PART IIIG. PHARMACOKINETICS

1. INTRODUCTION

Amprenavir (APV, 141W94) is a small peptidomimetic inhibitor of HIV-1 and HIV-2 protease (protease inhibitor, or PI), and is marketed as AGENERASE Capsules or AGENERASE Oral Solution. The marketing application for AGENERASE Capsules and AGENERASE Oral Solution was submitted 7th December 1998 and APV was approved for use in the EU in 2000. GW433908 is a phosphate ester prodrug of APV developed to enable a reduced pill burden and therefore better patient acceptability. The form proposed for clinical use is the calcium salt, GW433908G or Fosamprenavir Calcium.

The proposed clinical dosing regimen is a "PI-boosted" regimen where GW433908G is administered with an inhibitor of APV metabolism, ritonavir (a potent CYP3A4 inhibitor). GW433908G is delivered either via 700 mg tablets or an oral suspension. The proposed tablet therapeutic dosage is either 700 mg GW433908G (equivalent to 600 mg APV): 100 mg ritonavir BID, or 1400 mg GW433908G (equivalent to 1200 mg APV): 200 mg ritonavir QD. The suspension dosage is 30 mg/kg GW433908G (equivalent to 24 mg/kg APV): 6 mg/kg ritonavir QD.

Human exposure (arithmetic mean $AUC_{24,ss}$ and $C_{max,ss}$) to APV or GW433908X was calculated in clinical studies using 1395 mg GW433908G : 200 mg ritonavir QD (APV10009) or 700mg GW433908G: 100 mg ritonavir BID (APV10010). However a further study (APV10006) demonstrated 1395 mg GW433908G and 1400 mg GW433908G were bioequivalent. Maximum arithmetic mean C_{max} and $AUC_{24,ss}$ values following these dose regimens are 7.57 µg/mL and 83.2 µg*h/mL, and 0.015 µg/mL and 0.024 µg*h/mL for APV and GW433908X, respectively.

As GW433908 is extensively hydrolysed to APV during intestinal absorption (see Pharmacology Written Summary) and a comprehensive pharmacokinetics program was carried out with APV as part of its nonclinical development, a more limited program of pharmacokinetic studies has been carried out with GW433908A or GW433908G. However, to complete the data package for this submission, relevant APV pharmacokinetic from the AGENERASE application are included in this application, and hence the Pharmacokinetics Written Summary for the original AGENERASE application is included as APPENDIX G3 to this summary. Additionally, brief summary of the previously submitted APV data is provided in Section 2 in this summary.

New information submitted with this application includes data generated following both APV and GW433908G administration to animals. The APV data includes information carried out to complete the pharmacokinetic profile of APV in mice, toxicokinetic studies as part of carcinogenicity studies with APV in rats and mice, studies to confirm the structure of the major human and rat metabolites, and in vitro protein binding and drug interaction studies.

Studies with GW433908G include in vivo studies to characterise the absorption, biotransformation and excretion of both APV and GW433908 in mice, rats and dogs following single dose oral administration of radiolabelled GW433908G. In vitro investigations have also been conducted to determine protein binding and cytochrome

P450 interactions. These studies were conducted in a facility that operates in accordance with the principles of Good Laboratory Practice, and where the facility is the subject of routine independent quality assurance process and facility audits. These studies were not the subject of a specific quality assurance audit. Nothing occurred to affect adversely the quality or integrity of these experimental data.

Blood samples were also obtained during toxicity studies in each species to investigate the pharmacokinetics of APV and GW433908 after repeated administration and to provide an assessment of exposure to APV and GW433908X during toxicity studies. All investigations conducted as a part of definitive toxicological investigations were performed in full compliance with GLP regulations.

The new information has shown that:

- APV was rapidly absorbed in mice, rats and dogs following oral administration GW433908; however, exposure was generally not proportional to dose. Bioavailability of APV was lower after administration of GW433908G compared to administration of equivalent doses of APV. This effect was small and variable in dogs, but more pronounced in rats where exposure was up to 50% lower.
- Systemic exposure, in terms of C_{max} and AUC, to APV after repeat oral administration of GW433908A or GW433908G to mice, rats or dogs generally increased with increases in dose, but not dose-proportionally. Exposure decreased on repeat dosing in mice and rats, but increased slightly in dogs.
- In general, the exposure ratios of GW433908X to APV were 2% or less. Systemic exposure to APV and GW433908X was similar after oral dosing of pregnant and non-pregnant rats.
- In pregnant rabbits, systemic exposure increased in a greater than dose-proportional manner. GW433908X to APV exposure ratios were variable, ranging from 2.9 to 39.8%, indicating that conversion of GW433908X to APV may be less efficient in the rabbit.
- Hepatic clearance was the principal route of excretion for APV after administration of GW433908G. Quantitatively, the main routes of metabolism in rats were a di-oxidation on the tetrahydrofuran moiety of the molecule and an additional site of oxidation on the aniline ring portion of the molecule.
- On administration of GW433908G, 3-13 % of the dose was excreted in urine in mice, rats and dogs, with APV being a minor component in the urine in all cases. The balance of the dose was excreted in feces, with APV being the major component in dog feces, but only a secondary component of both mice and rat feces.
- Repeat administration of APV or GW433908G induces cytochrome P450 3A4 in the mouse and rat, but the inhibitory effect of APV on this enzyme may partially mask this effect when assessed by enzyme activity in vitro.
- Plasma protein binding studies indicate displacement of 4 to 6% by selected anti-HIV drugs at high doses. This displacement may affect the pharmacokinetics of APV in vivo.

This section contains a brief summary of the previously submitted pharmacokinetic information regarding APV (Subsection 2), followed by summaries of the new studies carried out with APV and GW433908 and a discussion of the results from these studies. Earlier studies were initiated under the auspices of Glaxo Wellcome, however the company name has subsequently changed to GlaxoSmithKline.

1.1. Species

The species and strains used in the present studies reflected those employed in the toxicological testing of GW433908G, to enable meaningful assessment of the exposure levels in the toxicity studies and provide confidence in the conclusions drawn regarding the safety of GW433908G. The species and strains used were CD-1 mice, Han Wistar or Sprague Dawley rats, New Zealand White rabbits and beagle dogs. In some rat studies, the strain designation is Wistar Han; this is the same strain as Han Wistar, but the animal supplier changed the strain name. The Sprague Dawley rat was used in reproductive toxicity studies as there is considerable background data for this strain in these types of studies and Han Wistar rats have relatively small litter sizes.

1.2. Methodology

A detailed summary of the validated methods of analysis is included as APPENDIX G1 to this summary, and the validation reports are included in this submission as reference reports.

Plasma concentrations of APV and GW433908X were determined by high-pressure liquid chromatography tandem mass spectrometry (LC/MS-MS) with detection by positive electrospray ionisation (ES+) in multiple reaction monitoring mode (MRM). Levels of APV and GW433908X present in biological samples were determined by using calibration curves with internal standards ($^{13}C_6$-GW433908 and $^{13}C_6$-APV) constructed from analysis of samples spiked with known concentrations of APV and GW433908X, with linear regression applied in each case. The suitability of each analytical method was confirmed by determining the accuracy, precision, linearity, range and limits of quantification. In addition, quality control samples were analysed in parallel to establish the suitability of the method at the time of analysis.

In studies following APV administration, the validated range was 10 – 5000 ng/mL in mice (Reference Report RD1998/01974/00) and rats (Reference Report R1998/02888/00). APV was stable in frozen plasma (-30°C) from mice and rats for 5 or 6 months, respectively.

In studies following GW433908A or GW433908G administration, the validated ranges for assays were 5 to 1000 ng/mL for GW433908X, and 10 to 5000 ng/mL for APV in plasma samples from mice (Reference Report RD2002/00232/00), rats (Reference Report RD1999/02681), rabbits (Reference Report RD1999/02498) and dogs (Reference Reports RD1999/00101/00 and RD2002/00142/00). Additionally, a safety pharmacology study in dogs with GW433908A assessed exposure to APV by using a validated method with a range of 25 – 5000 ng/mL (Reference Report RD1996/00211/00). APV and

GW433908X were stable in frozen plasma (-20°C) from rats, rabbits or dogs for at least 6, 3 or 12 months, respectively. Stability in frozen mouse plasma is to be determined.

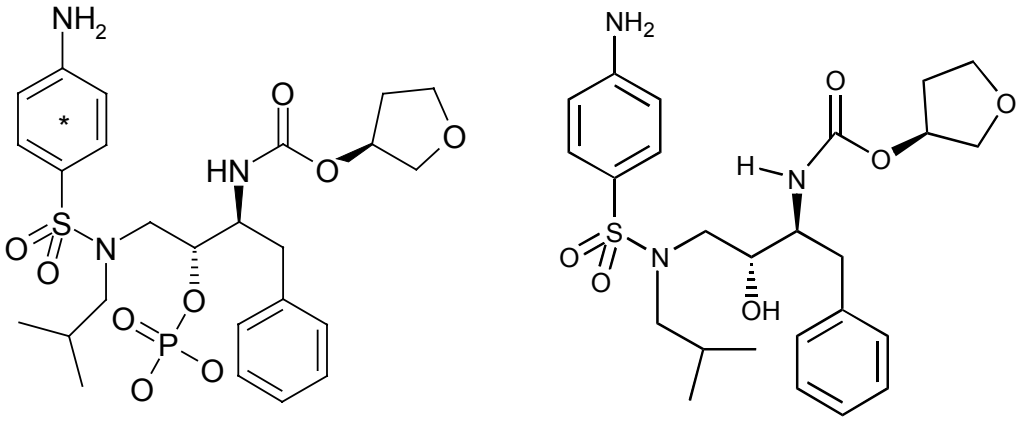
Determination of the radiolabelled material in biological samples, following the administration of ^{14}C-radiolabelled GW433908G (see below) was carried out by liquid scintillation counting. The profiling and identification of metabolites of APV were performed, after solid phase extraction, by LC-MS and nuclear magnetic resonance (NMR) analysis.

A time-resolved immunofluorometric assay was developed and validated to determine levels of recombinant human CYP3A4-like immunoreactivity (rHuCYP3A4-LI) in rat liver microsomes (Reference Report RD1999/02148/00). The assay had acceptable precision and accuracy over a range of 3.4 to 250 ng/mL, with rHuCYP3A4-LI stable in rat microsomes for 1 freeze-thaw cycle.

1.3. Test Substance

The single dose pharmacokinetic studies and those investigating the metabolism and excretion of APV and GW433908 were performed with the ^{14}C-radiolabelled GW433908G, labelled uniformly in the aniline ring (see Figure G1 for structure, and also APV structure). The desired specific activity of radioactivity was achieved by diluting radiolabelled drug with unlabelled GW433908G. Repeat-dose toxicokinetic analyses were carried out by measuring non-radiolabelled GW433908X in plasma samples taken during repeat-dose toxicity studies.

Figure G1. The Structure of ^{14}C-GW433908X and Amprenavir



GW433908X Amprenavir

* Denotes position of ^{14}C-radiolabel.

Initial development studies used the disodium salt of GW433908 (GW433908A), however this was superseded by the calcium salt (GW433908G) which is the form proposed for use in man. The salt form used in each study is detailed in the relevant study

summary, and dose levels are expressed in terms of both the salt and the APV-equivalent. The dosage conversion from GW433908 to APV is based on correction for both molecular weight and the impurity content of the relevant batch unless otherwise indicated. Plasma concentrations are expressed in terms of the free ester, GW433908X. Generally, GW433908A was formulated in water and GW433908G was formulated in aqueous 0.5% (w/w) hydroxypropyl methylcellulose/ 0.1% (w/w) Tween® 80, unless otherwise indicated in the study summaries.

