycoprotein or cytochromes P450. No adverse effects of peramivir on the cardiovascular,

respiratory, GI or CNS were noted during safety pharmacology studies. Peramivir did not show significant inhibitory activities on hERG potassium channels.

NONCLINICAL TOXICITY PROFILE OF PERAMIVIR

Major toxicity findings and key target organ of toxicities are highlighted below.

NEPHROTOXICITY:

Acute tubular necrosis accompanied by abnormal renal function parameters (e.g., BUN, creatinine etc.) occurred uniquely in rabbits after iv or oral peramivir administration. Rabbit appeared to be the sensitive species for the nephrotoxic effects of peramivir. Kidney may also be the target organ in rats and monkeys, as was reported in a one-month monkey iv study and the 2-year rat oral study, in which renal toxicity was induced at high drug exposures (monkeys) or with longer treatment duration (rats). Additionally, fetal findings in the rat embryofetal study (continuous iv infusion) and the juvenile rabbit study both showed renal system as a primary target as well (see below). The toxicity profile is provided below.

STUDY & DOSE mg/kg iv	Renal Function	Histopathology	NOAEL/AUC (mg/kg; ug·h/ml)	Notes
Rabbit, 4-Day, 0, 200, 300 iv	↑BUN/creatinine	Acute tubular necrosis & dilatation in cortex (multifocal) with protein casts, mineralizations in dilated tubules in corticomedullary junction (multifocal), plus ongoing tubular regeneration (multifocal)	No NOAEL	
Rabbit, 1-Week, 0, 50, **100**, 200 iv (male)	↑BUN/ urine protein, creatinine Na, K, Cl	Tubular dilatation with hyaline casts, tubular epithelial necrosis, tubules regeneration	100/219	Male only.
Rabbit, 14-day (0, 200 iv)	↑BUN/creatinine	Acute tubular necrosis	No NOAEL At 200mg/kg: 519-593 (Day 1, end of injection)	Actual dosing: 9 days for males, 7 days for females due to mortalities.
Rabbit, 1-Week, 0, 10, 25, 50, **100** iv	↓ Urine volume ↑urine protein, creatinine Na, K, Cl	Unremarkable	100/309 males 366 female	
Rabbit, 4-Week, 0, 50, **300**, 900, 1200 po	↓ Urine volume, Na, K, Cl, creatinine	Tubular necrosis	300/76 male 58 female Lethal: 1200.	A 13-week oral study (50, 150, 900) showed no findings (NOAEL: 900/214 male 270 female).
Monkey, 1-Month, 0, 120, **360**, 720 iv		↑Kidney weight, tubular dilatation, tubular regeneration	360/1450 male, 1510 female	Highest continuous iv infusion dose achievable.
Rat, 4-Week, 0, 100, **300**, 600, 3000 po	↓ Urine creatinine, Na, K, Cl	Unremarkable	300/5 male, 8 female	
Rat, 2-Yr, 0, **150**, 1000, 3000 po		Mineralization of renal pelvis (both sexes), tubular dilatation & vacuolation (females)	150/2.5 male 4.5 female (Week 26)	

HEPATOTOXICITY

Abnormal liver functions were observed concurrently with nephrotoxicity in rabbits at high iv doses (≥200 mg/kg). Significant liver toxicity was not reported in any other animal species.

STUDY & DOSE mg/kg iv	Live Function Test	Liver Pathology	NOAEL/AUC (mg/kg; ug·h/ml)	Toxicological Implications
Rabbit, single-dose 0, 200 iv	↑AST, ALT, GGT and or bilirubin	Hepatocellular vacuolation	No NOAEL At 200mg/kg: 519-593	Hepatic fatty change in males not seen in 100mg/kg study
Rabbit, 4-Day, 0, 200, 300 iv	↑AST, ALT, GGT and or bilirubin	No histology findings.	No NOAEL	

REPRODUCTIVE TOXICITY

Reproductive toxicology of peramivir was studied in rats and rabbits. The key findings are as follows.

- *Rat*. While there is no finding in the iv bolus study at the maximum feasible dose (600 mg/kg), a continuous iv infusion that attempted to escalate drug exposures did elicit dose-related increases in incidences of reduced renal papilla and dilated ureters without any maternal toxicity. Both anomalies are neither definitive malformations nor variations, but listed under grey-zone category according to literature (Federal Institute for Risk Assessment, Federal Ministry of Food and Agriculture, Germany). The findings may suggest potential delays in the development of the urinary tract, and impact of this finding is unclear. No other remarkable findings in regard to malformations, skeletal anomalies or skeletal variants were observed. No maternal toxicity was observed.
  Rabbits. Peramivir caused nephrotoxicity and related maternal toxicity in rabbits at ≥100 mg/kg (decreased body weight gain; reduced food consumption, renal tubular necrosis). There were fetal body weight losses and pre-implantation losses at maternally nephrotoxic doses and lower. However, no teratogenic findings were reported at all doses studied.

STUDY & DOSE mg/kg/day	Maternal	Fetal	NOAEL/AUC (mg/kg; ug·h/ml)	Toxicological Implications
Rat, Embryofetal study, 0, **50,** 400, **1000** continuous infusion GD6-17	None	↑Incidence of reduction of the renal papilla(e) and dilatation of the ureters	Fetal: 50/84 Maternal: 1000/1475	Delayed development of urogenital system. NOAEL was lower than iv bolus study (600 mg/kg) because no additional doses were studied between 50 & 400mg/kg.
Rabbit, Embryofetal study, 0, 200, 300, 400, 500, 600 iv	Body weight loss, absence of urine, cortical tubular epithelial necrosis) ↑BUN/creatinine/GGT	Body weight loss	No NOAEL (lethal=300, sublethal=200)	
Rabbit, Embryofetal study, 0, **50**, 100, **200** iv	Body weight loss, pale renal cortex, ↑abortions	Body weight loss, ↑pre-implantation loss	Maternal: 50 Fetal: 200	

GENOTOXICITY Peramivir tested negative in AMES, chromosome aberration assay using Chinese hamster ovary cells, and in vivo mouse bone marrow micronucleus assay following iv administration.

CARCINOGENICITY A rat carcinogenicity study was performed using the oral, instead of, the iv route of drug administration. The study is not a requirement for this NDA. However, the study revealed no significant tumor findings, partly because of low drug exposures via the oral gavage route of drug administration. Non-tumor lesions observed in this rat study included mineralization of the renal pelvis (in both sexes), tubular dilatation and vacuolation (females), which occurred following 2-year dosing at 1000 mg/kg. The NOAEL for this renal effect was 150 mg/kg (AUC=2.5 [males] and 4.5ug.h/ml [females]).

JUVENILE TOXICOLOGY In a 4-week iv study performed in the 9-day old rats, IV peramivir did not cause any organ toxicity or affect physical development or behavior of the neonates, except that body weight reductions occurred at 240 mg/kg (NOAEL=120 mg/kg, AUC=158-177 ug.h/ml).

During earlier drug development for the oral formulation, 2-week juvenile rat and rabbit studies were completed via oral gavage and the findings were as below:
(1). Juvenile rats: liquid feces (500 and 1500 mg/kg), lower body weight (1500 mg/kg) and dose-dependent increases of segmented neutrophils (500 and 1500 mg/kg, males), RBC/Hct/Hb (1500 mg/kg, males) and low urinary pH/high specific gravity (1500 mg/kg, both sexes). NOAEL=50 mg/kg (AUC=1.2 ug.h/ml) for both sexes (these findings, especially for the GI and body weight changes, might not be relevant to this NDA because oral formulation, instead of iv was employed).
(2). Juvenile rabbits: tubular nephrosis and cystic dilatation (300 and 1200 mg/kg). The NOAEL was 50 mg/kg/day (although this oral rabbit study was deemed invalid due to toxicokinetics inconsistency, the neonatal renal toxicity is worthwhile to note).

A summary of systemic exposures, NOAELs, and margins of safety (safe nonclinical exposures vs clinical exposure ratios) for potential toxicities is presented in tabular form below.

Animal Species	**Study Type**	**NOAEL Dose (mg/kg)**	**AUC (µg*hr/ml)**	**Margin of Exposure M - F**
Rat	7-day	200	263 (female) -355 (male)	*2.6-3.5*
	14-day continuous infusion	1152	1668[b]	*16.2*
	28-day	120	338 (male), 428 (female)	*3.3-4.2*
	1-month continuous infusion	1440	2330 (male)[b], 1620 (female)[b]	*22.7-15.8*
	Reproductive Toxicity Paternal	600	1065[c]	10.4

Animal Species	Study Type	NOAEL Dose (mg/kg)	AUC (µg*hr/ml)	Margin of Exposure M - F
	Reproductive Toxicity Maternal (study 1)	600	789[c]	7.7
	Reproductive Toxicity Maternal (study 2)	1000 (continuous infusion)	1475 [b]	14.4
	Reproductive Toxicity Fetal (developmental, teratogenic) (study 1)	600	789[c]	7.7
	Reproductive Toxicity Fetal (developmental, teratogenic) (study 2)	50 (continuous infusion)	84 [b]	0.8
	Reproductive Toxicity (Postnatal) Maternal & F1	600	789[c]	7.7
	Carcinogenicity	3000 (oral)	46.7(male), 24.3 (female)	>0.5-0.2
	Juvenile	120	158-177	1.5-1.7
Rabbit	Reproductive Toxicity Maternal	50	200 ug.hr/ml[d]	1.9
	Reproductive Toxicity Fetal (developmental)	200	800 ug.hr/ml[d]	7.8
	Nephrotoxicity Special Study (male)	100 (male)	219 (male)	2.1 (male)
	Nephrotoxicity Special Study	100	309 (male), 366 (female)	3.0-3.6
Monkey	2-week	45	249	2.4
	28-day	90	473 (male), 610 (female)	4.6-5.9
	1-month continuous infusion	Sponsor 720 FDA 360	Sponsor 2945 FDA 1450-1510	Sponsor 28.7 FDA 14.1-14.7

[a] *Based on exposure comparisons between safe animal exposures (NOAEL) and human exposures. Peramivir, at 600 mg single clinically recommended dose, produced a mean systemic exposure of approximately 102.7 µg*hr/ml.*

[b] *Approximation, based on Css or end of infusion levels.*

[c] *Approximation, based on study DS99318 (*200 mg/kg AUC=263 for females and 355 *µg*hr/ml* for males) *extrapolated linearly to 600 mg/kg AUC*=789 for females and 1065 *µg*hr/ml* for males.

[d] *Approximation, based on study DS99318 (10mg/*/kg AUC=40 *µg*hr/ml*) *extrapolated linearly to 50 and 100 mg/kg AUC=200, and 400 µg*hr/ml*.

In conclusion, the sponsor has adequately explored nonclinical safety profile of IV peramivir. The non-clinical safety information presented above shows reasonable and adequate exposure margins and no relevant human adverse events reported so far have corroborated any of the animal findings. In view of the single dose indication proposed, the significant nonclinical toxicity findings observed

are not likely to occur in human patients. Based on all the assessments provided above, this NDA should be approved from the non-clinical pharmacology/toxicology perspective.

12 APPENDIX/ATTACHMENTS

ORAL TOXICITY STUDIES:

1. Five day oral toxicity study of RWJ-270201 (b) (4) in rats (Study No. DS98009; R.W. Pharmaceutical Research Institute, Spring House, PA/Raritan, NJ; August, 1998).

Method. This is a GLP study. Twenty-five Crl:CD® (SD) IGS BR, VAF/Plus® rats (5/sex/group) were orally administered either vehicle (sodium carboxymethyl cellulose, 0.5% solution), or 25, 50, 500, or 1000 mg/kg/day of RWJ-270201 (b) (4) (batch# 15486-281) for 5 days. Mortality checks were performed at least once daily. Clinical observations were performed daily. Body weights were recorded twice predose and on Days 1, 5, and 6 (to assess relative organ weights). At study termination, blood samples were for analyses of hematology, coagulation, and clinical chemistry parameters. Urinalysis was performed at termination. After 5 days on test, all rats were killed and necropsied. The following selected organs/tissues were weighed and examined histologically.

Adrenal Gland (both)[a]	Esophagus
Aorta, thoracic	Eye (both)
Bone and Bone Marrow, stifle joint including distal femur and proximal tibia	Heart[a]
	Ileum
	Jejunum
Brain[a]	Kidney (both)[a]
Cecum	Lacrimal/Harderian Gland
Cervix	Liver[a]
Colon	Lung
Duodenum	Lymph Node, mandibular
Epididymis (both)[c]	Lymph Node, mesenteric
Mammary Gland, inguinal	
Ovary (both)[a]	Spleen
Pancreas	Stomach
Parathyroid Gland	Testis (both)[a,c]
Pituitary Gland[b]	Thymus
Prostate	Thyroid Gland[b]
Salivary Gland, submaxillary	Tongue
Sciatic Nerve	Trachea
Seminal Vesicle[d]	Urinary Bladder
Skeletal Muscle, quadriceps femoris	Uterus
Skin, inguinal	Vagina
Spinal Cord, thoracic	

[a] Weighed
[b] Weighed fixed
[c] Fixed in Bouin's solution at scheduled necropsy
[d] Collected, but evaluated microscopically as deemed necessary.

Results. No deaths occurred during the study. Clinical signs related to RWJ-270201 (b) (4) administration were: discolored (white) feces observed prior to dosing on Days 2 - 5 for most rats in the 500- and 1000-mg/kg/day dosage groups. RWJ-270201 (b) (4) had no effect on body weight gains, clinical pathology parameters, urinalysis, organ weights, or gross and microscopic evaluations. Based on the results of this study, NOAEL was designated at ≥1000 mg/kg/day for 5 days. The presence of discolored feces (white) at 500 and 1000 mg/kg/day might be due to unabsorbed test article. Toxicokinetic analysis showed that on day 5, the drug exposures as

expressed in AUCs (0-24hr) for the 25, 50, 500 and 1000 mg/kg doses were respectively 2.1/0.9, 1.7/1.5, 1.2/1.0, 2.0/2.1 ug.h/ml (male/female).

2. Two-week oral toxicity study of RWJ-270201 (b) (4) in Sprague-Dawley rats (Study No. DS99001; R.W. Pharmaceutical Research Institute, Spring House, PA/Raritan, NJ; January, 1999).

Method. This is a GLP study. Ten male and 10 female Crl:CD® (SD) IGS BR, VAF/Plus® rats were dosed by oral gavage with either the vehicle (sodium carboxymethylcellulose 0.5%), or 50, 1000, or 2000 mg/kg/day of RWJ-270201 (b) (4) (batch# A09001) for 2 weeks. An additional 4 rats/sex were included in the 50-, 1000-, and 2000-mg/kg/day treatment groups for evaluation of plasma concentrations. Clinical observations for signs of toxicity and body weights were recorded at scheduled intervals during the study. Ophthalmological examinations were performed predose and at study termination. Blood samples for hematology, coagulation, clinical chemistry, and urine samples for analysis were evaluated at study termination. At the end of the dosing period, all rats were necropsied and microscopic evaluations were performed on selected tissues (see below).

Adrenal Gland (both)[a]	Mammary Gland, inguinal
Aorta, thoracic	Ovary (both)[a]
Bone and Bone Marrow, stifle joint including distal femur and proximal tibia	Pancreas
	Parathyroid Gland
Brain[a]	Pituitary Gland[b]
Cecum	Prostate
Cervix	Salivary Gland, submaxillary
Colon	Sciatic Nerve
Duodenum	Seminal Vesicle
Epididymis (both)[c]	Skeletal Muscle, quadriceps femoris
Esophagus	Skin, inguinal
Eye (both)	Spinal Cord, thoracic
Heart[a]	Spleen
Ileum	Stomach
Jejunum	Testis (both)[a,c]
Kidney (both)[a]	Thymus
Lacrimal/Harderian Gland	Thyroid Gland[b]
Liver[a]	Tongue
Lung	Trachea
Lymph Node, mandibular	Urinary Bladder
Lymph Node, mesenteric	Uterus
	Vagina

[a] Weighed
[b] Weighed fixed
[c] Fixed in Bouin's solution at scheduled necropsy

Results.

