CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-814

Pharmacology Review(s)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-814
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/22/04
PRODUCT:	Aptivus (Tipranavir)
INTENDED CLINICAL POPULATION:	HIV-1 infected patients who are protease inhibitor treatment experienced.
SPONSOR:	Boehringer Ingelheim Pharmaceuticals, Inc. 900 Ridgebury Road P.O. Box 368 Ridgefield, CT 06877
DOCUMENTS REVIEWED:	Modules 1, 2 and 4
REVIEW DIVISION:	Division of Antiviral Drug Products (HFD-530)
PHARM/TOX REVIEWER:	Anita Bigger, Ph.D.
PHARM/TOX SUPERVISOR:	James Farrelly, Ph.D.
DIVISION DIRECTOR:	Debra Birnkrandt, M.D.
PROJECT MANAGER:	Tanima Sinha, M.S.

Date of review submission to Division File System (DFS): June 22, 2005

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The recommendation from the pharmacology/toxicology perspective is to approve.

B. Recommendation for nonclinical studies

Carcinogenicity studies are ongoing and will be completed as a Post-Marketing Commitment.

C. Recommendations on labeling

The wording agreed upon by the NDA team and the sponsor for the pharmacology/toxicology portions of the label can be found at the end of this document (see Overall Conclusions and Recommendations).

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Safety pharmacology assays assessed TPV effects on a number of organ systems, including cardiovascular, central nervous, pulmonary, renal and gastrointestinal (GI) systems. TPV was well-tolerated with some effects in the renal and GI. In renal studies in rats, females exhibited increases in sodium excretion at all doses and decreases in potassium excretion at the high dose. Male rats exhibited decreases in potassium excretion at all doses. Although the changes in urinary electrolyte excretion were considered TPV-related, neither of these findings was correlated with any significant observation in a 4-week oral dose toxicity study of TPV in rats. In GI studies in rats, gastric emptying and GI propulsion were significantly decreased at all doses in males and at middle and high doses in females. Gastric fluid volume was increased at the high dose in males and acid concentration of gastric fluid was decreased at the high dose in males and females. These changes were considered TPV-related. Results of these studies suggest that TPV may elicit some effects on renal and/or GI function at therapeutic doses.

TPV was assessed both in vitro and in vivo for cardiovascular toxicity potential. TPV showed an inhibitory effect in vitro on the HERG-associated potassium channel (IC_{50} = 2.9 μM) but no effect on action potential duration in guinea pig papillary muscle tissue at concentrations up to 10 μM. TPV demonstrated no drug related effects in vivo on mean arterial pressure, heart rate or ECG (including QT interval) in the beagle dog dosed with TPV up to 160 mg/kg. In addition, no evidence of cardiovascular effects was observed in toxicity studies of up to 26 weeks in dogs with TPV/RTV or up to 39 weeks in dogs with TPV alone and no prolongation of QT interval has been observed in multiple clinical studies.

The primary TPV target organs identified through repeat-dose toxicology studies in mice, rats, dogs and/or monkeys are the liver and gastrointestinal tract. Additional organs that were affected included the thyroid gland, testes and to a lesser extent the adrenal gland, kidneys, spleen and heart. Co-administration of TPV and RTV in rats and dogs revealed only target organs or signs of toxicity seen when each compound was administered alone. Neither drug exacerbated the toxicity of the other.

It should be noted that in rats and dogs, TPV exposure in animals at the NOAEL doses is equivalent to or slightly above exposure in humans at the clinical dose of 500/200 TPV/RTV BID. Early toxicity studies were performed with TPV alone. Once the decision was made for co-administration with RTV in the clinic, bridging toxicity studies with both compounds were performed. Co-administration does increase the exposure of TPV, especially at low doses, but the effect is lower at higher doses. In animals, the boosting effect of co-administration is generally lower than in humans (11-fold increase) and in particular much lower at NOAEL doses in repeat-dose toxicology studies: mice (12- to 22-fold), rats (6- to 7-fold), dogs (3- to 13-fold) and monkeys (2-fold). However, toxicities seen in repeat-dose toxicology studies are not considered to preclude chronic administration of TPV to the intended patients, even though plasma levels are equivalent to or below human exposure. These toxicities are reversible, manageable, species specific and/or considered secondary to species-specific hepatic enzyme-inducing effects of TPV in the rodent.

Gastrointestinal effects included emesis, soft stools, diarrhea and/or excessive salivation after dosing and were observed in all species tested. These effects probably effect local actions since no correlative macroscopic or microscopic changes in the GI tract were observed in mice, rats or dogs.

TPV is a microsomal enzyme inducer and has been shown to increase activity of CYP 3A and CYP 3B in rats and dogs. Changes associated with hepatic enzyme induction, increased liver weights, hepatocellular hypertrophy and increases in smooth endoplasmic reticulum have been seen in nonclinical studies in mice, rats and dogs. Additional changes considered secondary to enzyme induction and specific to rodents were seen generally at high doses and included hepatocellular degeneration, vacuolation, necrosis and mineral deposition in mice and multinucleated hepatocytes in rats. Karyomegaly, an effect of RTV in rats, was observed at a low incidence in the 26-week TPV/RTV study. Histological changes specific to dogs included bile duct hyperplasia and gallbladder cystic hyperplasia at a high dose in the 39-week TPV alone study. This effect was not seen in the 26-week TPV/RTV dog study. These effects were reversible and enzyme induction caused by TPV with resultant hepatocellular hypertrophy is considered an adaptive response and not evidence of toxicity.

The rat is the more inducible species, with males showing more activity than females. This is reflected in the fact that females have higher plasma concentrations of TPV than males after repeated doses of TPV. Additional effects seen in the rat could be secondary to hepatic microsomal enzyme induction. These include increased metabolism and clearance of thyroid

hormones, slight increase in plasma proteins and potentialy effects on coagulation parameters. Increases in plasma proteins were seen in rat studies as increases in total protein, albumin (accompanied by a slight increase in plasma calcium in several studies) and/or globulin. Increased thyroid gland weights, thyroid follicular hypertrophy, increases in TSH and decreases in T3 and T4 were seen in rat studies and are considered to reflect a rodent specific increase in thyroid hormone metabolism secondary to induction of hepatic enzymes. Changes in thyroid parameters were monitored in the clinic in early trials. The changes seen in rodents in nonclinical studies were not seen in humans. TPV clearly increases coagulation parameters (prothrombin time and activated partial thromboplastin time) in rodents but the mechanism is unknown. It may be caused by an indirect mechanism related to hepatic enzyme induction in rodents. No similar changes were seen in dog studies. Monitoring of PT was performed in clinical trials and no significant changes in this parameter were observed in humans.

Dogs exposed to TPV or TPV/RTV exhibited mild increases in liver alkaline phosphatase (AP) isoenzymes and this suggests an effect on the liver. Histopathological effects in dogs included gallbladder cystic glandular hyperplasia and bile duct hyperplasia in long term repeat dose studies. These effects are common in older beagle dogs but were increased in TPV treated dogs. In the absence of more severe histopathology, such as biliary stasis or cholestasis, these changes raise little concern for humans. This is also in contrast to the observation that rats exhibited decreased serum AP at higher dose levels in a number of studies.

In mice, enzyme leakage (ALT, AST) at high dose levels was correlated with hepatocellular necrosis. Increases in AST and/or ALT were observed minimally or not at all in toxicology studies on rats and dogs. Based on this difference in species, the importance for humans is not clear. However, liver function can be easily monitored in humans and the nonclinical studies support monitoring as a way of managing this potential human toxicity.

The safety of the self-emulsifying drug delivery system (SEDDS) formulation was explored in a 26-week TPV/RTV dog study to eliminate concerns over the high dose [ ] from 500/200 mg/kg TPV/RTV BID) of Cremophor EL (CrEL) contained in this formulation. Of concern was the possibility that CrEL might pass from the GI tract into systemic circulation and thereby pose a risk of anaphylactoid reactions since CrEL is known to cause these reactions if given in high levels IV. Consequently, exposures to the SEDDS formulation were chosen to achieve 1, 10 and 30-fold exposure to CrEL in humans. CrEL plasma levels were detectable 2 hours after the first or second dose in several animal administered high dose SEDDS and one animal intermediate dose SEDDS. Plasma CrEL levels ranged from [ ] mg/ml. These levels are unlikely to cause anaphylactoid reactions. The NOAEL for SEDDS is considered to be 910 mg/kg/day SEDDS which supports a 10-fold safety factor for the SEDDS vehicle in the recommended human dose.

TPV was tested for the ability to induce point mutations in DNA (mutagenicity) and the ability to damage chromosomes (clastogenicity) in five in vitro and in vivo assays including the battery of assays specificed in the ICH S2B guidance on genotoxicity. These included the in vitro bacterial

reverse mutation assay, unscheduled DNA synthesis (UDS) in rat hepatocytes, induction of gene mutation in Chinese hamster ovary cells and a chromosome aberration assay in human peripheral lymphocytes, as well as an in vivo bone marrow micronucleus assay in mice. TPV was negative in these assays, indicating that TPV has no mutagenic or clastogenic potential.

A male and female fertility and early embryonic development study (oral) in Sprague-Dawley rats demonstrated that TPV did not affect spermatogenesis, estrous cycle, copulation, conception, fertility, implantation or early embryonic development at doses up to 1000 mg/kg/day. This corresponds to a Cmax of 258 μM which is approximately two-fold the human Cmax at the proposed clinical dose of 500/200 TPV/RTV BID.

In an embryo-fetal development studies in Sprague-Dawley rats, there was no evidence of TPV-related embryolethality or teratogenicity at doses of 40 to 1000 mg/kg/day. However, the NOAEL for both maternal and developmental toxicity was 40 mg/kg/day, based on findings of postdose salivation, decreased body weight and food consumption in dams and decreased body weight and sternebrae ossification in fetuses. This NOAEL corresponds to a mean Cmax 30.4 μM and a mean AUC of 340 μM.h, which is 0.2-fold of the expected human exposure at the proposed dose of 500/200 TPV/RTV BID.

In embryo-fetal development studies in pregnant rabbits treated with TPV (on gestation days 6 through 20), maternal toxicity (death of one female, abortions, decreased body weight and food consumption and increased clinical signs) and developmental toxicity (slightly decreased fetal body weights, fetuses with wavy ribs and bent femurs and increased incidence of fetuses with gross malformations) were observed at the high dose. Interpretation of these fetal findings is complicated by maternal toxicity and a litter effect. These gross malformations were not observed in fetuses at 375 and 759 mg/kg/day TPV in the dose range-finding study in rabbits. Therefore, it is unlikely that TPV was teratogenic at 375 mg/kg/day. The NOAEL for maternal toxicity was 75 mg/kg/day while the NOAEL for developmental toxicity was 150 mg/kg/day. The AUCs associated with these NOAEL doses correspond to 0.04-fold and 0.08-fold, respectively, the human exposure at the proposed clinical dose of 500/200 TPV/RTV BID.

In a pre- and post-natal development study in rats, TPV was toxic to dams and suckling pups at 400 and 1000 mg/kg/day with dose-relationship. Maternal toxicity was restricted to adverse effects on body weight and food consumption. Pup toxicity consisted of slight to marked progressive growth inhibition throughout lactation, resulting in persistent adverse influence on the growth of the pups up to maturity. However, none of the postweaning functions examined in F1 offspring, including reproductive ability, were compromised up to the 1000 mg/kg/day dose and there was no evidence of teratogenicity at any dose. The 40 mg/kg/day dose was an NOAEL for both the dams and offspring.

The results from these reproductive toxicology studies indicate that TPV is not teratogenic. However, since human exposure levels are above the rat and rabbit NOAEL exposure levels

derived from these studies, TPV should be given during pregnancy only if the benefit to the mother and the fetus outweighs the risk to the fetus. Distribution studies in rats administered radiolabeled TPV demonstrated that radioactivity is excreted into the milk of rats. Therefore, women should be cautioned to avoid breastfeeding while taking TPV.

The immunotoxic potential of TPV was assessed in a series of immunotoxicology assays. In a mouse immune function study, a single dose of TPV inhibited anti-CD3-dependent T-cell stimulation as measured by IL-2 concentrations. Also a minimal, albeit statistically significant, T cell-driven delayed type hypersensitivity (DTH) was observed as measured in [redacted]. Although there were no additional appropriate assays for further investigation of immunostimulatory potential of TPV, an additional assay, the T-dependent antigen response to sheep red blood cells was performed to confirm or negate TPV immunosuppressive potential. The results of this study demonstrated lack of immunosuppressive potential for TPV and/or RTV under the conditions of the study.

B. Pharmacologic activity

See review by Microbiology Reviewer.

C. Nonclinical safety issues relevant to clinical use

Major targets organs for TPV in nonclinical studies are the gastrointestinal tract and the liver. The nonclinical studies support monitoring of GI and liver function in the clinic.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

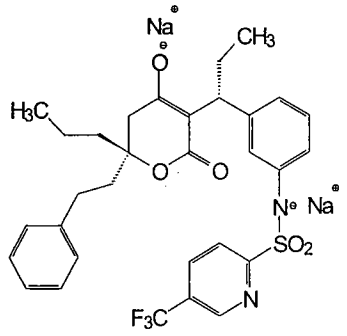
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-814
Review number:
Sequence number/date/type of submission: 000/12-22-04/IT
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road
P.O. Box 368
Ridgefield, CT 06877

Reviewer name: Anita Bigger, PhD
Division name: Division of Antiviral Drug Products
HFD #: 530
Review completion date: June 22, 2005.

Drug:

Trade name: Aptivus (tipranavir)
Generic name:
Code name: TPV; PNU-140690
Chemical name: N-[3-[(1R)-1-[5,6-dihydro-4-hydroxy-2-oxo-6(R)-(2-phenylethyl)-6-propyl-2H-pyran-3-yl]propyl]phenyl]-5-(trifluoromethyl)-2-pyridinesulfonamide
CAS registry number: 174484-41-4
Molecular formula/molecular weight: $C_{31}H_{33}F_3N_2O_5S/602.7$
Structure:



Relevant INDs/NDAs/DMFs: IND 51,979

Drug class: Protease Inhibitor

Intended clinical population: Treatment of HIV-1 infection in patients who are protease inhibitor treatment experienced.

Clinical formulation: Self Emulsifying Drug Delivery System (SEDDS)

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

TPV was investigated in safety pharmacology assays, a series of secondary pharmacodynamic immune function tests and a biochemical receptor assay screen.

Safety pharmacology assays assessed TPV effects on a number of organ systems, including cardiovascular, central nervous, pulmonary, renal and gastrointestinal (GI) systems. TPV was well-tolerated with some effects in the renal and GI. In renal studies in rats, females exhibited increases in sodium excretion at all doses and decreases in potassium excretion at the high dose. Male rats exhibited decreases in potassium excretion at all doses. Although the changes in urinary electrolyte excretion were considered TPV-related, neither of these findings was correlated with any significant observation in a 4-week oral dose toxicity study of TPV in rats. In GI studies in rats, gastric emptying and GI propulsion were significantly decreased at all doses in males and at middle and high doses in females. Gastric fluid volume was increased at the high dose in males and acid concentration of gastric fluid was decreased at the high dose in males and females. These changes were considered TPV-related. Results of these studies suggest that TPV may elicit some effects on renal and/or GI function at therapeutic doses.

TPV was assessed both in vitro and in vivo for cardiovascular toxicity potential. TPV showed an inhibitory effect in vitro on the HERG-associated potassium channel (IC_{50} = 2.9 μM) but no effect on action potential duration in guinea pig papillary muscle tissue at concentrations up to 10 μM. TPV demonstrated no drug related effects in vivo on mean arterial pressure, heart rate or ECG (including QT interval) in the beagle dog dosed with TPV up to 160 mg/kg. In addition, no evidence of cardiovascular effects was observed in toxicity studies of up to 26 weeks in dogs with TPV/RTV or up to 39 weeks in dogs with TPV alone and no prolongation of QT interval has been observed in multiple clinical studies.

TPV was evaluated for effects on immune function to determine if it possessed immunomodulatory potential. Evidence from repeat-dose studies in mice, rats and dogs suggested that TPV might have immunostimulatory and/or immunosuppressive potential. In a mouse immune function study (185) a single dose of TPV inhibited anti-CD3-dependent T-cell stimulation as measured by IL-2 concentrations. Also a minimal, albeit statistically significant, T cell-driven delayed type hypersensitivity (DTH) was observed as measured in [redacted] Although there were no additional appropriate assays for further investigation of immunostimulatory potential of TPV, an additional assay, the T-dependent antigen response to sheep red blood cells was performed to confirm or negate TPV immunosuppressive potential. The results of this study showed that treatment with TPV alone or TPV/RTV did not adversely affect the functional ability of the humoral component of the immune system in female CD-1 mice, as evaluated in the IgM antibody-forming cell response to the T-dependent antigen, sheep red blood cells. Thus, the potential for TPV to be immunosuppressive was negated.

TPV was also evaluated in a biochemical receptor screen [redacted] and in general showed a low inhibitory profile against a variety of receptor targets at concentrations up to 10μM. The only exception was the cholecystokinin-A (CCK-A) receptor binding assay where TPV was shown to bind with some affinity (82%) at 10μM. The relevance of this finding is unknown.

2.6.2.2 Primary pharmacodynamics
(Reviewed by Microbiology Reviewer)

Mechanism of action:

Drug activity related to proposed indication:

2.6.2.3 Secondary pharmacodynamics

See IND 51,979 (474) Summary of Immunotoxicity Findings for Tipranavir in Appendix.

Study title: In vivo assessment of Tipranavir (EXRS 1406 XX) effects on murine immune function.

Key study findings: Tipranavir showed modest to marginal effects at oral doses up to 300 mg/kg on assays designed to examine T cell, B cell and complex inflammatory responses.

Study no.: U02-3010
Volume # and page #: Module 4, M-002, vol. 1.2, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc.

Pharmacology Department
900 Ridgebury Road
Ridgefield CT 06877

Date of study initiation: Unknown. Report Date 2-11-02
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: Not reported.

Methods and Results

1. In Vivo Anti-CD3 Assay

A single dose (30, 100 or 300 mg/kg) of tipranavir was administered orally to female BALB/c mice prior to injection with a monoclonal antibody to mouse CD3 to measure the direct effects of the compound on T cell activation as assessed by plasma IL-2 levels.

In the first assay IL-2 was inhibited 46% by tipranavir at 300 mg/kg. These mice had been fasted overnight and exhibited marked diarrhea at the high dose. Since diarrhea can impair immune responses in this model, the assay was repeated using unfasted mice. In the repeat assay, IL-2 was inhibited 39%, 25% and 27% by 300, 100 and 30 mg/kg of tipranavir, respectively.

2. In Vivo Delayed Type Hypersensitivity (DTH)

Male BALB/c mice were immunized intradermally at the tail base with [illegible] Seven days later, DTH responses were elicited by subcutaneous injection of [illegible] into the dorsal surfaces of each ear, eliciting the maximal swelling response at 24 hours post-injection. In the treatment groups, a single dose (30, 100 or 300 mg/kg) was administered 1 hour prior to ear injection. Ear thickness is before and 24 hours after the injection. The change in ear thickness is calculated as a measure of the DTH response.

There was a statistically significant effect at the high dose. The sponsor notes that the mice were still able to mount a robust T cell and inflammatory response even at the high dose.

3. In Vivo T Cell Independent B Cell Activation

Trinitrophenyl (TNP)-conjugated lipopolysaccharide (LPS) was injected into mice to stimulate the polyclonal activation of B cells. Upon activation, these B cells produce TNP-specific IgM at levels which can be detected in the plasma by day 3. Tipranavir (30, 100 or 300 mg/kg) was administered orally 1 hour prior to TNP-LPS injection and 24 hours later. Tipranavir showed no detectable effects on B cell activation.

4. Efforts to address the ability of tipranavir to induce compound-specific immune response (i.e. allergic response to the compound itself) were hindered due to solubility and irritation. In the popliteal lymph node assay, five mg of soluble compounds are injected into the footpads of mice and seven days later the increase in draining lymph node size is measured as an indication of compound-triggered immune responses. Only 3 mg of tipranavir could be injected per paw. Even at this level, the paws were extremely swollen and showed evidence of scabbing, suggesting an irritant response. Thus, immunogenicity could not be adequately assessed for tipranavir.

Study title: T-Cell dependent antigen response to Tipranavir and Ritonavir in female CD-1 mice (BIPI Toxicology Study No. 04R103).

Key study findings: The results of this immunotoxicological evaluation demonstrate that treatment with TPV alone or co-administered with RTV did not adversely affect the functional ability of the humoral component of the immune system in female CD-1 mice. Therefore, the study demonstrated lack of immunosuppressive potential for TPV and/or RTV under the conditions of the study.

Study no.: 04R103

Volume # and page #: Submitted separately to NDA 21-814 on March 3, 2005. Also, submitted to IND 51,979 under submission number N-621.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877-0368

Date of report: January 4, 2005

Objective: The study was performed to assess the potential effects of TPV alone and when co-administered with RTV on the humoral immune component of the immune system, when evaluated in the antibody-forming cell response to the T-dependent antigen, sheep red blood cells. This study was initiated in response to a FDA request on August 30, 2004.

Materials and methods: Female CD-1 mice were administered TPV and/or RTV by oral gavage for 28 days at dose levels of 30/8, 100/26.7 and 300/80 mg/kg/day TPV/RTV, 300 mg/kg/day TPV or 80 mg/kg/day RTV. RTV was administered in propylene glycol at 5 ml/kg, followed approximately 1 hour later by TPV in an aqueous solution, pH 10.5, at 10 mg/kg. The study included a sham-dosed group as well as a vehicle group to control for stress induced by handling and vehicle. Sham treated Controls received deionized water and vehicle Controls received vehicles corresponding to the two drugs. A positive control group received 50 mg/kg/day cyclophosphamide IP for 4 days prior to terminal sacrifice. Clinical signs, mortality, body weights food consumption were monitored during the study. Four days prior to sacrifice, all animals received an IV injection of sheep red blood cells. At termination of the study, each animal was weighed, then sacrificed and the spleen was removed Blood was collected from the

vena cava and then the liver and thymus were removed. These organs were weighed. Spleens were processed for immunological evaluation, while livers and thymuses were retained in formalin for potential histopathologic evaluation.

Results and Discussion: Clinical signs included rough and stained hair. No effects were observed on body weight or food consumption during the course of the study. Terminal body weight measurements showed an increase (+8%) in mean body weights of sham control animals and animals treated with RTV alone, as compared to vehicle control mean body weights. No statistically significant effects were observed in either spleen or thymus weight in TPV and/or RTV treated groups but weights of both organs were decreased significantly with administration of the positive control. Liver weights were increased in animals treated with TPV or RTV.

Treatment with TPV/RTV, TPV or RTV did not result in significant changes in spleen cell number or in the IgM antibody-forming cell (AFC) response to the T-dependent antigen, sheep red blood cells, when evaluated as either specific activity (AFC/10^6 spleen cells) or as total spleen activity (AFC/spleen). The positive control decreased spleen cell number as well as specific activity and total spleen activity.

Dr. Steve Kunder reviewed report and found the study valid. Dr. Kunder agreed with the conclusion of the draft report that the study demonstrated lack of immunosuppressive potential for TPV and/or RTV.

Study title: HIV-1 and HIV-1 protease inhibitor U-140690 tested in 32 assays

Key study findings: TPV was tested in 31 receptor binding assay and 1 phosphoinositide turnover assay as an assessment of its broader selectivity. TPV was inactive in the assays used with the possible exception of the CCK-A receptor binding assay were TPV produced an apparent dose-related inhibition suggestive of a potency (IC_{50}) in the 0.1 to 10 μM range. Quantitative determination of the compound's affinity for the CCK-A site would require a complete competition binding study using a full range of doses. The relevance of this finding is unknown.