1.4. Pharmacokinetic Parameter Definitions

AUC: Area under the plasma concentration-time curve

C_{max}: Maximum observed plasma concentration

T_{max}: Time at which C_{max} occurred

t½: Half-life

2. SUMMARY OF PREVIOUSLY SUBMITTED NONCLINICAL PHARMACOKINETIC INFORMATION FOR AMPRENAVIR

- APV was rapidly absorbed in mice, rats and dogs following oral administration; however, the exposure was generally not proportional to the dose. Bioavailability depends on formulation, and was experimentally defined as approximately 40% in rats and approximately 100% in dogs at clinically relevant doses in formulations similar to that used clinically. After oral doses, APV was found primarily in the organs of excretion and metabolism.

- APV was highly bound to plasma proteins, especially α_1-acid glycoprotein (AAG), and differences in AAG concentrations or changes in disease state that affect AAG concentrations could have an effect on the pharmacokinetics of APV.

- Systemic exposure to APV was similar after oral dosing of pregnant and non-pregnant rats; plasma concentrations of APV were significantly higher after oral administration in juvenile rats compared to mature rats.

- Placental transfer studies in rats confirmed that fetuses were exposed to drug-related material following administration to the dams. APV and APV-related material were also present in the milk of lactating rats.

- Hepatic clearance was the principal route of excretion for APV. Quantitatively, the main routes of metabolism in rats and humans were a di-oxidation on the tetrahydrofuran moiety of the molecule and an additional site of oxidation on the aniline ring portion of the molecule. Metabolism in dogs was different than that in rats and humans, with unchanged APV the principal component in excreta and the product of di-oxidation on the tetrahydrofuran moiety only a minor component.

- Approximately 15% of an oral dose in humans and 4-11% of an oral dose in rats and dogs was excreted in urine. Unchanged APV was a minor component in the urine in all cases and no single urinary metabolite accounted for more than 1% of the dose in any species. The balance of the dose was excreted in faeces, with unchanged APV being a major component in dog faeces, and a minor component in both rat and human faeces.

- APV showed a small inductive effect on CYP3A in rats. Drugs that affect, or are affected by, CYP3A4 have a potential to interact with APV. APV inhibited CYP3A4 at concentrations similar to those seen with the marketed HIV protease inhibitors indinavir and nelfinavir (K_i = 0.6 μM).

- APV interacts with the transmembrane efflux pump, P-glycoprotein, and this may be an important factor in the disposition of APV.

3. PHARMACOKINETICS

Studies investigating the absorption and excretion of APV and GW433908X after single and repeated administration of APV or GW433908G have been performed in mice, rats, rabbits and dogs. The locations of these reports in the submission are listed in Table G1. An inter-species comparison of plasma levels following repeat oral administration is presented in Subsection 8, below.

In all repeat-dose studies, APV, GW433908A or GW433908G was administered daily in two equal portions to increase exposure to APV. The second portion was given 6 hours after the first. The 6-hour interval was selected to aid technical efficiency and was not based on the kinetics of APV or GW433908G. Where GW433908X:APV exposure ratios are quoted, the comparison used APV exposure multiplied by 1.158 to normalise to molecular weight of GW433908X, and the ratio is presented as a % value.

3.1. Mouse

3.1.1. Oral administration

The pharmacokinetics of GW433908X and APV have been assessed during single- and repeat-dose studies in CD-1 mice following oral administration. Additionally, exposure to APV was assessed during a 104-week carcinogenicity study with APV in mice.

Pharmacokinetics after single doses

The results from these studies are summarised in Table G2.

To complete the pharmacokinetic profile of APV in mice, a single-dose study was carried out where male CD-1 mice received ^{14}C-APV formulated in Vitamin E-TPGS/PEG 400/propylene glycol by oral gavage at a dose of 150 mg/kg (Report RD2001/00558/00). Blood samples were taken from 4 males per timepoint by cardiac puncture from 0 (predose) to 24 hours post-dose, and analysed for APV and total radiocarbon (TRC) in plasma and whole blood, respectively.

Values of T_{max} were 1.0 hour post-dosing for both APV and TRC, indicating rapid absorption. Exposure to APV, in terms of AUC, was 48% of that for total radiocarbon indicating circulating metabolites. Plasma TRC levels were greater than whole blood levels, indicating minimal association of TRC with formed elements of the blood.

The pharmacokinetics of APV and GW433908X have been assessed following a single oral dose of 150 mg/kg ^{14}C-GW433908G (105 mg/kg APV-equivalents) to male CD-1 mice (Report RD2001/00560/00). Blood samples were taken from 4 males per timepoint by cardiac puncture from 0 (predose) to 24 hours post-dose, and analysed for GW433908X and APV in plasma, and TRC in whole blood.

Table G1. List of Single and Repeat Dose Pharmacokinetic Studies Performed With GW433908 or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage† (mg/kg)	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Mouse (CD-1)	M	Oral (gavage)	APV	[150]	Single Dose	Pharmacokinetic study.	No	/GSK	RD2001/00558/00 (01AVV0003)	Vol 28 p001
Mouse (CD-1)	M	Oral (gavage)	G	150	Single Dose	Pharmacokinetic study.	No	/GSK	RD2001/00560/00 (01AVV0004)	Vol 29 p074
Mouse (CD-1)	M+F	Oral (gavage)	APV	[250] [500] [750] [1000]	13 weeks	Toxicokinetics during repeat dose toxicity study	Yes		RD1998/00926/00 (M40220) GSK Appendix 3	Vol 20 p581
Mouse (CD-1)	M+F	Oral	APV	[150] [300/275]^ [600/500]^	104 weeks	Toxicokinetics during carcinogenicity study	Yes		RD1998/02066/01 (M40221) GSK Appendix 2	Vol 23 p689
Mouse (CD-1)	M+F	Oral (gavage)	G	400 800 1600 3200	13 weeks	Toxicokinetics during repeat dose toxicity study	Yes		RD2000/02408/00 (M40725) GSK Appendix 2	Vol 19 p665
Rat (Han Wistar)	M+F	Oral (gavage)	G	110	Single Dose	Pharmacokinetic study.	No	/GSK	RD2002/00725/00 (98AVV0018)	Vol 29 p141

Key:
† = In terms of the relevant salt
[ ] = APV dose
^ = Dose levels reduced during Week 3.

Salt:
APV = Amprenavir
G = calcium salt

Testing Facility:
GSK = GlaxoSmithKline Inc, RTP, NC, USA

Table G1 (Continued). List of Single and Repeat Dose Pharmacokinetic Studies Performed With GW433908 or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage† (mg/kg)	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Rat (Han Wistar)	M	Oral (gavage)	A	150 550 2000	Single Dose	Toxicokinetics during safety pharmacology study	No	GSK	RD1998/00541/00 (R40357) Appendix 3	Vol 28 p214
Rat (Han Wistar)	M	Oral (gavage)	G	748 1495 2990	Single Dose	Toxicokinetics during micronucleus study	Yes	GSK	RD1999/00412/00 (R40476) Appendix 1	Vol 18 p240
Rat (Han Wistar)	M+F	Oral (gavage)	APV	[50] [50]* [190] [750]	104 weeks	Toxicokinetics during carcinogenicity study	Yes		RD1998/01521/02 (R40222) GSK Appendix 2	Vol 27 p484
Rat (Han Wistar)	M	Oral (gavage)	A	50 190 750	14 days	Toxicokinetics during repeat dose toxicity study	Yes	GSK	RD1998/00711/00 (R40364) Appendix 3	Vol 2 p082
Rat (Han Wistar)	M+F	Oral (gavage)	G	149 478 1493 2240	4 weeks	Toxicokinetics during repeat dose toxicity study	Yes	GSK	RD1998/02573/00 (R40427) Appendix 3	Vol 3 p614
Rat (Han Wistar)	M+F	Oral (gavage)	G	149 478 1493 2240	26 weeks	Toxicokinetics during repeat dose toxicity study	Yes		RD1998/02858/01 (R40417) GSK Appendix 2	Vol 7 p974

Key:
† = In terms of the relevant salt
* = High excipient concentration

Salt:
G = calcium salt
A = disodium salt

Testing Facility: GSK = GlaxoSmithKline Inc. RTP. NC. USA

Table G1 (Continued). List of Single and Repeat Dose Pharmacokinetic Studies Performed With GW433908 or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage† (mg/kg)	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Rat (Wistar Han)	M+F	Oral (gavage)	G	1942 1942‡	14 days	Toxicokinetics during repeat dose toxicity study with impurities	Yes	GSK	RD2000/01884/00 (R40857) Appendix 2	Vol 2 p408
Rat (Wistar Han)	M+F	Oral (gavage)	G	2240 2240‡	14 days	Toxicokinetics during repeat dose toxicity study with impurities	Yes	GSK	RD2001/0212/01 (R40917) Appendix 2	Vol 3 p125
Rat (Wistar Han)	M+F	Oral (gavage)	G	50 150 450 900	15 Days	Toxicokinetics during juvenile toxicity study.	No		RD2000/02506/00 (R40877) GSK Appendix 2	Vol 8 p629
Rat (Wistar Han)	M+F	Oral (gavage)	G	5 10 20 40 80 160	4 weeks	Toxicokinetics during juvenile toxicity study.	Yes		RD1999/02344/00 (R40576) GSK Appendix 2	Vol 9 p507
Rat (Wistar Han)	M+F	Oral (gavage)	G	100 175 300	13 weeks	Toxicokinetics during juvenile toxicity study.	Yes		RD2002/00045/00 (R40860) GSK Appendix 2	Vol 11 p573

Key: † = In terms of the relevant salt
‡ = Spiked with drug substance impurities
[ ] = APV dose

Salt:
G = calcium salt
APV = amprenavir

Testing Facility: GSK = GlaxoSmithKline Inc, RTP, NC, USA

Table G1 (Continued). List of Single and Repeat Dose Pharmacokinetic Studies Performed With GW433908 or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage† (mg/kg)	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Rat (Sprague Dawley)	M+F	Oral (gavage)	G	300 820 2240	Up to 42 days (male) or 35 days (females)	Toxicokinetics during fertility study.	Yes		RD1999/01281/00 (R40458) GSK Appendix 3	Vol 16 p317
Rat (Sprague Dawley)	F	Oral (gavage)	G	300 820 2240	Day 6 to 17 of pregnancy	Toxicokinetics during embryofetal study.	Yes		RD1999/02690/00 (R40470) GSK Appendix 2	Vol 16 p572
Rat (Han Wistar)	M	ID	A	150 550 2000	Single Dose	Toxicokinetics during safety pharmacology study	No	GSK(U)	WD1999/00154/00 (S22240) Appendix 1	Vol 28 p271
Rabbit (New Zealand White)	F	Oral (gavage)	G	149.5 373.8 747.5 1121.3 1495	14 Days	Dose range-finding in non-pregnant animals	No		RD1999/00465/00 (L40459) GSK Appendix 2	Vol 16 p656
Rabbit (New Zealand White)	F	Oral (gavage)	G	74.8 149.5 224.3 299	Day 7 to 20 of pregnancy	Dose range-finding in pregnant animals	No		RD1999/00716/00 (L40460) GSK Appendix 2	Vol 16 p802

Key:
† = In terms of the relevant salt
ID = Intraduodenal

Salt:
X = Free ester
G = calcium salt
A = disodium salt

Testing Facility:
GSK (U) = GlaxoSmithKline Research And Development (UK), Ware, UK

Table G1 (Continued). List of Single and Repeat Dose Pharmacokinetic Studies Performed With GW433908 or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage† (mg/kg)	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Rabbit (New Zealand White)	F	Oral (gavage)	G	74.8 224.3 672.8	Day 7 to 20 of pregnancy	Toxicokinetics during embryofetal development study.	Yes		RD1999/01035/00 (L40461) GSK Appendix 2	Vol 17 p312
Dog (Beagle)	M	Oral (gavage)	G	24	Single Dose	Pharmacokinetic study.	No	/GSK	RD2002/00724/00 (98AVV0017)	Vol 29 p216
Dog (Beagle)	M	Oral (gavage)	X A G	360 250 418 418 434	Single dose	Bioavailability of salts	No	GSK	RD1998/03011/01 (98APK0034)	Vol 29 p297
Dog (Beagle)	M	Oral (gavage)	G	800	Single dose	Pharmacokinetics of liquid formulations	No	GSK	RD1999/00927/00 (99APK0030)	Vol 29 p311
Dog (Beagle)	M	Oral (gavage)	A	150 550 2000	Single Dose	Toxicokinetics during safety pharmacology study	No	GSK (U)	WD1999/00155/00 (S22321) Appendix 3	Vol 28 p239
Dog (Beagle)	M	Oral (gavage)	A	150 550 2000	Single Dose	Toxicokinetics during safety pharmacology study	No		WD1998/00543/00 (S22241) GSK Appendix 1	Vol 28 p358