RWJ-270201 (b) (4) did not produce significant toxic effects on body weight gain, hematology, coagulation and urinalysis parameters. Discolored feces, exhibited by all rats in the 1000- and 2000-mg/kg dosage groups as early as Day 5, remained evident at end of dosing. Decreases in cholesterol and triglyceride (0.7 and 0.65 times, respectively) were noted (females, 2000 mg/kg), which may reflect slightly altered lipid metabolism. At necropsy, mean absolute and relative thyroid weights of 1000 and 2000 mg/kg dosed females (dose-related) were significantly higher than controls. No histopathological findings were remarkable. The NOAEL was 50 mg/kg based

on liver and thyroid glands finding. Toxicokinetic analysis showed that on day 14, the drug exposures as expressed in AUCs (0-24hr) for the 50, 1000, 2000 mg/kg doses were respectively 1.5/1.3, 13.2/9.7, 31.1/14.3 ug.h/ml (male/female).

3. Three-day oral toxicity study of RWJ-270201 (b) (4) in dogs (Study No. DS98316; R.W. Pharmaceutical Research Institute, Spring House, PA/Raritan, NJ; August, 1998).

Method. This is a GLP study. Two beagle dogs/sex were orally gavaged with vehicle (sodium carboxymethyl cellulose, 0.5% solution), or 25, 50, or 100 mg/kg/day RWJ-270201 (b) (4) (batch# 15486-281) for three days. Clinical observations, mortality, body weight, food consumption, drug exposure, hematology, coagulation, clinical chemistry, organ weights, and histopathology (see tissue list below) were evaluated.

Adrenal Gland (both)[a]	Mammary Gland, inguinal
Aorta, thoracic	Ovary (both)[a]
Bone and Bone Marrow, rib	Pancreas
Bone and Bone Marrow, proximal femur[b]	Parathyroid Gland
Brain[a]	Pituitary Gland[a]
Cecum	Prostate
Cervix	Salivary Gland, submaxillary
Colon	Sciatic nerve
Duodenum	Skeletal Muscle, quadriceps femoris
Epididymis (both)[c]	Skin, inguinal
Esophagus	Spinal Cord, thoracic
Eye (both)	Spleen
Gallbladder	Stomach
Heart[a]	Testis (both)[a,c]
Ileum	Thymus
Jejunum	Thyroid Gland[a]
Kidney (both)[a]	Tongue
Lacrimal Gland	Trachea
Liver[a]	Urinary Bladder
Lung	Uterus
Lymph Node, mandibular	Vagina
Lymph Node, mesenteric	

[a] Weighed
[b] Collected, but evaluated microscopically as deemed necessary.
[c] Fixed in Bouin's solution at scheduled necropsy.

Results. No deaths occurred during the study. There were no drug-related effects on body weight, estimated food consumption, hematology, coagulation, clinical chemistry parameters, organ weights, or morphologic pathology. Minor GI effects (all dosages) were evident by sporadic occurrences of abnormal feces (soft, mucoid, or decreased). The NOAEL was 100 mg/kg/day. AUC(0-24hr) at day 1 for the 25, 50, and 100 mg/kg doses were 21/21, 31/29, 66/38 ug.h/ml (male/female), respectively. Oral bioavailability was estimated to be 23%.

4. Two-week oral toxicity study of RWJ-270201 (b) (4) in cynomolgus monkeys (Study No. DS99007; (b) (4) January, 1999).

Method. This is a GLP study. Twenty-four cynomolgus monkeys were assigned to four treatment groups (three/sex/group) and received the control vehicle (sodium carboxymethylcellulose), or 50,

250, or 1000 mg/kg/day of RWJ-270201 (b) (4) (batch# A09001) at a dose volume of 10, 10, 1.67, and 6.67 ml/kg and at concentrations of 0, 5, 150, and 150 mg/ml for Groups 1 through 4, respectively. Clinical observations, mortality, body weight, food consumption, drug exposure, hematology, coagulation, clinical chemistry, organ weights, and histopathology (see tissue list below) were evaluated.

Adrenal Gland (both)	Mammary Gland, pectoral
Aorta, thoracic	Ovary (both)
Bone and Bone Marrow	Pancreas
Brain	Parathyroid Gland
Cecum	Pituitary Gland
Cervix	Prostate
Colon	Salivary Gland, mandibular
Duodenum	Sciatic nerve
Epididymis (both)	Skeletal Muscle, thigh
Esophagus	Skin, pectoral
Eye (both)	Spinal Cord, thoracic
Gallbladder	Spleen
Heart	Stomach
Ileum	Testis (both)
Jejunum	Thymus
Kidney (both)	Thyroid Gland
Lacrimal Gland	Tongue
Liver[a]	Trachea
Lung	Urinary Bladder
Lymph Node, mandibular	Uterus
Lymph Node, mesenteric	Vagina

Results. No treatment-related clinical observations, or any changes in body weights, appetite, or ophthalmology and ECG findings were reported. Clinical pathology, organ weight, and histopathology data were unremarkable. In conclusion, the NOAEL was determined to be 1000 mg/kg/day. AUCs (0-24hr) at day 5 for the 50, 250 and 1000 mg/kg doses were respectively 5/10, 39/27, 32/39 ug.h/ml (male/female). Oral bioavailability of the drug was estimated at 4/8% (male/female).

4. **Four-Week Oral Toxicity Study Of RWJ-270201 (b) (4) In Cynomolgus Monkeys With A Four-Week Recovery Period (RWJPRI GLP Study DS99303; (b) (4) Drug Lot# 99P0061)**

Methods. Twenty male and twenty female cynomolgus monkeys were assigned to 4 treatment groups (6/sex in Groups 1 and 4, and 4/sex in Groups 2 and 3) and received the control material (sodium carboxymethylcellulose, 0.5% solution) or 100, 500, or 3000 mg/kg/day of RWJ-270201-(b) (4) once daily via nasogastric intubation for 4 weeks. Clinical observations including blood chemistry and urinalysis, and histopathology (see tissue list below) were evaluated.

Adrenal Gland (both) Aorta, thoracic Bone and Bone Marrow, femur[c], (articular surface of the distal end) Bone and Bone Marrow, sternum Brain[a] Cecum Cervix Colon Duodenum Esophagus Eye (both) Heart Ileum Jejunum Kidney (both) Lacrimal Gland Lesions Liver and Gallbladder Lung Lymph Node, mandibular Lymph Node, mesenteric Mammary Gland, pectoral (females only)	Ovary (both) Pancreas Pituitary Gland Prostate Rectum[c] Salivary Gland, mandibular (both) Sciatic Nerve Seminal Vesicle Skeletal Muscle, thigh Skin, pectoral Spinal Cord, cervical, thoracic, and lumbar Spleen Stomach Testis and Epididymides (both) Thymus Thyroid and Parathyroid Glands Tongue Trachea Urinary Bladder Uterus Vagina Animal Identification[c]

Results.

No adverse effects or remarkable findings on body weights, ophthalmology/ECG, clinical pathology, organ weight, macroscopic and/or histopathology were reported. A dose-dependent increase in the incidence of nonformed feces was observed. During the recovery period, the nonformed feces were noted once for each female, and were considered reversible. In this study, NOAEL was considered to be 3000 mg/kg/day.

AUC and Cmax (at Days 1 and 28) increased with dose in both sexes (see below), with Tmax = 0.75 - 1.67 hours, T½ = 11.5 - 23.1 hours. The clearance ranged from 7268 to 151553 (ml/h.kg). No gender differences and no differences in parameters between Days 1 and 28 of dosing.

Summary of Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in Male and Female Cynomolgus Monkeys Following Single or Multiple Oral Doses (100, 500, or 3000 mg/kg) of RWJ-270201 (b) (4) (DM99304)

Period - Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (0-∞) (ng•h/mL)	$t_{½}$ (h)	CL/F (mL/h•kg)
Day 1 - Male	100[a]	1972	1.00	13513	12.35	7455
		(491)	(0.71)	(1297)	(5.96)	(762)
	500[a]	4070	1.25	32253	13.67	16035
		(1024)	(0.50)	(6591)	(1.37)	(3519)
	3000[b]	5381	1.33	44637	11.92	70575
		(1743)	(0.52)	(11120)	(4.15)	(16323)
Day 1 - Female	100[a]	2555	0.88	15312	12.70	7268
		(1918)	(0.25)	(6588)	(6.99)	(2313)
	500[a]	5530	0.75	27801	11.57	18433
		(2220)	(0.29)	(5383)	(3.16)	(3101)
	3000[b]	3712	1.33	33894	11.50	137268
		(1792)	(0.52)	(24230)	(3.76)	(87651)

[a] N=4

Summary of Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in Male and Female Sprague-Dawley cynomolgus monkeys (N=4) Following Single or Multiple Oral Doses (50, 1000, and 2000 mg/kg) of RWJ-270201 (b) (4) (DM99304) (Continued)

Period - Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (0-24 h) (ng•h/mL)	$t_{½}$ (h)	CL/F (mL/h•kg)
Day 28 - Male	100[a]	1875	1.63	10383	11.47	8437
		(842)	(0.75)	(2799)	(4.78)	(3856)
	500[a]	4809	1.50	34594	16.04	10348
		(989)	(0.58)	(8118)	(5.77)	(2036)
	3000[b]	6491	1.33	44546	16.02	51775
		(1808)	(0.52)	(15986)	(3.43)	(19030)
Day 28 - Female	100[a]	2445	1.50	8571	23.10	8127
		(3126)	(1.22)	(3143)	(22.16)	(4118)
	500[a]	6078	1.13	27925	16.29	12976
		(2683)	(0.63)	(5341)	(1.54)	(2902)
	3000[b]	5579	1.67	32718	15.85	151553
		(4479)	(0.52)	(25234)	(11.49)	(148709)

[a] N=4
[b] N=6

5. Four-Week Oral Toxicity Study Of RWJ-270201 (b) (4) In Rabbits With A Four-Week Recovery Period (RWJPRI GLP Study DS99025; RWJPRI Laboratories, Drug Lot# 99P0062)

Method. Twenty rabbits/sex were gavaged qd for 4 weeks with vehicle (sodium carboxymethyl cellulose, 0.5%) or 50, 300, or 900 mg/kg of RWJ-270201 (b) (4). Rabbits in the high dosage group received 1200 mg/kg/day of RWJ-270201 (b) (4) on test Days 1 through 7. The high dosage group was decreased to 900 mg/kg/day starting on test Day 8 and continued through the remainder of the study. Clinical observations including blood chemistry and urinalysis, and histopathology (see below).

Adrenal Gland (both)	Mammary Gland, inguinal
Aorta, thoracic	Ovary (both)
Bone and Bone Marrow, proximal femur	Pancreas
Bone and Bone Marrow, rib	Parathyroid Gland
Brain	Pituitary Gland
Cecum	Prostate
Cervix	Salivary Gland, submaxillary
Colon	Sciatic Nerve
Duodenum	Seminal Vesicle
Epididymis (both)	Skeletal Muscle, quadriceps femoris
Esophagus	Skin, inguinal
Eye (both)	Spinal Cord, thoracic
Gall bladder	Spleen
Heart[a]	Stomach
Ileum	Testis (both)
Jejunum	Thymus
Kidney (both)	Thyroid Gland
Lacrimal Gland	Tongue
Liver	Trachea
Lung	Urinary Bladder
Lymph Node, mandibular	Uterus
Lymph Node, mesenteric	Vagina

Results.
Two rabbits died (1200 mg/kg/day, 1 female on Day 7 and one male on Day 8). The female showed decreased activity, decreased rate of respiration, an increased depth of respiration, and decreased feces prior death. The female died from marked drug-induced nephrosis (histopathology: necrosis of proximal convoluted tubules in the cortex and presence of granular casts in the tubular lumens). There were ulceration of the pyloric stomach which was likely related to stress or occurred secondary to uremia due to renal dysfunction. The high dose male did not have nephrosis but gross pathology showed discoloration of kidney and red/brown appearance of the renal medulla. The sponsor claimed it died as a result of gavage error.

No treatment-related effects on hematology, coagulation or clinical chemistry parameters were reported in other animals. Urinary excretion in females (900mg/kg) was lower (~0.5×) in excreted urine volumes and excreted sodium, potassium, chloride, creatinine, and/or protein concentrations. However, when urine chemistry results are normalized to creatinine excretion, no consistent trend for increased ratios of electrolytes or protein to creatinine was apparent except for slightly higher potassium/creatinine (K/Cr) ratios for females dosed at 300- and 900-mg/kg. The sponsor stated that the small shifts in ratios of K/Cr were drug-related but the extent of which had not reached nephrotoxicity or that of adverse biological consequence.

AUCs and Cmax (Days 1 and 28) were dose-related in both sexes (see table below). The drug was rapidly absorbed (Tmax =0.67-2.67 hours), and was eliminated very slowly with T½ = 25.5 - 115 hours. The mean clearance ranged from 674 to 6926 (ml/h.kg). No gender differences in the pharmacokinetic parameters were evident. There were increases in Cmax from Day 1 to Day 28, but there were no consistent differences in the remaining parameters between Days 1 and 28 of dosing.

Summary of Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in Male and Female New Zealand White Rabbits (N = 3) Following Single or Multiple Oral Doses (50, 300, 900, or 1200 mg/kg) of RWJ-270201 [(b) (4)] (DM99046)

Period - Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (0-∞) (ng•h/mL)	$t_{½}$ (h)	CL/F (mL/h•kg)
Day 1 - Male	50	279	0.83	8453	31.65	5948
		(107)	(0.29)	(780)	(8.04)	(530)
	300	1230	2.00	46194	30.89	6498
		(447)	(1.73)	(1381)	(8.91)	(196)
	1200	4049	1.17	266692[a]	53.58[a]	6926[a]
		(1049)	(0.76)	-	-	-
Day 1 - Female	50	360	2.67	9291	26.66	6162
		(111)	(1.15)	(4574)	(19.57)	(2384)
	300	1229	2.00	75229	57.18	4600
		(619)	(1.73)	(36890)	(20.81)	(1909)
	1200	3975	1.83	724656[a]	137.06[a]	1743[a]
		(1473)	(1.89)	-	-	-

[a] N=2

Summary of Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in Male and Female New Zealand White Rabbits (N = 3) Following Single or Multiple Oral Doses (50, 300, 900, or 1200 mg/kg) of RWJ-270201 [(b) (4)] (DM99046) (Continued)

Period - Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (0-24 h) (ng•h/mL)	$t_{½}$ (h)	CL/F (mL/h•kg)
Day 28 - Male	50	659	0.67	9460	53.50	1503
		(97)	(0.29)	(2249)	(23.33)	(769)
	300[a]	4785	1.00	75670	115	674
		-	-	-	-	-
	900	9375	0.67	123514	36.95	2626
		(2357)	(0.29)	(33790)	(12.14)	(1496)
Day 28 - Female	50	1192	1.00	15915	25.52	1689
		(396)	(0.87)	(8439)	(6.46)	(840)
	300	3714	2.00	57465	68.93	1654
		(1199)	(1.73)	(16845)	(63.98)	(1087)
	900	10885	0.83	130455	36.29	2156
		(670)	(0.29)	(24755)	(3.06)	(492)

[a] N=2

In summary, oral administration of RWJ-270201 [(b) (4)] to rabbits for 4 weeks caused 2 deaths in the 1200/900 mg/kg/day group due to nephrosis. The sponsor considered the NOAEL to be 300 mg/kg/day under the conditions of this study.

6. Four-Week Oral Toxicity Study Of RWJ-270201 [(b) (4)] In Rats With A Four-Week Recovery Period (RWJPRI GLP Study DS99308; RWJPRI Laboratories, Drug Lot# 99P0062)

Methods. Ten male and ten female Crl:CD® (SD) IGS BR, VAF/Plus® rats per group were orally administered either vehicle (0.5% sodium carboxymentylcellulose solution) or RWJ-270201 [(b) (4)] at dosages of 100, 300, 600, or 3000 mg/kg/day for 4 weeks. An additional five rats per sex were

administered either vehicle or 3000 mg/kg/day of RWJ-270201 (b) (4) for 4 weeks and retained for a 4 week recovery period. Clinical pathology and histopathology (see tissue list below) were evaluated.