Study no.: U01-3033 (Pharmacia & Upjohn Technical Report 7295-96-034).
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of report: August 13, 1996

Objective: The objective of the study was to test TPV in a broad spectrum of binding and enzyme assays as part of its ongoing development as a potential treatment for AIDS.

Materials and methods: TPV was shipped to [ ] The compound was tested at three concentrations, 10^{-9}, 10^{-7} and 10^{-5} M in duplicate in 31 receptor binding assays and in a phosphoinositide (PI) turnover assay.

Results and Discussion: TPV was inactive in the assays even when tested at the highest concentration, 10^{-5} M, except for the CCK-A receptor. In this case a large degree of inhibition (82%) was observed only at the highest dose, suggesting a potency (IC_{50}) in the 0.1 to 10 μM range. Quantitative determination of the compound's affinity for the CCK-A site would require a complete competition binding study using a full range of doses.

2.6.2.4 Safety pharmacology

Neurological effects:

Study title: U-140690E: Evaluation of locomotor activity following a single oral dose in male and female Sprague-Dawley rats.

Key study findings: Single oral doses of 62.5, 200, 500 (females only) and 625 (males only) mg/kg TPV administered to Spraque-Dawley rats had no significant effect on any parameters of locomotor activity.

Study no.: U00-3102 (Pharmacia & Upjohn Technical Report No. 7228-96-130)
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI
Date of report: October 9, 1996

Objective: The objective of this non-GLP study was to determine the effect of a single oral dose of TPV on parameters of locomotor activity in male and female Sprague-Dawley rats.

Materials and methods: TPV [(A)5075-AS-1720, purity [ ] was administered to male and female — CD[SD]BR Sprague-Dawley rats as single oral doses of 0, 62.5, 200, 500 (females only) and 625 (males only) mg/kg in water adjusted to pH 10.5 with sodium hydroxide at a dose volume of 10 mg/kg. A total of 48 males and 48 females were assigned to the evaluation of locomotor activity with 12 rats/sex/dose. Each group was observed predose and 6 hours post dose.

Results and Discussion: There were no statistically significant changes in any of the parameters of locomotor activity following administration of TPV at all doses.

Study title: U-140690E: Modified Irwin's/Rectal Temperature Test following a single oral dose in male and female Sprague-Dawley rats.

Key study findings: Single oral administration of TPV at doses of 0, 62.5, 200, 500 (female) or 625 (male) mg/kg to rats had no biologically significant effects on any of the parameters of the Modified Irwin's/Rectal Temperature Test.

Study no.: U00-3101 (Pharmacia & Upjohn Technical Report No. 1470-96-016)
Volume # and page #: M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn Ltd., Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki, Japan

Date of report: October 14, 1996

Objective: The objective of this GLP-compliant study was to determine the effect of a single oral dose of TPV on parameters of the Modified Irwin's/Rectal Temperature Tests in male and female rats.

Materials and methods: TPV [(A)5075-AS-1768-J395, [ ] purity] was administered to respective groups of rats at doses of 0, 62.5, 200 and 500 (females) or 625 (males) mg/kg in aqueous vehicle adjusted to pH 10.5 using sodium hydroxide or hydrochloric acid at a volume of 10 ml/kg. Twenty-four males and 24 females were assigned to four groups (6/sex/group). Each group of rats was observed predose and six hours postdose.

Results and Discussion: Measurements made in the Modified Irwin's/Rectal Temperature Test were not affected at any dose level of TPV.

Cardiovascular effects:

Study title: Influence of Tipranavir on HERG-mediated potassium current in stably transfected HEK293 cells. Amendment No. 1

Key study findings: The IC_{50} of TPV on the HERG-mediated potassium channel was 2.9 μM. Together with a previous study which had tested the effect of TPV on action potential parameters in guinea pig papillary muscle in vitro, these studies indicate a low proarrhythmic potential.

Study no.: U02-1175

Volume # and page #: Module 4, M002, vol. 1.2, page 1.

Conducting laboratory and location: Boehringer Ingelheim Pharma KG, Birkendorfer Strase 65, 88397 Biberach an der Riss, Germany

Date of report: September 3, 2002

Objective: This non-GLP study was conducted to determine the influence of TPV on HERG (human ether-a-go-go-)-mediated potassium current as a marker for potential effects on myocardial repolarization.

Materials and methods: Experiment on HERG-mediated potassium current were performed using HEK293 (human embryonic kidney) cells stably expressing the HERG-mediated potassium current. Whole-cell experiments were carried out by means of the patch-clamp technique at room temperature. Effects on the HERG-channel current amplitude by TPV were measured for each concentration (0.1, 3.0, 10.0 μM) over 5 minutes. Control experiments were performed on every experimental day to confirm the stability of the preparation and to correct for the current run-down of the test system.

Results and Discussion: The IC_{50} for TPV on the HERG channel was 2.9 μM. Previous results with TPV on action potential configuration in guinea pig papillary muscle had revealed no effects up to 10 μM.

Study title: Effects of tipranavir (0.1 to 10 μM) on action potential configuration in isolated guinea pig papillary muscle.

Key study findings: TPV has no effect on action potential parameters in concentrations up to 10 μM, indicating a lack of effect on cardiac ion channels over a wide range of concentrations.

Study no.: U01-1226
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharma KG, Birkendorfer Strase 65, 88397 Biberach an der Riss, Germany

Date of report: February 13, 2001

Objective: The objective of this non-GLP study was to test the influence of TPV on myocardial action potential configuration.

Materials and methods: Action potentials were measured in isolated guinea pig papillary muscles. Three groups of experiments were performed: The first group (n=5) received TPV in cumulative doses of 0.1, 0.3, 1.0, 3.0 and 10.0 μM, the second group (n=5) received cisapride as a positive control in cumulative concentrations of 0.1, 0.3, 1.0, 3.0 and 10.0 μM and the third group (n=5) served as a control group and received equivalent volumes of the vehicle (DMSO).

Measurements were taken at a stimulation frequency of 0.33 Hz (20 cycles/min) and included action potential duration to 10, 30 and 90% repolarization (APD10, APD30 and APD90, respectively), resting membrane potential (RMP), maximal velocity of phase 0 upstroke (V-max), AP overshoot (OS), AP amplitude (APA) and the force of contraction (FOC).

Results and Discussion: No changes were observed in concentrations up to 10 μM TPV. Cisapride markedly prolonged the action potential by approximately 13.2%. These results indicate that TPV has no effect on cardiac ion channels over the entire concentration range tested.

Study title: U-140690E: Cardiovascular profile following a single oral dose in conscious Sprague-Dawley rats.

Key study findings: TPV administered at single oral doses of 62.5, 200 and 625 (males) or 500 (females) to Sprague-Dawley rats had no effect on mean arterial pressur or heart rate in either sex at doses of 62.5 and 200 mg/kg. There were small drug-related increases in mean arterial pressure in males and females at the high dose but these were not considered toxicologically relevant.

Study no.: U00-3103 (Pharmacia & Upjohn Technical Report 7228-96-143)
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of report: October 116, 1996

Objective: The objective of this non-GLP study was to determine the effect of a single oral dose of TPV on mean arterial pressure and heart rate in conscious Sprague-Dawley rats.

Materials and methods: The effects of oral administration by gastric intubation of TPV on mean arterial pressure and heart rate were evaluated in male and female Sprague-Dawley rats at dose levels of 0, 62.5, 200 and 625 (males) or 500 (females) mg/kg in aqueous solution, pH 10.5. A total of four males and four females were chronically instrumented with radiotelemetry transmitters to provide measurements of mean arterial pressure and heart rate. Each animal was treated with vehicle and each dose of drug in a Latin Square Cross-Over design. Each day of dosing was separated by a minimum of 72 hours. Heart rate and mean arterial pressure were measured twice prior to dosing and at 15 minute intervals postdose for seven hours.

Results and Discussion: TPV had no effect on mean arterial pressure or heart rate in either sex at doses of 62.5 and 200 mg/kg. A small increase in mean arterial pressure was observed in females dosed at 500 mg/kg. This effect was sustained for six hours. It was considered drug-related but the magnitude was too small to be viewed as toxicologically relevant.

A statistically significant increase in mean arterial pressure was observed in males at 625 mg/kg for the first 45 minutes following dosing. This change was not considered drug-related as it did not coincide with the maximum plasma concentration of TPV. This effect may have been a response to the high dose formulation.

Study title: U-140690E: Cardiovascular profile following a single oral dose in conscious beagle dogs.

Key study findings: Single oral administration of TPV at doses of 37.5, 80 and 160 mg/kg to male and female beagle dogs had no TPV-related effects on heart rate, mean arterial pressure, respiratory rate or on parametersof the electrocardiogram.

Study no.: U00-3104 (Pharmacia & Upjohn Technical Report 7228-96-144)
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of report: October 14, 1996

Objective: The objective of this non-GLP study was to determine the effect of a single oral dose of TPV on mean arterial pressure, heart rate, electrocardiographic parameters and respiratory rate in conscious beagle dogs.

Materials and methods: Four male and four femal young adult beagle dogs were evaluated with vehicle and three doses of TPV using a Latin Square Cross-Over design. Dose levels were 0, 37.5, 80 and 160 mg/kg of TPV (Lot No. (A)5075-AS-1768, 97.44% purity) in aqueous solution pH 10.5. Each animal received a bolus oral dose, followed by a flush of 15 ml sterile water, once per day on four separate days (different dose levels each day) with a washout period of at least 72 hours between doses. Mean arterial pressure, heart rate, electrocardiogram parameters and respiratory rate data were obtained twice predose and every 30 minutes postdose for nine hours.

Results and Discussion: There were no TPV-related effects on heart rate, mean arterial pressure, respiratory rate or on the parameters of the electrocardiogram at any of the doses tested.

Pulmonary effects:

Study title: Effect of tipranavir on pulmonary function in the conscious Sprague-Dawley rat after single dose administration

Key study findings: TPV had no effect on tidal volume or respiratory frequency at dose up to 500 mg/kg in female rats and 625 mg/kg in male rats.

Study no.: U01-3013
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of report: January 10, 2001

Objective: The objective of this non-GLP study was to evaluate the effects of a single dose of TPV on pulmonary function (respiratory frequency and tidal volume) in conscious Sprague-Dawley rats.

Materials and methods: Respiratory frequency and tidal volume were monitored using BUXCO whole body unrestrained plethysmograph chambers. TPV in aqueous solution pH 10.5 was studied at oral doses of 0, 62.5, 200 and 500 mg/kg (female rats) and 625 mg/kg (male rats) (n=3/sex/group). Blood samples were collected at 375 minutes postdose for plasma level determinations.

Results and Discussion: TPV exhibited no effects on tidal volume or respiratory frequency. No gross observations were noted for any animals in the study. Analysis of blood samples demonstrated detectable mean plasma levels of TPV.

Renal effects:

Study title: U-140690E: Renal profile following a single oral dose in male and female Sprague-Dawley rats.

Key study findings: Single oral administration of TPV at doses of 62.5, 200, 500 (females) and 625 (males) mg/kg to Sprague-Dawley rats did not affect water consumption or urine volume. Females exhibited increases in sodium excretion at all doses and decreases in potassium excretion at the high dose. Males exhibited decreases in potassium excretion at all doses. Although the changes in urinary electrolyte excretion were considered TPV-related, neither of these findings was correlated with any significant observation in a 4-week oral dose toxicity study of TPV in rats.

Study no.: U00-3098 (Pharmacia & Upjohn Technical Report 1470-96-018)
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn Ltd., Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki, Japan

Date of report: October 14, 1996

Objective: The objective of this GLP compliant study was to determine the effect of a single oral dose of TPV on water consumption, urine volume and urinary electrolyte excretion in male and female Sprague-Dawley rats.

Materials and methods: TPV was administered at single oral doses of 0, 62.5, 200, 500 (females) and 625 (males) in aqueous solution pH 10.5. A total of 48 rats (24 males and 24 females) were assigned to four groups (6/sex/group). A bolus of 0.9% sodium chloride (20 ml/kg) was given orally approximately two hours after dosing. Animals were placed in metabolic cages immediately after administration of sodium chloride and urine was collected over a period of five hours. Parameters of renal function evaluated included water consumption, urine volume and the urinary excretions of sodium, potassium and chloride.

Results and Discussion: No notable changes in water consumption and urine volume were observed in any animals. Males exhibited statistically significant decreases in potassium excretion of 24%, 19% and 28% at low, middle and high doses. Females exhibited statistically significant increases in sodium excretion of 46%, 38% and 42% at low, middle and high doses and a decrease in potassium excretion of 39% at the high dose.

Gastrointestinal effects:

Study title: U-140690E: Isolated ileum profile.

Key study findings: TPV at 10^{-6} M did not contract or relax the tissue nor did it affect concentration-response curves for acetylcholine, histamine and barium chloride, indicating that TPV is devoid of significant agonist activity or antogonist effects on muscarinic or histamine receptors or membrane-mediated depolarization in the isolated guinea pig ileum..

Study no.: U00-3100 (Pharmacia & Upjohn Technical Report 1470-96-019)
Volume # and page #: Module 4, M002, vol.1.3, page 1.
Conducting laboratory and location: Pharmacia & Upjohn Ltd., Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki, Japan

Date of report: October 14, 1996

Objective: The objective of this GLP compliant study was to determine the effect of TPV on contractility and on agonist-induced contractions in ileum isolated from guinea pigs.

Materials and methods: The ileum was isolated from six male guinea pigs of the Hartley strain and cut into 1.5 cm sections. Isotonic contractions under 1 g tension were monitored in an organ bath filled with 10 ml of the modified Krebs solution, gassed with 95% O_2/5% CO_2 and maintained at 37° C. Concentration-response curves for acetylcholine, histamine and barium

chloride were obtained before and after 30 minutes of equilibration with TPV (Lot no. (A) 5075-AS-1768,[ ]purity) or vehicle (DMSO).

Results and Discussion: TPV at 10^{-6} M did not contract or relax the tissue nor did it affect concentration-response curves for acetylcholine, histamine and barium chloride.

Study title: U-140690E: Gastrointestinal profile following a single oral dose in Sprague-Dawley rats.

Key study findings: Gastric emptying and GI propulsion were significantly decreased at all doses in males and at middle and high doses in females. Gastric fluid volume was increased at the high dose in males and acid concentration of gastric fluid was decreased at the high dose in males and females. These changes were considered TPV-related.

Study no.: U00-3099 (Pharmacia & Upjohn Technical Report 1470-96-017)
Volume # and page #: Module 4, M002, vol. 1.3, page 1.
Conducting laboratory and location: Pharmacia & Upjohn Ltd., Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki, Japan

Date of report: October 14, 1996

Objective: The objective of this GLP compliant study was to determine the effect of a single oral dose of TPV on gastric emptying, gastrointestinal propulsion and gastric secretion.

Materials and methods: TPV (Lot No. (A)5075-AS-1768-J395,[ ]purity) in aqueous solution pH 10.5 was administered as single oral doses of 0, 62.5, 200, 500 (females) and 625 (males) to rats. Gastric emptying was studied in four groups with one group being euthanized without treatment. After dosing with vehicle or TPV, rats were fasted for four hours. All rats were euthanized and the weight of stomach contents was determined. Gastrointestinal propulsion was studied in four groups of rats. After an overnight fast without water, rats were dosed with vehicle or TPV. Six hours later, rats received a single oral dose of a liquid charcoal suspension as a marker of GI transit. Rats were euthanized 20 minutes after administration of the charcoal suspension. Gastric secretion was studied in four groups of rats. After an overnight fast without water, rats were dosed with vehicle or TPV. Three hours later, rats were subjected to pylorus ligation. Four hours later, rats were euthanized and gastric fluid volume, acid concentration and acid output were determined.

Results and Discussion: Gastric emptying and GI propulsion were significantly decreased at all doses in males and at middle and high doses in females. Gastric fluid volume was increased at the high dose in males and acid concentration of gastric fluid was decreased at the high dose in males and females. These changes were considered TPV-related.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

TPV, co-administered with RTV, will be indicated for treatment of HIV-1 infection. Nonclinical pharmacokinetic studies have been conducted with and without RTV. This summary will focus on TPV/RTV study results but will also include TPV alone studies when appropriate.

TPV is primarily metabolized in humans by CYP3A4 and is a substrate for Pgp. The boosting effect of RTV in humans may be due to inhibition of CYP3A4 and to inhibition of Pgp. RTV also boosts levels of TPV in animals but, as discussed in the Toxicology Summary, the boosting effect of RTV on TPV in animals is not predictive of the level of boosting in humans.

The pharmacokinetics of TPV/RTV has been investigated in mice, rats, dogs and monkeys. With RTV co-administration, following IV dosing, TPV demonstrated low to moderate clearance ranging from 0.0182 L/h/kg in rats to 3.00 L/h/kg in mice. The steady state volume of distribution ranged from 0.112 L/kg in dogs to 7.36 L/kg in mice. TPV terminal elimination half-life ranged from 1.93 h in rats to 13.0 h in dogs. Following oral dosing, TPV mean Cmax were 22.7 and 30.5 μM in male and female rats, respectively, at doses of 10/10 mg/kg TPV/RTV and 28.6 and 20.9 μM in male and female dogs, respectively, at doses of 15/4 mg/kg TPV/RTV. The corresponding AUC values were 231 and 464 μM.h in male and female rats, respectively, and 121 and 85 μM.h in male and female dogs, respectively. In general, TPV exposure increased in a less than proportional manner. With repeated dosing, TPV plasma levels declined relative to Day 1 by 93% in mice, 60% in rats and marginally in dogs. Induction of CYP450 (CYP2B and CYP3A) was observed in rats and dogs.

The exposure of total drug-related radioactivity in all species following oral or IV administration of radiolabeled TPV was similar, with and without RTV co-administration. With RTV co-administration, TPV, rather than metabolites, accounted for most of the drug-related radioactivity in plasma, feces and urine in rat and human, indicating suppression of TPV metabolism by RTV. After oral dosing, drug-related radioactivity was primarily associated with the liver and GI tract and did not easily cross the blood:brain barrier. There was no apparent melanin binding. RBC partitioning was low. Similar results were obtained in TPV studies without RTV co-administration.

Drug-related radioactivity was secreted in the milk of lactating rats after oral dosing with ^{14}C-TPV with RTV co-administration. Radioactivity also crossed the placenta of pregnant rats. Radioactivity did not readily cross the blood:brain barrier in pregnant rats or fetuses.

The major route of elimination of radiolabeled TPV in mouse, rat and dog was via feces (≥87%, ≥75% and ≥68%, respectively). Urinary excretion of radioactivity was minor, less than 10%. In rats approximately 40% of an oral dose was excreted in bile over 48 hours. Enterohepatic

recirculation of radioactivity was also seen in rats. Similar results were seen in studies of TPV without RTV co-administration.

In rats and dogs, TPV exposure was higher following TPV only dosing when TPV was administered orally as a SEDDS formulation rather than an aqueous solution.

In vitro and in vivo metabolism studies were conducted on TPV without RTV co-administration. In vitro studies with hepatic microsomes of rat, dog, monkey and human and with hepatocytes of rat and human identified a number of metabolites including several mono-hydroxylated metabolites, a glucuronide conjugate of the parent, desaturated metabolites and a cleavage product formed via N-S bond cleavage. In vivo plasma samples from rats, dogs, monkeys and humans dosed orally with TPV revealed four major metabolites. Two metabolites were apparently formed by intramolecular cyclization and the additional two metabolites were secondary oxidation products of these primary metabolites. These were formed in the absence of RTV co-administration and since RTV inhibits metabolism of TPV, the relevance of these metabolites is unclear. In studies of rats and humans administered radiolabeled TPV with RTV, TPV was the major drug-related material. Excreted metabolites accounted for 6% or less of the radioactivity administered. This observation is consistent with the mechanism of inhibition of TPV metabolism by RTV.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

Study title: Oral pharmacokinetics of tipranavir in rats when administered alone or in combination with ritonavir.

Key study finding: Plasma concentrations of TPV were six- to seven-fold higher for rats receiving both TPV and RTV compared to rats receiving TPV alone.

Study no.: U01-3316
Volume # and page #: Module 4, M002, vol. 1.5, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of report: November 15, 2001

Objective: The objective of the study was to describe the pharmacokinetics of tipranavir in male Sprague Dawley rats after administration of tipranavir alone or when co-administered with ritonavir.

Materials and methods: Ten rats were administered TPV alone at 40 mg/kg/day for four consecutive days. Beginning on Day 5, five of the rats received RTV at 10 mg/kg/day in addition to TPV. The remaining five rats continued with TPV alone. On Day 9, serial blood samples were drawn out to 24 hours and plasma concentrations of TPV were measured by LC/MS/MS. The LOQ of the assay was — ng/ml.

Results and Discussion: Plasma concentrations of TPV were substantially higher for rats receiving both TPV and RTV (TPV + RTV) compared to rats receiving tipranavir alone (TPV). Mean AUC_{0-24} and Cmax values were six- to seven-fold greater for the TPV + RTV rats compared to the TPV rats.

Study title: Pharmacokinetics of tipranavir after intravenous (5 mg/kg) and oral (10 mg/kg) dosing with ritonavir co-administration in Sprague-Dawley rats.

Key study findings: After IV dosing, clearance of TPV and volume of distribution was low. The elimination half-life averaged 4.60 hours in males and 7.80 hours in females. $AUC_{0-\infty}$ in females was approximately two-fold higher compared to males.

After oral dosing, TPV exposure tended to be higher in females compared to males. Apparent bioavailability of TPV was 58.7% in males and 50.9% in females.

Study no.: U04-3030
Volume # and page #: Module 4, M002, vol. 1.5, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of report: Not provided in hard copy.

Objective: The objectives of this study were to investigate the pharmacokinetics of TPV after intravenous and oral dosing following five consecutive daily oral doses of RTV in Sprague-Dawley rats.

Materials and methods: RTV was administered orally at 10 mg/kg/day for five days. Immediately after RTV dosing on Day 5, [^{14}C]TPV was administered intravenously at 5 mg/kg or orally at 10 mg/kg. TPV was administered as a solution in 80% PEG 400/20% water (v:v) and RTV in propylene glycol. Blood samples were collected serially at selected time points following [^{14}C]TPV dosing on Day 5 in both groups. The samples were analyzed for total radioactivity and following radioanalyses, male and female rat plasma samples were pooled separately for each time point. The pooled plasma samples were assayed for TPV and RTV concentrations using LC/MS/MS.

Results and Discussion: After IV dosing, clearance of TPV was low in both sexes. Volume of distribution was low. The elimination half-life averaged 4.60 hours in males and 7.80 hours in females. $AUC_{0-\infty}$ in females was approximately two-fold higher compared to males.

After oral dosing, TPV exposure tended to be higher in females compared to males. Apparent bioavailability of TPV was 58.7% in males and 50.9% in females.

Exposures to parent drug and to drug-related radioactivity were similar to each other in each dose group.

Study title: Pharmacokinetics of tipranavir in beagle dogs with and without ritonavir co-administration.

Key study findings: Co-administration of TPV and RTV resulted in a five-fold and seven-fold increase in TPV Cmax and AUC_{0-24}, respectively, relative to Drug Day 10 when dogs received TPV only. The effect was more evident on Drug Day 18 as TPV Cmax and AUC_{0-24} both increased approximately nine-fold, relative to Drug Day 10 Cmax and AUC_{0-24}.

Study no.: U03-3086
Volume # and page #: Module 4, M002, vol. 1.7, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of report: Not provided in hard copy.

Objective: The objectives of this study were to obtain a pharmacokinetic profile of TPV after oral dosing in beagle dogs after single and repeated drug administration and to determine the effect of co-administration of RTV on the pharmacokinetics of TPV.

Materials and methods: TPV was administered by gavage in an aqueous, pH 10.5, solution to two beagle dogs per sex per group at an initial dose of 75 mg/kg/day, given as a single dose. Due to emesis, the dose was reduced to 3715 mg/kg/day and then to 18.75 mg/kg/day on Drug Days 4 and 9, respectively. On Drug Day 15, RTV at a dose of 6.4 mg/kg/day was added to the regimen and dosing continued through Drug Day 18. Serial blood samples were drawn out to 24 hours and plasma concentrations of TPV and RTV were measured by LC/MS/MS. The LOQ of the assay was — ng/ml for both analytes.