Key:
† = In terms of the relevant salt

Salt:
X = Free ester
G = calcium salt
A = disodium salt

Testing Facility:
GSK = GlaxoSmithKline Inc, RTP, NC, USA
GSK (U) = GlaxoSmithKline Research And Development (UK), Ware, UK

Table G1 (Continued). List of Single and Repeat Dose Pharmacokinetic Studies Performed With GW433908 or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage† (mg/kg)	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Dog (Beagle)	M	Oral (gavage)	A	50 130 350	14 days	Toxicokinetics during toxicity study	Yes	GSK	RD1998/00487/00 (D40350) Appendix 1	Vol 12 p089
Dog (Beagle)	M	Oral (gavage)	G	75 194 523 747	4 weeks	Toxicokinetics during toxicity study	Yes	GSK	RD1998/02605/00 (D40436) Appendix 1	Vol 12 p461
Dog (Beagle)	M	Oral (gavage)	G	75 195 337	40 weeks	Toxicokinetics during toxicity study	Yes		RD1998/02861/01 (D40418) GSK Appendix 2	Vol 15 p544
Dog (Beagle)	M	Intravenous	A	30 100	Single Dose	Toxicokinetics during safety pharmacology study	No		WD1998/00588/01 (S22365) GSK Appendix 1	Vol 28 p

Key:
† = In terms of the relevant salt

Salt:
G = calcium salt
A = disodium salt

Testing Facility: GSK = GlaxoSmithKline Inc, RTP, NC, USA
GSK (U) = GlaxoSmithKline Research And Development (UK), Ware, UK

Table G2. Single Oral Dose Pharmacokinetics in the CD-1 Mouse

Dose (mg/kg) [amprenavir equivalents]	Sex	Analyte	Pharmacokinetic Parameters			
			C_{max} (µg/mL)	T_{max} (h)	AUC_{24} (h*µg/mL)	APV:GW433908X AUC ratio
Report RD2001/00558/00						
[150]	M	APV	17.4	1.0	45.2	-
		TRC	26.0	1.0	98.7	-
Report RD2001/00560/00						
150 [105]	M	GW433908X	0.0063	1.0	0.0047	0.02
		APV	9.88	1.0	21.7	
		TRC	16.1	1.0	45.4	-

Key: APV = Amprenavir; TRC = Total Radiocarbon

The T_{max} for all three analytes was 1-hour post-dose, the only timepoint GW433908X was detectable. The calculated AUC for GW433908X was 0.02% of the AUC for APV, indicating extensive conversion of GW433908X to APV. Similar to the APV study in mice above, a higher TRC AUC compared to APV indicated circulating metabolites. Generally, TRC concentrations were higher in the plasma compared to blood, suggesting minimal association with formed elements of the blood.

3.1.1.1. Pharmacokinetics after repeated administration

Exposure to GW433908X and APV was assessed during a 13-week pilot study in mice carried out with GW433908G. Additionally, exposure to APV was assessed in mice during a 13-week dose-ranging study and a carcinogenicity study carried out with APV. For clarity of review, the APV studies are summarised first, followed by the GW433908G study. Results are presented in Table G3 and Table G4, respectively.

Amprenavir studies

In the 13-week study with APV, male and female CD-1 mice received APV at 250, 500, 750 and 1000 mg/kg/day (Report RD1998/00926/00). APV was formulated as a 12.2% w/w solution in PEG400 (60%w/w), TPGS (23%w/w) and propylene glycol (PG, 4.6%w/w). Blood samples for toxicokinetic analysis were collected on Days 1 and 90. APV was not detected in any control samples.

Due to a dosing error, mice received only the first of their two daily doses on Day 90. Pharmacokinetic parameters were therefore only calculated to 6 hours post first dose to enable direct comparison of Day 1 and 90 data. Estimates of T_{max} were generally 1 to 2 hours after the last dose for the day. Both C_{max} and AUC estimates were dose-related, but not dose-proportional. After allowing for the halving of doses on Day 90, both C_{max} and AUC values were reduced on Day 90, a finding similarly to the 13-week study with GW433908G and indicating possible enzyme induction (see Subsection 6.1.5).

Table G3. Repeat Dose Pharmacokinetics in the CD-1 Mouse following Administration of Amprenavir

Dose (mg/kg/day)	Day/ Week^	Mean Amprenavir Pharmacokinetic Parameters					
		AUC (h*µg/mL) ‡		C_{max} (µg/mL)		T_{max} (h)	
		M	F	M	F	M	F
Report RD1998/00926/00							
250	1	27.9	31.0	8.30	9.46	1	2
	90†	10.0	10.2	2.75	3.67	2	1
500	1	39.9	37.4	21.8	16.3	1	2
	90†	9.50	27.6	3.39	7.41	1	2
750	1	57.7	56.5	35.4	26.7	2	4
	90†	18.2	22.8	6.53	9.08	1	1
1000	1	53.4	79.9	55.6	45.8	2	2
	90†	33.1	31.6	8.60	9.41	2	2
Report RD1998/02066/01							
150	1	29.5	77.1	3.30	8.36	3	3
	52	28.0	52.9	10.3	8.65	1	1
300/275	1	85.6	114	7.52	10.2	3	3
	52	68.0	100	12.7	13.0	1	1
600/500	1	86.9	66.2	7.44	9.10	3	1
	52	62.9	95.3	11.0	15.5	1	1

Key:
† = First half of dose administered only, therefore dose levels halved on Day 90;
‡ = 0-6 hours for the 13 week study, 0-24 for the carcinogenicity study
^ = Day (13 week study)/ Week (carcinogenicity study)

In the APV carcinogenicity study, groups of male and female CD-1 mice were administered APV, formulated in PEG/TPGS/PG, at 150, 300 and 600 mg/kg/day for 104 weeks (Report RD1998/02066/01). Due to high mortality at 600 mg/kg/day, the high dose was reduced to 500 mg/kg/day during Week 3. To maintain dose proportionality, the mid dose was reduced to 225 mg/kg/day. Blood samples for toxicokinetic analysis were taken during Weeks 1 and 52 (24 hour exposure data), and Weeks 26 and 78 (7 hours post dose only, the approximate T_{max}). Samples were also taken 7 and 24 hours after the first dose during Week 104; however these were not available for analysis.

APV was detected in 9 out of 12 control samples in Week 52 and 1 out of 12 control samples in Week 26. The presence of APV in these samples could have been due to either misdosing or sample contamination. However, plasma APV concentrations in the control groups were very low, and were not considered to have effected the conclusion or validity of the study.

During Weeks 1 and 52, APV C_{max} and AUC_{24h} generally increased, but not dose proportionally, between the low and mid dose groups, and generally decreased or remained unchanged between the mid and high dose groups. APV T_{max} values were 1.0 to 4.0 hours after the first or second daily dose. Values of $t_{½}$ were 1.7 to 3.7 hours. Plasma APV concentrations at 7 hours after the first dose were generally similar within each dose group during Weeks 26 and 78, and similar to 7 hour APV concentrations determined during Weeks 1 and 52.

GW433908G study

In the 13-week study with GW433908G (Report RD2000/02408/00), male and female CD-1 mice were administered GW433908G at dose levels of 400, 800, 1600, and 3200 mg/kg/day (281, 562, 1125, or 2250 mg/kg/day APV-equivalents). Blood samples for toxicokinetic analysis were collected on Days 1 and 90.

GW433908X was detected in 2 out of 6 control samples on Day 1, and 1 out of 6 control samples on Day 90 at concentrations just above the 5 ng/mL lower limit of quantification. APV was not detected in control samples on Day 1 or Day 90. Since concomitant APV was not detected, the GW433908X present in the samples was considered due to contamination, and not misdosing or mishandling.

Exposure to both APV and GW433908X was demonstrated in all treated groups during the study. Day 1 exposure (AUC) to APV only increased at 3200 mg/kg/day in males and ≥1600 mg/kg/day in females. At steady state (Day 90), exposure decreased compared to Day 1, particularly at the lower doses such that AUC was dose-related in both males and females. GW433908X exposure showed no relationship to dose on Day 1, particularly in females where extremely high values were noted at 400, 1600 and 3200 mg/kg/day. By Day 90, exposure was generally dose-related in males and females. Exposure to GW433908X generally increased on repeat dosing in males; due to the high variability on Day 1, any effect of repeat dosing could not be determined in females. This high variability also precluded meaningful evaluation of Day 1 GW433908X: APV exposure ratios. On Day 90, when GW433908X exposure was less variable, exposure ratios were generally dose-related and <3%, except in females at 1600 mg/kg/day, where a ratio of 6.6% was seen. There were no discernible sex differences in either APV or GW433908X exposure.

3.2. Rat

3.2.1. Oral Administration

3.2.1.1. Studies in adult nonpregnant rats

The pharmacokinetics of GW433908X and APV have been assessed during single and repeat dose studies carried out with GW433908G in nonpregnant rats following oral administration. Additionally, exposure to APV was assessed during a 104-week carcinogenicity study with APV in rats.

Table G4. Repeat Dose Pharmacokinetics in the CD-1 Mouse following administration of GW433908G (Report RD2000/02408/00)

Sex	Dose (mg/kg/day) [amprenavir equivalents]	Day	Mean Pharmacokinetic Parameters						
			AUC† (h*µg/mL)		C_{max}(µg/mL)		T_{max} (h) ‡		GW433908X: Amprenavir ratio (%)^
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
M	400 [281]	1	0.492	141	0.014	11.8	2	1	0.30
		90	0.095	57.5	0.027	12.7	1	1	0.14
	800 [562]	1	0.063	79.0	0.030	12.6	1	1	0.07
		90	0.240	72.5	0.061	12.7	1	1	0.29
	1600 [1125]	1	0.227	138	0.025	19.7	2	1	0.14
		90	2.738	143	0.444	13.4	6	2	1.7
	3200 [2250]	1	1.861	377	0.154	15.4	2	1	0.43
		90	11.90	359	1.056	29.2	4	2	2.9
F	400 [281]	1	140.0	108	16.30	12.5	4	1	112
		90	0.194	55.5	0.031	9.02	1	2	0.30
	800 [562]	1	0.235	110	0.033	10.5	1	2	0.18
		90	0.440	78.7	0.103	11.6	1	2	0.48
	1600 [1125]	1	526.7	287	65.77	21.9	4	4	158
		90	14.19	187	1.597	16.3	4	1	6.6
	3200 [2250]	1	440.4	410	62.70	31.1	18	18	93
		90	10.10	352	0.954	29.1	2	2	2.5

Key: † = AUC_{∞} on Day 1 and AUC_{24h} on Day 90; ‡ = time to C_{max} after first or second daily dose; ^ = Calculated using mean AUC data

Pharmacokinetics after single doses

The results from these studies are summarised in Table G5.

The pharmacokinetics of APV and GW433908X have been assessed following a single oral dose of 110 mg/kg ^{14}C-GW433908G (approximately 78 mg/kg APV-equivalents, uncorrected for impurities) to male Han Wistar rats (Report RD2002/00725/00). Blood samples were taken according to a composite sampling regimen from 3 groups of 3 males. Samples were taken from 0 (predose) to 24 hours post-dose, and analysed for GW433908X, APV and total radiocarbon (TRC).