Adrenal Gland (both)	Mammary Gland, inguinal
Aorta, thoracic	Ovary (both)
Bone and Bone Marrow, stifle joint including distal femur and proximal tibia	Pancreas
	Parathyroid Gland
Brain	Pituitary Gland
Cecum	Prostate
Cervix	Salivary Gland, submaxillary (both)
Colon	Sciatic Nerve
Duodenum	Seminal Vesicle (both)
Epididymis (both)	Skeletal Muscle, quadriceps femoris
Esophagus	Skin, inguinal
Eye (both)	Spinal Cord, thoracic
Heart	Spleen
Ileum	Stomach
Jejunum	Testis (both)
Kidney (both)	Thymus
Lacrimal/Harderian Gland	Thyroid Gland
Liver[a]	Tongue
Lung	Trachea
Lymph Node, mandibular	Urinary Bladder
Lymph Node, mesenteric	Uterus
	Vagina

Results.

Discolored feces (white, after drying) were observed in all animals at 600 and 3000 mg/kg/day. These effects were not observed during the recovery period. A statistically significant decrease in body weight gain was observed in males (3000 mg/kg during weeks 2, 4, and 6), whereas females in the same group experienced a statistical decrease in body weight gain during Week 2. At end of recovery, body weight changes were unremarkable (3000 mg/kg)

Lower creatinine (300, 600, and 3000 mg/kg/day, both sexes) and lower sodium and potassium (females, 3000 mg/kg/day female) were observed. The magnitudes ranged from ~0.91 to 0.60 (week 5) and $p \leq 0.05$ for 600 and 3000 mg/kg groups (both sexes). When these excretions were normalized to creatinine (sodium/creatinine, potassium/creatinine, chloride/creatinine, and protein/creatinine ratios) the values were higher (x1.5) in males of 300, 600, and 3000 mg/kg/day. The findings indicated that the site of electrolyte imbalance is not pre-renal but the renal (i.e., decreased renal function due to true acute kidney injury). At the end of recovery, effects were unremarkable (recovery Week 9). The sponsor stated that these were drug-related effects. Findings on hematology, coagulation, clinical chemistry, gross and microscopic exams were unremarkable, except that organ weights were lower for the liver and adrenals in males of 3000 mg/kg dose.

AUCs and Cmax (Days 1 and 29) were dose-related in both sexes (see table below). The drug was rapidly absorbed (Tmax =1-1.6 hours), and was eliminated very slowly with T½ =7.5-33.5 hours. No gender differences in the PK parameters were evident. T½ increased from Day 1 to Day 29, but there were no consistent differences in the remaining parameters between Days 1 and 28 of dosing.

Summary of Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in Male and Female Rats (N = 5) Following Single or Multiple Oral Doses (100, 300, 600, or 3000 mg/kg) of RWJ-270201 (b) (4) (DM99002) (Continued)

Period - Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (0-24 h) (ng•h/mL)	$t_{½}$ (h)	CL/F (mL/h•kg)
Day 29-Male	100	692	1.40	2794	11.53	30488
		(184)	(0.55)	(385)	(8.56)	(6564)
	300	1629	1.00	5418	12.93	43823
		(217)	(0.00)	(1115)	(2.44)	(10278)
	600	2071	1.00	6979	16.35	64966
		(630)	(0.00)	(1647)	(10.25)	(30707)
	3000	3992	1.00	17235	22.90	109989
		(1089)	(0.00)	(3909)	(9.77)	(35663)
Day 29-Female	100	598	1.00	2809	24.76	19036
		(152)	(0.00)	(741)	(10.58)	(9207)
	300	2133	1.00	8074	18.25	21976
		(557)	(0.00)	(1813)	(4.97)	(5249)
	600	2391	1.00	10602	26.47	31560
		(965)	(0.00)	(2508)	(7.28)	(12045)
	3000	6263	1.00	36187	33.54[a]	65729[a]
		(2327)	(0.00)	(23695)	(15.28)	(49065)

[a] N=4

Summary of Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in Male and Female Rats (N = 5) Following Single or Multiple Oral Doses (100, 300, 600, or 3000 mg/kg) of RWJ-270201 (b) (4) (DM99002)

Period - Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (0-∞) (ng•h/mL)	$t_{½}$ (h)	CL/F (mL/h•kg)
Day 1-Male	100	845	1.00	3197	7.95	37037
		(125)	(0.00)	(1557)	(3.57)	(16114)
	300	2136	1.20	5888	7.54	51832
		(691)	(0.45)	(836)	(2.40)	(7807)
	600	4164	1.60	15313	8.09	52588
		(1096)	(1.34)	(11412)	(1.96)	(25076)
	3000	3745	1.00	20447	12.42	153163
		(523)	(0.00)	(4936)	(2.17)	(33615)
Day 1-Female	100	873	1.20	2742	9.78	36640
		(50)	(0.45)	(207)	(2.86)	(2914)
	300	1894	1.00	6530	10.80	46326
		(276)	(0.00)	(676)	(3.12)	(4641)
	600	2730	1.20	10593	9.03	56827
		(403)	(0.45)	(675)	(4.69)	(3625)
	3000	5054	1.00	22875	10.55	137995
		(1269)	(0.00)	(5349)	(2.92)	(37011)

In summary, oral administration of RWJ-270201 (b) (4) to rats for 4 weeks resulted in minimal renal signs and effects at 3000 and 600 mg/kg/day. Body weight gain effects and organ weight changes were observed at 3000 mg/kg/day. The NOAEL was considered by the sponsor to be 300 mg/kg/day.

7. **Thirteen-Week Oral Toxicity Study of RWJ-270201 (b) (4) in Cynomolgus Monkeys With A Four-Week Recovery Period**

STUDY NO.:	DS99032
VOLUME/PAGE:	EDR
LABORATORY:	(b) (4)
STUDY INITIATION:	12/14/1999
GLP:	Yes (x) no ()
QA REPORT:	Yes (x) no ()
OBJECTIVE	To assess the toxicity of RWJ-270201 (b) (4) when administered via nasogastric intubation to cynomolgus monkeys for 13 weeks and to assess the reversibility of any effects following a 4-week recovery period.
LOT #/PURITY	(b) (4) Lot 99P0236. The doses were calculated using a potency value of 85.1% based on the water content reported in the (b) (4) Certificate of Analysis.
VEHICLE:	Sodium Carboxymethylcellulose, 7L2P, 0.5% solution
DOSING:	Control vehicle or 100, 500, or 3000 mg/kg/day of RWJ-270201 (b) (4) The monkeys received the control or test article at a dose volume of 10 mL/kg (Groups 2 and 3) or 20 mL/kg (Groups 1 and 4) and at concentrations of 0, 10, 50, and 150 mg/ml for Groups 1 through 4, respectively.
SPECIES/STRAIN:	Cynomolgus monkeys
NO./SEX/GROUP	6/sex in Groups 1 and 4; 4/sex in Groups 2 and 3
ROUTE	Nasogastric intubation
OBSERVATIONS:	
MORTALITY:	Observations for moribund or dead animals were made 2 times daily.
CLINICAL SIGNS:	1/day
BODY WEIGHTS:	Checked once/week
FOOD CONSUMPTION:	Checked once/week
OPHTHALMOLOGY:	During pretest and during weeks 13
EKG:	During pretest and during weeks 13
HEMATOLOGY:	During pretest and during weeks 5, 9, and 13 (Week 17, recovery)
CLINICAL CHEMISTRY:	During pretest and during weeks 5, 9, and 13 (Week 17, recovery)
URINALYSIS:	During pretest and during weeks 5, 9, and 13 (Week 17, recovery)
TOXICOKINETICS	During pretest, day 1, and 91
GROSS PATHOLOGY:	Macroscopic examinations were conducted on all tissues and lesions from the control and high-dose monkeys (Groups 1 and 4) at the terminal sacrifice, on all lesions noted at the terminal necropsy of the low-and mid-dose monkeys (Groups 2 and 3),

and on all lesions from the recovery monkeys (Groups 1 through 4).

ORGANS WEIGHED: At the end of the treatment period.

HISTOPATHOLOGY: Adequate.

RESULTS:

MORTALITY None

CLINICAL SIGNS A dose-related increase in the incidence (number of monkeys/group) and occurrence (total number of observations/group) of nonformed feces at 100 (males only), 500, and 3000 mg/kg/day, as well as with an increased incidence of liquid feces at 500 and 3000 mg/kg/day. At end of recovery, these effects were unremarkable.

BODY WEIGHTS: Unremarkable

FOOD CONSUMPTION Unremarkable

OPHTHALMOLOGY: Unremarkable

EKG: Unremarkable

HEMATOLOGY: Unremarkable

CLINICAL CHEMISTRY Unremarkable

URINALYSIS: Unremarkable

ORGAN WEIGHTS: Unremarkable

HISTOPATHOLOGY: Unremarkable

TOXICOKINETICS Drug was rapidly absorbed (Tmax = 0.88-2.00 hours), and very slowly eliminated (mean T½ = 18.43 - 30.16 hours on Day 1, and 12.83 - 24.29 hours on Day 91). Dose-related increases in AUC and Cmax were observed in both males and females.

Table DM5: Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in Male and Female Cynomolgus Monkeys Following Single or Multiple Oral Doses (100, 500, or 3000 mg/kg/day) of RWJ-270201 (b) (4) (DM99405)

Day 1 Sex	Dose (mg/kg)	N	C_{max} (ng/mL)	t_{max} (h)	AUC (0-∞) (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h·kg)
Male	100	4	2679	1.50	25027	24.61	4336
			(683)	(0.58)	(8837)	(19.74)	(1324)
	500	4	4228	2.00	51432[a]	23.36[a]	10126[a]
			(565)	(0.00)	(13217)	(5.59)	(2374)
	3000	6	6257	1.33	71351	18.43	46682
			(2066)	(0.52)	(22001)	(9.01)	(18964)
Female	100	4	2182	1.75	22140	21.69	4846
			(351)	(0.50)	(7219)	(1.02)	(1366)
	500	4	4796	0.88	55123	30.16	9522
			(162)	(0.25)	(13383)	(17.74)	(2502)
	3000	6	5722	1.50	69267	20.47	53546
			(1912)	(0.55)	(46463)	(10.28)	(19082)

Day 91 Sex	Dose (mg/kg)	N	C_{max} (ng/mL)	t_{max} (h)	AUC (0-24) (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h·kg)
Male	100	4	2789	1.25	15507	12.83	5207
			(678)	(0.50)	(4393)	(4.71)	(668)
	500	4	4590	1.25	34645	22.70	8958
			(285)	(0.50)	(8003)	(7.58)	(3629)
	3000	6	6712	1.25	52051	24.29	35538
			(2089)	(0.88)	(15834)	(10.78)	(15309)
Female	100	4	1905	1.00	11307	22.65	5572
			(577)	(0.00)	(1733)	(5.94)	(1389)
	500	4	6074	1.25	42316	14.95	8718
			(992)	(0.50)	(5539)	(4.37)	(1934)
	3000	6	6883	1.08	48305	20.96	41195
			(837)	(0.49)	(15378)	(8.25)	(11847)

[a] N = 3

SUMMARY Because of liquid feces and reduced appetite at 3000 mg/kg/day, the sponsor designated 500 mg/kg/day as the NOAEL for this 13-week monkey study.

8. Thirteen-Week Oral Toxicity Study Of RWJ-270201 (b) (4) In Rats With A Four-Week Recovery Period

STUDY NO.: DS99033
VOLUME/PAGE: EDR
LABORATORY: R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ
STUDY INITIATION: 3/13/2000
GLP: Yes (x) no ()
QA REPORT: Yes (x) no ()
OBJECTIVE To assess the toxicity of RWJ-270201 (b) (4) when administered orally (gavage) to Crl:CD ® (SD) IGS BR, VAF/Plus ® rats for 13 weeks
LOT #/PURITY (b) (4) Lot 99P0236
VEHICLE: Sodium Carboxymethylcellulose, 7L2P, 0.5% solution
DOSING: Ten male and 10 female rats were dosed by oral gavage with either vehicle (sodium carboxymethylcellulose 0.5%) or 300, 750, 1500, or 3000 mg/kg/day of RWJ-270201 (b) (4) for 13 weeks. An additional 5 rats/sex were administered the vehicle or 3000 mg/kg of RWJ-270201 (b) (4) and were allocated for a 4-week recovery period.
SPECIES/STRAIN: Crl:CD ® (SD) IGS BR, VAF/Plus ® rats
NO./SEX/GROUP 10
ROUTE Orally (gavage)
OBSERVATIONS:

Group	1 (Control)	2 (Low)	3 (Low-Mid)	4 (Mid)	5 (High)
	Rat Numbers (DS99033-)				
Males	1001-1015	2001-2010	3001-3010	4001-4010	5001-5015
Females	1501-1515	2501-2510	3501-3510	4501-4510	5501-5515
	RWJ-270201 (b) (4)				
Dosage[a] (mg/kg/day)	0	300	750	1500	3000
Concentration (mg/mL)	0	75	75	150	150
Volume (mL/kg/day)	20	4	10	10	20
	Number of Rats/Sex				
On Study	15	10	10	10	15
Clinical Pathology					
Week -1 (Predose n = 140)					
Weeks 5, 9, 14	AS	AS	AS	AS	AS
Week 18 (Recovery)	5	0	0	0	5
Necropsy					
Week 14 (Terminal)[b]	AS	AS	AS	AS	AS
Week 18 (Recovery)	AS	0	0	0	AS
Histopathology					
Week 14 (Terminal)[b]	AS	AR	AR	AR	AS
Week 18 (Recovery)	AR	0	0	0	AR

[a] Calculated as the anhydrous, nonsalt form.
[b] All survivors excluding rats allocated for recovery.
AR = as required (determined by the study director and pathologist)
AS = all survivors

MORTALITY: See schedule of events above.

CLINICAL SIGNS: See schedule of events above.

BODY WEIGHTS: See schedule of events above.

FOOD CONSUMPTION: See schedule of events above.

OPHTHALMOLOGY: See schedule of events above.

HEMATOLOGY: See schedule of events above.

CLINICAL CHEMISTRY See schedule of events above.

URINALYSIS: See schedule of events above.

TOXICOKINETICS

Group	6 (Low)	7 (Low-Mid)	8 (Mid)	9 (High)
		Rat Numbers (DS99033-)		
Males	6001-6005	7001-7005	8001-8005	9001-9005
Females	6501-6505	7501-7505	8501-8505	9501-9505
		RWJ-270201 (b) (4)		
Dosage[a] (mg/kg/day)	300	750	1500	3000
Concentration (mg/mL)	75	75	150	150
Volume (mL/kg/day)	4	10	10	20
		Number of Rats/Sex		
On Study	5	5	5	5
Drug Exposure				
Day 1	5	5	5	5
Week 13	AS	AS	AS	AS

[a] Calculated as the anhydrous, nonsalt form.
[b] All survivors excluding rats allocated for recovery.
AR = as required (determined by the study director and pathologist)
AS = all survivors

GROSS PATHOLOGY: At the end of the treatment period.

ORGANS WEIGHED: At the end of the treatment period.

HISTOPATHOLOGY: Adequate.

RESULTS:

MORTALITY None

CLINICAL SIGNS Unremarkable

BODY WEIGHTS: Unremarkable

FOOD CONSUMPTION Unremarkable

OPHTHALMOLOGY: Unremarkable

EKG: Unremarkable

HEMATOLOGY: Unremarkable

CLINICAL CHEMISTRY Unremarkable

URINALYSIS: Unremarkable

ORGAN WEIGHTS: Liver weights were increased (males, 300 mg/kg) and heart weight decreased in females (750, 1500, and 3000 mg/kg). Heart weights could not be correlated with any microscopic changes and were reversible. Higher pituitary weights were noted in females from the 3000 mg/kg dosage group at recovery but not at terminal necropsy and could not be correlated with any microscopic changes.

HISTOPATHOLOGY: Unremarkable

TOXICOKINETICS: AUC and Cmax increased dose-proportionally in both males and females. Drug was rapidly absorbed (Tmax=1.00 - 1.40 hours) and slowly eliminated (T½ = 4.63 to 10.57 hours). T½ increased at all doses in both sexes, ranging from 6.61 to 16.68 hours, over the 13-week study period. No gender differences were apparent.