Results and Discussion: No mortality occurred; TPV-induced clinical signs included emesis and diarrhea. No additional clinical signs were noted with addition of RTV to the regimen.

Co-administration of TPV and RTV resulted in a five-fold and seven-fold increase in TPV Cmax and AUC_{0-24}, respectively, relative to Drug Day 10 when dogs received TPV only. The effect

was more evident on Drug Day 18 as TPV Cmax and AUC_{0-24} both increased approximately nine-fold, relative to Drug Day 10 Cmax and AUC_{0-24}.

There was a trend toward increased exposure to RTV after repeated dosing. RTV Cmax and AUC_{0-24} were increased three-fold and four-fold, respectively, on Drug Day 18 versus Drug Day 15. However, these parameters were quite variable and do not support a strong conclusion on RTV accumulation after multiple administration.

Study title: Pharmacokinetics, excretion and mass balance of radioactivity following single oral and intravenous doses of ^{14}C-tipranavir to dogs after an oral dosing regimen of ritonavir and/or tipranavir.

Key study findings: There were no substantial differences in the pharmacokinetic parameters due to gender. Concentrations of radioactivity in blood were consistently lower than concentrations in plasma regardless of dose route and gender. Absorption of radioactivity after the oral dose of ^{14}C-TPV was estimated to be 26.6% in males and 17.8% in females.

The elimination of radioactivity was similar in males and females regardless of the route of administration of ^{14}C-TPV. The major route of elimination (68% to 93% range) was via feces with small amounts (1.7% to 3.6% range) via urine. Mean total recoveries after IV dosing were 96.8% for males and 89.6% for females and after oral dosing, 84.8% for males and 81.5% for females.

Study no.: U04-3034-02
Volume # and page #: Module 4, M002, vol. 1.7, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of report: December 23, 2003

Objective: The objective of the study was to assess the pharmacokinetics, absorption, elimination and mass balance of ^{14}C-TPV given to dogs by IV and oral routes of administration following treatment regimens with RTV and RTV/TPV, respectively.

Materials and methods: Beagle dogs (3/sex/group) were administered ^{14}C-TPV IV or orally after five daily oral doses of RTV. The RTV treatment continued an additional three days after administration of ^{14}C-TPV. Animals given an oral dose of ^{14}C-TPV were also given four daily oral doses of TPV before the ^{14}C-TPV dose. For pharmacokinetic analysis, blood and plasma were collected at selected times through 48 hours postdose (IV) and 96 hours postdose (oral). Excretion of radioactivity in urine and feces for both administration routes of ^{14}C-TPV was determined through 168 hours.

Results and Discussion: There were no substantial differences in the pharmacokinetic parameters due to gender. There was no retention of radioactivity in whole blood and concentrations of radioactivity in blood were consistently lower than concentrations in plasma regardless of dose route and gender. Absorption of radioactivity after the oral dose of ^{14}C-TPV was estimated to be 26.6% in males and 17.8% in females.

The elimination of radioactivity was similar in males and females regardless of the route of administration of ^{14}C-TPV. The major route of elimination was via feces within the first 24 to 48 hours after dosing. Mean total excretion of radioactivity in feces after IV dosing was 93.3% and 83.9% for males and females respectively. After oral dosing, the mean total excretion of radioactivity in feces was 68.0% in males and 75.9% in females. The mean total urinary excretions of radioactivity after IV dosing were 2.66% for males and 2.62% for females. After oral dosing, the mean total radioactivity in the urine was 1.71% for males and 3.61% for females. Mean total recoveries after IV dosing were 96.8% for males and 89.6% for females and after oral dosing, 84.8% for males and 81.5% for females.

Study title: Pharmacokinetics of tipranavir in rhesus monkeys with and without ritonavir co-administration.

Key study findings: A two-fold increase in AUC_{0-24} of TPV in rhesus monkeys was observed after co-administration of RTV. The increase in exposure appears to be caused by a decrease in TPV metabolism after addition of RTV as evidenced by a decrease in TPV metabolite M1 formation.

Study no.: U02-3080
Volume # and page #: Module 4, M002, vol. 1.7, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of report: March 12, 2002

Objective: The objectives of the study were to describe the steady-state pharmacokinetics of TPV in rhesus monkeys after administration of TPV alone or when co-administered with ritonavir.

Materials and methods: Control group monkeys (one/sex) received vehicle only. The treated group (one/sex) was orally dosed with TPV at 40 mg/kg/day. Initially, TPV was administered as a suspension in CMC/Tween 80. Due to low TPV plasma concentrations, the vehicle for TPV was changed to an aqueous solution (pH 10.5) beginning on Day 7. Beginning on Day 16, ritonavir was co-administered with TPV at 6.4 mg/kg/day up to 23 days. Serial blood samples were drawn on Days 1, 7, 11, 16 and 23. Planma concentrations of TPV and RTV were

measured by LC/MS/MS. The LOQ of the assay was —ng/ml for both analytes. A TPV plasma metabolite, M1, was measured using an LC/MS/MS method by comparison with a TPV standard curve.

Results and Discussion: Due to the change in formulation, mean Cmax and AUC_{0-24} were much greater on Day 11 compared to Day 1. This is probably due to better absorption from the aqueous solution vehicle.

Mean TPV Cmax values were similar before and after RTV co-administration. However, by Day 23, TPV AUC_{0-24} values increased by two-fold after four days of co-administration with RTV. M1:TPV ratios for both Cmax and AUC_{0-24} decreased substantially after initiation of RTV co-administration on Day 16.

Plasma concentrations of RTV were generally below quantifiable limits — ng/ml). RTV was only detected in two plasma samples.

2.6.4.4 Distribution

Study title: In vitro protein binding of tipranavir in mouse, rat, rabbit, dog and human plasma and in 4% human serum albumin, 0.07% $\acute{\alpha}_1$-acid glycoprotein, and 6% fetal bovine serum.

Key study findings: Binding was very high (>99.9%) in plasma of all species studied. Binding in plasma showed only a slight tendency to decrease as concentration increased. Binding was also very high for HSA, AAG and FBS. There was only a slight trend towards saturation of binding in HSA but clear saturation was observed for AAG and FBS.

Study no.: U03-3213
Volume # and page #: Module 4, M002, vol. 1.7, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of report: Not provided.

Objective: The objectives of the study were to determine the in vitro protein binding of TPV in plasma from several species, including human, as well as in 4% human serum albumin (HSA), 0.07% $\acute{\alpha}_1$-acid glycoprotein (AAG), and 6% fetal bovine serum (FBS).

Materials and methods: The in vitro protein binding of TPV was determined by equilibrium dialysis in several matrices: mouse, rat, rabbit, dog and human plasma, 4% HSA, 0.07% AAG and 6% FBS. For each species, plasma from several individuals was pooled. TPV was quantitated using an LC/MS/MS method with a lower limit of quantitation of —ng/ml. Equilibrium was achieved with human plasma with a six hour incubation. Therefore, a six hours incubation was used throughout the study. A TPV concentration range of 10 to 100 μM was

chosen for this study because 20 μM is the target clinical trough concentration and also this range covers a large portion of the range of concentration in toxicology studies.

Results and Discussion: Binding was very high (>99.9%) in plasma of all species studied. Binding in plasma showed only a slight tendency to decrease as concentration increased. Binding was also very high for HSA, AAG and FBS. There was only a slight trend towards saturation of binding in HSA but clear saturation was observed for AAG and FBS.

Study title: The determination of tipranavir (PNU-140690) in cerebrospinal fluid and brains of male Sprague-Dawley rats after oral administration (DMR Protocol 1999-0105).

Key study findings: Penetration of TPV into brain tissue and CSF was low. The ratio of TPV concentration in brain to blood plasma ranged from 0.0006 to 0.0081. The ratio of TPV concentration in CSF to blood plasma ranged from 0 to 0.0017.

Study no.: U00-3206 (Pharmacia & Upjohn Study Report a0056253)
Volume # and page #: Module 4, M002, vol. 1.7, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of report: September 17, 1999

Objective: The objective of the study was to determine whether tipranavir crosses the blood-brain barrier in rats after sustained, elevated plasma levels.

Materials and methods: Three male Sprague-Dawley rats were administered a single oral solution dose of 400 mg/kg TPV. Approximately six hours after dosing, the animals were anesthetized, administered a bolus IV injections of [^{14}C]-sucrose to mark the plasma water volume, sacrificed and exanguinated. A sample of cerebral spinal fluid (CSF) was then obtained from the base of the skull after which the entire brain was excised. TPV was determined in blood plasma, CSF and brain tissue by specific HPLC-UV methods. [^{14}C]-sucrose was measured by scintillation counting. The presence of radiolabel in brain and CSF was used to correct for the contamination of these samples by blood, since sucrose does not cross the blood-brain barrier.

Results and Discussion: Penetration of TPV into brain tissue and CSF was low. The ratio of TPV concentration in brain to blood plasma ranged from 0.0006 to 0.0081. The ratio of TPV concentration in CSF to blood plasma ranged from 0 to 0.0017. Plasma concentrations of TPV at the time of sacrifice ranged from [ ] and the volume of residual blood in brain tissue ranged from [ ]

Study title: Tissue distribution of [^{14}C]-PNU-140690 in male Long-Evans rats by quantitative whole-body autoradiography following single oral administration at 10 mg/kg (DMR Protocol No. 96-415)

Key study findings: The highest concentrations of TPV-related radioactivity were found in liver and variably in lung, blood, adrenal gland, intestines and kidney. The lowest levels were observed in skeletal muscle, thymus, testes and brain. Non-pigmented eye tissues contained no measurable radioactivity. Lymph nodes contained low to moderate amounts of drug-related radioactivity. The AUC values and half-lives for pigmented tissues were similar to those of other tissues, indicating that TPV does not bind to melanin.

Study no.: U00-3181 (Pharmacia & Upjohn Technical Report 7256-97-038)
Volume # and page #: Module 4, M002, vol. 1.8, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Drug Metabolism Research, Kalamazoo, MI

Date of report: September 22, 1997

Objective: The objectives of the study were to 1) determine the extent to which drug-related radioactivity is distributed to various tissues and organs, 2) evaluate localization of radioactive materials in melanin-containing tissues and 3) obtain data for the determination of human radiolabel dosimetry.

Materials and methods: A single dose of 10 mg/kg [^{14}C]-TPV was administered to Long-Evans rats. Thirty tissues and organs were analyzed through quantitative whole-body autoradiography for radioactive content at time points ranging from one hour to 72 hours after drug administration.

Results and Discussion: Drug-related radioactivity was slowly absorbed and distributed, such that maximum levels in tissues were generally observed at four hours postdose with a steady decline after four hours. The highest concentrations were found in liver and variably in lung, blood, adrenal gland, intestines and kidney. The lowest levels were observed in skeletal muscle, thymus, testes and brain. Non-pigmented eye tissues contained no measurable radioactivity. Lymph nodes contained low to moderate amounts of drug-related radioactivity. The AUC values and half-lives for pigmented tissues were similar to those of other tissues, indicating that TPV does not bind to melanin.

Study title: Quantitative whole-body autoradiography of rats after single oral doses of ^{14}C-tipranavir administered to rats following 5 consecutive daily oral doses of ritonavir.

Key study findings: Radioactivity was primarily associated with the liver and tissues and contents of the GI tract. For both groups, levels in other tissues were much lower (below 5000 ng equivalents ^{14}C-TPV/g). Concentrations of radioactivity were low or below the level of

detection in tissues of brain and central nervous system, indicating that TPV did not readily cross the blood-brain barrier, although radioactivity was present in the CSF.

Study no.: U03-3061
Volume # and page #: Module 4, M002, vol. 1.8, page 1.
Conducting laboratory and location: [illegible]

Date of report: January 24, 2003

Objective: The objective of the study was to assess the tissue distribution of TPV-derived radioactivity in rats administered ^{14}C-TPV orally following five consecutive daily doses of RTV.

Materials and methods: Male albino Sprague-Dawley rats (Group 1) and male pigmented Long Evans rats (Groups 2) were given single 10 mg/kg oral doses of ^{14}C-TPV immediately after the fifth of five consecutive daily oral doses of RTV (10 mg/kg). For each group, two animals were euthanized at 8, 24, 48, 96 and 168 hours after dosing and blood and plasma were collected for radioanalysis and carcasses were collected for quantitative whole-body autoradiography (WBA) analysis.

Results and Discussion: Radioactivity was primarily associated with the liver and tissues and contents of the GI tract. For both groups, levels in other tissues were much lower (below 5000 ng equivalents ^{14}C-TPV/g). By 96 hours postdose, the concentrations of radioactivity in most tissues were not discernable. Concentrations of radioactivity were low or below the level of detection in tissues of brain and central nervous system, indicating that TPV did not readily cross the blood-brain barrier, although radioactivity was present in the CSF at 8 and 24 hours postdose with levels up to 1350 ng equivalents ^{14}C-TPV/g.

Blood and plasma exposures between the two groups were very similar. The pharmacokinetic parameters in tissues for ^{14}C-TPV-derived radioactivity were not remarkably different between albino and pigmented rats. The terminal elimination half-life values in tissues ranged from 3.24 hours (rudimentary teeth) to 59.5 hours (liver) for Sprague-Dawley rats and from 4.07 hours (small intestine) to 38.6 hours (liver) for Long Evans rats. There was no apparent melanin binding.

Study title: Lacteal secretion and placental transfer of radioactivity following oral administration of ^{14}C-tipranavir to Sprague-Dawley rats after an oral dosing regimen with ritonavir.

Key study findings: TPV-associated radioactivity was secreted into the milk of lactating rats and crossed the placenta of dams dosed on Gestation Days 13 and 19. Higher concentrations were observed in fetus of dams dosed on Gestation Day 19. Tissue concentrations of

radioactivity in the dams were well below those found in plasma except for liver. Radioactivity also crossed the blood:brain barrier of the dams and the fetal rats but only to a limited extent.

Study no.: U04-3037
Volume # and page #: Module 4, M002, vol. 1.8, page 1.
Conducting laboratory and location:

Date of report: December 23, 2003

Objective: The objective of the study was to assess the secretion of radioactivity in milk from lactating rats and the placental transfer of radioactivity in pregnant rats following oral administration of ^{14}C-TPV after an oral dosing regimen with RTV.

Materials and methods: ^{14}C-TPV was administered orally to lactating rats after five daily doses of RTV. ^{14}C-TPV was administered orally to pregnant rats on Gestation Days 13 and 19 following five daily oral doses of RTV. Concentrations of radioactivity in blood, plasma, milk and tissues were determined at time points up to 48 hours postdose in thh lactating rats and up to 96 and 48 postdose in the pregnant rats dosed on Gestation Days 13 and 19, respectively. Concentrations of radioactivity in blood, plasma and milk were determined by liquid scintillation counting. Quantitative whole-body autoradiography analysis was used to determine concentrations of radioactivity in the blood and tissues of pregnant rats.

Results and Discussion: The maximum mean concentration of radioactivity in milk (1.43 µequivalents/g) was observed at the initial sampling time at six hours postdose. For pregnant rats dose with ^{14}C-TPV on Gestation Day 13, radioactivity was present in the fetus at all time points sampled. For pregnant rats dosed on Gestation Day 19, radioactivity was generally present in the fetus and fetal tissues at all time points sampled and higher concentrations were observed than for those dosed on Gestation Day 13. Tissue concentrations of radioactivity in the dams were well below those found in plasma except for liver. Radioactivity also crossed the blood:brain barrier of the dams and the fetal rats but only to a limited extent.

2.6.4.5 Metabolism

Study title: Characterization of PNU-140690 (tipranavir) metabolites in human, monkey, dog and rat plasma.

Key study findings: Four major metabolites were identified in in vivo plasma samples from rat, dog, monkey and human dosed orally with TPV. Two metabolites (M3 and M4) were formed by intramolecular cyclization and the additional two metabolites (M1 and M2) were secondary oxidation products of these primary metabolites.

Study no.: U01-3135 (Pharmacia & Upjohn Study Report a0073317)

Volume # and page #: Module 4, M002, vol. 1.9, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of report: April 25, 2000

Objective: The objective of the study was to characterize four metabolites of TPV in human plasma and across species.

Materials and methods: The major in vivo TPV metabolites were characterized in plasma from rat, dog, monkey and humans. Following protein precipitation with acetonitrile, the organic supernatants were profiled directly by HPLC with UV detection. Metabolites of TPV were also isolated from human and monkey plasma for structural characterization.

Results and Discussion: Chromatographic plasma profiles from each species revealed two nonpolar metabolites, M3 and M4. Two additional metabolites (M1 and M2) were also present in human and monkey plasma. Among human volunteers treated with TPV, metabolite M1 was approximately 20% of the total exposure to parent drug. These four metabolites were characterized as two pairs of tricyclic regioisomers (M1/M2 and M3/M4). Metabolites M3 and M4 were formed by an intramolecular cyclization between the dihydropyran and aromatic amine of TPV to yield tricyclic pyrano-benzopyran regioisomers. Oxidation of TPV resulted in formation of a ketene acetal (M3) and a lactone (M4) via substitution at the arylamine C-4 position in each case. Metabolites M1 and M2 were determined to be tricyclic pyrano-benzoopyran regioisomers also, similar to metabolites M3 and M4, but M1 and M2 were each further oxidized to a ketone at the benzylic position of the 6'-phenethyl substituent. Based upon structural similarities between the two pairs of tricycli regioisomers, the data are consistent with the sequential oxidative metabolism of in vivo metabolites M3 and M4 to metabolites M2 and M1, respectively.

2.6.4.6 Excretion

Study title: Excretion of radioactivity in urine, feces, expired air and bile after single oral and intravenous doses of ^{14}C-tipranavir administered to Sprague-Dawley rats following 5 consecutive daily oral doses of ritonavir.

Key study findings: The main route of excretion after IV and oral administration of ^{14}C-TPV was via feces. Over the 168 hours study, mean totals excreted via the feces ranged from 75.0% to 86.7%. Totals of 4.06% to 9.71% were excreted via urine over 168 hours. Only a small fraction (less than 1%) of the dose of ^{14}C-TPV was metabolized to $^{14}CO_2$ after oral or IV administration. No radioactivity was found in organic volatile traps and 1% or less of the radioactivity remained in animal carcasses at 168 hours postdose.

Study no.: U03-3060
Volume # and page #: Module 4, M002, vol. 1.9, page 1.
Conducting laboratory and location: [redacted]

Date of report: January 24, 2003

Objective: The purpose of the study was to assess the extent of absorption, distribution and elimination of TPV-derived radioactivity in rats following single oral and IV doses of ^{14}C-TPV, following five consecutive daily oral doses of RTV.

Materials and methods: Sprague-Dawley rats (4/sex/group) were given single 5 mg/kg IV or 10 mg/kg oral doses of ^{14}C-TPV after five consecutive daily oral doses of RTV (10 mg/kg/day). A third group of male rats, with surgically implanted bile duct cannuli, was treated similarly with RTV and an oral dose of ^{14}C-TPV. Appropriate samples (urine, feces, expired air, organic volatiles, bile, cage wash, cage wipe and residual carcass) were collected at specified intervals over 168 hours in bile duct intact animals and over 48 hours in bile duct-cannulated rats. All samples were analyzed for radioactivity by liquid scintillation counting.

Results and Discussion: The main route of excretion after IV and oral administration of ^{14}C-TPV was via feces. Over the 168 hours study, mean totals of 86.7% and 82.4% (IV) and 75.0% and 82.0% (oral) were excreted via feces in males and females, respectively. Totals of 4.06% and 6.73% (IV) and 9.71% and 8.28% (oral) were excreted via urine over 168 hours in males and females, respectively. Only a small fraction (less than 1%) of the dose of ^{14}C-TPV was metabolized to $^{14}CO_2$ after oral or IV administration. No radioactivity was found in organic volatile traps and 1% or less of the radioactivity remained in animal carcasses at 168 hours postdose. The total mass balance of radioactivity was similar after IV or oral administration of ^{14}C-TPV and ranged from 91.0% to 93.5%.

2.6.4.9 Discussion and Conclusions ***See 2.6.4.1 Brief summary***

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: The minimum lethal doses of TPV identified in single dose toxicology studies were 3000 mg/kg free acid equivalents (FAE) in mice, 2330 mg/kg FAE in male rats and 1500 mg/kg FAE in female rats and >500 mg/kg FAE in beagle dogs. Gastrointestinal symptoms (emesis, soft stools and/or diarrhea) were common findings among the species tested. In rats, elevations of coagulation parameters were noted in females after single administration of 1500 to 3000 mg/kg.

The primary TPV target organs identified through repeat-dose toxicology studies in mice, rats, dogs and/or monkeys are the liver and gastrointestinal tract. Additional organs that were affected included the thyroid gland, testes and to a lesser extent the adrenal gland, kidneys, spleen and heart. Co-administration of TPV and RTV in rats and dogs revealed only target organs or signs of toxicity seen when each compound was administered alone. Neither drug exacerbated the toxicity of the other.

It should be noted that in rats and dogs, TPV exposure in animals at the NOAEL doses is equivalent to or slightly above exposure in humans at the clinical dose of 500/200 TPV/RTV BID. Early toxicity studies were performed with TPV alone. Once the decision was made for co-administration with RTV in the clinic, bridging toxicity studies with both compounds were performed. Co-administration does increase the exposure of TPV, especially at low doses, but the effect is lower at higher doses. In animals the boosting effect of co-administration is generally lower than in humans (11-fold increase) and in particular much lower at NOAEL doses in repeat-dose toxicology studies: mice (12- to 22-fold), rats (6- to 7-fold), dogs (3- to 13-fold) and monkeys (2-fold). However, toxicities seen in repeat-dose toxicology studies are not considered to preclude chronic administration of TPV to the intended patients, even though plasma levels are equivalent to or below human exposure. These toxicities are reversible, manageable, species specific and/or considered secondary to species-specific hepatic enzyme-inducing effects of TPV in the rodent.

Gastrointestinal effects included emesis, soft stools, diarrhea and/or excessive salivation after dosing and were observed in all species tested. These effects probably effect local actions since no correlative macroscopic or microscopic changes in the GI tract were observed in mice, rats or dogs. GI effects in rodents included soft stool, diarrhea, increased salivation after dosing (attributed to the bitter taste of TPV during gavage administration) and decreases in food consumption. In dogs and monkeys, soft stool, diarrhea, as well as emesis were observed. The frequency of these GI effects increased with TPV dose level and these signs decreased or ceased completely when TPV dosing was stopped. There was a decrease in emesis and post-dose salivation when TPV was administered in the SEDDS formulation to dogs by capsule, rather than by oral gavage. This supports the theory that the bitter taste of TPV leads to post-dose salivation and emesis. It should be noted that in a 26-week safety study in dogs with varying amounts of a SEDDS vehicle equivalent to the bulk fill solution, an increase in soft stools was noted during the Pretest Phase when only SEDDS vehicle was administered to dogs. This occurrence of soft stools was related to the volume of SEDDS vehicle which was approximately 30-fold (mg/kg basis) that of humans at a dose level of 500/200 mg/kg TPV/RTV BID.

TPV is a microsomal enzyme inducer and has been shown to increase activity of CYP 3A and CYP 3B in rats and dogs. Changes associated with hepatic enzyme induction, increased liver weights, hepatocellular hypertrophy and increases in smooth endoplasmic reticulum have been seen in nonclinical studies in mice, rats and dogs. Additional changes considered secondary to enzyme induction and specific to rodents were seen generally at high doses and included hepatocellular degeneration, vacuolation, necrosis and mineral deposition in mice and

multinucleated hepatocytes in rats. Karyomegaly, an effect of RTV in rats, was observed at a low incidence in the 26-week TPV/RTV study. Histological changes specific to dogs included bile duct hyperplasia and gallbladder cystic hyperplasia at a high dose in the 39-week TPV alone study. This effect was not seen in the 26-week TPV/RTV dog study. These effects were reversible and enzyme induction caused by TPV with resultant hepatocellular hypertrophy is considered an adaptive response and not evidence of toxicity.