The T_{max} for all three analytes was 0.5 to 1-hour post-dose, indicating rapid absorption. GW433908X was only detected at 0.5 and 1.0 hours post-dose, and the calculated AUC for GW433908X was 0.07% the AUC for APV, indicating extensive conversion of GW433908X to APV. The AUC for TRC was approximately 2-fold that for APV, suggesting circulating metabolites. The ratio of blood to plasma TRC concentrations increased at later timepoints, particularly 12 and 24 hours post-dose. These data suggest some association of radiocarbon with formed elements at these later timepoints.

Table G5. Single Dose Pharmacokinetics in the Han Wistar Rat.

Dose (mg/kg) [amprenavir equivalents]	Sex	Pharmacokinetic Parameters				
Report RD2002/00725/00						
110 [78]	M	Analyte	C_{max} (µg/mL)	T_{max} (h)	AUC (h*µg/mL)	GW433908X:APV AUC ratio
		GW433908X	0.0125	0.5	0.0105	0.07
		APV	4.31	1.0	13.4	-
		TRC	8.99	0.5	28.6	-
Report RD1998/00541/00						
		3 Hour Plasma Concentration(µg/mL)				GW433908X: Amprenavir ratio (%)~
		GW433908X	Amprenavir			
150 [112]	M	0.0087	3.31			0.22
550 [410]	M	0.095	6.92			1.19
2000 [1493]	M	0.603	9.53			4.47
Report RD1999/00412/00						
		8 Hour Plasma Concentration(µg/mL)				GW433908X: Amprenavir ratio (%)~
		GW433908X	Amprenavir			
748 [500]	M	0.0097	8.16			0.10
1495 [1000]	M	0.041	10.1			0.31
2990 [2000]	M	0.057	11.9			0.41

Key: ~ = calculated using individual animal data; APV = Amprenavir; TRC = Total Radiocarbon

Plasma concentrations of GW433908X and APV were determined 3-hours post-dose during safety pharmacology study in male Han Wistar (Report RD1998/000541/00). In this study, rats received GW433908A orally at doses of 150, 550 or 2000 mg/kg (112, 410 or 1493 mg/kg APV-equivalents).

Plasma concentrations of APV and GW433908X increased with increasing dose, but not in a dose-proportional manner. The GW433908X: APV ratio was also dose-related and are similar to values observed in the 14-day repeat dose study in male rats (Report RD1998/00711/00, see below).

Exposure to APV and GW433908X was determined during a micronucleus study where male Han Wistar rats received a daily dose of GW433908G at 748, 1495 or 2990 mg/kg (500, 1000 and 2000 mg/kg APV-equivalents). Animals received GW433908G in two equal portions, the second portion given 6 hours after the first. Plasma samples were collected 8 hours following the first dose (Report RD1999/00412/00).

Plasma APV and GW433908X concentrations 8 hours after the first dose were dose-related, but not dose-proportional. The GW433908X:APV ratio increased with dose, but was lower than those seen during the safety pharmacology study at similar doses.

Pharmacokinetics after repeated administration

Exposure to GW433908X and APV to rats was assessed in repeat dose-studies. Additionally, exposure to APV was assessed in rats during a carcinogenicity study carried out with APV. For clarity of review, the APV study is summarised first, followed by the GW433908A or GW433908G studies. Results are presented in Table G6 and Table G7, respectively.

Amprenavir study

In the APV carcinogenicity study, groups of male and female Han Wistar rats were administered APV, formulated in PEG/TPGS/PG, twice daily at 50, 190 and 750 mg/kg/day for 104 weeks (Report RD1998/01521/02). An additional group of animals received APV at 50 mg/kg/day in a formulation containing the same concentration of excipients as the high-dose group. Blood samples for toxicokinetics analysis were taken during Weeks 1 and 52 (24-hour exposure), and during Weeks 26, 72 and 104 (7 hours after the first daily dose only).

APV was detected in 32 out of 53 control plasma samples. The presence of APV in these samples could have been due to either misdosing or sample contamination. However, plasma APV concentrations in the control groups were generally very low, except for a Week 26 vehicle control female (2.26 µg/mL) and the Week 104 water controls (means of 0.33 and 0.29 µg/L, for males and females, respectively). The presence of low levels of APV in these control samples was not considered to have effected the conclusion or validity of the study.

Table G6. Repeat Dose Pharmacokinetics in the Han Wistar Rat following Administration of Amprenavir

Dose (mg/kg/day)	Week	Mean Amprenavir Pharmacokinetic Parameters					
		AUC_{24} (h*µg/mL)		C_{max}(µg/mL)		T_{max} (h)	
		M	F	M	F	M	F
Report RD1998/01521/02							
50	1	18.1	25.8	2.77	3.08	1	1
	52	22.0	30.1	2.89	3.34	1	1
50^	1	10.0	25.0	1.79	3.42	2	1
	52	16.6	26.8	2.36	2.47	2	1
190	1	46.4	53.5	4.46	4.18	1	2
	52	62.3	62.4	7.11	7.97	2	1
750	1	73.0	69.0	7.06	6.27	1	2
	52	123	117	14.8	12.3	1	1

Key: ^ = Higher excipient concentration.

Exposure generally increased between doses in a less than dose-proportional manner. APV T_{max} values were 1 to 2 hours after first daily doses, and estimates of $t_{1/2}$ ranged from 2.4 to 6.7 hours. Generally, exposure (AUC or C_{max}) was similar throughout the study, except in the Week 1 mid- and high-dose groups, when lower exposure was noted compared to other time points in the study. However, Week 1 samples were taken on Day 4 for all dose groups and the mid- and high-dose values were similar to those obtained on Day 31 in a 26-week study with APV at similar dose levels (see Report RD1996/00584/00 summary, Appendix G3). As autoinduction was observed in the 26-week study, these results indicate that autoinduction may have occurred by Day 4 in the mid- and high-dose groups.

GW433908A or GW433908G studies

Plasma concentrations of GW433908X and APV were determined on Days 1 and 14 of a 14-day repeat dose study in male Han Wistar rats (Report RD1998/00711/00). Rats received daily doses of GW433908A at 50, 190 or 750 mg/kg/day (37.3, 142 or 560 mg/kg/day APV-equivalents). Blood samples for toxicokinetic analysis were collected at intervals between 0 and 24 hours after the first portion of the daily dose on Days 1 and 14. Plasma concentrations of GW433908X were determined only for the high-dose group, whereas APV concentrations were determined for all dose groups.

Exposure (C_{max} and AUC) to APV increased with increasing dose, and was lower on Day 14 compared to Day 1 at 190 or 750 mg/kg/day. GW433908X exposure at the high dose increased on Day 14 compared to Day 1. These changes in exposure on repeat dosing resulted in the GW433908X:APV exposure ratio increasing on Day 14 compared to Day 1. The decrease in APV exposure on repeat dosing is consistent with enzyme induction, and was seen following repeat dosing with APV.

Table G7. Repeat Dose Pharmacokinetics in the Non-pregnant Adult Han Wistar Rats

			Mean Pharmacokinetic Parameters						
14-Day Study with GW433908A (Report RD1998/00711/00)									
Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	AUC (h*µg/mL) †		C_{max}(µg/mL)		T_{max} (h) ‡		GW433908X: Amprenavir AUC ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
50 [37.3]	M	1	-	3.78	-	0.59	-	2	-
		14	-	4.06	-	0.71	-	2	-
190 [142]		1	-	26.0	-	4.20	-	2	-
		14	-	11.5	-	2.21	-	2	-
750 [560]		1	0.388	105	0.078	7.34	1	1	0.32
		14	0.637	50.2	0.167	3.82	1	1	1.1
4-Week Study with GW433908G (Report RD1998/02573/00)									
Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	AUC (h*µg/mL) †		C_{max}(µg/mL)		T_{max} (h) ‡		GW433908X: Amprenavir AUC ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
149 [100]	M	1	15.269	32	2.148	3.00	18.0	2.0	41.2
		23	0.546	19.9	0.144	1.63	6.0	4.0	2.37
478 [320]		1	2.832	62.4	0.341	5.61	18.0	2.0	3.92
		23	1.636	33.8	0.570	3.48	6.0	1.0	4.18
1493 [1000]		1	1.569	224	0.146	9.60	18.0	2.0	0.60
		23	0.786	47.2	0.276	5.38	1.0	1.0	1.44
2240 [1500]		1	1.419	184	0.122	11.3	1.0	2.0	0.67
		23	1.200	53.1	0.147	4.54	2.0	6.0	1.95

Key: † = AUC_{∞} on Day 1 or AUC_{24} on succeeding days, except for the 2-week study when all as AUC_{∞}; ‡ = time to C_{max} after first or second daily dose; ~ = Calculated using mean AUC data

Table G7 (Continued). Repeat Dose Pharmacokinetics in Non-pregnant Adult Han Wistar Rats

			Mean Pharmacokinetic Parameters						
4-Week Study with GW433908G (Report RD1998/02573/00) (Continued)									
Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	AUC (h*µg/mL) †		C_{max}(µg/mL)		T_{max} (h) ‡		GW433908X: Amprenavir AUC ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
149 [100]	F	1	3.182	31.2	0.438	2.42	18.0	1.0	8.80
		23	0.067	23.0	0.010	1.76	1.0	1.0	0.25
478 [320]		1	0.993	83.1	0.132	7.12	6.0	2.0	1.03
		23	0.636	49.9	0.062	4.30	2.0	1.0	1.10
1493 [1000]		1	1.771	133	0.488	11.1	1.0	1.0	1.15
		23	1.245	68.1	0.281	4.89	2.0	4.0	1.58
2240 [1500]		1	1.773	234	0.174	10.4	18.0	6.0	0.66
		23	1.578	80.8	0.420	5.38	1.0	1.0	1.69
26-Week Study with GW433908G (Report RD1998/02858/01)									
Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	AUC (h*µg/mL) †		C_{max}(µg/mL)		T_{max} (h) ‡		GW433908X: Amprenavir AUC ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
149 [100]	M	1	0.052	12.7	0.021	2.55	1.0	2.0	0.36
		92	0.124	15.5	0.022	1.98	1.0	2.0	0.69
		179	0.030	19.8	0.015	2.90	2.0	2.0	0.13
478 [320]		1	0.156	84.4	0.036	7.54	2.0	2.0	0.16
		92	0.367	48.0	0.045	3.97	1.0	4.0	0.66
		179	0.229	46.2	0.032	5.09	1.0	2.0	0.43

Key: † = AUC_{∞} on Day 1 or AUC_{24} on succeeding days, except for the 2-week study when all as AUC_{∞}; ‡ = time to C_{max} after first or second daily dose; ~ = Calculated using mean AUC data

Table G7 (Continued). Repeat Dose Pharmacokinetics in Non-pregnant Adult Han Wistar Rats

			Mean Pharmacokinetic Parameters						
26-Week Study with GW433908G (Report RD1998/02858/01) (continued)									
Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	AUC (h*µg/mL) †		C_{max}(µg/mL)		T_{max} (h) ‡		GW433908X: Amprenavir AUC ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
1493 [1000]	M	1	1.575	154	0.050	9.70	2.0	1.0	0.88
		92	1.432	57.8	0.152	6.44	1.0	2.0	2.14
		179	0.930	57.0	0.102	5.33	1.0	2.0	1.41
2240 [1500]		1	0.616	243	0.047	8.57	1.0	2.0	0.22
		92	2.597	63.0	0.353	5.71	1.0	2.0	3.56
		179	1.079	54.9	0.117	5.28	1.0	2.0	1.70
149 [100]	F	1	0.034	33.3	0.0097	3.32	1.0	2.0	0.09
		92	0.130	19.2	0.029	2.23	1.0	2.0	0.60
		179	0.271	22.7	0.102	2.31	2.0	2.0	1.03
478 [320]		1	0.299	74.1	0.132	8.52	1.0	1.0	0.35
		92	0.368	51.7	0.047	5.01	1.0	2.0	0.61
		179	3.267	54.3	0.381	5.23	4.0	1.0	5.20
1493 [1000]		1	4.719	237	0.069	13.1	4.0	4.0	1.72
		92	1.681	76.5	0.233	6.84	1.0	2.0	1.90
		179	1.001	62.3	0.184	7.28	1.0	1.0	1.39
2240 [1500]		1	1.256	253	0.124	9.85	2.0	1.0	0.43
		92	2.421	93.1	0.322	7.29	1.0	1.0	2.25
		179	1.815	107	0.143	7.68	1.0	2.0	1.46

Key: † = AUC_{∞} on Day 1 or AUC_{24} on succeeding days, except for the 2-week study when all as AUC_{∞}; ‡ = time to C_{max} after first or second daily dose; ~ = Calculated using mean AUC data

The pharmacokinetics of APV and GW433908X were determined during a 4-week repeat dose toxicity study in male and female Han Wistar rats (Report RD1998/02573/00). In this study, rats received GW433908G at 149, 478, 1493 or 2240 mg/kg/day (100, 320, 1000 or 1500 mg/kg/day APV-equivalents). Blood samples for toxicokinetic analysis were collected at intervals between 0 and 24 hours after the first portion of the daily dose on Days 1 and 23 for pharmacokinetic analysis.