Table DM9: Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in M and Female Rats (N=5) Following Single or Multiple Daily Oral Doses (3 750, 1500 or 3000 mg/kg/day) of RWJ-270201 (b) (4) DM99409)

Day 1 Sex	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	AUC (0-___) (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h·kg)
Male	300	2469 (261)	1.00 (0.00)	9291 (1311)	4.63 (1.62)	32891 (5353)
	750	4870 (3618)	1.40 (0.55)	15599 (7881)	6.23 (2.44)	57703 (26643)
	1500	6725 (3454)	1.20 (0.45)	20751 (4882)	6.46 (2.09)	75265 (16042)
	3000	8438 (3860)	1.20 (0.45)	31248 (19781)	7.52 (1.27)	122096 (57173)
Female	300	2960 (1061)	1.40 (0.55)	10965 (1911)	5.68 (2.50)	28200 (6050)
	750	4346 (1536)	1.00 (0.00)	12746 (1863)	7.21 (3.89)	59837 (8597)
	1500	6892 (3448)	1.00 (0.00)	19840 (7705)	9.45 (3.99)	87390 (38528)
	3000	7178 (2407)	1.20 (0.45)	27766 (17343)	10.57 (6.46)	132832 (52298)

Week 13 Sex	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	AUC (0-24) (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h·kg)
Male	300	2093 (672)	1.00 (0.00)	8866 (2553)	6.61 (2.26)	33737 (9036)
	750	2693 (1004)	1.00 (0.00)	9650 (2954)	11.28 (5.28)	69828 (25266)
	1500	2792 (1281)	1.00 (0.00)	12067 (6134)	16.04 (3.47)	95988 (28455)
	3000	4086 (2570)	1.00 (0.00)	22583 (17933)	15.53 (5.00)	132039 (74131)
Female	300	2196 (974)	1.20 (0.45)	7497 (2055)	8.56 (2.21)	38023 (7833)
	750	4006 (1307)	1.00 (0.00)	13341 (5697)	10.92 (4.74)	55091 (16391)
	1500	3720 (997)	1.00 (0.00)	14705 (3669)	16.68 (8.83)	75422 (33812)
	3000	4904 (2543)	1.00 (0.00)	16127 (6406)	16.37 (6.29)	162009 (72361)

SUMMARY In conclusion, based on the results of this rat study the NOAEL was considered to be 3000 mg/kg.

9. **A Thirteen-Week Oral Toxicity Study of RWJ-270201 in Rabbits with a 14-Week Recovery Period (Study Number: DS-99034, (b) (4)**

Key Findings: A 13-week rabbit toxicity study (50, 150, 900 mg/kg) did not show significant toxicity findings except that (1) thyroid weights were reduced approximately 40% and 30% compared to control in male rabbits receiving 50 and 150 mg/kg of RWJ-270201 (b) (4) respectively (no remarkable changes in T4 and thyroid histopathology in any group; no thyroid weight changes at 900 mg/kg), (2) both male and female animals receiving the 900 mg/kg dose exhibited moderate increases in cholesterol levels (17-82%) compared to control (reversible). The significance of reduction in thyroid weight is not known.

Methods: RWJ-270201 (b) (4) was administered orally to rabbits for three months and the reversibility of any potential toxic effects was assessed by a one-month recovery period. Doses of 50, 150, and 900 mg/kg were administered to 7 animals/sex for Groups 2 (low dose) and 3 (mid dose), and 9 animals/sex for the high dose group. The test article was administered by daily oral intubation (gavage), at a volume of 6 ml/kg, for Groups 3 and 4 and 5 mL/kg for Group 2. Six control animals/sex received the vehicle, Sodium Carboxymethycellulose Grade 7L2P 0.5% solution. Viability checks were made twice daily while clinical signs (cage-side) were made once daily. Physical examinations were performed twice pretest and once weekly during the study period.

Body weights were measured twice pretest and weekly during the study. Ophthalmoscopic observations were performed pretest and at the end of dosing. Clinical pathology studies were performed at Weeks 5, 9, and 13 (termination) and at the end of the recovery period. Urinalysis and urine chemistry were performed at termination and at the end of the recovery period. Three animals/sex/Groups 2, 3, and 4 were designated as toxicokinetic animals. Blood for determination of plasma concentrations of RWJ-270201 (b) (4) was obtained from toxicokinetic animals predose then 30 minutes and 1, 2, 4, 8, and 24 hours post-dose on Days 0 and 91. Toxicokinetic animals were euthanized without necropsy following the Day 91 sample collection. After three months of treatment, 4 animals/sex/group were sacrificed and after a one-month recovery period the remaining animals were sacrificed. At necropsy, macroscopic examinations were conducted on all animals. Histopathology were performed on the control and 900mg/kg groups.

Results:

All animals survived until their scheduled sacrifice (one female at 50 mg/kg sacrificed for dosing errors). Clinical observations, body weights, ophthalmology, hematology and coagulation were unremarkable.

Both male and female animals receiving the 900 mg/kg dose exhibited moderate increases in cholesterol (17-82%) at Weeks 5 (males only), Week 9 and termination (recovered at end of recovery period).

Thyroid weights were reduced by 40% and 30% in males (50 and 150 mg/kg, respectively). The thyroid/ body weight and thyroid/brain weights ratios were also reduced. Thyroid weights in the 900 mg/kg group were unremarkable. No changes in thyroxine or cholesterol levels were found in these groups. Because of the lack of a dose response, no change in thyroxine levels, presence in one sex, and lack of pathology findings in the highest dose group, the sponsor concluded that it was difficult to associate the decrease in thyroid weight with administration of the test article.

AUC and Cmax showed dose-related increases in both sexes. The drug was rapidly absorbed (Tmax=1-2.33 hours), whereas, with the exception of the high dose females, absorption was slower following 92 daily doses of 150 or 900 mg/kg/day (Tmax >8 hours). T½ =29.55 - 69.54 hours on Day 1 (excluding high dose males where one value was exceptionally high) and 110 - 280 hours on Day 92. There were increases in Cmax, AUC, and T½ from Days 1 to 92, indicating that there may be some accumulation of the drug after 13 weeks of dosing. Gender differences were not apparent in the pharmacokinetic parameters (see below).

Table 2: Mean (SD) Plasma Pharmacokinetic Parameters for RWJ-270201-000 in Male and Female New Zealand White Rabbits (N=3) Following Single or Multiple Oral Doses (50, 150, or 900 mg/kg) of RWJ-270201 (b) (4) (DM99407)

Sex – Interval	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	AUC (0-∞) (ng*h/mL)	$t_{1/2}$ (h)	CL/F (mL/h*kg)
Male – Day 1	50	185	2.33	9113[a]	37.87[a]	6109[a]
		(83.05)	(1.53)	-	-	-
	150	1279	2.33	21468	35.52	7420
		(901)	(1.53)	(6185)	(25.67)	(2282)
	900	3590	1.67	946180[a]	325[a]	2659[a]
		(2177)	(0.58)	-	-	-
Female – Day 1	50	176	2.00	14135	69.54	6173
		(43.72)	(1.73)	(14142)	(52.99)	(4002)
	150	624	2.33	19325	29.55	8197
		(249)	(1.53)	(5522)	(16.14)	(2313)
	900	2801	1.00	152726	55.84	6427
		(810)	(0.00)	(59663)	(19.96)	(2049)
Male – Day 92	50	780	1.00	13251	128[a]	1218[a]
		(148)	(0.00)	(2378)	-	-
	150	2451	8.67	36101	139[a]	544[a]
		(645)	(13.28)	(9033)	-	-
	900	13284	8.33	214071	110[a]	1013[a]
		(8979)	(13.58)	(149410)	-	-
Female – Day 92	50	720[a]	1.00[a]	14031[a]	191[a]	296[a]
		-	-	-	-	-
	150	4108	8.17	58522	120[a]	306[a]
		(2161)	(13.71)	(16836)	-	-
	900	17327	0.00	270112	280[a]	174[a]
		(6818)	(0.00)	(112912)	-	-

[a]N = 2

In conclusion, this 13-week oral rabbit study showed slight to moderate, reversible, increases in cholesterol (900 mg/kg, both sexes). Additionally, decreased thyroid weights (males, 50 and 150 mg/kg) were noted. Toxicokinetic analysis showed that the drug was rapidly absorbed and slowly eliminated in rabbits and that AUC and Cmax were increased in a dose dependent manner. Based on changes in TK parameters between Days 1 and 92, there appeared to be accumulation of drug during 13 weeks dosing.

10. Toxicokinetics of A Thirteen-Week Range-Finding Oral Toxicity Study Of RWJ-270201 (b) (4) In Rats (RWJPRI Report Number DM99360)

Methods. The objectives of this study were to assess the systemic exposure to RWJ-270201 in male and female rats on Days 1 and 91 in support of a 13-week oral toxicity study, and to determine the relationship of exposure to dose, gender, and multiple dosing. The Sprague-Dawley rat (four/sex/group) received 150, 750, 1500, or 3000 mg/kg/day doses of RWJ-270201 (b) (4) (trihydrate; (b) (4) Lot 99P0062) at 10 mL/kg/day for the 150, 750, and 1500 mg/kg doses and 20 mL/kg/day for the 3000 mg/kg doses by oral gavage.

Results. AUC and Cmax increased dose-proportionally in both sex. The drug was rapidly absorbed (Tmax =1-1.5 hours), and eliminated slowly (T½=7.2-31.7 hours). No significant gender differences in PK parameters and no PK difference between Days 1 and 91 of dosing (see below).

Interval	Sex	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	AUC (0-∞) (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h·kg)
Day 1	Male	150	1286	1.00	3388	7.24	45598
			(341)	(0.00)	(617)	(1.84)	(9708)
		750	3217	1.00	9070	9.01	87111
			(476)	(0.00)	(2530)	(1.95)	(21568)
		1500	3962	1.50	18512	9.67	129353
			(1526)	(0.58)	(12213)	(2.77)	(111501)
		3000	4991	1.00	60596	31.68	93967
			(2217)	(0.00)	(56219)	(36.69)	(84072)
	Female	150	1167	1.00	4538	11.99	39744
			(583)	(0.00)	(2144)	(4.43)	(19003)
		750	2254	1.00	11951	22.20	65643
			(815)	(0.00)	(2891)	(7.10)	(15918)
		1500	3935	1.00	26571[a]	22.15[a]	78813[a]
			(3121)	(0.00)	(19992)	(16.49)	(46718)
		3000	17131	1.00	38034	15.64	155894
			(25098)	(0.00)	(44773)	(10.19)	(93506)
[a] N = 3							
Day 91	Male	150	1019	1.00	4176	12.18	31620
			(368)	(0.00)	(1155)	(4.92)	(8536)
		750	1303	1.00	5588	12.66	113067
			(278)	(0.00)	(1576)	(4.94)	(30565)
		1500	2944	1.00	10750	25.26	74906
			(1419)	(0.00)	(3161)	(10.93)	(17836)
		3000	3641	1.00	17614	19.46[a]	111647[a]
			(1833)	(0.00)	(3073)	(6.69)	(41418)
	Female	150	1498	1.00	4132	7.64	38390
			(876)	(0.00)	(1847)	(2.22)	(15983)
		750	1783	1.00	6685	15.32	83176
			(378)	(0.00)	(1055)	(2.72)	(21169)
		1500	3209	1.00	12026	22.43	79059
			(1265)	(0.00)	(1119)	(17.92)	(32774)
		3000	3965	1.00	19267	20.98	116145
			(2268)	(0.00)	(9787)	(11.18)	(43462)

[a] N = 3

11. 13-Week Range Finding Oral Study of RWJ-270201 (b) (4) in Mice (11/16/99; GLP Study No. DS99108; RWJPRI, Lot No. S-990072)

Methods. Fifty male and female CrI:CD-I® (ICg) BR, VAF/Plus® mice (10/sex/group) were orally administered either vehicle (sodium carboxymethylcellulose 7L2P, 0.5% solution), or 150, 750, 1500, or 3000 mg/kg/day of RWJ-270201 (b) (4) for 91 or 92 days. Mortality checks were performed at least once daily starting 16 days prior to dosing. Clinical observations were performed twice predose and weekly thereafter through Week 13. Body weights were recorded three times predose and weekly thereafter through Week 13. Blood samples were collected from the orbital sinus of anesthetized mice for hematology and clinical chemistry measurements during Week 14. Ophthalmologic examinations were performed once predose and during Week 13. Mice were necropsied on Days 92 and 93. Selected tissues were examined microscopically. Drug absorption was performed on a satellite group of mice, which will be reported separately.

Results.

Clinical signs, body weights, ophthalmology, hematology and clinical chemistry findings were unremarkable, except that increases in triglyceride (<35%) and AST (<25%) were reported. There were no drug-related gross or microscopic changes.

AUC and Cmax increased in dose-related fashion in both sexes (except that no difference between 1500 and 3000 mg/kg for the AUC). The drug was rapidly absorbed (Tmax=0.5 -1hour), and was eliminated with T½= 2.6 - 6.8 hours. No gender differences in PK parameters were noted and there were no differences in PK parameters between Days 1 and dosing. The NOAEL for this 13-week mouse study was considered 3000 mg/kg (see below, mouse TK study DM99080, n=3/sex/timepoint/group).

Day 91 Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (0-24) (ng·h/mL)	$t_{½}$ (h)	CL/F (mL/h·kg)
Male	150	1299	1.0	4912	3.20	30410
	750	2844	0.5	20087	3.57	37075
	1500	4379	0.5	44226	3.12	33787
	3000	7196	0.5	35481	6.75	78242
Female	150	1031	0.5	4469	3.17	33434
	750	2745	0.5	19658	3.08	38009
	1500	4218	0.5	39287	4.34	37879
	3000	6059	0.5	40168	3.17	74272

12. 13-Week Range Finding Oral Study of RWJ-270201 (b) (4) in Rats (11/16/99; GLP Study No. DS99434; RWJPRI, Lot No. S-990072)

Methods. Ten male and ten female CrI:CD®(SD) IGS BIL VAF/Plus® rats per group were orally administered either vehicle (0.5% sodium carboxymethylcellulose solution) or RWJ-270201 (b) (4) at dosages of 150, 750, 1500, or 3000 mg/kg/day for 13 weeks. Evaluations were made of mortality, clinical observations, body weight, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, organ weights, gross pathology, and microscopic pathology. Drug exposure assessment

was performed on satellite groups of 5 rat/sex/group at dosages of 150, 750, 1500, or 3000 mg/kg/day.

Results.

Discolored feces (white, after drying) were observed (≥ 750 mg/kg). Body weights were increased in males (all dose groups, dose-related; no effects in females). There were no treatment-related findings on mortality, ophthalmology, organ weight, gross/microscopic exams. Further there were no effects on hematology, coagulation, or urinalysis parameters except that slight increases in ALT, AST, glucose, and triglyceride were observed in males (at Week 9, return to baseline at Week 14) for which the sponsor was not certain whether it was drug-related.

AUC and Cmax increased dose-proportionally in both sexes (see below, TK study DM99360, n=4). Drug was rapidly absorbed (Tmax =1-1.5 hours), and eliminated slowly (T½=7.2-31.7 hours). No gender differences were noted and no remarkable differences in PK parameters between Days 1 and 91 of dosing were observed.

Day 91 Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (0-24) (ng·h/mL)	$t_{½}$ (h)	CL/F (mL/h·kg)
Male	150	1019 (368)	1.00 (0.00)	4176 (1155)	12.18 (4.92)	31620 (8536)
	750	1303 (278)	1.00 (0.00)	5588 (1576)	12.66 (4.94)	113067 (30565)
	1500	2944 (1419)	1.00 (0.00)	10750 (3161)	25.26 (10.93)	74906 (17836)
	3000	3641 (1833)	1.00 (0.00)	17614 (3073)	19.46[a] (6.69)	111647[a] (41418)
Female	150	1498 (876)	1.00 (0.00)	4132 (1847)	7.64 (2.22)	38390 (15983)
	750	1783 (378)	1.00 (0.00)	6685 (1055)	15.32 (2.72)	83176 (21169)
	1500	3209 (1265)	1.00 (0.00)	12026 (1119)	22.43 (17.92)	79059 (32774)
	3000	3965 (2268)	1.00 (0.00)	19267 (9787)	20.98 (11.18)	116145 (43462)

In summary, this 13-week rat study did not reveal remarkable findings except that increased body weight for males at all dosages, and discolored feces (white, after drying) for both sexes at dosages ≥ 750 mg/kg were reported. The NOAEL for this study was considered by sponsor to be 3000 mg/kg.