The rat is the more inducible species, with males showing more activity than females. This is reflected in the fact that females have higher plasma concentrations of TPV than males after repeated doses of TPV. Additional effects seen in the rat could be secondary to hepatic microsomal enzyme induction. These include increased metabolism and clearance of thyroid hormones, slight increase in plasma proteins and potentially effects on coagulation parameters. Increases in plasma proteins were seen in rat studies as increases in total protein, albumin (accompanied by a slight increase in plasma calcium in several studies) and/or globulin. Increased thyroid gland weights, thyroid follicular hypertrophy, increases in TSH and decreases in T3 and T4 were seen in rat studies and are considered to reflect a rodent specific increase in thyroid hormone metabolism secondary to induction of hepatic enzymes. Changes in thyroid parameters were monitored in the clinic in early trials. The changes seen in rodents in nonclinical studies were not seen in humans. TPV clearly increases coagulation parameters (prothrombin time and activated partial thromboplastin time) in rodents but the mechanism is unknown. It may be caused by an indirect mechanism related to hepatic enzyme induction in rodents. No similar changes were seen in dog studies. Monitoring of PT was performed in clinical trials and no significant changes in this parameter were observed in humans.

Dogs exposed to TPV or TPV/RTV exhibited mild increases in liver alkaline phosphatase (AP) isoenzymes and this suggests an effect on the liver. Histopathological effects in dogs included gallbladder cystic glandular hyperplasia and bile duct hyperplasia in long term repeat dose studies. These effects are common in older beagle dogs but were increased in TPV treated dogs. In the absence of more severe histopathology, such as biliary stasis or cholestasis, these changes raise little concern for humans. This is also in contrast to the observation that rats exhibited decreased serum AP at higher dose levels in a number of studies.

In mice, enzyme leakage (ALT, AST) at high dose levels was correlated with hepatocellular necrosis. Increases in AST and/or ALT were observed minimally or not at all in toxicology studies on rats and dogs. Based on this difference in species, the importance for humans is not clear. However, liver function can be easily monitored in humans and the nonclinical studies support monitoring as a way of managing this potential human toxicity.

Testicular effects consisting of decreased weights and bilateral seminiferous tubule degeneration and/or atrophy were observed in a 26-week TPV/RTV study in rats and a 39-week TPV study in dogs. In the 26-week TPV/RTV study in rats, mean testicular weights were decreased 19% in males receiving the highest dose of 1200/320 mg/kg/day TPV/RTV but not at 1200 mg/kg/day TPV or 160 mg/kg/day RTV. Bilateral seminiferous tubule degeneration was seen in 3/15 male

rats. The 1200/320 mg/kg/day TPV/RTV dose level caused high mortality. No other studies in rats, including the 26-week study with TPV alone, resulted in testicular changes. The low incidence of testicular findings together with the high incidence of mortality in the 1200/320 TPV/RTV group, coupled with hemorrhagic events, decreased food consumption and body weight gain, suggest that the testicular degeneration seen in rats in this study is related to stress. However, a direct effect of the drugs cannot be discounted. Testicular changes consisting of vacuolar degeneration of the epithelial lining and atrophy of the seminiferous tubules were noted in 3/4 male dogs after administration of 320 mg/kg/day TPV for 39 weeks. Following a recovery period, 1/3 males at this dose level still displayed degeneration of the seminiferous tubules as well as abnormal germ cells and decreased number of sperm within the ducts of the epididymides. No testicular changes were noted in other studies in dogs with TPV or TPV/RTV. After the submission of the NDA, the sponsor submitted Study Number U04-3531 which gives the results of a re-evaluation of the data on testicular degeneration and/or atrophy by an expert panel. The panel concluded that the findings in the dog were within normal limits of variation. Based on the above information, morphologically unrelated testicular findings in one rat study in three animals at a high dose associated with high mortality are not considered to be a cause of concern in humans.

Effects were seen sporadically in adrenal gland, kidneys, spleen and heart are not considered to have predictive value for humans for the following reasons: 1) Adrenal gland effects consisted of increased adrenal weights without correlative microscopic changes, with the exception of one 4-week study in mice where hypertrophy of the zona fasciculata was observed at the highest TPV and TPV/RTV levels. These were minimal to mild effects seen at high doses and similar changes were not seen in dogs. Thus, these adrenal effects in rats were attributed to stress and not a direct effect of TPV. 2) Changes in the kidney were an increased urinary protein and exacerbation of chronic progressive nephropathy, a rodent specific spontaneous change, seen in a 26-week rat study. Kidney changes were not seen in other species or in rats in the 26-week TPV/RTV study. 3) Increased extramedullary hematopoeisis was observed in the spleen in mice, rats and dogs but was considered secondary to mildly reduced red blood cell parameters in rats and dogs and hemorrhage in the 26-week TPV/RTV rat study. 4) Minimal to mild myocardial degeneration was observed in one study in mice when TPV was administered by diet over 13 weeks. No heart changes were seen in any gavage administration study in mice up to 13-weeks nor have heart changes been seen in any study in rats or dogs, up to 26- and 39-weeks, respectively. The significance of this finding is unclear but it is assumed that if TPV had cardiotoxic liability cardiac changes would have been seen in multiple species or consistently in one species rather than in one study.

The safety of the self-emulsifying drug delivery system (SEDDS) formulation was explored in a 26-week TPV/RTV dog study to eliminate concerns over the high dose ([redacted]) from 500/200 mg/kg TPV/RTV BID) of Cremophor EL (CrEL) contained in this formulation. Of concern was the possibility that CrEL might pass from the GI tract into systemic circulation and thereby pose a risk of anaphylactoid reactions since CrEL is known to cause these reactions if given in high levels IV. Consequently, exposures to the SEDDS formulation were chosen to achieve 1, 10 and

30-fold exposure to CrEL in humans. Toxicities in two animals and the death of one animal treated with the high dose of SEDDS were deemed due to the effect of the formulation. The target organs noted in the early death female were the stomach, intestine and mesenteric lymph nodes. CrEL plasma levels were detectable 2 hours after the first or second dose in several animal administered 2720 mg/kg/day SEDDS and one animal receiving 910 mg/kg/day SEDDS. Plasma CrEL levels ranged from[redacted] to [redacted] ng/ml. These levels are unlikely to cause anaphylactoid reactions. The NOAEL for SEDDS is considered to be 910mg/kg/day SEDDS which supports a 10-fold safety factor for the SEDDS vehicle in the recommended human dose.

Genetic toxicology: TPV was tested for the ability to induce point mutations in DNA (mutagenicity) and the ability to damage chromosomes (clastogenicity) in five in vitro and in vivo assays including the battery of assays specificed in the ICH S2B guidance on genotoxicity. These included the in vitro bacterial reverse mutation assay, unscheduled DNA synthesis (UDS) in rat hepatocytes, induction of gene mutation in Chinese hamster ovary cells and a chromosome aberration assay in human peripheral lymphocytes, as well as an in vivo bone marrow micronucleus assay in mice. TPV was negative in these assays, indicating that TPV has no mutagenic or clastogenic potential.

In addition, a number of genetic toxicology assays were performed to qualify impurities. Bacterial reverse mutation assays were performed on TPV plus unqualified impurities including degradation products of the SEDDS formulation and specific drug substance impurities [redacted] with unqualified impurities was also evaluated in a UDS rat primary hepatocyte assay and a three-day bone marrow micronucleus assay in rats. TPV SEDDS formulation containing degradation products was also evaluated for induction of micronuclei in a 13 week oral micronucleus assay in rats. TPV and all impurities and degradation products were negative in all of the assays. These studies reinforce the conclusion from the results of the standard battery assays on TPV alone that TPV, as well as impurities and degradation products tested, are not mutagenic or clastogenic.

Carcinogenicity: Carcinogenicity studies in mice and rats are ongoing.

Reproductive toxicology: A male and female fertility and early embryonic development study (oral) in Sprague-Dawley rats demonstrated that TPV did not affect spermatogenesis, estrous cycle, copulation, conception, fertility, implantation or early embryonic development at doses up to 1000 mg/kg/day. This corresponds to a Cmax of 258 μM which is approximately two-fold the human Cmax at the proposed clinical dose of 500/200 TPV/RTV BID.

In an embryo-fetal development study in Sprague-Dawley rats, there was no evidence of TPV-related embryolethality or teratogenicity at doses of 40 to 1000 mg/kg/day. However, the NOAEL for both maternal and developmental toxicity was 40 mg/kg/day, based on findings of postdose salivation, decreased body weight and food consumption in dams and decreased body weight and sternebrae ossification in fetuses. This NOAEL corresponds to a mean Cmax 30.4

μM and a mean AUC of 340 μM.h, which is 0.2-fold of the expected human exposure at the proposed dose of 500/200 TPV/RTV BID.

In embryo-fetal development studies, when TPV was administered to pregnant rabbits (gestation days 6 through 20) in daily doses up to 375 mg/kg, maternal toxicity (death of one female, abortions, decreased body weight and food consumption and increased clinical signs) and developmental toxicity (slightly decreased fetal body weights, fetuses with wavy ribs and bent femurs and increased incidence of fetuses with gross malformations) were observed at the high dose. Interpretation of these fetal findings is complicated by maternal toxicity and by the fact that a single litter was responsible for the majority of the developmental toxicities, suggesting a litter effect. These gross malformations were not observed in fetuses at 375 and 759 mg/kg/day TPV in the dose range-finding study in rabbits. Therefore, it is unlikely that TPV was teratogenic at 375 mg/kg/day. Maternal toxicity (abortions) occurred at 150 mg/kg/day but no developmental toxicity was observed at that dose level. The NOAEL for maternal toxicity was 75 mg/kg/day while the NOAEL for developmental toxicity was 150 mg/kg/day. The AUCs associated with these NOAEL doses correspond to 0.04-fold and 0.08-fold, respectively, the human exposure at the proposed clinical dose of 500/200 TPV/RTV BID.

In a pre- and post-natal development study in rats, TPV was toxic to dams and suckling pups at 400 and 1000 mg/kg/day with dose-relationship. Maternal toxicity was restricted to adverse effects on body weight and food consumption. Pup toxicity consisted of slight (400 mg/kg/day) or marked (1000 mg/kg/day) progressive growth inhibition throughout lactation, resulting in persistent adverse influence on the growth of the pups up to maturity. However, none of the postweaning functions examined in F1 offspring, including reproductive ability, were compromised up to the 1000 mg/kg/day dose and there was no evidence of teratogenicity at any dose. The 40 mg/kg/day dose was an NOAEL for both the dams and offspring.

Special toxicology: Local irritation studies in rabbits demonstrated that TPV was minimally irritating to the eye and mildly irritating to abraded skin with open wound.

2.6.6.2 Single-dose toxicity

Study title: U-109112 and U-140690: Preliminary single-dose oral safety/toxicity and toxicokinetics study in male Sprague-Dawley rats.

Study no.: U00-3082 (Pharmacia & Upjohn Technical Report 7227-96-016)
See Appendix, IND 51,979 (original) for review of this study.

Study title: U-140690E: One-day oral dose preliminary toxicokinetic study in female Sprague-Dawley rats.

Study no.: U00-3083 (Pharmacia & Upjohn Technical Report 7270-96-015)
See Appendix, IND 51,979 (original) for review of this study.

Study title: U-140690E: A series of preliminary one-day oral formulation/safety/toxicity and toxicokinetic study segments in male beagle dogs.

Study no.: U00-3084 (Pharmacia & Upjohn Technical Report 7270-96-013)
See Appendix, IND 51,979 (original) for review of this study.

2.6.6.3 Repeat-dose toxicity

Study title: PNU-14069E: Four-week preliminary oral safety/toxicity and toxicokinetics study with male and female Non-Swiss Albino (CF-1) mice.
(See Appendix Review of Carcinogenicity Study Design/Dose Selection Proposals, IND 51,979 Submission 217 (SX), March 24, 2003, for additional comments.)

Key study findings: TPV, when orally administered to male and female Non-Swiss Albino (CF-1) mice at doses of 0, 80, 400 or 800 mg/kg/day as twice daily doses of 0, 40, 200 or 400 mg/kg/dose 8 hours apart for at least 28 days, was fairly well tolerated up to 400 mg/kg/day since there were no deaths. Administration of doses of 1200 mg/kg/day was terminated after 4 days due to severe toxicity. Three mice administered 800 mg/kg/day died or were euthanized early. Dose-related clinical signs included decreased activity, dyspnea, soft/no stool, ptosis and changes in haircoat. There were increases in APTT, fibrinogen, T3, T4 and ALT and a slight increase in TSH was noted. Liver weights were increased. Livers had white foci and/or a nutmeg appearance, grossly, and hepatocellular hypertrophy accompanied by degenerative changes including necrosis, microscopically. Cmax and AUC increased with dose for both sexes. TPV was severely toxic at 1200 mg/kg/day, markedly toxic at 800 mg/kg/day and moderately toxic at 400 mg/kg/day. The NOAEL was considered to be 80 mg/kg/day which gives an exposure 0.002 to 0.003-fold the expected human exposure at the proposed clinical dose of 500/200 TPV/RTV BID.

Study no.: U00-3193 (Pharmacia & Upjohn Technical Report 7228-97-051)
Volume #, and page #: Module 4, M002, vol. 1.28, page # 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI
Date of study initiation: January 9, 1997
GLP compliance: No.
QA report: yes ( ) no (x)
Drug, lot #, and % purity: TPV, Lot No. (A) 5075-AS-1768, [redacted] purity.

Methods

Doses: 0, 80, 400, 800 or 1200 mg/kg/day (0, 40, 200, 400 or 600 mg/kg/dose with twice daily dosing).

Species/strain: Mouse/Non-Swiss Albino (CF-1)
Number/sex/group or time point (main study): 9/sex/group
Route, formulation, volume, and infusion rate: Oral by gavage, aqueous solutions of TPV in purified water adjusted to pH 10.5 with sodium hydroxide, 6 ml/kg/dose.
Satellite groups used for toxicokinetics or recovery: 15/sex/group
Age: Mature
Weight: Males, 23.25 to 32.75 g; Females, 20.25 to 27.90 g
Sampling times:
Unique study design or methodology (if any): TPV expressed as free acid equivalents. Total daily doses were equally divided and administered eight hours apart.

Observations and times:

Mortality: Observations were performed at least once daily throughout the study.

Clinical signs: Observations were performed at least once daily throughout the study.

Body weights: Body weights were measured on Days -1, 7, 14 21 and 28.

Food consumption: Not determined.

Ophthalmoscopy: Not determined.

EKG: Not performed.

Hematology: During the scheduled necropsy only, blood from the first three mice/sex/group was collected for clinical chemistry, blood from the second three mice/sex/group was collected for coagulation indices and blood from the last three mice/sex/group was collected for hematology.

Clinical chemistry: During the scheduled necropsy only, blood from the first three mice/sex/group was collected for clinical chemistry, blood from the second three mice/sex/group was collected for coagulation indices and blood from the last three mice/sex/group was collected for hematology.

Blood samples were also obtained from five Satellite Group animals/sex/group for measurement of TSH, T3 and T4 on Day 28.

Urinalysis: Not performed.

Gross pathology: All main study mice were necropsied at the end of the study or if found dead or moribund.

Organ weights (specify organs weighed if not in histopath table):

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x) – non-GLP study

Tissues indicated in the table were evaluated for the control and high dose groups. Additionally, because all animals in the 1200 mg/kg/day dose group were necropsied by Day 5, tissues from the 800 mg/kg/day dose group were also evaluated. Identified target organs (liver and thyroid glands) from the remaining dose groups and the lungs from animal 40 (which died on study) were also evaluated.

Results

Mortality: In the 1200 mg/kg/day group four males and two females in the main study group and five males and five females in the satellite study group were found dead or euthanized by Day 4. The surviving mice in this group exhibited signs of severe distress and all remaining mice were euthanized on Day 5. These early deaths and euthanasias were considered related to TPV. In addition, the deaths or early euthanasias of two males and one female in the 800 mg/kg/day satellite study may have been due to TPV. The deaths of one main study male and one satellite study female in the 400 mg/kg/day group were considered due to instillation errors.

Clinical signs: Clinical observations associated with TPV treatment noted in the 80, 400 and 800 mg/kg/day groups included decreased activity, dyspnea, soft or no stool, ptosis and changes in haircoat. In addition animals with distended abdomens were noted in the 400 and 800 mg/kg/day groups. Unsteady gait and dehydration were also noted in a few animals in the 400 mg/kg/day dose group. Generally, the incidence and severity of these clinical signs increased with dose and were greater in males than females. The 1200 mg/kg/day mice had a more pronounced complement of these clinical signs and also exhibited signs of cool-to-the-touch and a moribund appearance.

Body weights: Body weights in the 400 mg/kg/day group were decreased compared to beginning weights. All other dose groups had gains in body weight but the weight gains were suppressed when compared to control body weight gains.

Food consumption: Not determined.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: There were very slight decreases as compared to controls in hematocrit values in males and females at all dose levels. There were slight dose-related decreases compared to controls in mean cell hemoglobin in males at all dose levels and females at the 400 and 800

mg/kg/day dose levels. There were slight dose-related decreases in mean cell volume in males and females at all dose levels with statistical significant decreases in males at 400 and 800 mg/kg/day dose groups and in females in the 800 mg/kg/day dose group.

There were slight dose-related increases compared to controls in APTT in males and females at all dose levels. These increases were statistically significant for males and females at doses of 400 and 800 mg/kg/day. There were also slight treatment-related increases in fibrinogen in males and females at all dose levels, with statistical significance in males in the 400 and 800 mg/kg/day dose groups.

Clinical chemistry: There were treatment-related increases in ALT which were dose-related in males at all dose levels and treatment-related in females. Group mean ALT value were 2- to 4-fold greater than control mean ALT values ALT increases were statistically significant in females at all dose levels. There were no pathologic changes in any livers from mice administered the low dose. In contrast, ALT increases in the 400 and 800 mg/kg/day dose groups correlated with hepatocellular necrosis.

There were treatment-related increases in AST in males and females. The group mean AST values in females were higher than historical control values at all dose levels. The increases in male group mean AST values were still within the historical control range.

Other slight changes considered related to a drug-induced adaptive effect in the liver were increases in albumin, alkaline phosphatase, cholesterol, total protein, triglycerides and calcium and decreased in blood urea nitrogen.

There were dose-related increases in T3 and T4 which were considered toxicologically relevant. These changes correlate with follicular cell hypertrophy of the thyroid glands noted microscopically at the 800 mg/kg/day dose level There was a slight increase in mean TSH levels also. These changes are considered to be rodent-specific and have not been seen in clinical trials of TPV.

Urinalysis: Not performed.

Gross pathology: The only treatment-related gross observation noted was the presence of white foci and/or a nutmeg appearance of the liver in mice in the 400 and 800 mg/kg/day dose groups.

Organ weights (specify organs weighed if not in histopath table): There was a dose-related increase in mean absolute and relative (% body weight) liver weights in all dose groups of both sexes. Mean relative liver weights were increased over controls by 9%, 62% and 131% in males and 11%, 72% and 141% in females at 80, 400 and 800 mg/kg/day, respectively.

There was a slight dose-related increase in mean absolute and relative (% body weight) adrenal gland weight in all dose groups of both sexes with the exception of absolute adrenal gland

weights of males in the 80 mg/kg/day dose group. There were no histopathological correlates in the adrenal glands.

There was a slight dose-related decrease in mean absolute and relative (%body weight) spleen weight in females in all dose groups and in males at 400 mg/kg/day or greater. There were no microscopic changes noted in the spleen.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x) – non-GLP

The only treatment-related microscopic observations noted were hepatocellular hypertrophy and follicular cell hypertrophy of the thyroid glands. All mice in the 400 and 800 mg/kg/day dose groups had hepatocellular hypertrophy, commonly located in the periacinar region and edges of the liver lobes. The severity of this change was moderate to marked. Where the hypertrophy was most prominent, it was accompanied by degenerative changes of hepatocellular vacuolation, haphazard distribution of multifocal necrosis and mineral deposition. Changes were more severe in the 800 mg/kg/day dose group and similar to those noted for the animals in the 1200 mg/kg/day dose group. The hepatocellular necrosis could account for the low grade increases in ALT values of animals administered 400 and 800 mg/kg/day TPV.

The follicular cell hypertrophy of the thyroid gland was noted in 4 of 8 males and 4 of 9 females evaluated in the 800 mg/kg/day dose group only.

No treatment-related changes were noted in these target tissues of animals administered the 80 mg/kg/day dose.

Toxicokinetics: For first (0, 8 hours) and second (8, 24 hours) dosing intervals maximum plasma concentrations were observed between 1 and 4 hours after dosing, except for the first dosing interval for males administered 80 mg/kg where the tmax was at 8 hours. The Cmax (0, 24) occurred during the second dosing interval for all gender and dose groups. In general, Cmax and AUC values were similar for male and female mice and these values increased as the doses increased throughout the dose range of 80 to 800 mg/kg/day.

Toxicokinetic parameters on Day 28.

Dose (mg/kg/day)	80		400		800	
Sex: Number Examined	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
$Cmax_{0-24}$ (μM)	0.621	0.811	27.0	9.59	41.6	37.0
AUC_{0-24} (μM.h)	2.51	4.45	59.8	43.1	126	193
Fold versus Human AUC*	0.002	0.003	0.039	0.028	0.082	0.125

*Based on 500/200 mg TPV/RTV BID and an AUC of 1542 μM.h.

Other:

Histopathology inventory

Study U00-3193	Dose (mg/kg/day)				
Species CF-1 Mice	0	80	400	800	1200
Adrenals	X*	*	*	X*	X*
Aorta	X			X	X
Bone Marrow smear					
Bone (femur)					
Brain	X*	*	*	X*	X*
Cecum	X			X	X
Cervix	X			X	X
Colon	X			X	X
Duodenum	X			X	X
Epididymis	X			X	X
Esophagus	X			X	X
Eye	X			X	X
Fallopian tube					
Gall bladder	X			X	X
Gross lesions	X			X	X
Harderian gland					
Heart	X*	*	*	X*	X*
Ileum	X			X	X
Injection site					
Jejunum	X			X	X
Kidneys	X*	*	*	X*	X*
Lachrymal gland					
Larynx					
Liver	X*	X*	X*	X*	X*
Lungs	X			X	X
Lymph nodes, cervical					
Lymph nodes mandibular	X			X	X
Lymph nodes, mesenteric	X			X	X
Mammary Gland	X			X	X
Nasal cavity					
Optic nerves					
Ovaries	X			X	X
Pancreas	X			X	X
Parathyroid	X			X	X
Peripheral nerve					
Pharynx					

Pituitary	X			X	X
Prostate	X			X	X
Rectum					
Salivary gland	X			X	X
Sciatic nerve	X			X	X
Seminal vesicles	X			X	X
Skeletal muscle	X			X	X
Skin	X			X	X
Spinal cord	X			X	X
Spleen	X*	*	*	X*	X*
Sternum	X			X	X
Stomach	X			X	X
Testes	X			X	X
Thymus	X			X	X
Thyroid	X	X	X	X	X
Tongue	X			X	X
Trachea	X			X	X
Urinary bladder	X			X	X
Uterus	X			X	X
Vagina	X			X	X
Zymbal gland					
Additional Tissues					
Knee joint	X			X	X
Diaphragm	X			X	X
Psoas muscle	X			X	X

X, histopathology performed
*, organ weight obtained

Study title: A 4-week range-finding toxicity study in the FVB/N mouse administered tipranavir by oral gavage.
(See Appendix Review of Carcinogenicity Study Design/Dose Selection Proposals, IND 51,979 Submission 217 (SX), March 24, 2003, for discussion of this study.)