GW433908X was detected in 5 out of 6 control animals on Day 1 at concentrations similar to those seen at 149 or 478 mg/kg/day, which may have resulted in over-estimation of plasma GW433908X concentrations in treated animals. GW433908X was detected in 1 out of 6 control samples on Day 23 at a concentration close to the limit of detection. As no APV was detected in any control samples on either day, the presence of GW433908X in control samples was considered due to contamination and not misdosing or mishandling. As there was effectively insignificant contamination at steady-state, safety margins for human exposure could be drawn from this study. Furthermore, definitive margins were calculated by using data from the 26-week study.

Exposure to APV increased with increasing dose in a less than dose-proportional manner on Days 1 and 23, except for the 2240 mg/kg/day male dose group on Day 1 where a fall in exposure compared to 1493 mg/kg/day was noted. Exposure to APV decreased on repeat dosing suggestive of autoinduction, consistent with previous studies with APV and the other repeat-dose studies with GW433908. On Day 1, exposure to GW433908X was inversely proportional to dose, whereas on Day 23, exposure to GW433908X was unrelated to dose in male rats, and increased in relation to dose, but not dose-proportionally, in female rats. Exposure ratios were generally less than 2%, with exceptions (males and females at 149 mg/kg/day on Day 1, and males at 478 mg/kg/day on Days 1 and 23) due to anomalously high plasma GW433908X levels at 6 or 24 hours. No sex-related differences in exposure were seen for either analyte.

Exposure to GW433908X and APV were determined during a 26-week repeat dose study in male and female Han Wistar rats (Report RD1998/02858/01) where rats received daily doses of 149, 478, 1493 or 2240 mg/kg/day GW433908G (100, 320, 1000 or 1500 mg/kg/day APV-equivalents). Blood samples for toxicokinetic analysis were collected at time points between 0 and 24 hours after the first portion of the daily dose on Days 1, 92 and 179. GW433908X was detected in 1 out of 8 control samples on Day 92 at a concentration at the limit of quantification. APV was not detected in any control samples.

Exposure to APV (C_{max} and AUC) was generally dose-related, but increased in a less than dose-proportional manner on Days 1, 92 and 179, with the exception of AUC_{∞} values in males in all dose groups on Day 1 and males in the 478 mg/kg/day GW433908G group on Day 92 where the increase was dose-proportional. Exposure decreased from Day 1 to Day 179, except in the 149 mg/kg/day males where exposure increased. The decrease in exposure is consistent with the previous APV and GW433908G repeat dose studies and indicative of autoinduction. Values of T_{max} for APV were between 1 to 2 hours, except in males at 478 mg/kg/day on Day 92 and females at 1493 mg/kg/day on Day 1 when T_{max} was 4 hours. Values of t½ were dose-related at between 1.1 to 2.5 hours at 149 mg/kg/day, to between 4.3 to 19.1 hours at 2240 mg/kg/day.

These results were consistent with the previous 14-day and 4-week studies (see summaries for Reports RD1998/00711/00 and RD1998/02573/00). When adjusted for APV dose, exposure to APV in the GW433908G studies was approximately 50% lower than the exposure observed following administration of APV during a 26-week oral toxicity study (see APPENDIX G3).

On Days 1 and 92, exposure to GW433908X generally increased between doses in a less than dose-proportional manner. On Day 179, AUC values for GW433908X generally increased in a greater than dose-proportional manner. There were no instances of anomalously high GW433908X plasma levels on Day 1 to correspond to the findings during the 4-week study (see Report RD1998/02573/00 summary). Values of T_{max} were between 1 and 4 hours on Days 1 and 179, at were 1 hour on Day 92 at all doses. Values for t½ were 1 to 45 hours on Day 1, between 1 and 24 hours on Day 92 and 4 to 14 hours on Day 179. Exposure estimates for GW433908X from the present study are similar to those from the previous 14-day and 4-week oral toxicity studies with GW433908. Exposure ratios (GW433908X:APV) were generally less than 2%. There were no sex-related differences in the pharmacokinetics of either analyte.

Impurity Studies

Two 14–day repeat dose toxicity studies have been carried out to assess the influences of various added drug substance impurities on the toxicology and toxicokinetics of GW433908G in Wistar Han rats. The results from these studies are summarised in Table G8.

In the first study, male and female rats received either GW433908G alone or GW433908G with added potential drug substance impurities 02, 13, ※ 14, 15, 08, 16, 03 and 09 (Report RD2000/01884/00). Animals received GW433908G with or without impurities at 1942 mg/kg/day (1575 mg/kg/day APV-equivalents). GW433908X was detected in 1 out of 8 control samples on Day 13 at a concentration similar to those seen in samples from rats administered GW433908G. As APV was not detected in any control samples, the presence of GW433908X in one control sample was considered due to sample or analytical contamination.

In the second study animals received either GW433908G alone or GW433908 with added drug substance impurity 09 at a higher concentration to that used in the first ※ study (Report RD2001/00212/01). Animals received GW433908G with or without 09 at 2240 mg/kg/day (1816 mg/kg/day APV-equivalents). GW433908X or APV were not detected in any control samples.

In both studies, blood samples for toxicokinetic analysis were taken 7 hours after the first daily dose (i.e. 1 hour after the second dose) on Days 1 and 13.

※ 新薬承認情報提供時に置き換えた

Table G8. Plasma GW433908X and Amprenavir Concentrations in Han Wistar Rats Following Administration With or Without Drug Substance Impurities

Dose (mg/kg) [Amprenavir equivalents]	Sex	7-Hour Plasma Concentration (μg/mL)			
Report RD2000/01884/00					
		GW433908X		Amprenavir	
		Day 1	Day 13	Day 1	Day 13
1942 [1575]	M	0.282	0.103	12.0	4.81
	F	0.133	0.157	10.3	5.43
1942 [1575]†	M	0.185	0.173	13.8	5.37
	F	0.071	0.088	9.42	7.11
Report RD2001/00212/01					
		GW433908X		Amprenavir	
		Day 1	Day 13	Day 1	Day 13
2240 [1816]	M	0.217	0.140	13.0	4.41
	F	0.094	0.135	13.9	5.61
2240 [1816]†	M	0.286	0.349	13.4	3.27
	F	0.057	0.141	7.98	5.73

Key: † = Spiked with potential drug substance impurities

The results from both studies demonstrated that the presence of impurities had no effect on either plasma APV or plasma GW433908X concentrations. Plasma levels were consistent with those from previous repeat-dose studies with GW433908G at similar dose levels. Plasma APV concentrations were lower on Day 13 compared to Day 1, consistent with autoinduction. Plasma levels of GW433908X were similar between Day 1 and 13. There were no sex differences noted for either analyte in either study.

3.2.1.2. Juvenile Studies

The results from toxicokinetic studies in juvenile rats are summarised in Table G9.

In a 15-day pilot study, male and female Wistar Han rat pups (Day 4 post partum on Dose Day 1) received GW433908G at 61, 184, 553 or 1105 mg/kg/day (43, 130, 389 or 777 mg/kg/day APV-equivalents). Blood samples for toxicokinetic analysis were taken 7 hours after the first daily dose (i.e. 1 hour after the second dose) on Dose Days 1 and 15 (Report RD2000/02506/00). GW433908X was not detected in any control samples. APV was detected in a control male on Day 18 at a low concentration (324 ng/mL) that may have resulted from either contamination or misdosing.

Exposure to both APV and GW433908X was demonstrated in all treated groups on Day 1, however high mortality at 553 or 1105 mg/kg/day meant plasma samples were not available from these groups on Day 15. Plasma concentrations of both APV and GW433908X were dose-related, but not dose-proportional. The GW433908X:APV ratio on Day 1 increased from <2.0% at 61 and 184 mg/kg/day to 19% at 1105 mg/kg/day. On

Day 14 the ratio was <1.0% in both surviving dose groups (61 and 184 mg/kg/day). No sex-related differences in plasma levels were noted for either analyte.

In a 30-day pilot study, male and female Han Wistar rat pups (Day 5 post partum on Dose Day 1) received GW433908G twice daily at 5, 10, 20, 40, 80, 160 mg/kg/day (3.3, 6.7, 13.4, 26.8, 53.5, 107 mg/kg/day APV-equivalents). Blood samples for toxicokinetic analysis were taken 7 hours after the first daily dose (i.e. 1-hour after the second dose) on Dose Days 6 and 30 (Report RD1999/02344/00).

Dose-related, but not dose-proportional, exposure to APV was demonstrated at all dose levels on Days 6 and 30. Plasma APV concentrations were generally lower on Day 30 than on Day 6, consistent with the adult repeat-dose studies with GW433908G. Plasma APV concentrations in this study were similar to those in the 15-day study at comparable dose levels. Plasma GW433908X levels were generally below quantification limit (BQL) at ≤10 mg/kg/day, and highly variable and unrelated to dose at ≥20 mg/kg/day. Similarly to the 15-day study, GW433908X: APV ratios varied greatly on Day 6 (Day 11 post partum), ranging from BQL at lower dose levels to 36 and 15% in females at 80 and 160 mg/kg/day, respectively. On Day 30 (Day 35 post partum), ratios were ≤5% at all dose levels. No sex-related differences in plasma levels were noted for either analyte.

GW433908X was detected in one control male on Day 6 at levels similar to those seen in treated animals. As APV was not detected in any control samples, the presence of GW433908X in one control sample was considered due to sample or analytical contamination.

Exposure to GW433908X and APV was assessed during a 13-week study where male and female Han Wistar rat pups (Day 4 post partum on Dose Day 1) received GW433908G a 100, 175 or 300 mg/kg/day (71, 124 or 213 mg/kg/day APV-equivalents). Blood samples for toxicokinetic analysis were taken 7 hours after the first daily dose (i.e. 1 hour after the second dose) on Dose Days 6 and 90 (Report RD2002/00045/00). APV or GW433908X were not detected in any control samples.

Plasma APV concentrations were dose-related and dose-proportional on Day 6. On Day 90, plasma levels increased less than dose-proportionally at 300 mg/kg/day. GW433908X concentrations were also dose-related on both days and were generally dose-proportional on Day 6. On Day 90, plasma concentrations increased greater than dose-proportionally in males and less than dose-proportionally in females. The plasma concentrations of both APV and GW433908X were similar to those obtained in the previous juvenile studies with GW433908G. However, on Day 6 APV concentrations were up to twice those on Day 8 in a juvenile study carried out with APV at similar dose levels, with the difference greatest at the higher dose levels (see Report RD1998/00618/00 summary, Appendix G3). By Day 30, plasma APV levels were similar to the Day 32 levels in the APV study. A decrease in APV concentration was seen on repeat dosing, consistent with the previous juvenile and adult studies with GW433908G and APV, and consistent with autoinduction. GW433908X:APV ratios were ≤1% at all dose levels on both sampling occasions.