INTRAMUSCULAR TOXICITY STUDIES:

STUDY TITLE:	**14-DAY SINGLE DOSE INTRAMUSCULAR TOXICITY STUDY WITH PERAMIVIR 75 MG /ml AND 150 MG /ml IN RATS**
KEY FINDINGS:	This single-dose (IM) local irritation study in rats showed that peramivir at a concentration of 150 mg/ml was as well tolerated as the 75 mg/ml concentration when given IM. AUCs achieved were 48/51 (males/females, 75 mg/kg) and 55/77 ug.h/ml (150 mg/kg).
STUDY NO.:	806-030

LABORATORY: (b) (4)
STUDY INITIATION: 3/08
GLP: Yes (x) no ()
QA REPORT: Yes (x) no ()

OBJECTIVE To evaluate possible peramivir-concentration effects on tissue irritation and TK.

LOT #/PURITY No lot number provided. The pre-formulated dosing solutions at 75 and 150 mg/ml were administered undiluted. Formulations of were provided by the sponsor.

DOSING: Single dose: 75 mg/kg.
Two groups (10/sex) of rats were administered IM the test article one injection at respective dose levels of 75 mg/kg (Peramivir; 75 mg /ml) or 75 mg/kg (Peramivir; 150 mg /ml).
Two additional groups (10/sex) were administered the respective placebo articles (Placebo 1; 75 mg /ml) or (Placebo 2; 150 mg /ml)(vehicle:0.9% NaCl, USP Saline).

Dose volume: 1 ml/kg (0.5 ml/kg at two different sites) except that the 75 mg/kg (150 mg /ml) Peramivir group received the drug at one site and the matching placebo at the other site (0.5 ml/kg at each site).

Two groups of nine animals per sex per group served as TK animals and received the Peramivir in the same manner, volume, and dose levels as the main study groups.

The first five main study animals per sex per group were necropsied at 24 hours postdose (Day 2), while the remaining five main study animals per sex per group were necropsied at 14 days postdose (Day 15).

The injection sites are provided below:

Control, Placebo, or Test Article Administration		
Group	Control, Placebo, or Test Article	Injection Site (0.5 mL/kg site)
1	Saline	Left hind limb
2	Placebo 1; 75 mg/mL	Right and left hind limb
3	Placebo 2; 150 mg/mL	Right and left hind limb
4	Peramivir; 75 mg/mL	Right and left hind limb
5	Peramivir; 150 mg/mL	Left hind limb
	Placebo 2; 150 mg/mL	Right hind limb
6	Peramivir; 75 mg/mL	Right and left hind limb
7	Peramivir; 150 mg/mL	Left hind limb
	Placebo 2; 150 mg/mL	Right hind limb

SPECIES/STRAIN: Male Crl:CD (SD0 IGS BR) Rats
NO./SEX/GROUP See above
ROUTE IM (hip or hind legs, shaved)
Observations Observations for morbidity, mortality, injury, and the availability of food and water were conducted 2/day. Clinical observations were conducted on main study animals predose and 0.5 to 1 hour postdose on Day 1 and once on Days 7 and 14. Evaluation

of skin reaction was conducted on main study animals at 0.5 to 1 hour postdose on Day 1 and once on Days 7 and 14. Body weights were measured and recorded on Day -1 and weekly thereafter. Food consumption was measured and recorded on main study animals on Day -1 and then weekly thereafter. Blood and urine samples for clinical pathology evaluations were collected from designated animals at the scheduled necropsies. On Days 2 and 15, necropsy examinations were performed and designated tissues were microscopically examined.

RESULTS: Unremarkable on survival, clinical findings, dermal irritation scoring at the injection sites, body weights, food consumption, clinical pathology evaluations (hematology, coagulation, clinical chemistry, and urinalysis), and macroscopic/microscopic evaluations. No differences were noted between the groups administered 75 mg/kg of Peramivir via the 75 mg /ml or 150 mg /ml concentrations, including TK (see below).

	Peramivir IM 75 mg/mL		Peramivir IM 150 mg/mL	
Gender	C_{max}	AUC_{0-24hr}	C_{max}	AUC_{0-24hr}
	ng/mL	hr.ng/mL	ng/mL	hr.ng/mL
Males	38033	48296	41367	54713
Females	34117	50851	62283	76903

<LLOQ=0 for calculations

The sponsor concluded that (b) (4)

STUDY TITLE: **14-Day Single Dose Intramuscular Toxicity Study With Peramivir 75 mg/ml and 150 mg/ml in Male Rats**

STUDY NO.: B08-002-004

KEY FINDINGS: This is a local irritation study in rats comparing two IM formulations of peramivir. Both IM formulations produced comparable degeneration/necrosis of myofibers at the injection sites. The new formulation (150 mg/ml) was not more irritating than peramivir 75 mg/ml as showed in this study.

LABORATORY: Biocryst Pharmaceuticals, Inc. 2190 Parkway Lake Drive, Birmingham, AL 35244

STUDY INITIATION: 2/08

GLP: Yes (x) no ()

QA REPORT: Yes (x) no ()

LOT # Peramivir IM, 75 mg/ml (Peramivir IM Injection, lot DTH-784-121, drug substance lot 07P0677) and Peramivir IM, 150 mg/ml (Peramivir IM Injection, lot DTH-784-136, drug substance lot 07P0677)

OBJECTIVE Peramivir IM 150 mg/ml was compared to Peramivir IM 75 mg/kg for local tissue tolerance in this rat study (at the same total peramivir dose.). The components included in the two formulations are similar and having similar pHs. They differed in peramivir concentration as shown in the table below:

Table 2 Specifications of Peramivir Drug Products, Placebos and Saline

Component	Function	0.9% Sodium Choride (Saline) (mg/mL)	Placebo 1 (mg/mL)	Peramivir IM 75mg/mL (mg/mL)	Placebo 2 (mg/mL)	Peramivir IM 150mg/mL (mg/mL)
Peramivir API	Active Ingredient	NA	NA	75	NA	150
						(b) (4)

NA, not applicable

DOSING:

Group Number	Test Article	Dosage Level (mg/kg)	Dosage Concentration (mg/mL)	Dosage Volume per Injection (µL)	Total Dosage Volume (µL)	Number of Animals[a]
1	Saline	0	0	150	150	10
2	Placebo 1[c]	0	0	150[b]	300	10
3	Peramivir	75	75	150[b]	300	10
4	Placebo 2[d]	0	0	150	150	10
5	Peramivir	75	150	150	150	10

[a]: 5 animals/sex/group will be euthanized 24 hr post dosing and 5 animals/sex/group will be euthanized 14 days post dosing; [b]: 0.5 mL/kg at two different sites (right and left hind leg); [c]: Placebo 1 is the control for peramivir IM 75 mg/ml; [d]: Placebo 2 is the control for peramivir IM 150 mg/ml

SPECIES/STRAIN: Rat, Crl:CD (SD0 IGS BR)

NO./SEX/GROUP See above.

ROUTE IM (hind legs, shaved; 5/group were sacrificed 24 hr and the tissue surrounding the injection site was examined histologically, with remaining animals sacrificed and examined histologically 14 days following treatment).

RESULTS: Post-dosing at the injection site of all animals showed erythema, edema, induration, and/or necrosis.

The injection sites at 24-hours post-injection exhibited acute degeneration/necrosis of myofibers and fascial and interstitial inflammation. The incidences and mean severities for these changes were comparable among the placebo and peramivir administered groups, but the 75 mg/ml peramivir group showed an increased mean severity for degeneration/necrosis of myofibers, suggesting that injection of 150 mg/ml of peramivir was no more irritating than injection of 75 mg/ml of peramivir.

The recovery changes at the injection site at 24 hours included fibroplasia/fibrosis and regeneration of myofibers, with Groups 3 and 5 having an increased severity of degeneration/necrosis of myofibers. Groups 3 and 5 also had an increased incidence of fibroplasia/fibrosis and regeneration of myofibers. The findings of placebo 2 and saline groups were all within normal limits by 14 days after injection.

Table 5 Incidences and Means of Selected Findings at the Injection Sites: 24-Hour Sacrifice

Observation	Saline	Placebo	Peramivir IM 75 mg/mL	Placebo 2	Peramivir IM 150 mg/mL
	n=5	n=5	n=5	n=5	n=5
Injection Site 1					
Degeneration/Necrosis/Myofiber	3(1.3)	4 (2.3)	4 (3.5)	5 (1.8)	5 (2.2)
Inflammation, Acute, Fascia	1 (1.0)	2 (2.0)	5 (2.0)	3 (1)	5 (2.2)
Inflammation, Acute, Interstitium	-	3 (2.3)	4 (2.0)	3 (1.7)	5 (1.8)
Injection Site 2					
Degeneration/Necrosis/Myofiber		4 (1.2)	2 (1.5)		
Inflammation, Acute, Fascia		3 (1.3)	3 (1.7)		
Inflammation, Acute, Interstitium		3 (1.7)	2 (1.0)		

( ) = Mean equals the sum of all severity grades divided by the number of animals affected

Observation	Saline	Placebo	Peramivir IM 75 mg/mL	Placebo 2	Peramivir IM 150 mg/mL
	n=5	n=5	n=5	n=5	n=5
Injection Site 1					
Degeneration/Necrosis/Myofiber	-	-	-	-	-
Inflammation, Acute, Fascia	-	-	-	-	-
Inflammation, Acute, Interstitium	-	-	-	-	-
Degeneration/Regeneration/Myofiber	-	1 (2.0)	-	-	-
Fibroplasia/Fibrosis, Fascia	-	-	2 (1.0)	-	4 (1.5)
Fibroplasia/Fibrosis, Interstitium	-	1 (1.0)	3 (1.0)	-	2 (1.5)
Regeneration, Myobiber	-	-	-	-	3 (1.0)
Injection Site 2					
Degeneration/Necrosis/Myofiber		-	-		
Inflammation, Acute, Fascia		-	-		
Inflammation, Acute, Interstitium		-	-		
Degeneration/Regeneration/Myofiber		-	1 (2.0)		
Fibroplasia/Fibrosis, Fascia		-	1 (2.0)		
Fibroplasia/Fibrosis, Interstitium		-	2 (1.5)		
Regeneration, Myobiber		-	-		

( ) = Mean equals the sum of all severity grades divided by the number of animals affected

SUMMARY AND CONCLUSIONS

The incidences and severities for injection site irritations were comparable among the placebo and peramivir groups. Peramivir IM 150 mg/ml was not more irritating than Peramivir 75 mg/ml in this rat study.

STUDY TITLE: **A 26-WEEK (ONCE EVERY TWO WEEKS DOSING) INTRAMUSCULAR TOXICITY STUDY OF BCX-1812 (PERAMIVIR) WITH A 4-WEEK RECOVERY PERIOD IN SPRAGUE DAWLEY RATS**

KEY FINDINGS: This is a once per 2 weeks im peramivir study (26 weeks) in rats, given in the hindlimb at 0, 12, 36 and 75 mg/kg/dose. No remarkable toxicity findings were reported, except slight increases in AST (36 mg/kg males and 75 mg/kg males and females; without histology changes), and some injection sites related irritation changes in muscles (dose-related, might be the cause of AST increases, reversible). The NOAEL of this study was 75 mg/kg (AUClast = 230,971 and 219,169 ng•h /ml [male]; and 206,837 and 211,361 ng•h /ml [females] for days 84 and 183.) The AUC were approximately 2.3 fold higher than human AUC of 90 ug.h/ml at 600 mg iv.

STUDY NO.: (b) (4) 196046

LABORATORY: (b) (4)

STUDY INITIATION: 4/26/07

GLP: Yes (x) no ()

QA REPORT: Yes (x) no ()

LOT #/PURITY Peramivir IM, 75 mg /ml (Lot no. A03581-2)

VEHICLE: 0.9% NaCl

DOSING: Once every 2 weeks for 26 weeks (14 doses) to 3 groups (Groups 2-4) and 3 TK groups (Groups 2A-4A). Dosage: 0 (group 1 and 1A), 12, 36 and 75 mg/kg/dose (dose volumes= 1, 0.16, 0.48 and 1 mL/kg for Groups 1/1A, 2/2A, 3/3A and 4/4A, respectively). Groups 1 and 4 each had 30 rats/sex and Groups 2 and 3 had 20 rats/sex (TK: Group 1A 3/sex and 2A-4A 9/sex.

Toxicology Groups (b) (4) 196046)

Group Number	Test Article	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals [a] Males	Females
1	Placebo	0	0	1	30	30
2	BCX-1812	12	75	0.16	20	20
3	BCX-1812	36	75	0.48	20	20
4	BCX-1812	75	75	1	30	30

a = ≤ 20 rats/sex/group were euthanized after 14 doses (primary necropsy). The remaining ≤ 10 rats/sex in Groups 1 and 4 were euthanized following a 4 -week nondosing (recovery) period.

Toxicokinetic Groups (b) (4) 196046A)

Group Number	Test Article	Dose Level (mg/kg/dose) [a]	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals Males	Females
1A	Placebo	0	0	1	3	3
2A	BCX-1812	12	75	0.16	9	9
3A	BCX-1812	36	75	0.48	9	9
4A	BCX-1812	75	75	1	9	9

a = Toxicokinetic group rats were dosed in the same manner as the toxicology group rats.

Necropsy: ≤ 20 rats/sex/toxicology group (study days 183, 184 and 185) and remaining ≤ 10 rats/sex in the control and high dose groups (Groups 1 and 4) were euthanized at end of 4-week recovery (recovery necropsy; study day 213).

SPECIES/STRAIN: Crl:CD (SD IGS BR) Rats

NO./SEX/GROUP see above.

ROUTE IM (hind legs, shaved). Injection locations were in the hindlimb in the caudal area of the thigh relative to the femur, alternated between the left and right hindlimbs.

MEASUREMENTS: 2/day for mortality and moribundity, 1/day for clinical examinations 1/week for detailed physical examinations, body weights and food consumption. Functional observational battery (FOB) parameters and locomotor activity were assessed for 20 rats/sex/group (end of the dosing, study week 24/25) and for ≤ 10 rats/sex in Groups 1 and 4 at end of the recovery, study week 30). Ophthalmology exams: study weeks -1, 12, 25 and 30. Hematology, coagulation, serum chemistry and urinalysis: week 12 and prior to necropsies. Gross macroscopic exams: all rats. Histopathology: control and high-dose groups.

RESULTS: Deaths: None.

Unremarkable: physical/clinical observations, body weights, food consumption, hematology, coagulation/urinalysis parameters, ophthalmology, effects on FOB and motor activity parameters, organ weights, macroscopic/histologic examinations

Increased in AST were noted for the 36 mg/kg/dose group male rats and the 75 mg/kg/dose group male and female rats at the study week 12 and/or 26 evaluation(s) (fully recovered)(might have been due to injuries to the skeletal muscle at the injection sites).

Injection Sites Changes: hemorrhage and edema, muscle degeneration, regeneration and myofiber atrophy, and subacute, chronic or chronic active inflammation (more severe and frequent in treatment than controls)(muscle irritation largely resolved following recovery period.)

TK:
No evidence of accumulations was observed. Exposures increased between Day 0 and Day 183 in both genders; AUC increased by 14-68%, whereas Cmax increased by 7-60%. Peak increases in Cmax occurred between Day 84 and 183. Tmax = 15 minutes whereas T½ ranged from 3.1-5.0 hours.

TOXICOKINETIC RESULTS FOR BCX-1812

BCX-1812 (mg/kg)	AUC_{last} (ng·h/mL)	AUC* (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)
	Males				
Day 0					
12	24766	24809	31450	0.25	1.0 †
36	64414	64507	75600	0.25	4.8
75	145508	145738	144000	0.25	5.0
Day 84					
12	27621	30761	18900	0.25	1.7 †
36	80580	80686	58200	0.25	3.9
75	230971	231132	158333	0.25	3.3
Day 183					
12	41568	41612	36150	0.25	4.1
36	103919	104432	87883	0.25	1.1 †
75	219169	219407	178667	0.25	3.5
	Females				
Day 0					
12	25602	25633	26383	0.25	5.0
36	68101	68121	76317	0.25	3.5
75	129412	129738	132333	0.25	5.5 †
Day 84					
12	26447	26496	28383	0.25	4.4 †
36	79413	79497	62100	0.25	3.8
75	206837	206937	154167	0.25	3.1
Day 183					
12	34877	35540	42317	0.25	1.1†
36	77690	77731	81283	0.25	3.2
75	211361	211512	163000	0.25	3.6

*AUC_{inf} on Day 0, AUC_{τ} on Days 84 and 183.
† Approximated value excluded from further consideration.