Study no.: U03-3574

Study title: Toxicokinetics of tipranavir in a 4-week range finding oral toxicity study in FVB/N mice.
(See Appendix Review of Carcinogenicity Study Design/Dose Selection Proposals, IND 51,979 Submission 217 (SX), March 24, 2003, for comments on this study.)

Study no.: U02-3410

Study title: 4-Week oral (gavage) interaction/toxicity study in the CD-1 mouse on Tipranavir and Ritonavir.

(See Appendix Review of Carcinogenicity Study Design/Dose Selection Proposals, IND 51,979 Submission 217 (SX), March 24, 2003, for additional comments.)
(See IND 51,979 (474) Summary of Immunotoxicity Findings for Tipranavir in Appendix for discussion of findings related to immunotoxicity in this study.).

Key study findings: The principal target organ of TPV and/or RTV in mice after 4 weeks of treatment was the liver, where hepatocellular hypertrophy, hepatocellular vacuolation and hepatocellular necrosis were observed. Effects suggestive of immune stimulation were also seen in the bone marrow (granulocytic hyperplasia) and spleen (lymphoid follicular hyperplasia). Effects were also seen in the adrenals (hypertrophy of the zona fasciculata) which may have been secondary to stress. A no toxic effect level was not achieved among the TPV/RTV groups due to drug-induced liver changes. A maximum tolerated dose was considered to be 300/80 mg/kg/day TPV/RTV based on effects seen in the liver as well as adrenal changes at the 600/160 mg/kg/day TPV/RTV dose level.

Study no.: U03-3027
Volume #, and page #: Module 4, M002, vol. 1.29, page # 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., Toxicology & Safety Assessment, 900 Ridgebury Road, Ridgefield, CT 06877-0368
Date of study initiation: August 7, 2002
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: TPV, Lot No. 113010, purity not given; RTV, Lot No. TSA-02-001, purity not given.

Methods

Doses: 0, 150/40 TPV/RTV, 300/80 TPV/RTV, 600/160 TPV/RTV, 600 TPV or 160 RTV mg/kg/day.
Species/strain: Mouse/CD-1
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Oral (gavage), aqueous solution, pH 10.5 (TPV) and propylene glycol (RTV)
Satellite groups used for toxicokinetics or recovery: 5/sex/group
Age: 7 weeks
Weight: 20 – 37 g
Sampling times:
Unique study design or methodology (if any): TPV administered 1 hour after RTV.

Observations and times:

Mortality: Room checks for morbidity and mortality were performed once daily during the Pretest Phase and twice daily during the Drug Phase.

Clinical signs: Clinical observations were made at least daily during the Pretest Phase and prior to dosing and 1 to 2 (Day 1) or 2 to 3 (all other days) hours after dosing during the Drug Phase.
Body weights: Body weights were measured once weekly in the Pretest and Drug Phases and on Drug Day 28.
Food consumption: Food consumption was measured weekly in the Pretest and Drug Phases. A measurement was also taken on Drug Day 28.
Ophthalmoscopy: Not performed.
EKG: Not performed
Hematology: Not measured.
Clinical chemistry: Not measured.
Urinalysis: Not measured.
Gross pathology: Necropsies were performed on all main study animals.
Organ weights (specify organs weighed if not in histopath table):
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Results

Mortality: The following table shows animals that died or were sacrificed moribund.

Dose (mg/kg/day)	0 Control		150/40 TPV/RTV		300/80 TPV/RTV		600/160 TPV/RTV	
Sex: No. of Animals	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
	2	7	2	3	0	4	1	5

In addition, 1 female died in the 600/0 mg/kg/day TPV/RTV dose group and 5 females died in the 0/160 mg/kg/day TPV/RTV dose group.

Although a large number of animals died before the end of the study, half of the early deaths were due to bleed-related complications in the satellite group for toxicokinetics and another nine main study animals deaths were due to dosing accidents. The cause of death of six toxicokinetic animals was undetermined.

Clinical signs: The clinical signs observed were rough hair, matted hair and stained hair in the TPV/RTV treatment groups. In the low dose TPV/RTV groups, only males showed clinical signs of matted and stained hair. In the two higher RPV/RTV dose groups, males continued to show those signs, while females showed the signs of rough matted hair.

Body weights: No drug-related changes in body weights were observed.

Food consumption: No drug-related changes in food consumption were observed.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: Not measured.

Clinical chemistry: Not measured.

Urinalysis: Not measured.

Gross pathology: Macroscopic findings considered related to drug treatment were observed in the liver and included discoloration, enlargement and disseminated foci.

Organ weights (specify organs weighed if not in histopath table): Both absolute and relative (to body and brain) liver weights increased in the drug treated groups in a dose-related manner relative to control liver weights. The percent differences in liver weight from control relative to brain weight were +59% (males) and + 48% (females) in the 150/40 TPV/RTV group, +130% (males) and +122% (females) in the 300/80 TPV/RTV group and +156% (males) and +145% (females) in the 600/160 TPV/RTV group.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Microscopic findings considered to be related to treatment were seen in the liver. Liver changes were characterized by hepatocellular hypertrophy, hepatocellular vacuolation (centrilobular or midzonal) and hepatocellular necrosis. Although drug-related minimal or mild hypertrophy was seen in the livers of most animals in the 600 mg/kg/day TPV and 160 mg/kg/day RTV groups, a dose-dependent increase in incidence and/or severity (mild to moderate) was seen in all three TPV/RTV groups. A similar dose-dependent increase in the incidence and/or severity of hepatocellular vacuolation (centrilobular or midzonal) was also seen in the TPV/RTV groups. Hepatocellular necrosis, characterized by focal areas of coagulation necrosis with or without inflammatory cells, was seen generally in subscapular locations of the liver of 3 to 6 out of 20 mice in all treatment groups except Controls.

Spontaneous microscopic changes which were exacerbated in the drug treatment groups were mixed cell infiltrates and focal hepatocellular mineralization in the parenchyma of the liver, granulocytic hyperplasia in the bone marrow and extramedullary hematopoiesis and lymphoid follicular hyperplasia in the spleen. The incidence of these findings was as follows: 1) mixed cell infiltrates (2 or 3 out of 10 or 20 in the Control, 150/40 TPV/RTV and 160 RTV groups and 5 to 7 out of 20 in the 300/80 and 600/160 TPV/RTV and 600 TPV groups); 2) focal

hepatocellular mineralization (1 out of 19 in the Control group, 6 out of 20 in the 600/160 TPV/RTV and 2 out of 20 in the 600 TPV groups); 3) granulocytic hyperplasia in bone marrow (1 to 4 out of 20 in all groups except 6 out of 20 in the 600 TPV group); 4) extramedullary hematopoiesis (5 to 6 out of 19 or 20 in the control and 150/40 TPV/RTV groups but 8 to 13 out of 20 in all other groups); 5) lymphoid follicular hyperplasia (1 out of 19 in the Control group but 3 to 6 out of 20 in all other groups).

A number of microscopic findings unique to the drug-treated groups were considered to be of an uncertain nature due to a lack of dose relationship and/or histomorphologic nature of the changes. These findings included chronic pyelonephritis (2 mice in the 600/160 TPV/RTV group) and alveolar fibrosis of the lungs (1 or 2 mice in the 150/40 and 600/160 TPV/RTV and 600 TPV groups). Hypertrophy of the zona fasciculata of the adrenal gland was seen in the majority of male mice in the 600/160 TPV/RTV group and in 3 of 10 mice in the 600 TPV group. This change may be secondary to stress and not direct toxicological change.

Toxicokinetics: Exposure in all animals in the treatment groups was confirmed by measurements of TPV and RTV in plasma samples taken on Drug Days 1 and 28 eight hours postdose. On Drug Day 1, TPV plasma concentrations were similar over the dose range from 150 to 600 mg/kg/day in both males and females with RTV co-administration. On Drug Day 28, there were no clear trends indicating an effect of dose level on TPV plasma levels in both genders with RTV co-administration. TPV plasma concentrations tended to decline by about 20% to 93% over the course of the study. TPV plasma levels were higher in females compared to males. Co-administration of RTV caused approximately 3- to 4-fold and 12- to 22-fold increases in TPV plasma concentrations on Drug Days 1 and 28, respectively. There were no clear trends indicating an effect of dose level, repeated dosing and gender on RTV plasma levels with TPV co-administration. TPV co-administration caused a 71% to 99% reduction in RTV plasma concentrations.

Other:

Histopathology inventory

Note: All tissues were examined for main study animals that died or were sacrificed. All tissues were examined from the Control, High dose TPV/RTV, High dose TPV and High dose RTV at terminal sacrifice, but selected tissues were examined for the Low dose and Middle dose TPV/RTV groups.

Study U03-3027	Dose TPV/RTV (mg/kg/day)					
Species CD-1 mouse	0/0	150/40	300/80	600/160	600/0	0/160
Adrenals	X			X	X	X
Aorta	X			X	X	X
Bone Marrow, femur	X	X	X	X	X	X

Bone Marrow, sternum	X			X	X	X
Bone (femur)	X	X	X	X	X	X
Bone (sternum)	X			X	X	X
Brain	X*	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X	X
Cervix	X			X	X	X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymis	X	X	X	X	X	X
Esophagus	X			X	X	X
Eye	X	X	X	X	X	X
Fallopian tube						
Gall bladder	X*	*	*	X*	X*	X*
Gross lesions	X	X	X	X	X	X
Harderian gland	X	X	X	X	X	X
Heart	X			X	X	X
Ileum	X	X	X	X	X	X
Injection site	X	X	X	X	X	X
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*
Lachrymal gland						
Larynx						
Liver	X*	X*	X*	X*	X*	X*
Lungs	X	X	X	X	X	X
Lymph nodes, bronchial	X			X	X	X
Lymph nodes mandibular	X			X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X			X	X	X
Nasal cavity						
Optic nerves	X	X	X	X	X	X
Ovaries	X*	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X	X
Parathyroid	X	X	X	X	X	X
Peripheral nerve						
Pharynx						
Pituitary	X			X	X	X
Prostate	X			X	X	X
Rectum	X	X	X	X	X	X
Salivary gland	X			X	X	X
Sciatic nerve	X			X	X	X
Seminal vesicles	X			X	X	X
Skeletal muscle	X			X	X	X
Skin	X			X	X	X
Spinal cord	X	X	X	X	X	X

Spleen	X	X	X	X	X	X
Sternum	X	X	X	X	X	X
Stomach	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X*
Thymus	X	X	X	X	X	X
Thyroid	X	X	X	X	X	X
Tongue	X			X	X	X
Trachea	X			X	X	X
Urinary bladder	X	X	X	X	X	X
Uterus	X			X	X	X
Vagina	X			X	X	X
Zymbal gland						

X, histopathology performed
*, organ weight obtained

Study title: Toxicokinetics of tipranavir in a 4-week range finding oral (gavage) interaction/toxicity study in CD-1 mouse with ritonavir co-administration.
(Reviewed under U03-30274-Week oral (gavage) interaction/toxicity study in the CD-1 mouse on tipranavir and ritonavir.)

Study no.: U04-3028

Study title: PNU-140690E: 13-Week Oral Toxicity Study in the Mouse (dose range finding).
(See IND 51,979 (474) Summary of Immunotoxicity Findings for Tipranavir in Appendix for discussion of findings related to immunotoxicity in this study.).

Key study findings: PNU-140690E given orally to mice for 13 weeks at 40, 120 and 360 mg/kg/day induced liver toxicity mainly at the high dose in both sexes, as evidenced by functional and morphological changes with slight to moderate increases in hepatic enzymes, increased liver weight, minimal to moderate hypertrophy and vacuolation of hepatocytes and necrotic areas in the liver. At 120 mg/kg/day slight functional alterations in ALT, AST and cholesterol and increased liver weight were seen indicating that the maximum tolerated dose (MTD) is between 120 and 360 mg/kg/day. Both AUC and Cmax increased linearly with dose; the linear increase was proportional to dose for males while it was more than proportional for females.

Study no.: U00-3209
Volume #, and page #: Module 4, M002, vol. 1.30, page # 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, B. le Pasteur 10, 20014 Nerviano, Italy
Date of study initiation: August 29, 1997.
GLP compliance: Yes

QA report: yes (x) no ( )
Drug, lot #, and % purity: TPV, Batch No. (A1)5134-AS-1216-N1, purity unknown as copy of Certificate of Analysis provided was unreadable.

Methods

Doses: 40, 120 and 360 mg/kg/day (expressed as free acid equivalents)
Species/strain: CD-1/mice
Number/sex/group or time point (main study): 20
Route, formulation, volume, and infusion rate: Purified water adjusted to pH 10.5 with sodium hydroxide diluted aqueous solution; 12 ml/kg/day; treatment given in two equally divided doses (20, 60 and 180 mg/kg/dose) at an interval of eight hours.
Satellite groups used for toxicokinetics or recovery: 15/sex/group for toxicokinetics; 3/sex/group for lymphocyte subsets.
Age: 36 days
Weight: males 22.8 g to 32.6 g & females 20.4 g to 28.2 g
Sampling times:
Unique study design or methodology (if any): TPV disodium salt was administered; dose levels were expressed as free acid equivalents. Dosing occurred twice daily 8 hours apart. Lymphocyte subsets were determined for 3 animals/sex/group.

Observations and times:

Mortality: Animals were observed daily.

Clinical signs: Animals were monitored for clinical signs daily.

Body weights: Body weights were recorded once during the pre-test period, immediately before the first day of treatment and weekly thereafter throughout the study.

Food consumption: Food consumption was measured weekly during the treatment period.

Ophthalmoscopy: Ophthalmoscopy was not performed.

EKG: EKG measurements were not determined.

Hematology: Hematology measurements were performed on the first five mice/sex/group. Coagulation parameters were measured on the second five mice/sex/group and the lymphocyte subset analysis, on the first three animals/sex/group. Blood samples were collected from the orbital sinus.

Clinical chemistry: Clinical chemistry measurements were performed on the second five animals/sex/group. Thyroid parameter measurements were performed on the last five animals/sex/group. Blood samples were collected from the abdominal aorta.

Urinalysis: Urinalysis measurements were not performed.

Gross pathology: At the end of the treatment period all mice were fasted overnight and killed by exsanguination from the abdominal aorta. A complete examination was done on all animals.

Organ weights (specify organs weighed if not in histopath table):

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Results

Mortality: No mortality occurred in any treatment group.

Clinical signs: No drug-related clinical signs were noted.

Body weights: Body weights were decreased minimally (2% - 9%) in all dose groups. The only statistically significant decrease was 9% in males in the 120 mg/kg/day group.

Food consumption: Food consumption decreased (8% - 18%) in all groups except males (+1%) in the 360 mg/kg/day group. The only statistically significant decrease was 18% in females in the 360 mg/kg/day group.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: No treatment related changes were seen in the hematological parameters or in the lymphocyte subsets. No changes in APTT occurred at any dose tested.

Clinical chemistry: Sporadic increases were observed in ALT and AST in males in the 120 mg/kg/day and females in the 360 mg/kg/day groups. A mild increase only in ALT occurred in the majority of males in the 360 mg/kg/day group. A few instances of increased cholesterol were seen in female mice in the 360 mg/kg/day group. No treatment-related changes in thyroid parameters (T3, T4 and TSH levels) were seen.

Dose mg/kg/day	0 (Control)		40		120		360	
Sex: no. of animals	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
AST (IU/L)[a]	53	68	54	76	66	59	58	81

			+ (2%)	+ (12%)	+ (25%)	+ (13%)	+ (9%)	+ (19%)
ALT (IU/L)	25	36	29 + (16%)	31 - (14%)	39 + (56%)	31 - (14%)	45 + (80%)	49 + (36%)
Cholesterol (mg/dl)	-	79	-	84 + (6%)	-	97 + (23%)	-	129* + (63%)

a % increase or decrease versus control.
Dunnett's test *p<0.05, **p<0.01.

Urinalysis: Not performed.

Gross pathology: Loss of general condition without dose response relationship was seen in 2 males and 9 females in treated groups. Ovarian cysts were observed in treated and control animals and are common in mice at this age. Other changes which occurred in single instances in treated animals were sporadic in nature and not treatment-related and consisted of enlarged spleen and lymph nodes, a subcutaneous ulcerated mass and abscess-like masses in the pleural cavity and throat region.

Organ weights (specify organs weighed if not in histopath table): Liver weight was increased significantly in the middle and high dose groups (120 mg/kg/day, males +9% and females +11% for absolute organ weight; 360 mg/kg/day, males and females +64% for the absolute organ weight.) There were no statistically significant differences in absolute or relative organ weights for spleen and thymus.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

The only changes related to treatment occurred in the livers of males and females in the 360 mg/kg/day group. Hepatocytic hypertrophy (minimal to moderate) was present in a majority of animals in the high dose group and was generally centrilobular in distribution and at times extended into other lobular areas. Hepatocytic vacuolation was also present in this dose group and occurred more frequently in males than in females. Against a background of small foci of necrosis in the livers of a few animals from most groups, more prominent areas of necrosis were noted in a total of seven high dose animals. This was considered to be secondary to the hypertrophy and an indirect toxicological effect.

Dose mg/kg/day	0 (Control)		40		120		360	
Sex: no.	M: 20	F:20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20

of animals								
LIVER **Hepatocytic Hypertrophy** Minimal							8/20	13/20
Slight							10/20	7/20
Moderate							2/20	
Hepatocytic Vacuolation Minimal							11/20	4/20
Slight							5/20	1/20
Moderate							1/20	
Focus/Foci of Necrosis Minimal	1/20		1/20		1/20		2/20	1/20
SLIGHT			1/20	3/20				1/20
Area(s) of Necrosis Minimal							4/20	1/20
Slight							1/20	1/20

Immunotoxicity: There were no findings in lymphocyte subsets (n =3), in spleen, thymus or bone marrow indicative of immunotoxicity.

In mandibular lymph node, lymphoid hyperplasia was seen in males (minimal, 4/19 in controls vs 7/20 in high dose) and females (minimal, 6/19 in controls vs 9/20 in high dose; slight, 1/1 low dose; moderate, 1/20 high dose). Lymphoid depletion was seen in 1 male (slight, 1/20 in high dose).

In mesenteric lymph node, lymphoid hyperplasia was seen in males (minimal, 2/20 in controls vs 4/20 in high dose). Lymphoid depletion was seen in males (slight, 2/20 in high dose; moderate, 1/20 in control).

In bone marrow, myeloid hyperplasia was seen in one male (moderate, 1/20 in high dose).

These changes, i.e. exacerbation of granulocytic hyperplasia in bone marrow and exacerbation of lymphoid follicular hyperplasia, suggest that TPV had an immunostimulatory effect. This is discussed further under the NDA section on immunotoxicology.

Group: Dose (mg/kg/day): Test Article: Formulation: Aqueous pH 10.5	Control 0		Low-Dose 40 TPV		Mid-Dose 120 TPV		High-Dose 360 TPV	
Gender:	M	F	M	F	M	F	M	F
No. in Group	20	20	20	20	20	20	20	20
Mandibular Lymph Node Lymphoid Hyperplasia	4/19 +	6/19 +	0/0	1/1 ++	0/0	0/0	7/20 +	9/20 + 1/20 +++
Mandibular Lymph Node Lymphoid Depletion	0/19	0/19	0/0	0/1	0/0	0/0	0/20	1/20 ++
Mesenteric Lymph Node Lymphoid Hyperplasia	2/20 +	3/20 + 1/20 ++	0/0	0/0	0/0	0/0	4/20 +	3/20 +
Femur, Bone Marrow Myeloid hyperplasia	0/20	0/20	0/20	0/20	0/20	0/20	1/20 +++	0/20

Number affected/Number examined; NE = None Examined
+ = Minimal; ++ = Mild; +++ = Moderate.

Toxicokinetics: Blood samples were drawn from the orbital sinus of 15 additional mice/sex/dose. Blood samples were collected from five mice/sex/dose on Day 30 before dosing and two hours after the second administration to determine the Cmax and from three mice/sex/dose at the end of the study (Day 93) prior to the first administration and after 2, 8 (just before the second administration), 10 and 14 hours.

AUC and Cmax increased with dose. For males, the increase was proportional to dose, but for females, the increase was slightly more than in proportion to dose. At 360 mg/kg/day, AUC for females was approximately twice the value determined for males. no gender difference was observed for the low and middle dose groups.

Dose (mg/kg/day)	Gender	Study Day	C_{2h} (• M)	C_{10h} (• M)	AUC_{0-24} (• M.h)
40	F	30	nd	0.43	nd
		93	0.31	0.28	2.42
	M	30	nd	0.22	nd
		93	0.287	0.28	1.99

120	F	30	nd	0.91	nd
		93	0.99	1.16	9.31
	M	30	nd	0.66	nd
		93	0.80	0.74	6.48
360	F	30	nd	3.8	nd
		93	1.90	3.53	36.1
	M	30	nd	2.9	nd
		93	1.49	2.5	19.3

C = concentration at indicated time. C at 2 h and 10 h correspond to the estimated maximum plasma concentrations for the first and second daily dose, respectively. nd = not determined.

Other:

Histopathology inventory

Study U00-3209 Dose (mg/kg/day)	0	40	120	360
Species Mouse				
Adrenals	X*	*	*	*
Aorta	X			X
Bone Marrow smear	X			X
Bone (femur)	X			X
Brain	X*	*	*	X*
Cecum	X			X
Cervix	X			X
Colon	X			X
Duodenum	X			X
Epididymis	X*	*	*	X*
Esophagus	X			X
Eye	X			X
Fallopian tube				
Gall bladder	X			X
Gross lesions	X	X	X	X
Harderian gland	X			X
Heart	X*	*	*	X*
Ileum	X			X
Injection site				
Jejunum	X			X
Kidneys	X*	*	*	X*
Lachrymal gland				
Larynx				
Liver	X*	X*	X*	X*

Lungs	X*	*	*	X*
Lymph nodes, cervical				
Lymph nodes mandibular	X			X
Lymph nodes, mesenteric	X			X
Mammary Gland				
Nasal cavity				
Optic nerves				
Ovaries	X*	*	*	X*
Pancreas	X			X
Parathyroid	X			X
Peripheral nerve				
Pharynx				
Pituitary	X*	*	*	X*
Prostate	X*	*	*	X*
Rectum	X			X
Salivary gland	X			X
Sciatic nerve	X			X
Seminal vesicles	X			X
Skeletal muscle	X			X
Skin	X			X
Spinal cord	X			X
Spleen	X*	*	*	X*
Sternum	X			X
Stomach	X			X
Testes	X*	*	*	X*
Thymus	X*	*	*	X*
Thyroid	X*	*	*	X*
Tongue	X			X
Trachea	X			X
Urinary bladder	X			X
Uterus	X*	*	*	X*
Vagina	X			X
Zymbal gland				
Diaphragm	X			X
Parotids	X			X

X, histopathology performed
*, organ weight obtained

Study title: Two-week preliminary oral safety/toxicity and toxicokinetics study in male and female Sprague-Dawley rats.

Study no.: U00-3085 (Pharmacia & Upjohn Technical Report 1470-96-015)
See Appendix, IND 51,979 (original) for review of this study.

Study title.: PNU-140690: 2-Week oral safety/toxicity study in Sprague-Dawley rats.

Study no.: U00-3269 (Pharmacia & Upjohn Study Report a0063181)
See Appendix, IND 51,979 (original) for review of this study.

Study title: PNU-140690: 2-Week oral toxicity study in the rat using a TRIS/ sedds-2 vehicle (Capmul MCM as a lipid component).

Study no.: U00-3171 (Pharmacia & Upjohn Study Report a0045169).
See Appendix, IND 51,979 (067) for review of this study.

Study title: PNU-140690: A two-week oral safety/toxicity study in Sprague-Dawley rats.