Table G9. Amprenavir and GW433908X Plasma Levels in Juvenile Wistar Han Rats

Dose (mg/kg) [Amprenavir equivalents]	Sex	Mean Pharmacokinetic Parameters					
Report RD2000/02506/00							
		7-Hour Plasma Concentration (µg/mL)				GW433908X: Amprenavir ratio (%)~	
		GW433908X		Amprenavir			
		Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
61 [43]	M	0.029	0.0059	1.79	1.27	1.85	0.54
	F	0.041	0.0055	3.46	1.20	1.37	0.53
184 [130]	M	0.038	0.026	6.96	4.19	0.63	0.73
	F	0.036	0.019	3.81	4.33	1.09	0.52
553 [389]	M	1.567	NS	22.6	NS	8.02	NS
	F	1.590†	0.086‡	29.2†	12.3‡	6.30	0.81
1105 [777]	M	0.978	NS	22.9	NS	4.95	NS
	F	1.399	NS	8.72	NS	18.6	NS
Report RD1999/02344/00							
		7-Hour Plasma Concentration (µg/mL)				GW433908X: Amprenavir ratio (%)~	
		GW433908X		Amprenavir			
		Day 6	Day 30	Day 6	Day 30	Day 6	Day 30
5 [3.3]	M	BQL	0.0015	0.16	0.05	BQL	2.56
	F	BQL	BQL	0.19	0.08	BQL	BQL
10 [6.7]	M	BQL	BQL	0.35	0.07	BQL	BQL
	F	BQL	BQL	0.25	0.16	BQL	BQL
20 [13.4]	M	0.011	0.0072	0.61	0.12	1.49	5.17
	F	BQL	0.0035	0.99	1.02	BQL	0.30
40 [26.8]	M	0.222	0.0032	2.40	0.97	7.99	0.29
	F	0.021	BQL	2.24	0.96	0.80	BQL
80 [53.5]	M	0.086	0.012	6.80	1.07	1.09	0.97
	F	2.076	0.0059	5.04	1.81	35.6	0.28
160 [107]	M	0.096	0.028	8.21	4.62	1.01	0.53
	F	1.114	0.014	6.55	3.64	14.7	0.32

Key: Day = Day of dosing; BQL = Below Quantification Limit; NS = No sample due to mortality; † = mean of 2; ‡ = single sample only; ~ = Calculated using mean plasma data

Table G9 (Continued). Amprenavir and GW433908X Plasma Levels in Juvenile Wistar Han Rats

Dose (mg/kg) [Amprenavir equivalents]	Sex	7-Hour Plasma Concentration (µg/mL)				GW433908X: Amprenavir ratio (%)~	
		GW433908X		Amprenavir			
		Day 6	Day 90	Day 6	Day 90	Day 6	Day 90
Report RD2002/00045/00							
100 [71]	M	0.050	0.0059	5.04	1.23	0.86	0.42
	F	0.039	0.0066	4.96	1.49	0.68	0.38
175 [124]	M	0.102	0.015	8.97	2.42	0.98	0.52
	F	0.075	0.011	9.56	2.44	0.68	0.40
300 [213]	M	0.108	0.034	14.9	2.95	0.62	1.01
	F	0.120	0.015	13.0	2.95	0.79	0.43

Key: Day = Day of dosing; ~ = Calculated using mean plasma data

3.2.1.3. Studies in Pregnant Rats

Pharmacokinetics after repeated administration

The results from toxicokinetic studies in pregnant adult rats are summarised in Table G10.

The toxicokinetics of APV and GW433908X were assessed during a male and female fertility study in CD-Sprague Dawley rats following administration of GW433908G at 300, 820 and 2240 mg/kg/day (201, 548 and 1498 mg/kg/day APV-equivalents). Males were dosed from 4 weeks prior to mating until successful mating and females were dosed from 2 weeks prior to mating until Gestation Day 6. Blood samples for toxicokinetic analysis were collected at time points between 0 and 24 hours after the first portion of the daily dose on Dose Day 1, 27 and 42 and Dose Days 1, 13 and 28 in males and females, respectively (Report RD1999/01281/00).

GW433908X was detected in most vehicle control samples at generally higher concentrations than the low-dose group. However, plasma GW433908X concentrations varied randomly over the 24 hours sampling time, rather than following the concentration-time profile expected following dosing with GW433908G. Additionally, correlating APV concentrations were not detected in most control samples, with only 3 male control samples having detectable APV on Day 42 at levels close to the limit of detection. It was therefore considered the GW433908X detected in controls was due to sample contamination during either sample handling or analysis, rather than misdosing. The presence of GW433908X in 7 out of 24 plasma samples taken just prior to dosing on Day 1 in the higher dose groups (also without concomitant APV concentrations) supports this theory. It was possible that animals treated with GW433908G may have been exposed to lower levels of GW433908X than indicated from plasma analysis, however, contamination with APV was likely to be very low. Even accounting for possible sample contamination with GW433908X, plasma concentrations of GW433908X in humans

following administration of GW433908G are still likely to be lower than those achieved in rats in the fertility study. The apparent sample contamination was therefore believed not to invalidate the pharmacokinetic analyses or the conclusions drawn in the fertility study.

Exposure (C_{max} and AUC) to APV and GW433908X generally increased between doses in a less than dose-proportional manner in males and females. A decrease in APV exposure following repeat dosing seen in this study was consistent with previous studies and with autoinduction. There was no difference between exposure in females on Day 28 compared to Day 13, indicating no effect of pregnancy on pharmacokinetics of GW433908X and APV. The GW433908X:APV exposure ratios were generally ≤3%. Values of APV T_{max} were between 1 and 4 hours, whereas GW433908X T_{max} values were between 0 and 6 hours. Values for t½ for APV were dose-related and varied between 2 and 22 hours. Values for t½ for GW433908X could only be determined at 300 and 820 mg/kg/day in males on Days 1 and 27, 300 mg/kg/day in females on Day 1 and all dose levels in females on Day 13. In samples where it was calculated, GW433908X $t_{½}$ values ranged from 4.0 to 19 hours.

APV and GW433908X exposures were generally higher than that seen in the repeat-dose toxicity studies (see Subsection 3.2.1.1) and the oral embryofetal development study (see summary below) at similar dose levels. However, exposure to APV was similar to that seen in the APV female fertility and embryofetal development study at similar APV-equivalent dose levels (see Report WD1997/00284/00 summary, Appendix G3).

Exposure to GW433908X and APV was determined during an embryofetal development study in pregnant CD-Sprague Dawley rats on Gestation Days 6 and 17 (Report RD1999/02690/00). In this study animals received GW433908G at 300, 820 and 2240 mg/kg/day (201, 549 and 1498 mg/kg/day APV-equivalents). Blood samples for toxicokinetic analysis were collected at time points between 0 and 24 hours after the first portion of the daily dose on Gestation Days 6 and 17. GW433908X and APV were not detected in any control samples.

Exposure (C_{max} and AUC) to APV increased in a less than dose-proportional manner on Days 6 and 17. Values of AUC for APV decreased on Day 17 compared to Day 6, consistent with autoinduction. Exposure to GW433908X increased in a greater than dose proportional manner on Day 6, but not dose-proportionally on Day 17. The AUC estimates for GW433908X increased during the dosing period. Values of T_{max} for APV were 1 to 4 hours after one of the two daily doses, while T_{max} for GW433908X were 1 to 18 hours after one of the two daily doses. Values of t½ for APV were dose-related and ranged from 3 to 16 hours on Day 6 and 2 to 6 hours on Day 17. Values of t½ for GW433908X were unrelated to dose and ranged from 1 to 8 hours. Exposure ratios (GW433908X:APV) were <3% in all dose groups. The results from this study were in general agreement with those determined during the repeat-dose toxicity studies, indicating that there was no effect of pregnancy on the pharmacokinetics of GW433908X and APV.

Table G10. Pharmacokinetics in Pregnant Han Wistar Rats

Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	Mean Pharmacokinetic Parameters						
			AUC (h*µg/mL) †		C_{max} (µg/mL)		T_{max} (h)‡		GW433908X: Amprenavir ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
Report RD1999/01281/00									
300 [201]	M	1	0.698	87.4	0.267	8.36	1.0	1.0	0.69
		27	0.843	46.0	0.84.1	3.66	2.0	2.0	1.58
		42	0.949	49.9	0.144	5.19	0	2.0	1.64
820 [548]		1	1.087	196	0.221	14.4	1.0	4.0	0.48
		27	1.561	61.8	0.153	4.77	2.0	1.0	2.18
		42	3.186	88.9	1.395	7.35	1.0	4.0	3.09
2240 [1498]		1	1.675	392	0.192	15.4	2.0	2.0	0.37
		27	5.253	89.6	0.536	5.73	2.0	2.0	5.06
		42	4.548	110	0.363	7.43	6.0	2.0	3.57
300 [201]	F	1	0.714	91.7	0.039	8.11	2.0	1.0	0.67
		13	0.544	57.8	0.101	4.47	6.0	4.0	0.81
		28	0.559	59.6	0.100	4.56	1.0	2.0	0.81
820 [548]		1	1.068	168	0.091	8.88	2.0	2.0	0.55
		13	0.738	57.0	0.061	4.52	1.0	2.0	1.12
		28	1.076	63.8	0.119	5.95	1.0	1.0	1.46
2240 [1498]		1	2.053	227	0.205	15.1	1.0	1.0	0.78
		13	3.378	111	0.380	8.66	1.0	1.0	2.63
		28	3.427	148	0.264	11.3	1.0	1.0	2.00

Key: † = AUC_{∞} on Day 1 or AUC_{24} on succeeding days; ‡ = time to C_{max} after first or second daily dose; ~ = Calculated using mean AUC data

Table G10 (Continued). Pharmacokinetics in Pregnant Han Wistar Rats.

Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day^	Mean Pharmacokinetic Parameters						
			AUC (h*µg/mL)†		C_{max} (µg/mL)		T_{max} (h)‡		GW433908X: Amprenavir ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
Report RD1999/02690/00									
300 [201]	F	6	0.055	68.5	0.013	4.71	1.0	3.0	0.07
		17	0.330	26.9	0.038	2.07	1.0	1.0	1.05
820 [548]		6	0.650	126	0.047	7.64	1.0	1.0	0.45
		17	0.668	43.2	0.131	3.55	2.0	4.0	1.33
2240 [1498]		6	1.322	229	0.145	8.52	18.0	2.0	0.50
		17	1.908	57.1	0.149	5.94	6.0	2.0	2.89

Key: † = AUC_{∞} on Day 1 or AUC_{24} on succeeding days; ‡ = time to C_{max} after first or second daily dose; ^ = Gestation Day; ~ = Calculated using mean AUC data

3.2.2. Intraduodenal administration

3.2.2.1. Pharmacokinetics after single doses

Plasma concentrations of GW433908 and APV were measured during a safety pharmacology in anaesthetised Han Wistar rats following intraduodenal administration (Report WD1999/00154/00). Male rats received GW433908A at doses of 150, 550 or 2000 mg/kg (111, 410 or 1493 mg/kg APV-equivalents) and blood samples were collected at approximately 2 hours post-dose. The results from this study are summarised in Table G11.

Table G11. Plasma Concentrations Following Single Dose Intraduodenal Administration in the Rat (Report WD1999/00154/00)

Dose (mg/kg) [Amprenavir equivalents]	Sex	3 Hour Plasma Concentration (µg/mL)		GW433908X: Amprenavir ratio (%)~
		GW433908X	Amprenavir	
150 [112]	M	0.256	10.1	2.2
550 [410]	M	2.897	10.3	24
2000 [1493]	M	112.733	37.4	260

Key: ~ = Calculated using mean data

GW433908X was detected in one control sample at a concentration close to the limit of quantification. As APV was not detected in any control samples, the presence of GW433908X in one control sample was considered due to sample or analytical contamination. Plasma concentrations of GW433908X and APV were dose-related, but APV concentrations were less than dose-proportional and GW433908X concentrations were greater than dose-proportional. The GW433908X:APV ratio was dose-related, with very high ratios at the high dose suggesting saturation of a pre-systemic metabolic conversion process, resulting in increased systemic exposure to GW433908X.