STUDY TITLE: **Single Dose Intramuscular Pharmacokinetic Study with Peramivir 75 mg/ml and 150 mg/ml in Male Rats**

KEY FINDINGS: This study was the same as previous IM study in rats (B08-002-002) except that the dosing volume was slightly lower (125ul/250 ul)(B08-002-002; 150ul/300 ul), and that the total dose was 18.7 mg/rat, instead of 22.5 mg/rat. No significant difference in time-concentration curves or the pk profiles between two groups. AUCs achieved were 65 (conc.: 75 mg/ml) or 69 (conc.:150 mg/ml)ug.h/ml.

STUDY NO.: B08-002-002

LABORATORY: Biocryst Pharmaceuticals, Inc. 2190 Parkway Lake Drive, Birmingham, AL 35244

STUDY INITIATION: 2/08

GLP: Yes (x) no ()

QA REPORT: Yes () no (x)

OBJECTIVE This study was designed to evaluate the pk of the same dose of Peramivir (mean 61 mg/kg) administered IM in rats using two different formulations and given in two different total volumes of injection.

LOT #/PURITY Peramivir IM, 75 mg/ml (Peramivir IM Injection, lot DTH-784-121, drug substance lot 07P0677) and Peramivir IM, 150 mg/ml (Peramivir IM Injection, lot DTH-784-136, drug substance lot 07P0677)

DOSING:

Group Number	Test Article	Dosage Level	Dosage Concentration	Total Dosage Volume	Number of Animals
		(mg)	(mg/mL)	(µL)	
001	Peramivir	18.7	75	250	5
002	Peramivir	18.7	150	125	5

SPECIES/STRAIN: Male Crl:CD (SD0 IGS BR) Rats

NO./SEX/GROUP 5 (see above)

ROUTE IM (hip or hind legs, shaved)

RESULTS: The PK of the same dose of Peramivir (50 mg/kg) in rats, using 2 formulations (Peramivir IM 75 mg/ml and Peramivir IM 150 mg/ml) given IM in two different total volumes of injection resulted in similar pharmacokinetic profiles in rats. The mean plasma concentration-time data for each group are shown below.

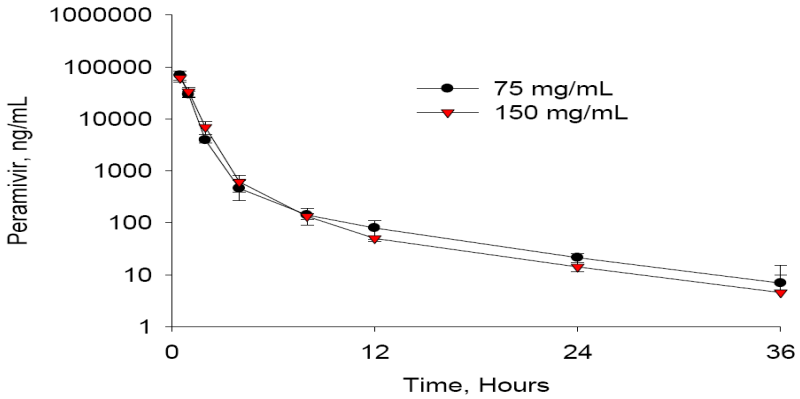


Figure 2. Log-Linear Plot of Plasma Peramivir

Table 2. Peramivir Mean Plasma Concentrations

Formulation	Time (hr)	N	Peramivir, ng/mL (mean ± SD)
75 mg/mL	0	4	2.6 ± 5.2
	0.5	4	69025.0 ± 13552.5
	1	4	29600.0 ± 4188.7
	2	4	3917.5 ± 424.1
	4	4	461.3 ± 195.7
	8	4	140.8 ± 49.9
	12	4	79.6 ± 31.3
	24	4	21.4 ± 4.3
	36	4	6.9 ± 8.3
	48	4	0.0 ± 0.0
150 mg/mL	0	4	0.0 ± 0.0
	0.5	4	61337.5 ± 10830.3
	1	4	33525.0 ± 6884.8
	2	4	6937.5 ± 1944.7
	4	4	613.3 ± 220.5
	8	4	133.3 ± 16.4
	12	4	50.1 ± 6.1
	24	4	14.2 ± 2.8
	36	4	4.5 ± 5.2
	48	4	0.0 ± 0.0

Table 3. Pharmacokinetics of Intramuscular Peramivir IM 75 mg/mL and Peramivir 150 mg/mL

	Animal Number	Formulation (mg/mL)	Cmax (ng/mL	AUC0-24 hr (hr·ng/mL)	AUC0-∞ (hr·ng/mL)	Weight (gm)	Injection Volume (µL/site)	Total Injection Volume (µL)	Total Dose (mg/kg)
	1M	75	74500	65397.9	65538.8	394.9	125	250	47.5
	2M	75	81600	73819.9	74266.3	354.2	125	250	52.9
	4M	75	50000	52058.2	52306.5	382.6	125	250	49.0
	5M	75	70000	69930.3	70233.5	381.7	125	250	49.1
Mean			69025	65301.6	65586.3	378.4			49.6
SD			13552.5	9476.0	9544.5	17.2			2.3
CV			20%	15%	15%	5%			5%

		Formulation (mg/mL)	Cmax (ng/mL	AUC0-24 hr (hr·ng/mL)	AUC0-∞ (hr·ng/mL)	Weight (gm)	Injection Volume (µL/site)	Total Injection Volume (µL)	Total Dose (mg/kg)
	6 M	150	73500	74365.5	74499.2	372.8	62.5	125	50.3
	7 M	150	51100	58959.0	59038.0	411.3	62.5	125	45.6
	8 M	150	53400	67966.4	68186.8	380.1	62.5	125	49.3
	9 M	150	67350	75017.1	75309.3	351.5	62.5	125	53.3
Mean			61337.5	69077.0	69258.3	378.9			49.6
SD			10830.3	7457.9	7520.7	24.8			3.2
CV			18%	11%	11%	7%			6%

STUDY TITLE: **2-Week IM Toxicity Study of Peramivir in Cynomolgus Monkeys With a 2-Week Recovery Period.**

STUDY NO.: 0527-06102

LABORATORY: (b) (4)

STUDY INITIATION: 4/06

GLP: Yes (x) no ()

QA REPORT: Yes (x) no (), in a draft report form without toxicokinetic results.

OBJECTIVE To assess the toxicity of IM peramivir in cynomolgus monkeys (2-week study).

LOT #/PURITY 6101-1 and -2, 75mg/ml

VEHICLE: 0.9% NaCl

DOSING:

Group	Dosage mg/kg	Conc. mg/ml	Volume mL/kg	Terminal Sacrifice		Recovery Sacrifice		Animal No.	
				M	F	M	F	M	F
CMU1	0*	0	0.36	3	3	2	2	1-5	22-26
CMU2	6	75	0.08	3	3	0	0	6-8	27-29
CMU3	18	75	0.24	3	3	0	0	9-11	30-32
CMU4	54*	75	0.36	3	3	2	2	12-16	33-37
CMU5	54*+	75	0.36	3	3	2	2	17-21	38-42

**Given as two separate injections, except Day 2 when there were three injections*
+Group 5 used a different test article lot than Group 4

SPECIES/STRAIN: Cynomolgus monkeys
NO./SEX/GROUP 3/sex (2/sex in recovery groups, see above)
ROUTE IM, 1/day for 14 days
OBSERVATIONS:
MORTALITY: Observations for moribund or dead animals were made 2 times daily.
CLINICAL SIGNS: 1/day
BODY WEIGHTS: Checked once/week
FOOD Checked once/week
CONSUMPTION:
OPHTHALMOLOGY: During pretest and at termination.
EKG: During pretest and at termination.
HEMATOLOGY: During pretest and at termination.
CLINICAL During pretest and at termination.
CHEMISTRY:
URINALYSIS: During pretest and at termination.
TOXICOKINETICS During pretest, day 1, 7 and 14
GROSS PATHOLOGY: Macroscopic examinations were conducted on all tissues and lesions from all monkeys.
ORGANS WEIGHED: At the end of the treatment period (see list below).
HISTOPATHOLOGY TISSUES/ORGANS COLLECTED, WEIGHTED AND EXAMINED LIST:

Tissue	Organ Weight Taken	Collected and Preserved in 10% NBF	Microscopic Examination
Adrenal glands*	X	X	X
Aorta (thoracic)		X	X
Brain	X	X	X
Cecum		X	X
Cervix		X	X
Colon		X	X
Duodenum		X	X
Epididymides*	X	X	X
Esophagus		X	X
Eyes		X	X

Femur, proximal		X	
Gall Bladder		X	X
Heart	X	X	X
Identification		X	
Ileum		X	X
Iliopsoas		X	X
Injection sites (Biceps femoris)		X	X
Jejunum		X	X
Kidneys*	X	X	X
Lesion(s)		X	X
Liver	X	X	X
Lungs		X	X
Lymph node - mandibular		X	X
Lymph node - mesenteric		X	X
Mammary gland with contiguous skin		X	X
Ovaries*	X	X	X
Pancreas		X	X
Pituitary		X	X
Prostate		X	X
Rectum		X	
Salivary gland		X	X
Sciatic nerve		X	X
Spinal cord – cervical, thoracic		X	X
Spleen	X	X	X
Sternum		X	X
Stomach		X	X
Testes*	X	X	X
Thymus	X	X	X
Thyroids/Parathyroids*	X	X	X
Trachea		X	X
Urinary bladder		X	X
Uterus	X	X	X
Vagina		X	X

RESULTS:

MORTALITY None

CLINICAL SIGNS Unremarkable

BODY WEIGHTS: Unremarkable

FOOD CONSUMPTION Unremarkable

OPHTHALMOLOGY: Unremarkable

EKG: Unremarkable

HEMATOLOGY: Decreased RBC/Hct in Group 4 (54 mg/kg, both sexes) were observed: 9.6% for

	RBC and 8.4% for HCT (male), 9.1% for RBC and 6.5% for Hct (female). Reticulocytes were increased in all dose groups in both sexes (males: 68-92%; females:69-143%). During the recovery period, for Group 4 and 5 males, HCT RBC, reticulocyte counts returned to levels similar to that seen in the Group 1 control males. For the females in Groups 4 and 5, HCT, RBC returned to levels similar to Group 1 control values by the end of the recovery period while the reticulocyte counts were still decreased. The sponsor considered these alterations in RBC, HCT and reticulocytes were likely to be related to blood loss from the multiple blood draws which were taken to obtain toxicokinetics samples the day before hematology samples were taken.
COAGULATION	Unremarkable
CLINICAL CHEMISTRY	Unremarkable
URINALYSIS:	Unremarkable
ORGAN WEIGHTS:	Unremarkable.
HISTOPATHOLOGY:	Hemorrhage, subacute inflammation and myocyte necrosis/degeneration were seen in both at control and high dose injection sites of both sexes. Acute inflammation was also seen at injection sites in one control animal of each sex and one female in one of the high dose groups, but not in any high dose males. The hemorrhage, subacute inflammation and myocyte degeneration tended to be more numerous and more severe in the two high dose groups compared to the controls. The sponsor indicated that 'there was evidence of recovery at the injection site as indicated by the lack of any subacute inflammation findings and incidences of myocyte regeneration in control and dose groups of both sexes.' and that 'The test article was well-tolerated at all doses tested in this study. '
TOXICOKINETICS	The AUC (54 mg/kg/day) at the high dose group were around 273-300 ug.h/ml. The dose response of the Cmax and AUC0-inf was linear over the course of the study. No differences were observed between the Cmax or AUC values of Group 4 and 5 (representing two different lots of test article). The Tmax was similar for all doses on all days with the range of Tmax values being between 0.25 and 0.33 hr. Cmax and AUC values were similar between days within doses. Cmax and AUC values increased with dose. Cmax and AUC values did not vary between differing lots (Groups 4 and 5). Tmax was not altered with dose.

Peramivir Pharmacokinetic Parameters for days 1, 7, and 14

	Group	Dose (mg/kg)	Day	R2	λz t½ (hr)	Tmax (hr)	Cmax (ng/mL)	AUC0-24 hr (hr·ng/mL)	AUC0-inf (hr·ng/mL)
Mean	2	60	1	0.931	2.952	0.33	165750	248477.1	249077.1
SD				0.046	0.969	0.129	39548	95678.8	95510.0
CV				4.9%	32.8%	38.7%	23.9%	38.5%	38.3%
Mean	2	6	7	1	4	0	25200	31873.7	31994.8
SD				0	1	0	3875	7517.3	7535.2
CV				5.4%	13.1%	0.0%	15.4%	23.6%	23.6%
Mean	2	6	14	0.848	4.2	0.25	25892	32279.9	32405.6
SD				0.044	0.5	0.0	4886.5	7588.9	7599.4
CV				5.2%	11.7%	0.0%	18.9%	23.5%	23.5%
Mean	3	18	1	0.913	3.4	0.25	73708	86929.6	87037.5
SD				0.021	0.156	0.000	10567	3921.8	3925.2
CV				2.2%	4.6%	0.0%	14.3%	4.5%	4.5%
Mean	3	18	7	0.869	4.1	0.25	75542	89980.7	90262.3
SD				0.026	0.1	0.0	8591.2	6455.7	6500.8
CV				0.030	0.022	0.0	0.114	0.072	0.072
Mean	3	18	14	0.854	4.2	0.25	65967	83022.3	83314.9
SD				0	0	0	8915	12232.1	12226.6
CV				6.4%	6.6%	0.0%	13.5%	14.7%	14.7%
Mean	4	27	1	0.91488	3.3	0.25	137600	157578.8	157779.1
SD				0.02	0.20	0	43262.6	26511.2	26547.4
CV				2.3%	6.1%	0.0%	31.4%	16.8%	16.8%
Mean	4	54	7	0.854	4.0	0.300	211550	283312.7	284297.8
SD				0.034	0.284	0.105	52026	65536.8	65669.3
CV				4.0%	7.1%	35.1%	24.6%	23.1%	23.1%
Mean	4	54	14	0.853	3.847	0.250	207350.000	299941.6	300823.3
SD				0.043	0.267	0.000	25376.115	43967.5	44137.3
CV				5.1%	6.9%	0.0%	12.2%	14.7%	1304.7%
Mean	5	27	1	0.897	3.4	0.25	119880	156085	156336
SD				0.038	0.2	0.0	22790	30105.5	30152.2
CV				4.2%	6.4%	0.0%	19.0%	19.3%	19.3%
Mean	5	54	7	0.847	4.1	0.25	207150	267426.0	268368.3
SD				0.037	0.3	0.000	39253	32431.6	32545.9
CV				4.3%	6.5%	0.0%	18.9%	12.1%	12.1%
Mean	5	54	14	0.846	4.1	0.25	188400	272600.3	273563.3
SD				0.037	0.3	0	13362	34286.9	34376.7
CV				4.4%	7.4%	0.0%	7.1%	12.6%	12.6%

SUMMARY The NOAEL was determined by the sponsor to be 54 mg/kg/day for this intramuscular study (AUC=273-300 ug.h/ml).