Key study findings: TPV was well tolerated when administered orally to rats by gastric intubation at 0, 20, 62.5, 200 and 500 mg/kg BID daily for two weeks. No deaths occurred and the only clinical sign noted was salivation at the 400 and 1000 mg/kg/day doses. There was a 10% suppression of body weight gain in males at the high dose. Plasma drug concentrations increased proportionally over the dose range for males and up to 400 mg/kg/day for females. There were no obvious gender differences in Cmax and AUC. Changes in most hematology and clinical chemistry measurements in the treated groups were minimal or not dose-related. Changes in coagulation factors were significant and dose-related and may be a direct drug-related toxic effect. Larger changes in clinical chemistry parameters, such as seen for ALT, triglycerides, total bilirubin, glucose, cholesterol and blood urea nitrogen, were considered secondary to effects of the drug on the liver. Effects on thyroid clinical chemistry parameters were significant and dose-related but are considered to be rodent specific effects which have not been seen in humans treated with TPV in clinical trials. Primary target organs of the drug were the liver and the thyroid, with organ weights increased for both accompanied by histopathological correlates (minimal to moderate hepatocellular hypertrophy and minimal to mild follicular cell hypertrophy, respectively). Liver necrosis was also seen in a one male at the 400 mg/kg/day dose and in three females at the two top doses.

Higher ALT values seen in females at 1000 mg/kg/day were considered to be an adverse effect possibly due to the presence of higher levels of unqualified impurities. All other changes noted in clinical chemistry parameters were similar to the findings from previous 4-week and 2-week toxicity studies in rats in which the unqualified impurities found in the bulk lot used in this study were at lower levels or absent.

The NOAEL for this study is 40 mg/kg/day and corresponds to 0.1 (males) and 0.2 (females) of the exposure expected in humans at the proposed dose of 500/200 mg TPV/RTV BID.

Study no.: U00-3279 (Pharmacia & Upjohn Technical Report 7226-97-024)
Volume #, and page #: Module 4, M002, Volume 1.34 and page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI 49001
Date of study initiation: January, 1997
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: TPV, (A1)5134-AS-0758, [redacted]

Methods

Doses: 0, 40, 125, 400, 1000 (½ dose twice daily) mg/kg/day
Species/strain: Rat/ (— CD[SD]BR) Sprague-Dawley
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: Oral, TPV in 0.25N sodium hydroxide, adjusted to approximately pH 10.5 with sodium hydroxide or hydrochloric acid as needed, ½ dose twice daily 8 hours apart.
Satellite groups used for toxicokinetics or recovery:
Age: 6 – 7 weeks
Weight: Males 229 – 288 g; Females 196 – 230 g
Sampling times:
Unique study design or methodology (if any): The bulk drug lot contained unqualified impurities.

Observations and times:
Mortality: Observations were conducted pretest on Days -8, -5 and -1 and at least once daily on all animals during the dosing phase.
Clinical signs: Observations were conducted pretest on Days -8, -5 and -1 and at least once daily on all animals during the dosing phase.
Body weights: Body weights were recorded for all pretest animals on Days -8, -5 and -1 and for all animals on Days 1, 3, 5, 7, 9, 11 and 13.
Food consumption: Not monitored.
Ophthalmoscopy: Not monitored.
EKG: Not monitored.
Hematology: Terminal blood samples were collected from the abdominal aorta while the rat was under anesthesia from all surviving rats following an overnight fast.
Clinical chemistry: Terminal blood samples were collected from the abdominal aorta while the rat was under anesthesia from all surviving rats following an overnight fast.
Urinalysis: Not monitored.

Gross pathology: A complete necropsy was conducted on all rats at the scheduled necropsy after overnight fasting.
Organ weights (specify organs weighed if not in histopath table): See table.
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

Results

Mortality: No deaths occurred during the study.

Clinical signs: TPV was well-tolerated with clinical signs limited to intermittent salivation in 1 male and 1 female given 400 mg/kg/day and 5 males and 3 females given 1000 mg/kg/day.

Body weights: When body weights from study day -1 were compared to those from study day 13, males at the 40, 125 and 400 mg/kg/day dose levels had a slightly greater gain (+2% to +7%).

Food consumption: Not monitored.

Ophthalmoscopy: Not monitored.

EKG: Not monitored.

Hematology: Most hematological changes in the treated groups were minimal or not dose-related. Treatment-induced changes in coagulation factors were large and dose-related. The sponsor argued that this effect was secondary to effects of treatment on the liver. While this may be correct, there is no definitive proof of this and, therefore, changes in coagulation factors are considered a possible drug-induced toxicity.

Hematology Parameters[a]

Dose mg/kg/day	0		40		125		400		1000	
Number Examined	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
WBC Count (Thou/μL)	8.406	5.294	-9%	+5%	-36%**	-18%	-11%	-15%	-35%**	+4%
RBC Count (Mill//μL)	6.442	6.312	-3%	-0.4%	-1%	-0.5%	-8%*	-6%	-2%	-0.4%
Hemoglobin (g/dL)	13.18	12.82	-3%	-1%	-1%	-1%	-5%	-9%*	-4%	-6%*
Hematocrit (%)	39.72	36.78	-2%	-1%	-2%	-2%	-5%	-7%	-4%	-5%

Mean Corp. Hemoblobin (pg)	20.42	20.30	-0.2%	-1%	-2%	-1%	-3%	-1%	-2%	-6%**
Platelet Count (Thou/µL)	1217.0	1102.6	-3%	-9%	-3%	-16%*	-15%	-11%	-3%	-30%**
Reticulocyte Count (Mill//µL)	0.22586	0.17294	+9%	-0.3%	-4%	+12%	+24%	+15%	-1%	+38%**
Reticulocyte (%)	3.508	2.732	+15%	+1%	-5%	+12%	+33%*	+22%*	+2%	+39%**
Lymphocytes (absolute) (Thou/µL)	6.302	4.068	-3%	+11%	-30%**	-12%	-3%	-13%	-30%**	+7%

a Group means are given for controls. Percent differences from controls are given for treatment groups.
* – Significantly different from Control (P≤0.05)
** – Significantly different from Control (P≤0.01)

Coagulation Parameters[a]

Dose mg/kg/day	0		40		125		400		1000	
No.examined	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Prothrombin Time (sec)	11.90	9.14	+14%	+12%	+70%**	+25%*	+102%**	+61%**	+144%**	+105%**
Activated Partial Thromboplastin Time (sec)	19.18	16.42	+19%	+18%	+59%**	+20%*	+74%**	+40%**	+82%**	+61%**

a Group means are given for controls. Percent differences from controls are given for treatment groups.
* – Significantly different from Control (P≤0.05)
** – Significantly different from Control (P≤0.01)

Clinical chemistry: Most serum chemistry changes in the treated groups are considered to be secondary to effects on the liver, e.g. changes seen for AST, ALT, triglycerides, total bilirubin, glucose, cholesterol and blood urea nitrogen. Statistically significant effects seen on triiodothyronine, thyroxine and thyroid-stimulating hormone are considered to be rodent specific and have not been seen in humans treated with TPV. This is probably due to differences in metabolism between rodents and humans regarding thyroid function. Other changes in clinical chemistry were minimal or not dose-related.

Higher ALT values seen in females at 1000 mg/kg/day were considered to be an adverse effect possibly due to the presence of higher levels of unqualified impurities. All other changes noted in clinical chemistry parameters were similar to the findings from previous 4-week and 2-week toxicity studies in rats in which the unqualified impurities found in the bulk lot used in this study were at lower levels or absent.

Clinical Chemistry Parameters[a]

Dose mg/kg/day	0		40		125		400		1000	
Number Examined	M: 5	F: 5	M: 5	F: 5	M: 5	M: 5	M: 5	F: 5	M: 5	F: 5
Aspartate Aminotransferase (AST) (U/L)	120.0	112.4	-11%	-12%	-17%	-20%	-29%**	-21%	-19%*	+67%
Alanine Aminotransferase (ALT) (U/L)	35.0	33.6	-5%	-15%	+0.5%	-10%	+22%	+21%	+43%*	+189%**
Alkaline Phosphatase (ALP) (U/L)	227.2	100.8	-28%	+4%	-18%	-19%	-38%**	+4%	-45%**	-12%
Blood Urea Nitrogen (BUN) (mg/dL)	12.2	14.8	-3%	-15%	+18%	+4%	+21%	-1%	+36%	+7%
Glucose (mg/dL)	130.8	121.0	-13%*	-12%	-15%**	-6%	-14%**	-8%	-18%**	-16%
Total Cholesterol (mg/dL)	23.6	30.6	-21%*	-26%*	-29%**	-13%	-26%**	+9%	+7%	+11%
Total Protein	5.62	5.96	-0.4%	-2%	+6%*	+5%	+10%**	+8%*	+14%**	+9%**
Albumin (g/dL)	3.04	3.20	-15	-3%	+6%	+3%	+9%*	+4%	+14%**	+9%**
Globulin (g/dL)	2.57	2.76	+0.8%	-2%	+2%	+7%	+12%**	+12%	+14%**	+10%
Triglycerides	23.0	12.4	-50%	+36%	-46%	+19%	-46%	+10%	-45%	+10%
Total Bilirubin (mg/dL)	0.32	0.30	-31%	-13%	-44%	-13%	-38%	-54%	-50%	0%
Triiodothyronine (T_3) (ng/ml)	1.276	1.436	-4%	-2%	-5%	-14%	-12%*	-25%**	-20%**	-26%**
Thyroxine (T_4) (µg/dL)	3.506	3.056	+0.5%	-0.8%	+11%	-9%	-6%	-21%*	-16%*	-19%
Thyroid-Stimulating Hormone (THS) (ng/mg)	1.452	0.710	+36%	+133%	+100%	+76%	+160%*	+277%**	+287%**	+243%*

a Group means are given for controls. Percent differences from controls are given for treatment groups.
* – Significantly different from Control ($P \leq 0.05$)
** – Significantly different from Control ($P \leq 0.01$)

Urinalysis: Not monitored.

Gross pathology: The only TPV-related gross observation was a nutmeg appearance to the liver of 1 of 5 female rats each at the 125 mg/kg/day and 1000 mg/kg/day groups.

Organ weights (specify organs weighed if not in histopath table): Mean liver weights were increased for males at 400 mg/kg/day or greater and for females at 125 mg/kg/day or greater and mean thyroid gland weights were increased for both sexes at 125 mg/kg/day or greater. Histopathological correlates were seen for the liver and thyroid weight increases. There were dose-related increases in adrenal glands in females at all doses and in males at 400 mg/kg/day or greater. There were slight decreases in thymus gland weights in males at all dose levels. There were no histopathological correlates for changes in the adrenals and thymus. These changes were thought to be stress-related.

Organ weights[a]

Dose mg/kg/day	0		40		125		400		1000	
Number Examined	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Thyroid/Parathyroid	0.019 g	0.016 g	+8%	+9%	+27%*	+22%	+29%*	+21%	+36%**	+47%**
Liver	10.73 g	6.97 g	+11%	+18%	+16%	+41%**	+65%**	+98%**	+69%**	+113%**
Adrenals	0.066 g	0.076 g	-0.7%	+4%	+0.3%	+28%*	+27%	+31%**	+6%	+51%**
Thymus	0.742 g	0.556	-5%	+0.3%	-4%	+8%	-6%	+7%	-30%	+8%

a Group means are given for controls. Percent differences from controls are given for treatment groups.
* – Significantly different from Control ($P \leq 0.05$)
** – Significantly different from Control ($P \leq 0.01$)

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no ( )

Minimal to moderate hepatocellular hypertrophy and minimal to mild follicular cell hypertrophy correlated with increases in organ weight of liver and thyroid. Liver necrosis was also observed in females treated with 400 (1/5) and 1000 (2/5) mg/kg/day and one male treated with 400 1/5) mg/kg/day.

Microscopic Observations

Dose mg/kg/day	0	40	125	400	1000

Number Examined	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Liver										
Hypertrophy, hepatocyte	-	-	-	-	-	2	5	5	5	5
Minimal	-	-	-	-	-	1	-	-	-	-
Mild	-	-	-	-	-	1	2	3	-	-
Moderate	-	-	-	-	-	-	3	2	5	5
Necrosis	-	-	-	-	-	-	1	1	-	2
Mild	-	-	-	-	-	-	1	-	-	-
Moderate	-	-	-	-	-	-	-	1	-	1
Marked	-	-	-	-	-	-	-	-	-	1
Thyroid										
Hypertrophy, follicular cell	-	-	-	2	3	3	5	5	5	5
Minimal	-	-	-	2	3	2	5	5	1	1-
Mild	-	-	-	-	-	1	2	4	4	4

Toxicokinetics: Blood samples were collected from the lateral tail vein from all animals at 2, 8, 10, 14 and 24 hours after the morning administration of TPV or vehicle. Plasma levels of TPV were assayed from the first 3 rats per sex per TPV treatment group.

Dose (mg/kg/day)	40		125		400		1000	
Number Examined	M: 5	F: 5	M: 5	F: 5	M: 5	M:5	M: 5	F:5
Cmax (μM)	21	33	48	79	68	91	131	78
AUC_{0-24} (μM.h)	164	262	390	880	880	1000	1610	1040
Fold versus Human AUC*	0.1	0.2	0.3	0.6	0.6	0.7	1.0	0.7

*Based on 500/200 mg TPV/RTV BID and an AUC of 1542 μM.h.

Other:

Histopathology inventory

Study U00-3279	Dose (mg/kg/day)				
Species Rat	0	40	125	400	1000
Adrenals	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X
Bone Marrow smear					
Bone (femur)					

Brain	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X
Cervix	X	X	X	X	X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	X*	X*	X*	X*	X*
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian tube					
Gall bladder					
Gross lesions	X	X	X	X	X
Harderian gland					
Heart	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X
Injection site					
Jejunum	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*
Lachrymal gland					
Larynx					
Liver	X*	X*	X*	X*	X*
Lungs	X	X	X	X	X
Lymph nodes, cervical					
Lymph nodes mandibular	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X
Mammary Gland	X	X	X	X	X
Nasal cavity					
Optic nerves					
Ovaries	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X
Parathyroid	X*	X*	X*	X*	X*
Peripheral nerve					
Pharynx					
Pituitary	X*	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*
Rectum					
Salivary gland	X	X	X	X	X
Sciatic nerve	X	X	X	X	X
Seminal vesicles	X	X	X	X	X
Skeletal muscle	X	X	X	X	X
Skin	X	X	X	X	X
Spinal cord	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*
Sternum	X	X	X	X	X
Stomach	X	X	X	X	X

Testes	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*	X*
Tongue					
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X*	X*	X*	X*	X*
Vagina	X	X	X	X	X
Zymbal gland					
Diaphragm	X	X	X	X	X
Knee joint	X	X	X	X	X
Tail tattoo	X	X	X	X	X

X, histopathology performed
*, organ weight obtained

Study title: U-140690E: Four-Week Oral Dose Toxicity Study with a four-week reversibility phase and toxicokinetic study in male and female Sprague-Dawley rats.

Key study findings: TPV given orally to rats for 28 days in equally divided doses of 0, 40, 125, 400, 1000 (females) and 1250 (males) mg/kg/day was well tolerated with no deaths due to drug and limited clinical signs (salivation and staining). Toxicity was minimal with respect to body weight effects, food consumption, hematology parameters, coagulation parameters and clinical chemistry parameters. Target organs of toxicity were liver and thyroid with organ weights of both increased and histopathological findings of hepatocellular hypertrophy and follicular cell hypertrophy, respectively. The hepatocellular hypertrophy correlated with electron microscopic observations of increased smooth endoplasmic reticulum and increases in hepatic microsomal cytochrome P450 enzyme activity. Thyroid effects were seen at the lowest dose but are not considered relevant to humans since metabolism of thyroid factor is different in rats as compared to humans and TPV effects on the thyroid gland have not been observed in clinical trials.

The NOAEL for this study is 40 mg/kg (mean Cmax (0, 24) 38 μM; mean AUC(0, 24) 211μM/h) and corresponds to 0.1-fold of the exposure expected in humans at the proposed dose of 500/200 mg TPV/RTV BID.

Study no.: U00-3087 (Pharmacia & Upjohn Technical Report 7270-96-017)
Volume #, and page: Module 4, M002, vol. 1.13, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI 49001
Date of study initiation: June, 1996.
GLP compliance: Yes
QA report: yes (x) no ( )

Drug, lot #, and % purity: TPV, lot number (A) 5075-AS-1720, [redacted] purity.

Methods

Doses: 0, 40, 125, 400, 1250 (M)/1000 (F) (½ dose twice daily) mg/kg/day

Species/strain: Rat/ CD[SD]BR) Sprague-Dawley

Number/sex/group or time point (main study): 15/sex/group

Route, formulation, volume, and infusion rate: Oral, TPV in 0.25N sodium hydroxide, adjusted to approximately pH 10.5 with sodium hydroxide or hydrochloric acid as needed, ½ dose twice daily 8 hours apart.

Satellite groups used for toxicokinetics or recovery: 3/sex/group for toxicokinetics and last 5/sex/group of the main study for recovery.

Age: 7 – 8 weeks

Weight: Males 206.34 – 303.80 g; Females 167.13 – 233.63 g

Sampling times:

Unique study design or methodology (if any): Hepatic microsomal cytochrome P450 enzyme activity was measured. Electron microscopy of the liver of selected animals was performed.

Observations and times:

Mortality: Observations were conducted twice daily during pretest and dosing phases and once daily during the reversibility phase.

Clinical signs: Observations were conducted twice daily during pretest and dosing phases and once daily during the reversibility phase.

Body weights: Body weights were recorded twice during the pretest phase and weekly during the dosing and reversibility phases. Terminal body weights were taken after an overnight fast on surviving rats scheduled for interim and final necropsies.

Food consumption: Food consumption was recorded weekly for a 7-day period, once during the pretest phase and for the duration of the study.

Ophthalmoscopy: Ophthalmic examinations were conducted once during the pretest phase, within the last week of dosing and during the last week of the reversibility phase for all animals.

EKG: Not monitored.

Hematology: Terminal blood samples were collected from the abdominal aorta prior to scheduled necropsies.

Clinical chemistry: Terminal blood samples were collected from the abdominal aorta prior to scheduled necropsies.

Urinalysis: Urine samples were collected overnight in metabolism cages once during the pretest phase and prior to interim and final necropsies.

Gross pathology: The first 10 rats/sex/group were necropsied at the interim necropsy. The last 5 rats/sex/group were necropsied at the final necropsy. Toxicokinetic group rats were necropsied on Day 35. All main study rats were subjected to a complete necropsy with tissue collection, while toxicokinetic group rats and rats found dead or killed in a moribund condition were subjected to a gross necropsy only.

Organ weights (specify organs weighed if not in histopath table): See table.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Results

Mortality: One male (1250 mg/kg/day) was found dead as a result of a dosing error. In the toxicokinetic group, two rats (1 male, 1250 mg/kg/day and 1 female, 40 mg/kg/day) were found dead on days 35 and 34, respectively. Moribund rats (1 male, 1250 mg/kg/day; 1 female, 400 mg/kg/day; 1 female, 1000 mg/kg/day were killed on Days 21, 24 and 31, respectively. These deaths appeared to be caused by complications from the chronic cannulation for toxicokinetic blood sampling and not to be drug-related.

Clinical signs: TPV was well-tolerated with clinical signs limited to increased salivation and red/orange or yellow staining on the face, mouth, forepaws/forelimbs, ventral surface, tail and/or anogenital area. All groups showed these signs and incidence increased with dose such that all animals in the highest dose exhibited these signs.

Body weights: Body weights in males decreased in a dose-dependent manner to -11% group mean versus the control in the highest dose groups. This effect was completely reversed during the recovery phase. Body weights in females were comparable to controls except for the highest dose group on Days 13 to 20 when the group mean was significantly lower than the control.

Food consumption: Food consumption was decreased significantly for males (8 – 10%) during the dosing phase but was comparable to controls during the recovery phase. There was no effect on food consumption in females.

Ophthalmoscopy: No treatment effects were observed.

EKG: Not monitored.

Hematology: The only statistically significant differences in hematological parameters which were considered possibly treatment-related involved minimal changes in hematocrit percentage and hemoglobin concentrations. Treatment-induced changes in coagulation factors were significant and dose-related. The sponsor suggests that these effects were possibly due to the effect of TPV on the liver, perhaps affecting the synthesis of several coagulation factors rather than directly interfering with the coagulation system. While this may be correct, there is no definitive proof of this and, therefore, changes in coagulation factors are considered a possible drug-induced toxicity.

Hematology Parameters[a]

Dose mg/kg/day	0		40		125	
Number Examined	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Hemoglobin (g/dL)	14.8	14.6	14.4	14.3	14.3*	14.1
Hematocrit (%)	42.5	40.93	41.7	40.36	41.2	39.99

a Group means are given for Day 29.
* – Significantly different from Control (P≤0.05)
** – Significantly different from Control (P≤0.01)

Hematology Parameters[a] continued

Dose mg/kg/day	400		1250	1000
Number Examined	M: 10	F: 10	M: 10	F: 10
Hemoglobin (g/dL)	13.7**	13.4**	13.6**	13.9**
Hematocrit (%)	40.0**	38.5	39.9**	39.64

a Group means are given for Day 29.
* – Significantly different from Control (P≤0.05)
** – Significantly different from Control (P≤0.01)

Coagulation Parameters[a]

Dose mg/kg/day	0		40		125	
No. examined	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Prothrombin Time (sec)	10.7	9.6	10.7	9.9	12.2	9.80
Activated Partial Thromboplastin Time (sec)	17.2	15.8	19.0	17.0	21.7*	18.0

a Group means are given for Day 29.
* – Significantly different from Control (P≤0.05)
** – Significantly different from Control (P≤0.01)

Coagulation Parameters[a] continued

Dose mg/kg/day	400		1250	1000
No. examined	M: 10	F:10	M:10	F: 10
Prothrombin	13.3	12.3**	21.1**	16.1**

Time (sec)				
Activated Partial Thromboplastin Time (sec)	24.5**	22.0**	33.8**	24.9**

a Group means are given for Day 29.
* – Significantly different from Control ($P \leq 0.05$)
** – Significantly different from Control ($P \leq 0.01$)

Clinical chemistry: Statistically significant effects seen on triiodothyronine, thyroxine and thyroid-stimulating hormone are considered to be rodent specific and have not been seen in humans treated with TPV. This is probably due to differences in metabolism between rodents and humans regarding thyroid function.

Statistically significant increases in serum total protein concentration, serum albumin and serum globulin concentrations were observed and are believed to be secondary to the effect of TPV on the liver. Minor increases in serum calcium concentrations were noted and considered to be the consequence of the increased serum albumin concentrations. Alkaline phosphatase activity decreased slightly with statistically significant differences compared to controls at all doses in males and at the two high doses in females. This decrease is not a concern but hypothyroidism has been reported to be associated with decreased serum alkaline phosphatase activity.

Clinical Chemistry Parameters[a]

Dose mg/kg/day	0		40		125	
Number Examined	M: 10	F: 10	M: 10	F: 10	M: 10	M: 10
Alkaline Phosphatase (ALP) (U/L)	155	91.5	131*	79.8	130*	74.3
Total Protein	5.89	6.14	6.06	6.32	6.14*	6.52*
Albumin (g/dL)	3.17	3.27	3.28	3.40	3.30	3.49*
Globulin (g/dL)	2.72	2.87	2.78	2.92	2.84	3.03
Calcium (mg/dL)	9.92	9.89	10.0	9.94	10.1	1.02
Triiodothyronine (T_3) (ng/ml)	1.40	1.51	1.34	1.43	1.34	1.34*
Thyroxine (T_4) (µg/dL)	4.05	3.43	4.07	3.00	3.97	2.97
Thyroid-						

Stimulating Hormone (THS) (ng/mg)	2.37	0.97	2.38	2.81**	2.97	2.82**

a Group means are given for Day 29.
* – Significantly different from Control ($P \leq 0.05$)
** – Significantly different from Control ($P \leq 0.01$)

Clinical Chemistry Parameters[a] continued

Dose mg/kg/day	400		1250	1000
Number Examined	M: 10	F: 10	M: 10	F: 10
Alkaline Phosphatase (ALP) (U/L)	104**	63.2**	118**	70.5*
Total Protein	6.45**	6.92**	6.61**	7.03**
Albumin (g/dL)	3.39**	3.66**	3.52**	3.74**
Globulin (g/dL)	3.06**	3.26**	3.09**	3.29**
Calcium (mg/dL)	10.3*	10.2	10.4**	10.5**
Triiodothyronine (T_3) (ng/ml)	1.21**	1.16**	1.14**	1.09**
Thyroxine (T_4) (µg/dL)	3.54*	2.86*	3.01**	2.73**
Thyroid-Stimulating Hormone (THS) (ng/mg)	3.41	5.19**	4.27	4.85**

a Group means are given for Day 29.
* – Significantly different from Control ($P \leq 0.05$)
** – Significantly different from Control ($P \leq 0.01$)

Urinalysis: There were no changes in the urinalysis data that were of toxicological concern.