3.3. Rabbit

3.3.1. Oral administration

3.3.1.1. Pharmacokinetics after repeated administration

Non-pregnant animals

Plasma concentrations of GW433908X and APV were determined on Days 1 and 14 of a dose range finding study in non-pregnant New Zealand white rabbits (Report RD1999/00465/00). In this study, female rabbits were dosed by oral gavage with GW433908G at 149.5, 373.8, 747.5, 1121.3 or 1495 mg/kg/day (100, 250, 500, 750 and 1000 mg/kg/day APV-equivalents). Blood samples were collected prior to dosing and 7 hours after the first daily dose (i.e., 1 hour after the second daily dose) on Days 1 and

14. The results from this study are summarised in Table G12. GW433908X and APV were not detected in any control samples.

Plasma concentrations of GW433908X and APV generally increased in a greater than dose-proportional manner. The GW433908X:APV ratios ranged from 2.98 to 8.62% on Day 1 to 12.3 to 64.2% on Day 14. The ratios were generally higher than those recorded in non-pregnant rats at similar dose levels, indicating conversion of GW433908X to APV maybe less efficient in the rabbit than other nonclinical species.

Table G12. Plasma Concentrations following Oral Administration to Non-Pregnant Rabbits (Report RD1999/00465/00)

Dose (mg/kg/day) [Amprenavir equivalents]	7 hours Plasma Concentrations (µg/mL)				GW433908X: Amprenavir ratio (%)~	
	GW433908X		Amprenavir			
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
149.5 [100]	0.0080	0.0084	0.26	0.14	2.98	28.7
373.8 [250]	0.036	0.117	0.97	0.39	3.44	26.4
747.5 [500]	0.147	0.278	2.13	2.09	5.48	12.3
1121.3 [750]	0.118	0.710	1.84	2.50	8.62	28.4
1495.0 [1000]	0.278	1.930	8.10	3.07	3.64	64.2

Key: ~ = Calculated using individual animal data

Pregnant animals

The results from toxicokinetic studies in pregnant adult rabbits are summarised in Table G13.

Exposure to GW433908X and APV was assessed during a dose range finding study in pregnant New Zealand white rabbits (Report RD1999/00716/00). In this study, rabbits received GW433908G by oral gavage at 74.8, 149.5, 224.3 or 299 mg/kg/day (50, 100, 150 or 200 mg/kg/day APV-equivalents) from Gestation Day 7 to 20. Blood samples were collected prior to dosing (0 hours) and 7 hours after the first daily dose (i.e., 1 hour after the second daily dose) on Gestation Day 7 and 20.

Average plasma concentrations of GW433908X and APV were dose-related, but increased in a greater than dose-proportional manner on Gestation Day 7 and a less than proportional manner on Gestation Day 20. The GW433908X:APV ratios ranged from 10 to 20% on Gestation Day 7 and from 3.2 to 8.3 on Gestation Day 20. Plasma concentrations were consistent with those seen in nonpregnant rabbits at similar doses, indicating no effect of pregnancy on the pharmacokinetics of GW433908X and APV in the rabbit (see Report RD1999/00465/00 summary).

Table G13. Pharmacokinetics in the Pregnant New Zealand White Rabbits.

Dose (mg/kg/day) [amprenavir equivalents]	Gestation Day	Mean Pharmacokinetic Parameters						
Report RD1999/00716/00								
		Mean Plasma Concentrations 7 hours post-dose (µg/mL)						GW433908X: Amprenavir ratio (%)~
		GW433908X		Amprenavir				
74.8 [50]	7	0.0028		0.02				11.9
	20	0.012		0.19				6.67
149.5 [100]	7	0.014		0.06				20.7
	20	0.015		0.42				3.21
224.3 [150]	7	0.019		0.22				11.6
	20	0.023		0.34				8.34
299.0 [200]	7	0.023		0.40				9.95
	20	0.017		0.54				6.81
Report RD1999/01035/00								
		AUC (µg*h/mL)†		C_{max} (µg/mL)		T_{max} (h)		GW433908X: Amprenavir ratio (%)~
		GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
74.8 [50]	7	0.015	0.03	0.0056	0.01	1.0	1.0	39.8
	20	0.043	1.81	0.013	0.22	1.0	1.0	2.94
224.3 [150]	7	0.069	2.11	0.017	0.59	1.0	1.0	9.86
	20	0.156	3.88	0.034	0.57	1.2	1.2	4.20
672.8 [450]	7	0.646	22.2	0.084	4.16	1.2	1.0	6.98
	20	0.881	25.8	0.190	3.33	1.0	1.0	3.73

Key: ~ = Calculated using individual animal data; † = AUC_{∞} on Day 1 or AUC_{24} on Day 20

The toxicokinetics of GW433908X and APV were determined during a definitive embryofetal development study in New Zealand white rabbits (Report RD1999/01035/00) where pregnant rabbits received GW433908G by oral gavage at 74.8, 224.3 and 672.8 mg/kg/day (50, 150, and 450 mg/kg/day APV-equivalents). Rabbits were dosed from Gestation Day 7 to Gestation Day 20. Blood samples were collected at time points between 0 and 24 hours after the first portion of the daily dose on Gestation Days 7 and 20. GW433908X and APV were not detected in any control samples.

Systemic exposure (AUC and C_{max}) to GW433908X and APV increased with dose in a generally greater than proportional manner and exposure was higher on Gestation Day 20 than Gestation Day 7. Values of T_{max} for both APV and GW433908X were generally 1.0 hour after the first daily dose. Values of t½ for APV ranged from 0.6 to 4.3 hours on Gestation Day 7 and 0.8 to 16.9 hours on Gestation Day 20. Values of t½ for GW433908X ranged from 0.5 to 7.6 hours on Gestation Day 7 and 0.4 to 7.3 hours on Gestation Day 20. The results from this study were consistent to those in nonpregnant rabbits at similar doses indicating no effect of pregnancy on the pharmacokinetics of GW433908X or APV. The GW433908X:APV ratio ranged from 2.9 to 39.8%, consistent with values seen in the previous studies with rabbits. These ratios are higher than those seen in other toxicity species (rat and dog) where ratios were generally <2%, consistent with findings in the nonpregnant rabbit and indicating less efficient conversion of GW433908X to APV in the rabbit compared to other nonclinical species.

Exposure to APV in this study at 50 and 150 mg/kg/day APV-equivalents were similar to estimates determined in pregnant New Zealand white rabbits after oral administration of 100 and 200 mg/kg/day APV. However, exposure to APV at 450 mg/kg/day APV-equivalents was approximately 2- to 3-fold lower than that seen in pregnant New Zealand white rabbits after oral administration of 315 or 500 mg/kg/day APV (see Report RD1997/04032/00 summary, Appendix G3).

3.4. Dog

3.4.1. Oral administration

The pharmacokinetics of GW433908X and APV have been assessed during single and repeat dose studies carried out with GW433908G in beagle dogs following oral administration. The results from these studies are summarised in Table G14 and Table G15, respectively.

3.4.1.1. Pharmacokinetics after single doses

The pharmacokinetics of APV and GW433908X have been assessed following a single oral dose of 24 mg/kg ^{14}C-GW433908G (approximately 17 mg/kg APV-equivalents, uncorrected for impurities) to male beagle dogs (Report RD2002/00724/00). Blood samples were taken from 0 (pre-dose) to 24 hours post-dose, and analysed for GW433908X, APV and total radiocarbon (TRC).

The T_{max} for all three analytes was 0.5 to 1-hour post-dose, indicating rapid absorption. GW433908X was only detected at 0.5 and 1.0 hours post-dose, and the calculated AUC

for GW433908X was 0.13% the AUC for APV, indicating extensive conversion of GW433908X to APV. The AUC for TRC was approximately 4-fold that for APV, suggesting circulating metabolites. Plasma TRC concentrations were generally greater than blood TRC concentrations, suggesting minimal association with formed elements in the blood.

A study has been carried out in beagle dogs to compare the relative bioavailability of salt forms of GW433908X phosphate ester (Report RD1998/03011/01). Three male dogs were used in the study, with each dog receiving each of the GW433908 formulations with at least a 7-day washout period between doses. Dogs were administered 360 mg/dog GW433908X (311 mg/dog APV-equivalents) and 250 mg/dog GW433908A (201 mg/dog APV-equivalents) orally in hand-filled soft gel capsules. GW433908G was administered in tablets at a dose of 418 mg/dog (294 mg/dog APV-equivalents), either alone or with a 100-mL gavage of 0.05N HCl 15 to 30 minutes prior to tablet administration. Finally, GW433908G was administered in tablets with citric acid at a dose of 434 mg/dog (305 mg/dog APV-equivalents). Following each administration, blood samples were taken from 0.25 to 24 hours post-dose for pharmacokinetic analysis. Relative bioavailability was calculated with reference to exposure following administration of APV in Vitamin E-TPGS:PEG400:propylene glycol at 300 mg/dog in the same three dogs (data from Report RD1998/00328/00, see Appendix G3).

Bioavailability of APV following administration of GW433908X relative to administration of APV was 64.2%, whereas that for GW433908A was 82.2%. When GW433908G was administered alone, relative bioavailability was 24.1%, but large inter-individual variation was noted. This variability was attributed to differences in gastric pH and associated limitations in GW433908G solubility, which decreases at higher pH (see Item 5, Section II, Pharmacology). To lower gastric pH and hence increase solubility, GW433908G was administered following HCl gavage, which resulted in a relative bioavailability of 59.4%. Administration with citric acid resulted in relative bioavailability of 28.2%. These results indicated bioavailability of APV was similar between the salt forms of GW433908 after adjustment for pH and solubility, and that the average bioavailability following GW433908 administration was 68.5 ± 21.1% of that following oral administration of APV.

As part of the development of an oral suspension for GW433908G, a study was carried out in male beagle dogs to compare the pharmacokinetics of APV following oral administration of either GW433908G in suspension formulations or APV (Report RD1999/00927/00). In this study, dogs received GW433908G at a dose equivalent to 800 mg/kg APV, formulated as oral suspensions either with or without sucrose. Prior to GW433908G administration, dogs received 50 mL of 0.1 N HCl. Dogs also received APV at a dose of 300 mg/kg, formulated in PEG400/TPGS/propylene glycol either as two 150-mg soft gel capsules or as APV oral solution. The same group of dogs was used for all dose groups, with a 7-day washout period between doses. Blood samples were collected from dogs at intervals from 0 to 24 hours post-dose administration for pharmacokinetic analysis. The parameters calculated for the GW433908G groups were adjusted to a 300-mg APV-equivalent dose for comparison.