Table 1.11.2-1. Summary of Treatment-Related Effects Following Intramuscular Injection for Two-Weeks in Cynomolgus Monkeys

Animal Species, Strain, Age (week), Sex, Body Weight	Cynomolgus monkey, 2.0–4.0 years, male and female, 1.572–2.946 kg (at study start)				
Dosing Route (dosing method)	Intramuscular (Test article prepared in placebo im injection. Administered once daily in the rear leg. Injection site alternated daily. Control and 54-mg/kg doses administered as a split dose of ~equal-sized injections to the same leg for each daily dose.)				
Dose (mg/kg/day)	Group 1 Vehicle control	Group 2 6 (Lot No. 6102-1)	Group 3 18 (Lot No. 6102-1)	Group 4 54 (Lot No. 6102-1)	Group 5 54 (Lot No. 6101-2)
Number of Animals	5 M, 5 F	3 M, 3 F	3 M, 3 F	5 M, 5 F	5 M, 5 F
Number of Dead Animals	—	—	—	—	—
Clinical Observations	—	—	—	—	—
Body Weight	—	—	—	—	—
Ophthalmology	—	—	—	—	—

Table 1.11.2-1.		Summary of Treatment-Related Effects Following Intramuscular Injection for Two-Weeks in Cynomolgus Monkeys				
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Food Consumption		—	—	—	—	—
ECG		—	—	—	—	—
Hematology		—	F: ↑ Retic	—	M: ↓ HCT, M/F: ↓ RBC, ↑ Retic	F: ↑ Retic
Coagulation		—	F: ↑ PT	—	—	F: ↑ PT
Blood Chemistry		—	—	—	—	—
Urinalysis		—	—	—	—	—
Necropsy Findings		—	—	—	—	—
Organ Weights		—	—	—	—	—
Histopathology		—	—	—	—	—
Plasma Concentrations						
n = 3–5/sex/group		M/F	M/F	M/F	M/F	M/F
Day 1	C_{max} (ng/mL)		165750[a]	73708	137600[b]	119880[b]
	$t_{½}$ (h)		2.952[a]	3.363	3.25[b]	3.4[b]
	$AUC_{(0-\infty)}$ (ng·h/mL)		249077.1[a]	87037.5	157779.1[b]	156336[b]
Day 7	C_{max} (ng/mL)		25200	75542	211550	207150
	$t_{½}$ (h)		4	4.1	4.0	4.1
	$AUC_{(0-\infty)}$ (ng·h/mL)		31994.8	90262.3	284297.8	268368.3
Day 14	C_{max} (ng/mL)		25892	65967	207350	188400
	$t_{½}$ (h)		4.2	4.2	3.847	4.1
	$AUC_{(0-\infty)}$ (ng·h/mL)		32405.6	83314.9	300823.3	273563.3
NOAEL		54 mg/kg/day				

— = No remarkable findings compared with the vehicle control group
[a] = following a dose of 60 mg/kg
[b] = following a dose of 27 mg/kg
ECG = Electrocardiogram
M = Male
F = Female
RBC = Red blood cell
$t_{½}$ = Half-life
NOAEL = No observed adverse effect level
Retic = Reticulocyte
HCT = Hematocrit
C_{max} = Maximum plasma concentration
AUC = Area under the plasma time curve

STUDY TITLE: **A 52-WEEK (ONCE WEEKLY DOSING) INTRAMUSCULAR TOXICITY STUDY OF BCX-1812 (PERAMIVIR) WITH A 4-WEEK RECOVERY PERIOD IN CYNOMOLGUS MONKEYS**

KEY FINDINGS: This is a once-a-week im peramivir study (52 weeks) in monkeys, given in the hindlimb at 0, 6, 18, and 54 mg/kg/dose. No remarkable toxicity findings were reported, except injection sites related irritation in local muscles and connective tissues that was not reversible and was significant (dose-related interstitial edema, myofiber degeneration and mixed inflammatory cell infiltrates, interstitial fibrosis, and myofiber regeneration). The NOAEL (systemic toxicity) of this study was 54 mg/kg. The AUClast was reported to be 247 and 231 ug•h /ml for male and female, respectively. However there is no accumulation because of infrequent dosing. The NOAEL for local irritation toxicity was 6 mg/kg/dose.

STUDY NO.: (b) (4)-196047

LABORATORY: (b) (4)

STUDY INITIATION: 4/26/07

GLP: Yes (x) no ()

QA REPORT: Yes (x) no ()

LOT #/PURITY Peramivir IM, 75 mg /ml (Peramivir IM Injection, Lot no. A03581-2, purity=98.5% (73.9 mg /ml) and 99.7% (74.9 mg /ml.)

VEHICLE: 0.9% NaCl

DOSING: BCX-1812 im was given once weekly, for a minimum of 364 days to 3 groups (Groups 2-4) of cynomolgus monkeys at 6, 18, and 54 mg/kg/dose (see table below). Controls (Group 1) received Peramivir Placebo IM for injection (placebo). The dose

volume for the control and 54 mg/kg/dose groups was 0.72 ml/kg, the 6 mg/kg/dose group was 0.08 ml/kg and the 18 mg/kg/dose group was 0.24mL/kg. Four animals/sex/group were scheduled for the primary necropsy at the end of the 52-week treatment period (remaining 2 animals/sex in the control and 54 mg/kg/dose groups were put in the 30-day recovery period and a 56-week recovery necropsy).

Group Number	Test Article	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals [a] Males	Females
1	Placebo	0	0	0.72	6	6
2	BCX-1812	6	75	0.08	4	4
3	BCX-1812	18	75	0.24	4	4
4	BCX-1812	54	75	0.72	6	6

[a] = 4 animals/sex/group were euthanized following a once weekly dose administration (52 weeks; 53 doses); the remaining 2 animals/sex/group were euthanized following a 30-day recovery period.

SPECIES/STRAIN: Cynomolgus monkeys (*Macaca fascicularis*; Vietnamese origin)

NO./SEX/GROUP see above.

ROUTE IM (hind legs, shaved). The injection sites were at the hindlimb in the caudal area of the thigh relative to the femur (a total of 53 doses, sites alternated between the left and right hindlimbs). To minimize trauma and bleeding, if the total dose volume was ≥ 1.5 mL, the total dose volume was injected into multiple areas at the site.

OBSERVATIONS: 2/day for mortality and moribundity, 1/day for clinical examinations 1/week for detailed physical examinations, body weights and food consumption.
Ophthalmic examinations: (study week -3) and during study weeks 26, 51, and 55 (recovery period).
EKG: (study week -2) and during study weeks 11, 24, 37, 52, and 56 (recovery period).
Hematology, coagulation, serum chemistry and urinalysis: (study week -2) and during study weeks 12, 25, 38, 52, and 56 (recovery period).
TK: prior to dosing and at 0.25, 1, 4, 8, and 24 hours after dose administration, on study day 0, and during study weeks 12, 25, 38, and 51.
Gross macroscopic examinations and microscopic examination: all animals

RESULTS: Death: None
Unremarkable: physical or clinical observations, body weights, hematology, coagulation, serum chemistry, and urinalysis.

Microscopic findings: None except at the injection sites (increased incidence and severity of interstitial edema, myofiber degeneration and mixed inflammatory cell infiltrates, and increased severity of interstitial fibrosis, and myofiber regeneration in animals from the 18 and 54 mg/kg/dose groups.) During recovery phase, there were still persistent histologic changes (increased incidence and severity of interstitial fibrosis, mild inflammatory cell infiltrates, and minimal to mild myofiber degeneration, indicating incomplete recovery test article-related irritations.) The sponsor considered that these injection site findings in the recovery phase were suggestive of systemic toxicity. (b) (4)

TK

Exposures increased with dose and were similar between genders. No evidence of accumulations was observed. Tmax≈ 15 minutes. T½≈0.8-4.2 hours

MALE EXPOSURES

BCX-1812 (mg/kg)	AUC_{last} (ng•h/mL)	AUC* (ng•h/mL)	C_{max} (ng/mL)	T_{max} (h)
Study Day 0				
6	19903	19953	20538	0.25
18	66605	66752	61213	0.25
54	188074	188496	168083	0.25
Study Day 84				
6	19305	19728	17588	0.25
18	79240	79423	66150	0.25
54	214815	215280	183583	0.25
Study Day 175				
6	21477	21896	22938	0.25
18	74820	74961	70438	0.25
54	216698	217094	184083	0.25
Study Day 266				
6	17600	18037	16163	0.25
18	82446	82600	64200	0.25
54	237770	238298	203000	0.25
Study Day 357				
6	23808	24056	22225	0.25
18	84513	84679	61313	0.25
54	246812	247276	196833	0.25

* = AUC_{inf} on Study Day 0, AUC_{τ} on Study Days 84, 175, 266, and 357.
N = 4 at 6 and 18 mg/kg
N = 6 at 54 mg/kg.

FEMALE EXPOSURES

BCX-1812 (mg/kg)	AUC_{last} (ng•h/mL)	AUC* (ng•h/mL)	C_{max} (ng/mL)	T_{max} (h)
Study Day 0				
6	22296	22357	22250	0.25
18	60821	60947	60588	0.25
54	187902	188343	166750	0.25
Study Day 84				
6	24136	24187	22163	0.25
18	69847	69995	62463	0.25
54	191442	202739 †[5]	176750	0.25
Study Day 175				
6	23258	23545	23100	0.25
18	67650	67772	59188	0.25
54	255174	255567	235417	0.25
Study Day 266				
6	27826	28398	25513	0.25
18	84641	84795	71863	0.25
54	240516	240908	203167	0.25
Study Day 357				
6	25796	25870	22813	0.25
18	78105	78232	63513	0.25
54	230708	231075	217000	0.25

* = AUC_{inf} on Study Day 0, AUC_{τ} on Study Days 84, 175, 266, and 357.
N = 4 at 6 and 18 mg/kg
N = 6 at 54 mg/kg, except where indicated as †[n].

MUTAGENICICITY STUDIES

1. **In vitro mutagenicity testing of RWJ-270201 (b) (4) in the bacterial/microsomal activation assay (Study No. DS98322; R.W. Pharmaceutical Research Institute, Spring House, PA/Raritan, NJ; April, 1999).**

Method. This is a GLP study. RWJ-270201 (b) (4) was tested in a bacterial/microsomal activation plate incorporation assay using *Salmonella typhimurium* Strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* Strain WP_2uvrA. This study included tests in the absence (buffer) and presence of metabolic activation by an Aroclor® 1254-induced rat liver microsomal preparation (S-9 mix). RWJ-270201 (b) (4) was tested in all strains under both metabolic conditions at doses of 50, 250, 500, 1000, 2500 and 5000 µg per plate. Mutations were detected by phenotypic reversion to amino acid prototrophy (histidine or tryptophan for *S. typhimurium* or *E. coli*, respectively) in two replicate tests for each strain, with the vehicle-treated plates serving as the

standards for comparison. Repeated and dose-related doublings of negative control values would have been considered significant mutagenic responses.
Acceptable negative control and positive controls (2-anthramine, 9-aminoacridine, sodium azide, and N-methyl-N-nitro-N-nitrosoguanidine) results were obtained for all strains in the absence and the presence of S-9 mix. This assures that the test system was functioning and responsive.

Treatment of all five strains of bacteria with RWJ-270201 (b) (4) under both of the metabolic conditions tested did not induce any notable increases in revertant colony counts as compared to the negative controls. Indications of toxicity (decreased colony counts or thin bacterial lawns) were not seen. These findings indicated that RWJ-270201was negative in the bacterial/microsomal activation mutagenesis assay.

2. **In vitro chromosome aberration assay of RWJ-270201 (b) (4) using CHO-K_1 cells (Study No. DS98321; (b) (4) December, 1999).**

Method. This is a GLP study. The test article, RWJ-270201 (b) (4) was tested in the chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the absence and presence of an Aroclor-induced S9-activation system (positive control: mitomycin C and cyclophosphamide). Based on preliminary toxicity assay findings, the concentrations chosen for the chromosome aberration assay ranged from 205 to 3280 μg/ml for the nonactivated and the S9-activated exposure groups.

Results. No substantial toxicity (≥50% cell growth inhibition) was observed at any concentration evaluated for chromosome aberrations in the nonactivated or S9 activated exposure groups. No statistically significant increases in structural and numerical and structural chromosome aberrations were observed in the nonactivated or S9-activated 4-hour exposure groups relative to the solvent control group, regardless of concentration ($p > 0.05$). Based on the findings of this study, RWJ-270201 (b) (4) was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells.

3. **Bone marrow micronucleus assay in mice dosed orally with RWJ-270201 (b) (4) (Study No. DS98320; R.W. Pharmaceutical Research Institute, Spring House, PA/Raritan, NJ; November, 1998).**

Method. This is a GLP study. Groups of five male and five female Crl:CD-1® (ICR) BR, VAF/Plus® mice were dosed orally with RWJ-270201 (b) (4) for 3 days at doses of 1250, 2500, and 5000 mg/kg/day. The mice were sacrificed 24 hours after the third dose for the collection of bone marrow. Bone marrow was also collected from a similar group of mice 24 hours after a single dose (0.3 mg/kg i.p.) of triethylene melamine (TEM), the positive indicator for the study. The vehicle control mice (10 males and 10 females) were dosed orally with vehicle (sodium carboxymethyl cellulose, 0.5% solution) for 3 days and sacrificed approximately 24 hours after the third dose. Smear preparations of the femoral bone marrow collected from all mice were evaluated for the incidence of micronuclei in polychromatic erythrocytes (PCEs), and for a PCE to normochromatic erythrocyte (NCE) ratio as an indicator of toxicity. Acceptable vehicle control results were obtained (male 0.10% and female 0.07% micronucleated PCEs), and statistically significant increases in micronucleated PCEs were seen with the positive indicator (male 6.98% and female 2.94%).

Results.

None of the RWJ-270201 (b) (4) dosed groups exhibited statistically significant increases in the incidence of micronucleated PCEs, yielding a negative result in this in vivo micronucleus assay. There were no notable decreases in the PCE/NCE ratio as compared with the vehicle control. All RWJ-270201 (b) (4) treated groups, male and female, exhibited white feces. The high dose of 5000 mg/kg is considered the limit dose for this type of assay and represents an adequate assessment of the potential genotoxic activity of RWJ-270201 (b) (4) in this assay. These findings indicate a lack of potential for the induction of chromosomal effects by RWJ-270201 (b) (4) in this mouse bone marrow micronucleus assay.

JUVENILE ORAL TOXICITY STUDIES:

STUDY TITLE:	**An Oral (Gavage) 2-Week Toxicity Study Of RWJ-270201 (b) (4) In Neonatal/Juvenile Albino Rabbits**
KEY FINDINGS:	This 14-day neonatal/juvenile oral rabbit study showed renal toxicity could be elicited at 1200 mg/kg (lethal dose). Renal toxicity was initially observed in adult rabbit studies (see Special Toxicology Section above). In this study, increased eosinophilic cytoplasmic materials in the epithelial cells of primarily proximal cortical tubules (minor degree) were seen for some male (3/11) and female (4/10) pups at 1200 mg/kg/day. NOAEL for renal toxicity was 300 mg/kg (AUC=60 [male] or 43 [female] ug.h/ml).
STUDY NO.:	DS00304
VOLUME/PAGE:	EDR
LABORATORY:	(b) (4)
STUDY INITIATION:	4/4/2000
GLP:	Yes (x) no ()
QA REPORT:	Yes (x) no ()
OBJECTIVE	The objective of this study was to determine the effects of RWJ-270201 (b) (4) when administered orally (gavage) to neonatal/juvenile NZW rabbits from postnatal days 21 to 34, inclusive.
LOT #/PURITY	99P0236
VEHICLE:	Sodium Carboxymethylcellulose, 7L2P, 0.5% solution
DOSING:	Pups (1/sex) from 10 litters each were assigned to the control group and each drug-treated group (Groups 2 to 4) and given drug at 0 (vehicle control), 50, 300 or 1200 mg/kg/day for 2 weeks (from Days 21 -34 postpartum*).* The control group received the vehicle 0.5% (w/v) carboxymethylcellulose, (10 mL/kg). From the two pups per sex per litter assigned to each group, 1 pup/sex/litter was assigned for toxicology assessments and 1 pup/sex/litter were assigned for TK used for Day 14 TK assessment. In addition, pups not selected initially for the main study or TK were selected from dams for the Day 1 TK assessments.
SPECIES/STRAIN:	NZW rabbits
NO./SEX/GROUP	10 litters of 1 male and 1 female/group
ROUTE	Oral gavage
OBSERVATIONS:	The animals were checked 2/day for mortality and signs of ill health (prior to and 1

hour after dosing). Physical exam was conducted on days of body weight assessment (Days 14, 17, 21, 24, 28, 32, and 35 postpartum)and culling were done on days 21/22 post-partum. Fundoscopic and biomicroscopic exams were done in all animals at week 2. On day 1 and day 14 of treatment, blood samples were obtained for TK were done on Day 1 and 14. Hematology, clinical chemistry and pathological examination were done at termination (day 35 postpartum).

RESULTS: 3 pups from one litter at 1200 mg/kg/day were either found dead or euthanized preterminally. Pups from all groups (control, 50, 300 and 1200 mg/kg/day), had soft feces between days 23 - 30. WBC counts were variable. There was a statistically significant decreasing dose related trend for WBC and segmented neutrophil counts (300, 1200 mg/kg females).

CLINICAL CHEMISTRY There was a statistically significant decrease in thyroxine (T4) for males at 1200 mg/kg/day, a slight statistically significant dose-related increasing trend in phosphorus levels for both males and females, and in sodium for females.

ORGAN WEIGHTS: There were no drug-related effects on organ weights.