Gross pathology: The only TPV-related gross observation was an enlarged liver in 1 male rat (1250 mg/kg/day) found dead (dosing error) on Day 23. This was consistent with the organ weight data at the interim necropsy.

There were numerous gross necropsy observations in the toxicokinetic group animals. These were associated with complications of implantation of jugular vein cannulas used for blood sampling.

Organ weights (specify organs weighed if not in histopath table): Alterations in relative organ weights (% brain weight) considered drug-related involved the liver, thyroid glands and adrenal glands. Histopathological correlates were seen for the liver and thyroid weight increases but not for the adrenal glands. Changes in relative weight of the adrenal glands were thought to be stress-related. Organ weights for these organs were still elevated, although reduced compared to the treatment phase, following the reversibility phase.

Organ weights[a] (Interim Necropsy)

Dose mg/kg/day	0		40		125	
Number Examined	M: 10	F: 10	M: 10	F: 10	M: 1010	F: 5
Thyroid Glands	1.04 % BR	0.90 % Brain	+6	+8	+16	+28**
Liver	561 % BR	383 % Brain	+11	+19	+21**	+60**
Adrenal Glands	3.04 % Brain	4.52 % Brain	+15	+6	+8	+12

a Relative weights compared to brain are determined from group means of controls. Percent differences from controls are given for treatment groups.
* – Significantly different from Control (P≤0.05)
** – Significantly different from Control (P≤0.01)

Organ weights[a] (Interim Necropsy) continued

Dose mg/kg/day	400		1250	1000
Number Examined	M: 10	F: 10	M: 10	F: 10
Thyroid Glands	+34**	+52**	+49**	+62**
Liver	+45**	+114**	+84**	+152**
Adrenal Glands	+15	+19*	+31**	+25**

a Relative weights compared to brain are determined from group means of controls. Percent differences from controls are given for treatment groups.
* – Significantly different from Control (P≤0.05)
** – Significantly different from Control (P≤0.01)

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Minimal to moderate hepatocellular hypertrophy and mild to marked follicular cell hypertrophy correlated with increases in organ weight of liver and thyroid. Moderate liver necrosis was seen in 1/10 females at the highest dose. These histopathological findings were not observed after the reversibility phase.

Microscopic Observations (Interim Necropsy)

Dose mg/kg/day	0		40		125		400		1250	1000
Number Examined	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F:10	M: 10	F: 10
Liver										
Hypertrophy, hepatocyte	-	-	-	-	-	10	10	10	9	10
Minimal	-	-	-	-	-	10	10	-	-	-
Mild	-	-	-	-	-	-	-	7	8	-
Moderate	-	-	-	-	-	-	-	3	1	10
Necrosis, moderate	-	-	-	-	-	-	-	-	-	1
Thyroid										
Hypertrophy, follicular cell	1	-	1	2	2	8	6	10	8	10
Mild	1	-	1	2	2	3	5	-	5	-
Moderate	-	-	-	-	-	5	1	10	3	7
Marked	-	-	-	-	-	-	-	-	-	3

Toxicokinetics: Blood samples were collected from the jugular vein via cannulas from all toxicokinetic group animals at predose, 2, 4, 6, 8 (just prior to pm dose), 10, 12, 14 and 24 hours after the morning administration of TPV on Days 1, 14 and 28.

Cmax and AUC were dose-related but less than dose proportional with repeated dosing, indicating induced metabolism. There were no gender differences in exposure.

Toxicokinetic parameters on Day 28.

Dose (mg/kg/day)	40		125		400		1250	1000
Number Examined	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F:3
Cmax (μM)	40	36	67	66	58	111	164	180
AUC_{0-24} (μM.h)	210	212	560	400	680	1060	2000	2300
Fold versus Human AUC*	0.1	0.1	0.4	0.3	0.4	0.7	1.3	1.5

*Based on 500/200 mg TPV/RTV BID and an AUC of 1542 μM.h.

Electron microscopy: Liver samples were examined from 3 rats/sex/group from the control and high dose groups. Hepatocyte samples from all high dose animals showed an increase in cell size and smooth endoplasmic reticulum content consistent with cellular hypertrophy. This increase is also consistent with the finding of increased microsomal cytochrome P450 enzyme

activity. Mitochondria appeared smaller in the high dose animals. The implication of this finding is not known.

Cytochrome P450 activity: Cytochrome P450 specific content, cytochrome P450 2B and cytochrome P450 3A activities were increased in all treatment groups. Cytochrome P450 2C11 was decreased in males at doses of 125 mg/kg/day and greater.

Histopathology inventory

Study U00-3087	Dose (mg/kg/day)				
Species Rat	0	40	125	400	1000
Adrenals	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X
Bone Marrow smear					
Bone (femur)					
Brain	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X
Cervix	X	X	X	X	X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	X*	X*	X*	X*	X*
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian tube					
Gall bladder					
Gross lesions	X	X	X	X	X
Harderian gland					
Heart	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X
Injection site					
Jejunum	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*
Lachrymal gland					
Larynx					
Liver	X*	X*	X*	X*	X*
Lungs	X	X	X	X	X
Lymph nodes, cervical					
Lymph nodes mandibular	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X
Mammary Gland	X	X	X	X	X
Nasal cavity					
Optic nerves					
Ovaries	X*	X*	X*	X*	X*

Pancreas	X	X	X	X	X
Parathyroid	X*	X*	X*	X*	X*
Peripheral nerve					
Pharynx					
Pituitary	X*	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*
Rectum					
Salivary gland	X	X	X	X	X
Sciatic nerve	X	X	X	X	X
Seminal vesicles					
Skeletal muscle	X	X	X	X	X
Skin	X	X	X	X	X
Spinal cord	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*
Sternum	X	X	X	X	X
Stomach	X	X	X	X	X
Testes	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*	X*
Tongue					
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X*	X*	X*	X*	X*
Vagina	X	X	X	X	X
Zymbal gland					
Diaphragm	X	X	X	X	X
Knee joint	X	X	X	X	X

X, histopathology performed
*, organ weight obtained

Study title: Thirteen Week Oral (Gavage) Toxicity Study in the Rat on Tipranavir SEDDS formulation, with and without degradation products.

Key study findings: TPV administered in SEDDS caused effects similar to those seen in previous studies, i.e. decreases in red blood cell parameters, increases in coagulation times and effects on clinical chemistry parameters (total protein, albumin, globulin, alkaline phosphatase, aspartate aminotransferase, triglycerides, cholesterol, calcium and potassium). No new targets of toxicity were introduced by the addition of impurities and degradants. Liver (hepatocellular hypertrophy) and thyroid (follicular cell hypertrophy) were target organs of toxicity. The effects on liver are thought to be adaptive and the effects on thyroid, rat-specific. Comparison of the two formulations, unstressed versus stressed, showed no difference in the toxicity profile at 125 mg/kg/day. At the high dose level, a decrease in body weight gain and food consumption was

seen in male rats receiving stressed TPV with impurities and degradation products. Therefore, the qualification dose for impurities and degradants is 125 mg/kg/day TPV.

Study no.: U04-3154
Volume # and page #: Module 4, M002, vol. 1.36, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., Toxicology & Safety Assessment, 900 Ridgebury Road, Ridgefield, CT 06877-0368
Date of study initiation: 5-6-2003
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: TPV drug substance, batch numbers 1004253 and 1002508, both [ ] purity. Unstressed TPV SEDDS lot no. 6380-1 and stressed TPV SEDDS lot no. NB4987/136.

Methods

Doses: Unstressed: 0, 125, 400 mg/kg/day; Stressed 0, 125, 400 mg/kg/day.
Species/strain: CD rat
Number/sex/group or time point (main study): 15/sex/group
Route, formulation, volume, and infusion rate: Oral (gavage) once per day
Satellite groups used for toxicokinetics or recovery: 5/sex/group
Age: 7 – 8 weeks
Weight: 150 – 300 g
Sampling times: See below.
Unique study design or methodology (if any): The purpose of the study was to compare the toxicity of TPV in SEDDS with and without degradation products/impurities for qualification of these degradants/impurities. The stressed formulation was [ ] formation of degradation products, and based on the results, additional impurities were added to achieve targeted exposure.

Observations and times:
Mortality: Checks for morbidity and mortality were performed twice daily during the Pretest and Drug Phases.
Clinical signs: Clinical observations were recorded once daily in the pretest phase, before dosing and 1 and 3 hours after dosing on Drug Day 1. During the rest of the Drug Phase, observation was made twice daily prior to dosing and 2 to 3 hours after dosing.
Body weights: Body weights were measured once weekly in Pretest and Drug Phases. A terminal fasted weight was obtained prior to necropsy.
Food consumption: Food consumption was measured once weekly in Pretest and Drug Phases.
Ophthalmoscopy: Eyes of all animals were examined by indirect ophthalmology in Pretest Week -1 and eyes of main study animals were examined in Drug Week 13.
Hematology: Blood samples were collected via the retro-orbital sinus from the first 10 animals/sex/group following an overnight fast in Drug Weeks 5, 9 and 13. Blood was analyzed for standard hematology parameters.

Coagulation parameters: Blood was collected from the vena cava from the first 10 animals/sex/group (fasted overnight) at terminal sacrifice and analyzed for PT, fibrinogen and APTT.
Clinical chemistry: Blood samples were collected via the retro-orbital sinus from the first 10 animals/sex/group following an overnight fast in Drug Weeks 5, 9 and 13. Blood was analyzed for standard clinical chemistry parameters.
Urinalysis: Urine was collected from metabolism units overnight without food from the first 10 animals/sex/group following an overnight fast in Drug Weeks 5, 9 and 13. Urine was analyzed for standard urinalysis parameters.
Micronucleus assay: At the time of terminal necropsy, at least two bone marrow smears were prepared from each surviving main study animal at scheduled necropsy. These bone marrow smears were used for micronucleus evaluation and the results were reported in U04-3013.
Gross pathology: A complete necropsy was performed on all animals. A standard battery of tissues were collected, fixed and examined macroscopically and microscopically.
Organ weights (specify organs weighed if not in histopath table):
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Results

Mortality: 9 animals died during the Drug Phase. Two males in the Control unstressed group and 1 male in the 125 mg/kg/day unstressed group died due to dosing accidents. Three males in the 400 mg/kg/day unstressed group and 2 males and 1 female in the 125 mg/kg/day stressed group possibly died from dosing accidents but clearly hemorrhage contributed to lethality.

Clinical signs: Signs considered due to TPV were increased salivation in all treatment groups.

Body weights: At the end of the dosing period, males in the stressed 400 mg/kg/day group showed a significant decrease (-15%) in body weight. Changes in all other groups were less than eight percent.

Food consumption: Food consumption was decreased in males in the stressed 400 mg/kg/day group (-7 to -13%) and in females in the stressed and unstressed 400 mg/kg/day groups (-10%).

Ophthalmoscopy: No drug-related changes were observed.

Hematology: Hematology changes considered related to drug treatment observed predominantly in females included dose-related decreases in mean red blood cell parameters (RBC counts, hemoglobin, hematocrit, MCV, MCH and MCHC). Increases in mean platelet counts were observed in both sexes. The addition of impurities/degradation products did not increase toxicities observed. No new hematology changes were observed.

Week 13 Hematology Parameters[a]

Dose mg/kg/day	0		125		400	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
RBC Count (10^6/µl) Unstressed	9.52	8.53	-6%	-3%	-6%	-19%*
Stressed	9.37	8.51	-5%	-8%*,[c]	-5%	-0.2%[b]
Hemoglobin (g/dL) Unstressed	15.9	15.6	-4%	-6%*	-5%	-14%*
Stressed	16.2	15.9	-6%*	-11%*,[c]	-9%*,[c]	-10%*,[b,c]
Hematocrit (%) Unstressed	49.6	47.1	-2%	-4%	-2%	-11%*
Stressed	50.7	47.8	-5%	-10%*,[c]	-8%[c]	-6%,[b,c]
Mean Red Cell Volume fL Unstressed	52.1	55.2	+4%	-2%	+4%	-4%*
Stressed	54.1	56.2	-4%	-3%	-2%	-6%*,[c]
Mean Corp. Hemoglobin (pg) Unstressed	16.7	18.3	+2%	-3%*	+1%	-6%*
Stressed	17.3	18.7	-1%	-9%[c]	-4%	-10%*,[c]
Mean Corp. Hemoglobin Concentration (g/dL) Unstressed	32.0	33.3	-2%	-2%	-3%	-4%*
Stressed	32.1	33.2	-1%	-1%	-2%	-4%*,[c]
Platelet Count (10^3/µl)						

Unstressed	1158	1141	+11%	+22%*	+33%*	+26%*
Stressed	1035	1085	+39%*	+20%	+45%*,c	+22%

a = percent differences are calculated against the control (Unstressed or Stressed) for the group.
* = statistically different than Unstressed Control, $p<0.05$.
b = statistically different than 400 mg/kg/day unstressed, $p\leq 0.05$.
c = statistically different than Stressed Control, $p\leq 0.05$.

Mean prothrombin time (PT) and activated partial thromboplastin times (APTT) were increased in male rats in all treatment groups. Increases in the 400 mg/kg/day stressed group were higher than in the unstressed group, but the differences were not statistically significant. Smaller increases were seen in PT in the high dose group; there were not differences between the unstressed and stressed groups. No significant changes in APTT were found in female groups. Fibrinogen levels were not affected in any group of either sex.

Week 14 Coagulation Parameters[a]

Dose mg/kg/day	0		125		400	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Prothrombin Time (sec) Unstressed	14.4	13.0	+22%	+1%	+59%*	+2%
Stressed	14.3	13.4	+22%	-1%	+77%*	+7%*,b
Activated Partial Thromboplastin Time (sec) Unstressed	25.8	22.1	+17%	+14%	+58%*	+13%
Stressed	25.4	21.3	+28%*	+20%	+81%*	+14%

a = percent differences are calculated against the control (Unstressed or Stressed) for the group.
* = statistically different than Unstressed Control, $p<0.05$.
b = statistically different than 400 mg/kg/day unstressed, $p\leq 0.05$.
c = statistically different than Stressed Control, $p\leq 0.05$.

Clinical chemistry: Clinical chemistry changes considered drug-related in both sexes were slight increases in total protein, globulin and albumin and decreases in alkaline phosphatase (AP) at both dose levels. Males had statistically significant increases in mean calcium, decreases in mean triglycerides and in mean aspartate aminotransferase (AST) at both dose levels and

decreases in potassium at the high dose. All these changes were comparable in unstressed and stressed groups. An increase in cholesterol was observed in high dose unstressed females only.

Mean BUN was increased significantly in both sexes in the high dose unstressed and stressed groups. One difference in the high dose unstressed group was due to a change in one early death male. This male was determined to have hydronephrosis which was considered spontaneous and unrelated to treatment. Thus, the significant difference in males treated at the high dose between unstressed and stressed groups was not considered biologically relevant. No differences were noted between unstressed and stressed groups with respect to changes in BUN in females.

Table: 13 Week Clinical Chemistry Parameters[a]

Dose mg/kg/day	0		125		400	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Aspartate Aminotransferase (AST) U/L						
Unstressed	95	85	-28%*	-14%	-17%	-27%
Stressed	90	94	-33%*,[c]	-34%[c]	-51%*,[b,c]	-26%[c]
Alkaline Phosphatase (AP) U/L						
Unstressed	98	51	-19%	-24%	-16%	-35%*
Stressed	92	50	-20%*	-46%*,[c c]	-3%	-20%
Blood Urea Nitrogen (BUN) mg/dl						
Unstressed	11	11	+18%	+10%	+18%	+18%*
Stressed	11	12	+18%	+8%	+36%*,[b,c]	+8%
Total Cholesterol mg/dl						
Unstressed	57	71	+26%	+14%	+37%	+44%*
Stressed	63	75	+33%	-8%	+16%	-12%[b]
Triglycerides mg/dl						
Unstressed	58	39	-36%	-13%	-52%*	-13%
Stressed	57	36	-16%	-22%	-42%*,[c]	-14%

Total Protein g/dl Unstressed	6.7	7/2	+7%	+8%*	+10%*	+18%*
Stressed	6.7	7.2	+4%	+8%*,c	+10%**,c	+6%b
Albumin g/dl Unstressed	3.5	4.0	+3%	+5%	+9%*	+15%*
Stressed	3.5	3.9	+3%	+8%	+11%*,c	+3%c
Globulin g/dl Unstressed	3.3	3.3	+9%	+12%*	+12%*	+18%*
Stressed	3.3	3.3	+3%	+12%*,c	+6%	+9%
Calcium mg/dl Unstressed	10.9	11.6	+6%*	+1%	+6%*	+3%
Stressed	11.3	11.4	+1%*	+4%	+4%*	+1%
Total Bilirubin mg/dl Unstressed	0.2	0.2	-50%*	-50%*	-50%*	-50%*
Stressed	0.2	0.2	-50%*	-50%*,c	0%	-50%*,c
Potassium mmol/L Unstressed	6.2	5.8	-8%	-5%	-21%*	-3%
Stressed	5.7	5.5	-2%	0%	-7%*	+6%

a = percent differences are calculated against the control (Unstressed or Stressed) for the group.
* = statistically different than Unstressed Control, $p<0.05$.
b = statistically different than 400 mg/kg/day unstressed, $p\leq 0.05$.
c = statistically different than Stressed Control, $p\leq 0.05$.

Urinalysis: No drug-related changes in urinalysis parameters were observed.

Gross pathology: Drug-related minimal to marked diffuse enlargement of the liver was present in all treated groups. This observation is correlated with increased liver weights and microscopic drug-related hepatocellular hypertrophy.

In early death males (3 in the 400 mg/kg/day unstressed group and 1 in the 125 mg/kg/day stressed group), macroscopic observations consistent with hemorrhages were present. The hemorrhages were considered to be more severe than those observed with dosing accidents or trauma and were considered be drug-related.

Organ weights (specify organs weighed if not in histopath table): Dose-related increases in liver and thyroid weights (analysis in relation to brain weights) were observed in drug-treated animals. No differences were noted between either sex or between unstressed and stressed groups at either dose. It was noted in the report that mean liver weights relative to body weights of males in the stressed 400 mg/kg/day group were increased 13% as compared to males in the high dose unstressed group. This likely resulted from the lower body weight of males in the high dose stressed group.

13 week Organ Weights: Percent Change as Compared to Unstressed Controls

Dose mg/kg/day	0		125		400	
Number of Animals	M 8/10	F 9/10	M 10/10	F 10/10	M 7/10	F 10/10
Liver Unstressed	14.01 g	8.09g	+44%*	+70%*	+79%*	+138*
Stressed	-8%	-4%	+57%*,c	+77%*,c	+83%*,c	+122%*,c
Thyroid Unstressed	0.030g	0.023g	+39%*	+8%	+38%*	+23%
Stressed	+8%	-1%	+25%	+19%	+34%*	+29

* = statistically different than Unstressed Control, $p<0.05$.
c = statistically different than Stressed Control, $p\leq 0.05$.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Drug-related observations were present in liver and thyroid of all unstressed and stressed groups.

Uncertain findings were present in one male and one female from the high dose unstressed group and consisted of a focus of cellular alteration in the adrenal gland (female) and histiocytic infiltration in the white pulp of the spleen (male).

Stress-related findings (related to dosing accidents/trauma) in one or more lymphoid tissues were present in animals that died from the unstressed Control (2/20), the 125 mg/kg/day stressed (1/20) and 400 mg/kg/day (3/20) unstressed groups. They consisted of lymphoid depletion in the spleen and lung and/or lymphocytolysis in the thymus and mesenteric lymph node.
Histopathological Observations

Dose mg/kg/day	0		125		400	
Number of Animals	M:10	F:10	M:10	F:10	M:10	F:10
Liver:						
Hepatocellular Hypertrophy						
Unstressed	0	0	10	10	10	10
Stressed	0	0	8	10	10	10
Thyroid Gland						
Follicular cell Hypertrophy						
Unstressed	0	0	8	2	8	3
Stressed	0	0	5	6	10	4
Adrenal Gland						
Focus of Alteration Cortex						
Unstressed	0	0	0	0	0	1
Stressed	0	0	0	0	0	0
Spleen						
Histiocytic infiltrate White Pulp						
Unstressed	0	0	0	0	1	0
Stressed	0	0	0	0	0	0

Toxicokinetics: Blood was collected via retro-orbital sinus bleed 3 hours after dose administration from the last 5 animals/sex/group for measurement of plasma levels of TPV on Drug Day 1 and in Drug Weeks 7 and 13.

TPV plasma levels were similar in stressed and unstressed groups. TPV decreased by approximately half during the course of the study. TPV plasma values were about 2-fold higher in females than in males.

Toxicokinetics of TPV (μM) 3 hour post-dose on Drug Day 1, and Drug Weeks 7 and 13.

Dose mg/kg/day	0		125		400	
Number of Animals	M: 5	F:5	M:5	F:5	M:5	F:5
Day 1 Unstressed	0	0	106	210	171	276
Day 1 Stressed	0	0	117	256	150	271
Week 7 Unstressed	0	0	35.7	53.4	35.8	84.7
Week 7 Stressed	0	0	40.0	96/2	40.1	64.7
Week 13 Unstressed	0	0	45.4	126	71.7	168
Week 13 Stressed	0	0	50.8	170	57.7	193

Other:

Histopathology inventory: Dose groups include stressed and unstressed groups.

Study: U04-3154	Dose Group mg/kg/day		
Species: Rat	0	125	400
Adrenals	X		X
Aorta	X		X
Bone Marrow femur	X	X	X
Bone Marrow sternum	X		X
Bone (femur)	X	X	X
Bone (femur) with stifle joint	X	X	X
Bone (sternum)	X		X
Brain	X*	X*	X*
Cecum	X	X	X
Cervix	X		X
Colon	X	X	X

Duodenum	X	X	X
Epididymis	X	X	X
Esophagus	X		X
Eye	X		X
Fallopian tube			
Gall bladder			
Gross lesions	X	X	X
Harderian gland	X		X
Heart	X	X	X
Ileum	X	X	X
Injection site			
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland			
Larynx			
Liver	X*	X*	X*
Lungs	X	X	X
Lymph nodes,bronchial	X		X
Lymph nodes mandibular	X		X
Lymph nodes mesenteric	X	X	X
Mammary Gland	X		X
Nasal cavity			
Optic nerves	X		X
Ovaries	X*	X*	X*
Pancreas	X	X	X
Parathyroid	X	X	X
Peripheral nerve			
Pharynx			
Pituitary	X		X
Prostate	X		X
Rectum	X	X	X
Salivary gland	X		X
Sciatic nerve	X		X
Seminal vesicles	X		X
Skeletal muscle	X		X
Skin	X	X	X
Spinal cord	X	X	X
Spleen	X	X	X
Sternum	X		X
Stomach	X	X	X
Testes	X*	X*	X*
Thymus	X	X	X
Thyroid	X*	X*	X*
Tongue	X		X

Trachea	X		X
Urinary bladder	X		X
Uterus	X		X
Vagina	X		X
Zymbal gland			

X, histopathology performed
*, organ weight obtained

Study title: Toxicokinetics of tipranavir in a 13-week oral (gavage) toxicity study in Sprague Dawley rats on tipranavir SEDDS formulations: with and without degradation products. *(Reviewed under U04-3154 Thirteen week oral (gavage) toxicity study in the rat on Tipranavir SEDDS formulation with and without degradation products.)*

Study no.: U04-3257

Study title: PNU-140690E: 26-Week oral toxicity study in the rat followed by a 13-week recovery period.