GROSS PATHOLOGY: No gross pathological changes were considered to be drug-related.

HISTOPATHOLOGY: Eosinophilic cytoplasmic material in the epithelial cells were increased (primarily proximal cortical tubules, minimal) in 3/11 males and 4/10 females at 1200 mg/kg. No effects were observed for the lower dose groups.

TOXICOKINETICS: AUCs increased dose-proportionally in both sexes. With the exception of one aberrant value, there were also dose-related increases in Cmax. Cmax, and AUC were slightly lower following 14 days of dosing at all dose levels and in both sexes. Following the first dose, drug was moderately absorbed with Tmax = 2-4hr, but was rapidly absorbed following Day 14 (Tmax=0.5-1 hr). The drug was eliminated slowly (T½= 7.43-43.03 hrs). No gender differences were apparent.

Table DM3: Summary of Pharmacokinetic Parameters for RWJ-270201-000 from Mean Plasma Concentration Data of Male and Female Neonatal/Juvenile New Zealand White Rabbits Following Single or Multiple Oral Doses (50, 300, or 1200 mg/kg) of RWJ-270201 (b) (4) (DM00352)

Interval	Sex	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	AUC (0-∞) (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h·kg)
Day 1	Male	50	1501	2.00	10826	7.43	4618
		300	4924	4.00	55116	10.11	5443
		1200	18726	4.00	257239	9.40	4665
	Female	50	1182	2.00	15374	10.85	3252
		300	7081	2.00	93641	9.05	3204
		1200	12813	2.00	319374	22.53	3757

Interval	Sex	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	AUC (0-24) (ng·h/mL)	$t_{½}$ (h)	CL/F (mL/h·kg)
Day 14	Male	50	581	0.50	6319	40.60	2805
		300	4647	1.00	59743	20.50	2632
		1200	13507	0.50	121822	42.53	2839
	Female	50	11601	0.50	12285	27.26	2230
		300	3301	1.00	43058	43.03	1630
		1200	13302	0.50	128297	22.29	4645

SUMMARY This rabbit juvenile toxicity study showed that: 1. dose-related decreases in WBC and neutrophil counts (300 and 1200 mg/kg, females), 2. Renal cortical tubular pathology (increased eosinophilic cytoplasmic material in the epithelial cells of primarily proximal cortical tubules) occurred at 1200 mg/kg/day (both sexes).

STUDY TITLE:	**An Oral (Gavage) 2-Week Toxicity Study Of RWJ-270201 (b) (4) In Neonatal/Juvenile Albino Rats**
KEY FINDINGS:	Toxicity profile revealed from this 14-day juvenile/juvenile rat toxicity study included clinical changes (liquid feces) (500, 1500 mg/kg), lower body weight (1500 mg/kg), dose-related increases in segmented neutrophils (500, 1500 mg/kg, males)/ RBC-Hct-Hb (1500 mg/kg, males), and low urinary pH/high specific gravity (1500 mg/kg, both sexes). NOAEL: 50 mg/kg (AUC=1.2 ug.h/ml) in both sex groups.
STUDY NO.:	DS00301
VOLUME/PAGE:	EDR
LABORATORY:	(b) (4)
STUDY INITIATION:	2/28/2000
GLP:	Yes (x) no ()
QA REPORT:	Yes (x) no ()
OBJECTIVE	The objective of this study was to determine the effects of RWJ-270201 (b) (4) when administered orally (gavage) to neonatal/juvenile Sprague-Dawley rats from postnatal days 13 to 26, inclusive.
LOT #/PURITY	99P0236
VEHICLE:	Sodium Carboxymethylcellulose, 7L2P, 0.5% solution
DOSING:	Pups from ten litters (4/sex/group); dose: 0 (vehicle control), 50, 500 or 1500 mg/kg/day oral gavage for 2 weeks (from Days 13 to 26 postpartum). The vehicle was 0.5% (w/v) aq. carboxymethylcellulose at 10 mL/kg. Five additional litters of 4/sex were used for TK analysis (Day 1 dosing [Day 13 postpartum] and 14). From the TK groups: 1 pup/sex/litter was assigned for toxicology assessments, 2 pups/sex/litter were TK, of which 1 was used for coagulation parameters and the other pup/sex/litter was a treated litter mate that was discarded at weaning.
SPECIES/STRAIN:	Sprague-Dawley rats
NO./SEX/GROUP	4
ROUTE	Oral gavage
OBSERVATIONS:	Observations on mortality (2/day), physical examination, body weight (2/week), hematology, clinical chemistry, urinalysis (at termination), TK (Day 1 and 14), Gross pathology/histopathology were performed.
MORTALITY:	There was no drug-related mortality.
CLINICAL SIGNS	Liquid feces were observed in all treated pups and were considered drug-related (Days 14 and 20 post-partum).
BODY WEIGHTS:	Body weight was reduced for animals dosed at 1500 mg/kg/day from Day 19 onwards. No drug-related effects were observed on body weight for the 50 and 500 mg/kg/day dose groups.
OPHTHALMOLOGY:	Unremarkable
HEMATOLOGY:	There were increased absolute and differential segmented neutrophils (males, 500 and 1500 mg/kg) and lower lymphocytes at 1500 mg/kg/day. There were also dose-dependent increases in absolute and differential segmented neutrophils and decreases in lymphocytes for females of 1500 mg/kg group. Dose-dependent increases in RBC/Hct/Hb and fibrinogen were observed (males, 500 and/or 1500 mg/kg).
CLINICAL	Increases in cholesterol, sodium, calcium and phosphorus and/or chloride were

CHEMISTRY AND URINALYSIS: observed (males, 500 and 1500 mg/kg). There were also increases in triglyceride (females, 50 mg/kg/day). Low urinary pH and high specific gravity occurred at 1500 mg/kg (both sexes).

ORGAN WEIGHTS: Unremarkable

GROSS PATHOLOGY: Unremarkable

HISTOPATHOLOGY: Unremarkable

TOXICOKINETICS: AUCs and Cmax increased dose-proportionally in both sexes. Cmax, and AUC were slightly lower following 14 days of dosing at all dose levels and in both sexes. Following the first dose, drug was moderately absorbed with Tmax = 2-8hr, but was rapidly absorbed following Day 14 (Tmax=1 hr). The drug was eliminated slowly (T½= 4.3-12.8 hrs). No gender differences were apparent in PK measurements.

Table DM5 Summary of Pharmacokinetic Parameters for RWJ-270201-000 from Mean Plasma Concentration Data of Male and Female Neonatal/Juvenile Sprague-Dawley Rats Following Single or Multiple Oral Doses (50, 500 or 1500 mg/kg) of RWJ-270201 (b) (4) (DM00315)

Interval	Sex	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	AUC (0-∞) (ng h/mL)	$t_{1/2}$ (h)	CL/F (mL/h kg)
Day 1	Male	50	2272	4 00	32833	8 67	1523
		500	7269	2 00	111175	8 56	4497
		1500	29648	8 00	398070	4 27	3768
	Female	50	1394	8 00	33491	12 80	1493
		500	8421	8 00	139961	7 05	3572
		1500	33682	8 00	433175	5 19	3463

Interval	Sex	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	AUC (0-24) (ng h/mL)	$t_{1/2}$ (h)	CL/F (mL/h kg)
Day 14	Male	50	342	1 00	1209	6 94	39158
		500	2097	1 00	9548	6 84	48573
		1500	5287	1 00	24163	7 29	59206
	Female	50	458	1 00	1287	11 44	32711
		500	2001	1 00	8137	7 04	57209
		1500	5588	1 00	20280	4 58	72745

SUMMARY This 14-day juvenile rat toxicity study showed clinical changes (liquid feces) at 500 and 1500 mg/kg/day and lower body weight at 1500 mg/kg/day. Dose-dependent increases for segmented neutrophils (males at 500 and 1500 mg/kg), and RBC/Hct/Hb (1500 mg/kg, males). Low urinary pH and high specific gravity were observed (1500 mg/kg, both sexes). NOAEL: 50 mg/kg (AUC=1.2 ug.h/ml) in both sex groups.

STUDY TITLE: **A 14-Day Oral Toxicity Study Of RWJ-270201 (b) (4) In Juvenile Sprague-Dawley Rats (Preliminary Study)**

STUDY NO.: DS99311

KEY FINDINGS: No remarkable toxicity was revealed from this preliminary non-GLP juvenile rat study. The NOAEL was 1500 mg/kg (see QA report below).

VOLUME/PAGE: EDR

LABORATORY: (b) (4)

STUDY INITIATION: 4/19/99

GLP: Yes () no (x)

QA REPORT: Yes () no (x)(This study was terminated by sponsor after completion of the in-life portion. The sponsor deemed this study invalid based on TK results [not submitted]).

OBJECTIVE The objective of this preliminary study was to assess the toxicity of RWJ-270201-(b) (4) following 14 days of oral gavage in juvenile CD® SD) albino rats.

LOT #/PURITY 99P0062

VEHICLE: Sodium Carboxymethylcellulose, 7L2P, 0.5% solution

DOSING: 20 pups/sex/group for the Main Study and 24 animals/sex/group (Groups 2, 3, 4) for the 2 TK groups.

Group Number	Dosage Level (mg/kg)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals					
				Males			Females		
				Main Study	Toxicokinetic		Main Study	Toxicokinetic	
					SD 1	SD 14		SD 1	SD 14
1	0	0	10	20	0	0	20	0	0
2	50	5	10	20	24	24	20	24	24
3	500	50	10	20	24	24	20	24	24
4	1500	150	10	20	24	24	20	24	24

SD = Study Day

SPECIES/STRAIN: Juvenile CD® SD albino rats

NO./SEX/GROUP 20

ROUTE Oral gavage

OBSERVATIONS: Day 1 (beginning on Lactation Day 14, Lactation Day 1=day of birth) - Day 14 (Lactation Day 27). Clinical observations (1/day) for the Main Study pups only; mortality and moribundity (2/day). Body weights (1/day) and necropsy were done for the pups only.

TOXICOKINETICS Performed on Study Days 1 and 14 from TK groups in Groups 2, 3, and 4.

GROSS PATHOLOGY, ORGANS WEIGHED AND HISTOPATHOLOGY 20/sex/group (included males reassigned from TK groups and a representative female from the Main Study groups. Histopathology was performed on all tissues in the control and high-dose groups, all tissues from pups found dead, all gross lesions in the selected animals, and the kidneys and thyroid/parathyroid from all selected animals.

RESULTS:

MORTALITY Death prior to scheduled necropsy occurred for two Group 2 male rats, one Group 1 female, and one Group 4 female rat. Two of these deaths were due to cannibalism, and the other two were possibly due to trauma or dosing error.

CLINICAL SIGNS Unremarkable.

BODY WEIGHTS: Body weight changes in high dose males and for the mid-dose females were increased.

HEMATOLOGY: Unremarkable

CLINICAL CHEMISTRY Unremarkable

URINALYSIS: Unremarkable

ORGAN WEIGHTS: There were no treatment-related organ weight differences.

GROSS PATHOLOGY At necropsy, the actual sex distribution was confirmed. There were no dose- or treatment-related gross necropsy findings.

HISTOPATHOLOGY: Unremarkable, including 3 rats that died prior to the terminal necropsy (due to trauma or a dosing accident).

TOXICOKINETICS Not provided.

STUDY TITLE: **A 14-Day Oral Toxicity Non-GLP Study Of RWJ-270201 (b) (4) In Juvenile New Zealand White Rabbits**

STUDY NO.: DS99312

VOLUME/PAGE: EDR

LABORATORY: (b) (4)

STUDY INITIATION: 4/21/99

GLP: Yes () no (x)

QA REPORT: Yes () no (x) (This study was terminated by sponsor after completion of the in-life portion. The sponsor deemed this study invalid based on TK results [not submitted]).

OBJECTIVE The objective of this study was to assess the toxicity of RWJ-270201 (b) (4) following 14 days oral garage in juvenile New Zealand White rabbits.

LOT #/PURITY 99P0062

VEHICLE: Sodium Carboxymethylcellulose, 7L2P, 0.5% solution

DOSING: The juvenile New Zealand White rabbits litters were culled to four male and four female kits/litter:

GP	Mg/kg (dose)	Mg/ml (conc)	Vol (ml/kg)	Main (male)	TK (day 1)	TK (day 14)	Main (female)	TK (day 1)	TK (day 14)
1	0	0	10	20	0	0	20	0	0
2	50	5	10	20	24	24	20	24	24
3	300	30	10	20	24	24	20	24	24
4	1200	120	10	20	24	24	20	24	24

Animal weight:139 to 332 grams.

SPECIES/STRAIN: New Zealand White rabbits

NO./SEX/GROUP 20 (24 for TK group)

ROUTE Oral gavage, 1/day

OBSERVATIONS: Clinical observations, body weight (1/day), mortality (2/day), hematology and clinical chemistry and urinalysis (prior to necropsy) were performed.

TOXICOKINETICS Days 1 and 14 (Groups 2, 3, and 4).

GROSS PATHOLOGY, ORGANS WEIGHED AND HISTOPATHOLOGY Main Study animals were necropsied (day 15). The following organs were weighed prior to fixation at necropsy: adrenal glands, brain, heart, kidneys, liver, ovaries, and testes. Histopathologic examination was performed on all tissues from the control and high dose groups and sacrificed moribund (also performed on all gross lesions, kidneys, and thyroid/parathyroids from all animals).

RESULTS:

MORTALITY Death prior to scheduled necropsy occurred for 5/7/2/2 males and 3/6/6/0 females in Groups 1/2/3/4, respectively. The sponsor indicated that the deaths were not test material-related, resulting from failure to thrive after being weaned or from dosing errors.

CLINICAL SIGNS Unremarkable

BODY WEIGHTS: On Study Days 13-15, body weights were greater at 300 mg/kg/day (Group 3, females). Body weight changes were reduced in Group 4 males (Days 8-15 and 1-

	15). Body weight changes in these periods were increased in Group 3 females.
HEMATOLOGY:	Increased RBC (Group 4 males and females), decreased clotting time PT, increased activated clotting time (Group 4 males), and decreased monocyte (Group 3, 4 females) were observed.
CLINICAL CHEMISTRY	GGT, globulin, cholesterol, blood urea nitrogen, and creatinine were increased in Group 4 males. Creatinine was increased in Group 4 females. These were considered to be treatment-related.
URINALYSIS:	Unremarkable
ORGAN WEIGHTS:	Dose-related increased heart weights were observed in females.
GROSS PATHOLOGY	Sex distribution was confirmed by necropsy. There was an increase in the incidence of pale kidneys for Group 4 males and females.
HISTOPATHOLOGY:	Tubular nephrosis and cystic dilatation were observed in 300 and 1200 mg/kg (Groups 3 and 4; males and females).
TOXICOKINETICS	Not submitted.
SUMMARY	This juvenile 14-day rabbits study showed: 1. Increased red cell counts for Group 4 male and female rabbits. 2. Decreased body weight gains, clotting times, and increased gamma-glutamyltransferase, globulin, cholesterol, BUN, and creatinine in Group 4 males. 3. Creatinine was increased in Group 4 females. 4. Kidneys were pale in group 4 males and females. 5. Tubular nephrosis and cystic dilatation were observed in the 300 and 1200 mg/kg groups. 6. NOAEL =50 mg/kg/day

APPEARS THIS WAY ON ORIGINAL

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/s/

KUEI MENG WU
08/20/2014

HANAN N GHANTOUS
08/20/2014
I concur with Dr. Wu's recommendation for approval of Peramivir from a non-clinical perspective.

Comments on NDA 206-426 peramivir

From: A. Jacobs, AD

Date: 8/12/14

1. I concur that there are no pharm/tox approval issues

2. I concur that the pregnancy category should be C.

3. I have conveyed other comments to the reviewer and he will address them as appropriate

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/s/

ABIGAIL C JACOBS
08/12/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 206426 **Applicant:** Biocryst Pharmaceuticals Inc. **Stamp Date:** 12/23/2013

Drug Name: Peramivir **(**RAPIVAB Injection) **NDA/BLA Type:** 505(b)(2) Standard

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
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 submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
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		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		
11	Has the applicant addressed any abuse potential issues in the submission?	x		No apparent abuse potential reported.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		x	

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

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

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

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

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/s/

KUEI MENG WU
02/03/2014

HANAN N GHANTOUS
02/03/2014