Key study findings: TPV was well-tolerated when administered orally to groups of male and female Sprague-Dawley rats for 26 weeks at doses of 0, 20, 40, 125 and 400 mg/kg/day (0, 10, 20, 62.5 and 200 mg/kg twice daily). The treatment period was followed by a recovery period of 13 weeks. Values for AUC and Cmax increased with dose for both sexes, but the increase was less than dose proportional and values decreased with repeated exposure. Steady state was achieved by Day 28. Exposure in females was approximately 2-fold greater than for males. The death of one high dose male was thought to be caused by treatment-related increases in clotting times. Reversible decreases in food consumption, body weight gain and several red blood cell parameters as well as reversible increases in coagulation parameters, serum proteins and thyroid function tests were attributed to treatment with TPV. Increased urinary protein was associated with treatment and was only partially reversible in the females. Partially reversible to reversible increases in liver, thyroid, kidney and adrenal weights were recorded in both sexes. Microscopic changes included hepatocellular hypertrophy, thyroid follicular epithelium hypertrophy, multinucleate hepatocytes and a higher than expected incidence of chronic progressive nephropathy (CPN). At the end of the 13-week recovery period, the presence of multinucleate hepatocytes and the higher incidence of CPN were not completely reversed.

Changes in thyroid function tests are considered to be a rat-specific effect and have not been seen in clinical trials. Treatment-induced changes in the liver are considered to be adaptive. Changes in coagulation factors may be secondary to this adaptive change in the liver; however, there is no definitive proof of this. TPV-induced changes in prothrombin time have not been seen in the clinic.

The NOAEL for this study is 40 mg/kg/day for males and 20 mg/kg/day for females and corresponds to 0.13- and 0.12-fold, respectively, of the exposure expected in humans at the proposed dose of 500/200 mg TPV/RTV BID.

Study no.: U00-3210 (Pharmacia & Upjohn Study Report a0011536)
Volume #, and page: Module 4, M002, Vol. 1.15, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI 49001
Date of study initiation: May 2, 1997
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: TPV, Lot # (A1)5134-AS-1650, [ ] purity.

Methods

Doses: 0, 20, 40, 125, 400 (½ dose twice daily) mg/kg/day
Species/strain: Rat/ ([ ]:CD[SD]BR) Sprague-Dawley
Number/sex/group or time point (main study): 30/sex/group
Route, formulation, volume, and infusion rate: Oral, TPV in 0.25N sodium hydroxide, adjusted to approximately pH 10.5 with sodium hydroxide or hydrochloric acid as needed, ½ dose twice daily 8 hours apart.
Satellite groups used for toxicokinetics or recovery: 5/sex/group for toxicokinetics and 10/sex/group for recovery.
Age: 9 weeks
Weight: Males 249 – 321 g; Females 121 – 223 g
Sampling times:
Unique study design or methodology (if any): Dosing was twice daily with an 8 hour interval between doses. TPV was administered as a disodium salt.

Observations and times:
Mortality: Observations were conducted at least twice a day.
Clinical signs: Detailed clinical examination of each main study animal was performed once weekly.
Body weights: Body weights were recorded weekly for all animals.
Food consumption: Food consumption was recorded weekly for main study animals.
Ophthalmoscopy: All animals received ophthalmoscopic examination in the predosing period and main study animals received examinations during Weeks 25 and 38.
EKG: Not monitored.
Hematology: Blood samples were collected after 13 weeks (14 weeks for thyroid parameters) of treatment by orbital sinus puncture from anesthetized animals (first 15/sex/group for hematology; last 15/sex/group for clinical chemistry). Terminal blood samples were collected from the abdominal aorta while the rat was under anesthesia from all surviving rats following an

overnight fast after 26 (20/sex/group) weeks of treatment and following 13 (up to 10/sex/group) weeks of recovery.
Clinical chemistry: Blood samples were collected after 13 weeks (14 weeks for thyroid parameters) of treatment by orbital sinus puncture from anesthetized animals (first 15/sex/group for hematology; last 15/sex/group for clinical chemistry). Terminal blood samples were collected from the abdominal aorta while the rat was under anesthesia from all surviving rats following an overnight fast after 26 (20/sex/group) weeks of treatment and following 13 (up to 10/sex/group) weeks of recovery.
Urinalysis: Animals were placed in metabolism cages for a collection period of 16 hours at Weeks 26 (20/sex/group) and 39 (up to 10/sex/group).
Gross pathology: A complete necropsy was conducted on all main study rats dying spontaneously or at the scheduled necropsy.
Organ weights (specify organs weighed if not in histopath table): See table.
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )
Electron microscopy: At all scheduled necropsies, samples of liver and kidney were taken for electron microscopy from the first 3 males and 3 females from each dose group.

Results

Mortality: A total of 8 animals died during the course of the study. The deaths of 5 animals were clearly associated with the scheduled orbital sinus bleeding procedure on Day 91. One male clotting times. Another male dosed at 400 mg/kg/day died on Day 184 but the cause of death was not determined. One control male died on Day 52 from urolithiasis with accompanying complication of pylonephritis. A male from the 400 mg/kg/day group died on Day 53 from possible treatment-related increases in hemorrhaging.

Clinical signs: Increased salivation was observed in rats of both genders in the 125 and 400 mg/kg/day groups.

Body weights: Reversible decreases (see summary tables below) in mean body weight gain were observed at 125 and 400 mg/kg/day.

Food consumption: Reversible decreases in food consumption were observed at 125 and 400 mg/kg/day.

Ophthalmoscopy: No drug-related effects were observed.

EKG: Not monitored.

Hematology: Reversible decreases in the group mean values for red blood cells, hemoglobin, and hematocrit were observed in rats receiving 125 and 400 mg/kg/day. Reversible increases in

coagulation parameters were noted for the males in the same two dose groups. (See summary tables below.)

Clinical chemistry:

Thyroid function tests showed reversible increases in triiodothyronine levels (males at 400 mg/kg/day). Reversible increases in total protein and globulin levels were noted in both sexes at 40, 125 and 400 mg/kg/day; reversible increases in albumin levels were observed in both sexes at 400 mg/kg/day. (See summary tables below.)

Urinalysis: Increased urinary protein was seen in the treated rats, especially at doses of 40 mg/kg/day and higher. At the end of the recovery period, a higher incidence of increased urinary protein was still evident in females at 125 and 400 mg/kg/day. Proteinuria was considered secondary to chronic progressive nephropathy and correlated with the increase seen in TPV-treated groups.

Gross pathology: There were no drug-related changes observed.

Organ weights (specify organs weighed if not in histopath table):

Organ weight changes included increased liver and thyroid weights in females at 40 mg/kg/day and in males and females at 125 and 400 mg/kg/day; increased kidney weights in both sexes at 125 and 400 mg/kg/day; and increased adrenal weights in both sexes at 400 mg/kg/day. Liver weight increases at 125 and 400 mg/kg/day (males and females) and kidney weight increases (males) at 400 mg/kg/day were only partially reversed at the end of the recovery period. All other organ weight changes were fully reversible. (See summary tables below.)

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Trace to moderate hepatocellular hypertrophy and follicular cell hypertrophy correlated with increases in organ weights of liver and thyroid. Multinucleate hepatocytes were also seen in both sexes at 125 and 400 mg/kg/day. Chronic progressive nephropathy (CPN), a naturally occurring lesion in the kidney of rats, was more prevalent in males at 400 mg/kg/day and in females at 125 and 400 mg/kg/day than in controls. At the end of the recovery period, the hypertrophic changes in the liver and thyroid were reversed; however, multinucleate hepatocytes were still present in both sexes at 400 mg/kg/day and the incidence of CPN in high-dose females was greater than in the controls. (See summary tables below.)

Summary table: Findings in male rats.

Parameter	Dose (mg/kg/day)				
	0	20	40	125	400
Body weight gain	322g	-3%	-8%	-12%	-14%
Absolute weight gain*	307	-4%	-9%	-14%	-17%
Erythrocytes (13 weeks)	9.18 mil	-1%	-1%	-9%	-6%
Erythrocytes (26 weeks)	8.41 mil	+1%	+2%	-3%	-3%
Hemoglobin (13 weeks)	15.8 g/dL	-1%	-1%	-6%	-7%
Hemoglobin (26 weeks)	14.7 g/dL	+1%	+2%	-3%	-5%
Hematocrit (13 weeks)	50.1%	0%	0%	-6%	-5%
Hematocrit (26 weeks)	42.8%	+2%	+3%	-2%	-3%
Total T_3 (13 weeks)	68.31 ng/dL	+14%	+8%	+13%	+29%
Total T_3 (26 weeks)	57.04 ng/dL	+10%	+15%	+13%	+29%
Total T_4 (13 weeks)	4.18 µg/dL	-4%	-8%	-9%	-12%
Total T_4 (26 weeks)	3.37 µg/dL	+2%	+10%	+6%	+1%
TSH (13 weeks)	2.56 ng/mL	+4%	+2%	+14%	+20%
TSH (26 weeks)	1.2 ng/mL	+23%	+36%	+53%	+44%
Liver weight	14.88 g	+8%	+9%	+28% NR	+51% NR
Hepatocyte hypertrophy	0/20	0/20	0/20	17/20	20/20
Multinucleated hepatocytes	0/20	0/20	0/20	3/20	10/20 NR
Thyroid weight	25 mg	-4%	+12%	+16%	+32%
Follicular cell hypertrophy	0/20	0/20	0/20	15/19	20/20
Kidney weight	3.78 g	+1%	+2%	+10%	+8% NR
Chronic progressive nephropathy	12/20	16/20	16/20	14/20 NR	18/20 NR
APTT (13 weeks)	19.5 sec	+4%	+7%	+14%	+47%
	24.3 sec	+5%	+5%	+23%	+28%

APTT (26 weeks)					
Prothrombin (13 weeks)	16.7	+1%	-2%	+2%	+18%
Prothrombin (26 weeks)	15.5 sec	-4%	-2%	0%	+16%

*Absolute weight gain = mean weight gain - mean liver weight
NR = not completely reversible during a 13-week recovery period

Summary table: Findings in female rats.

Parameter	**Dose (mg/kg/day)**				
	0	**20**	**40**	**125**	**400**
Body weight gain	113g	+9%	+21%	+7%	-17%
Absolute we ight gain*	105	+8%	+20%	+2%	-27%
Erythrocytes (13 weeks)	8.48 mil	-1%	-3%	-5%	-11%
Erythrocytes (26 weeks)	7.54 mil	-1%	-3%	-6%	-5%
Hemoglobin (13 weeks)	15.4 g/dL	-1%	-3%	-8%	-15%
Hemoglobin (26 weeks)	14.3 g/dL	-2%	-5%	-10%	-15%
Hematocrit (13 weeks)	48.7%	+1%	-1%	-6%	-14%
Hematocrit (26 weeks)	41.6%	0%	-2%	-8%	-12%
Total T_3 (13 weeks)	95.77 ng/dL	-5%	+3%	+7%	+4%
Total T_3 (26 weeks)	93.69 ng/dL	0%	+2%	0%	+1%
Total T_4 (13 weeks)	2.53 µg/dL	+6%	+9%	+1%	+2%
Total T_4 (26 weeks)	2.48 µg/dL	-14%	-6%	+1%	+6%
TSH (13 weeks)	1.96 ng/mL	0%	+17%	+56%	+59%
TSH (26 weeks)	0.9 ng/mL	+18%	+46%	+111%	+146%
Adrenal weight	75 mg	+5%	+4%	+9%	+20%
Liver weight	7.86 g	+16%	+39%	+77%	+115%
Hepatocyte hypertrophy	0/20	0/20	9/20	19/20	20/20

Multinucleated hepatocytes	0/20	0/20	0/20	3/20	7/20 NR
Kidney weight	2.19 g	+4%	+11%	+14%	+11%
Chronic progressive nephropathy	2/20	2/20	7/20	12/20 NR	11/20 NR
Thyroid weight	17 mg	+6%	+24%	+53%	+47%
Follicular cell hypertrophy	0/20	0/20	10/19	13/19	19/20
APTT (13 weeks)	17.7 sec	+5%	+6%	+11%	+12%
APTT (26 weeks)	25.5 sec	-9%	-3%	-3%	-15%
Prothrombin (13 weeks)	15.6 sec	0%	-2%	0%	+3%
Prothrombin (26 weeks)	14.1 sec	-2%	-3%	+3%	-3%

*Absolute weight gain = mean weight gain - mean liver weight
NR = not completely reversible during a 13-week recovery period

Toxicokinetics:

Blood samples were collected from all animals in the toxicokinetic groups by orbital sinus bleeding (Day 1) or the tail nick method (Days 28, 90 and 180). Blood was collected on Days 1, 90 and 180 at 0, 2, 8, 10 and 14 hours after the AM dose. On Day 28, collections were made at 0 hours and 10 hours after the AM dose.

Cmax and AUC values increased with dose but were less than proportional to the dose increases. Values for AUC and Cmax decreased with repeated exposure and achieved steady state by Day 28. The exposure of females was approximately 2-fold greater than males. There was no evidence of drug accumulation.

Toxicokinetic parameters on Day 180.

Dose (mg/kg/day)	20		40		125		400	
Number Examined	M: 5	F: 5	M: 5	F: 5	M: 5	M:5	M: 5	F:5
Cmax (μM)	12	23	30	47	38	114	90	209
AUC_{0-24} (μM.h)	103	190	207	290	300	770	910	2320
Fold versus Human AUC*	0.07	0.12	0.13	0.19	0.19	0.50	0.60	1.50

*Based on 500/200 mg TPV/RTV BID and an AUC of 1542 μM.h.

Histopathology inventory

Study U00-3210	Dose (mg/kg/day)				
Species Rat	0	20	40	125	400
Adrenals	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X
Bone (sternum)	X	X	X	X	X
Brain	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X
Cervix	X	X	X	X	X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	X*	X*	X*	X*	X*
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian tube					
Gall bladder					
Gross lesions	X	X	X	X	X
Harderian gland					
Heart	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X
Injection site					
Jejunum	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*
Lachrymal gland					
Larynx					
Liver	X*	X*	X*	X*	X*
Lungs	X*	X*	X*	X*	X*
Lymph nodes, mandibular	X	X	X	X	X
Lymph nodes mediastinal	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X
Mammary Gland	X	X	X	X	X
Nasal cavity					
Optic nerves	X	X	X	X	X
Ovaries	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X
Parathyroid	X*	X*	X*	X*	X*
Peripheral nerve					
Pharynx					
Pituitary	X*	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*

Rectum					
Salivary gland	X	X	X	X	X
Sciatic nerve	X	X	X	X	X
Seminal vesicles	X	X	X	X	X
Skeletal muscle	X	X	X	X	X
Skin	X	X	X	X	X
Spinal cord	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*
Sternum	X	X	X	X	X
Stomach	X	X	X	X	X
Testes	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*	X*
Tongue					
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X*	X*	X*	X*	X*
Vagina	X	X	X	X	X
Zymbal gland					
Ear	X	X	X	X	X
Joint, tibiofemoral	X	X	X	X	X
Preputial gland	X	X	X	X	X
Tail	X	X	X	X	X

X, histopathology performed
*, organ weight obtained

Study title: 26-Week Oral (Gavage) Interaction/Toxicity Study in the Rat on Tipranavir and Ritonavir.
(See IND 51,979 (474) Summary of Immunotoxicity Findings for Tipranavir in Appendix for discussion of findings related to immunotoxicity in this study.).

Key study findings: Co-administration of TPV and RTV by oral gavage to rats for 26 weeks resulted in toxicities common to the individual compounds administered separately. Target organs of TPV/RTV co-administration included the liver, lymphoreticular system, skin, stomach, testes, thyroid gland and multiple organs with hemorrhage. When TPV was administered alone at 1200 mg/kg/day, target organs were similar to those of TPV/RTV at the 1200/320 mg/kg/day dose level, with the exception that no hepatic karyomegaly was observed and there were no testicular findings. Hepatic karyomegaly and testicular degeneration are findings associated with RTV administration, with the former finding observed in this study at the 160 mg/kg/day RPV dose level and the latter finding not evident. No NOAEL was achieved in this study.

Study no.: U04-3111
Volume # and page #: Module 4, M002, vol. 1.15, page 1.

Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., Toxicology & Safety Assessment, 900 Ridgebury Road, Ridgefield, CT 06877
Date of study initiation: 12-03-2001
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Tipranavir lot no. 113010/ritonavir lot no. 66729 TL00 until Drug Week 13 and TSA-02-001 to end of study. Lot TSA-02-001 was a combination of the remaining drug substance from lot no. 66729 TL00 mixed with lot no. 79244 TL, purity not provided.

Methods

Doses: 0, 120/32, 600/160 and 1200/320 mg/kg/day TPV/RTV, 1200 mg/kg/day TPV or 160 mg/kg/day RTV
Species/strain: [illegible] CD(SD)IGS BR Sprague Dawley VAF+ albino rats.
Number/sex/group or time point (main study): 20
Route, formulation, volume, and infusion rate: Tipranavir in aqueous solution, pH 10.5; ritonavir in propylene glycol. 15 ml/kg for tipranavir and 5 ml/kg for ritonavir; animals receiving TPV and RTV were dosed first with a single dose of RTV, followed immediately by a single dose of TPV in the first six weeks of the study. Due to difficulties with the bitter taste of TPV and struggling animals, beginning in Drug Week 7 animals were dosed with RTV followed 1 hour later by a single dose of TPV.
Satellite groups used for toxicokinetics or recovery: last 5/sex/group for toxicokinetics.
Age: 7 - 8 weeks
Weight: 150 - 290 g
Sampling times:
Unique study design or methodology (if any):

Observation and Times:

Clinical signs: Clinical observations and mortality were noted at least once daily during the Pretest Phase. On Drug Day 1, observations were made prior to dosing and 1 and 4 hours after dosing. In the remainder of the Drug Phase, observations were initially made prior to dosing and 1 hour after dosing. After the change in dose regimen in Drug Week 7, observations were made prior to dosing, 1 hour after RTV or propylene glycol dosing and 1 hour after TPV or aqueous solution pH 10.5 dosing.
Body weights: Body weights were measured once weekly throughout the study.
Food consumption: Food consumption was measured once weekly throughout the study.
Ophthalmoscopy: Ophthalmology was performed in Pretest Week -1 and for Main study animals in Drug Weeks 6, 15 and 26.
EKG: Not performed.
Hematology: Hematology measurements were performed in Drug Weeks 4, 8, 12, 18 and 27.
Clinical chemistry: Clinical chemistry measurements were performed in Drug Weeks 4, 8, 12, 18 and 27.
Urinalysis: Urine samples were collected and analyzed in Drug Weeks 4, 8 and 12, 18 and 26.

Gross pathology: Necropsies were performed on all animals sacrificed moribund or found dead during the study and on all animals at terminal sacrifice.
Organ weights (specify organs weighed if not in histopath table):
Histopathology: specify groups examined , special stains, etc
Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )
Tissues were examined histopathologically for animals that died or were sacrificed moribund and for all terminal sacrifice Control, 1200/320 mg/kg/day TPV/RTV, TPV only and RTV only group animals.

Results

Mortality: The highest mortality occurred in males in the 1200/320 mg/kg/day TPV/RTV group (13 with 2 deaths due solely to dosing injury) and in the 1200 mg/kg/day group (13 with 1 death due solely to dosing injury). Based on clinical and macroscopic observations, excessive hemorrhage contributed to the majority of these deaths. Deaths were seen in all other groups except RTV only males. However, these early other deaths that were suspected to be solely due to drug treatment were comparable to those seen in the control group except for six females in the RTV only group (6 deaths versus 2 in the Control group).

Clinical signs: Clinical signs considered related to test article were decreased motor activity (all groups except Control females), increased salivation (all dosing groups) and excessive bleeding. The incidence of decreased motor activity and increased salivation was higher in drug treatment groups but was not proportional to increasing dose. Excessive bleeding was seen in middle and high TPV/RTV groups and in the TPV only group.

Body weights: Body weight group means in Drug Week 26 were decreased as compared to the Control group means as follows (* denotes statistical significance): 1) 120/32 TPV/RTV (M = -6%, F = -3%); 2) 600/160 TPV/RTV (M = -13%*, F = -6%); 3) 1200/320 TPV/RTV (M = -14%*, F = -12%*); 4) 1200 TPV (M = -34%*, F = -14%*); 5) 160 RTV (M = -5%, F = -5%).

Food consumption: Food consumption was reduced during the course of the study in middle (-12% to -20%) and high (-11% to -19%) TPV/RTV, TPV only (-13% to -29%) and RTV only (-7% to -16%) groups in males but not in females.

Ophthalmoscopy: No drug-related changes in ophthalmology were observed, except for changes attributed to eye bleeding.

EKG: Not performed.

Hematology: Drug-related statistically significant changes in TPV/RTV and TPV-treated groups included decreases of less than 13% hemoglobin, MCH and MCHC in females and increases of 24% to 58% in platelet counts observed in both sexes but to a greater extent in females.

Elevations in mean absolute neutrophil counts of 85% to 156% with no change in WBC counts were displayed by males and females of the 1200 mg/kg/day TPV group. In the 160 mg/kg/day RTV group, elevations in mean absolute monocyte counts of 96% to 125% were observed in both sexes and increases in mean WBC counts of 58% and reticulocyte counts of 45% to 55% were observed in males at certain time points.

Drug and dose-related significant increases in coagulation parameters PT (53 to 171%) and APTT (34% to 95%) were noted in males of all TPV/RTV and TPV treated groups as well as in females of the 1200 mg/kg/day TPV group (PT = 59% and APTT = 50%).

Clinical chemistry: Changes noted in groups treated with both TPV/RTV were decreases of 22% to 53% in alkaline phosphatase in females, as well as elevations in total protein (10% to 20%) and albumin (9% to 18%) in both sexes and globulin (25% to 34%) and cholesterol (39% to 113%) in females. Similar changes in the same parameters were noted in animals treated only with TPV only ; in addition, potassium was decreased 8% to 19% in males and increases in cholesterol of 52% to 113% were noted in both sexes of the TPV only group. In animals treated only with RTV, males exhibited increases of up to 128% and 289% in mean AST and ALT levels, respectively and elevations of up to 499% above Control were noted in mean SDH, while females of this group displayed increases in mean ALT of 76% to 115%. In females of the RTV only group, changes common to those observed in females of the TPV/RTV and TPV only groups included decreased alkaline phosphatase (22% to 33%) and increased cholesterol (44% to 137%), as well as elevations in total protein (25%), albumin (18%), and globulin (44%). Rodent specific changes in thyroid parameters that were observed in other rat studies were also noted in this study.

Urinalysis: There were no changes in urinalysis parameters attributed to drug administration in this study.

Gross pathology: Diminished size of the spleen and thymus in males and females in the TPV/RTV, TPV and RTV groups was observed.

Organ weights (specify organs weighed if not in histopath table): Group mean liver (up to + 124% in the low dose TPV/RTV group) and thyroid (up to +28% in the low dose TPV/RTV group) weights were increased in all drug-treatment groups compared to group mean weights in the Control groups. This has observed in other rodent studies of TPV and RTV. Spleen and thymus weights were not determined.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Administration of TPV, RTV or TPV/RTV resulted in drug-related microscopic changes in a number of target organs. These findings are summarized in the table below.