Table G14. Pharmacokinetic Parameters Following Single Oral Administration of GW433908 to Male Dogs

Dose (mg/kg) [Amprenavir Equivalents]	Pharmacokinetic parameters				
Report RD2002/00724/00					
	Analyte	AUC_{24} (h*µg/mL)	C_{max} (µg/mL)	T_{max} (h)	AUC ratio (%)
27 [17]	GW433908X	0.055	0.088	0.5	0.13
	APV	8.02	3.66	0.5 to 1.0	-
	TRC	34.2	6.06	0.5 to 1.0	

Report RD1998/03011/01						
	Formulation	AUC_{∞} (h*µg/mL) ^	C_{max} (µg/mL) ^	T_{max} (h)	$t_{½}$ (h)	Relative Bioavailability (%)
[300]	Amprenavir ‡	26.2	7.23	1 – 2	3.4	100
360 [311]	GW433908X	16.8	3.28	1 – 6	3.0	64.2
250 [201]	GW433908A	20.3	7.68	2 – 3	1.9	82.2
418 [294]	GW433908G	6.94	1.43	1 – 4	3.6	24.1
418 [294]	GW433908G with HCl gavage	15.8	4.48	2 – 4	1.4	59.4
434 [305]	GW433908G with citric acid	7.94	2.37	0.5 - 2	2.8	28.2

Key: APV = amprenavir; TRC = total radiocarbon; ‡ = Data from Report RD1998/00328/00 (see Appendix G3); ^ = Data adjusted to 25 mg/kg amprenavir equivalents

Table G14 (Continued). Pharmacokinetic Parameters Following Single Oral Administration of GW433908 to Male Dogs

Dose (mg/kg) [Amprenavir Equivalents]	Pharmacokinetic parameters				
Report RD1999/00927/00					
	Formulation	AUC_{∞} (h*µg/mL)	C_{max} (µg/mL)	T_{max} (h)	$t_{½}$ (h)
[800]†	GW433908G with sucrose	23.9	4.86	2.67	3.24
[800] †	GW433908G without sucrose	21.1	5.07	2.67	2.42
[300]	Amprenavir soft gel capsule	21.8	6.94	2.00	5.66
[300]	Amprenavir oral solution	21.2	7.72	1.00	9.61

Report WD1999/00155/00			
	1 Hour Plasma Concentration (µg/mL)		GW433908X:Amprenavir ratio (%)*
	GW433908X	Amprenavir	
150 [112]	0.165	11.6	1.2
550 [410]	0.205	10.0	1.8
2000 [1493]	1.836	13.4	11.8

Report WD1998/00543/00							
	AUC_{∞} (h*µg/mL)		C_{max} (µg/mL)		T_{max} (h)		GW433908X: Amprenavir ratio (%)*
	GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
150 [112]	0.562	29.1	0.688	9.14	0.4	1.3	1.61
550 [410]	8.584	56.1	14.782	13.8	0.3	1.0	12.0
2000 [1493]	60.380	62.3	105.847	22.0	0.4	0.8	79.5

Key: † = Data adjusted to 300 mg amprenavir dose; * = Based on mean plasma data

Exposure (AUC and C_{max}) and T_{max} values were similar across dose groups, but t½ increased when dogs were administered APV. However, these results indicate the choice of sweetener (sucrose or acesulfame potassium) for the GW433908G suspension formulation had no effect on pharmacokinetic parameters and that exposure to APV was similar following administration of either GW433908G or APV.

Plasma concentrations of GW433908X and APV were determined during a safety pharmacology study in beagle dogs where males received GW433908A at oral doses of 150, 550 or 2000 mg/kg (112, 410 or 1493 mg/kg APV-equivalents). Blood samples were collected at approximately one hour post-dose (Report WD1999/00155/00).

GW433908X was present in 1 control animal at a low concentration (30 ng/mL). As APV was not detected in any control animals, the presence of GW433908X was considered due to sample contamination rather than misdosing. Plasma concentrations of GW433908X were dose-related, but less than dose-proportional. Plasma concentrations of APV were similar across the dose range. The GW433908X:APV ratio was <2% at 150 and 550 mg/kg. However at 2000 mg/kg the ratio increased to 11.8%, indicating saturation of the pre-systemic metabolic conversion of GW433908X to APV. Emesis was noted in all dose groups soon after dose administration, and this may have affected plasma levels of both analytes (see Pharmacology Written Summary).

Exposure to GW433908X and APV was assessed during a further safety pharmacology study in the beagle dog where males received GW433908A orally at doses of 150, 550 or 2000 mg/kg (112, 410 or 1493 mg/kg APV-equivalents). Blood samples were collected at intervals from 0 to 4 hours after dosing (Report WD1998/00543/00).

Systemic exposure (AUC and C_{max}) to GW433908X was greater than dose-proportional, while exposure to APV was less than dose-proportional. Values of T_{max} for GW433908X were <0.5 hours, while T_{max} for APV was approximately 1 hour. GW433908X:APV exposure ratio was dose-related, suggesting saturation of the pre-systemic metabolic conversion process at higher dose levels.

3.4.1.2. Pharmacokinetics after repeated administration

The toxicokinetics of GW433908 and APV were assessed during a 14-day repeat-dose study in male beagle dogs (Report RD1998/00487/01) where animals received GW433908A by oral gavage at 50, 130 or 350 mg/kg/day (37.3, 97 or 261 mg/kg/day APV-equivalents). Blood samples were collected at various time points from 0 to 24 hours after the first daily dose on Days 1 and 13. Plasma concentrations of GW433908X were determined only for the high-dose group, whereas APV plasma concentrations were determined for all dose groups. APV was not detected in any control samples.

Exposure (AUC and C_{max}) to APV was dose-related, but increased less than dose-proportionally. Exposure was similar or slightly decreased on Day 13 compared to Day 1 at 50 or 130 mg/kg/day, but increased at 350 mg/kg/day. Values of T_{max} values for APV varied between 1 and 1.7 hours. In general, exposure to GW433908X at the 350 mg/kg/day increased on repeat dosing. The ratio of GW433908:APV was ≤0.3% on

Day 1 and ≤0.6% on Day 13, the increases on Day 13 were due primarily to increased GW433908X exposure in one dog compared to Day 1 values.

Exposure to GW433908X and APV was determined during a 4-week repeat dose toxicity study in male and female beagle dogs (Report RD1998/02605/00) where animals received GW433908G by oral gavage at 75, 194, 523 and 747 mg/kg/day (50, 130, 350 or 500 mg/kg/day APV-equivalents). Blood samples were collected at various time points from 0 to 24 hours after the first daily dose on Days 1 and 28 for toxicokinetic analysis. GW433908X and APV were not detected in any control samples.

Exposure (AUC and C_{max}) to APV increased with increasing dose in a less than dose-proportional manner up to 523 mg/kg/day. Exposure to APV at 747 mg/kg/day was generally similar to that at 523 mg/kg/day. On repeat dosing, systemic exposure to APV decreased in males receiving 523 mg/kg/day, but increased in females at 523 mg/kg/day and both sexes at 747 mg/kg/day. Estimates of APV C_{max} and AUC were similar (after adjustment for dose-equivalence to APV) to values obtained in the 26-week oral study with APV in beagle dogs (see Report RD1996/00752/00 summary, Appendix G3). Values of C_{max} for GW433908X generally increased with increasing dose in a greater than dose-proportional manner on Days 1 and 28. Values of AUC for GW433908X, although generally dose- related, were not dose-proportional on Days 1 and 28. Across the dose groups, there was no consistent effect of repeat dosing on exposure for both APV and GW433908X. The GW433908X:APV ratio varied between doses, but not dose-dependently, and was <1% in all dose groups on Day 1 and 28. Values of T_{max} for GW433908X and APV (approximately 1 and 1-2 hours, respectively) and $t_{1/2}$ (approximately 0.5-2 and 2-3 hours, respectively) were similar on Day 1 and Day 28. No sex differences in exposure were seen for either analyte.

Plasma concentrations of GW433908X and APV were determined on Days 1, 95, 180 and 273 of a 40-week repeat dose study in male and female beagle dogs (Report RD1998/02861/01). In this study, dogs were administered GW433908G by oral gavage at 75, 195, 525 and 750 mg/kg/day (50, 130, 350 and 500 mg/kg/day APV-equivalents). Due to severe intolerance, dosing in the high-intermediate and high-dose groups was suspended on Day 24 and resumed on Day 29 with the two dose groups combined and the dose of GW433908G reduced to 337 mg/kg/day (225 mg/kg/day APV-equivalents). Blood samples were collected at time points between 0 and 24 hours after the first portion of the daily dose and plasma concentrations of GW433908X and APV determined.

Very low concentrations of APV only were detected in one male control animal on Day 180 and two female control animals on Day 273. GW433908X only was detected in one female control animal on Day 1 and two control animals (one male and one female) on Day 273. Several of these concentrations were close to the limits of detection for APV and GW433908X, and were consequently at least 400 and 2.5 times lower, respectively than concentrations observed in plasma samples from dogs in the low-dose group. These findings are considered not to indicate a dosing error because either APV or GW433908X was detected but not both, hence these findings are considered to be due to analytical contamination. However, the plasma sample from one female control animal on Day 273 contained 9.45 µg/mL amprenavir and 251 ng/mL GW433908X, which indicate a possible dosing error on Day 273 in this animal. Given the above control data,

contamination of plasma samples taken from animals receiving GW433908G cannot be discounted, however any contamination with APV or GW433908X was likely to be very low and, thus, would not affect safety margins calculated for APV or the conclusions of this study.

Exposure (AUC and C_{max}) to APV was dose-related on all sampling days, with AUC values increasing generally less than dose-proportionally on Day 1 and generally greater than dose-proportionally on Days 95, 180 and 273. The proportionality of C_{max} increases was not consistent across dose groups, sexes or days. Exposure generally increased on repeat dosing at 195 and 337 mg/kg/day, but was similar throughout the study at 75 mg/kg/day. Values of APV T_{max} and $t_{½}$ were unaffected by repeat dosing, and ranged from 0.8 to 2.2 hours and 1.99 to 3.46 hours, respectively. On Days 1, 95, 180 and 273, exposure to GW433908X (AUC and C_{max}) generally increased with dose in a greater than dose-proportional manner. Exposure generally increased on repeat dosing except at 337 mg/kg/day in males where it was similar throughout the dosing period. GW433908X:APV ratios were <2% on all days. No sex differences in exposure were seen for either analyte.

Pharmacokinetic parameter estimates for APV and GW433908X from the present study are in general agreement with parameter estimates from the 14-day and 4-week oral toxicity studies. Estimates of APV C_{max} and AUC on Day 273 in males from the present study were approximately 50% lower than values observed on Day 364 after administration of APV in a 52-week oral toxicity study, and average estimates in females from the present study were approximately 25% lower (see Report RD1997/01249/00 summary, Appendix G3).

3.4.2. Intravenous administration

Pharmacokinetics after single doses

Plasma concentrations of GW433908 and APV were measured during a safety pharmacology study (Report WD1998/00588/01) where male beagle dogs received GW433908A by intravenous administration at 30 or 100 mg/kg (22.4 or 74.6 mg/kg APV-equivalents), and blood samples were collected before and up to 4 hours after dosing. The results are summarised in Table G16.

Peak plasma concentrations of GW433908X and APV increased dose-proportionally between the 30 and 100 mg/kg doses. Plasma concentrations of GW433908X 4 hours post-dose were close to the lower limit of quantitation in the 30 mg/kg dose group, and were decreasing rapidly in the 100 mg/kg dose group, suggesting that there was rapid systemic conversion of GW433908X to APV. Systemic exposure (AUC_{∞}) to GW433908X and APV increased greater than dose-proportionally. The GW433908:APV exposure ratio was >100% at both dose levels.

Table G15. Repeat Dose Pharmacokinetics in Beagle Dogs

Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	Mean Pharmacokinetic Parameters						
			AUC (h*µg/mL)†		C_{max} (µg/mL)		T_{max} (h)‡		GW433908X: Amprenavir ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
Report RD1998/00487/01									
50 [37.3]	M	1	-	28	-	4.69	-	1.0	-
		14	-	19	-	3.11	-	1.0	-
130 [97]		1	-	53	-	5.53	-	1.3	-
		14	-	30	-	4.12	-	1.7	-
350 [261]		1	0.289	95	0.224	14.1	1.0	1.7	0.26
		14	1.462	226	0.766	21.1	1.0	1.3	0.56
Report RD1998/02605/00									
75 [50]	M	1	0.023	27.51	0.010	5.40	1.0	1.0	0.07
		28	0.011	29.83	0.0087	5.60	1.0	1.0	0.04
194 [130]		1	0.041	86.38	0.016	10.4	1.3	1.5	0.04
		28	0.049	68.21	0.034	10.4	1.0	1.3	0.08
523 [350]		1	0.582	145.0	0.142	15.4	1.2	1.7	0.42
		28	0.622	95.57	0.257	18.4	1.2	1.0	0.59
747 [500]		1	0.386	133.2	0.187	15.5	1.0	1.7	0.27
		28	1.640	208.7	0.783	31.5	1.2	1.3	0.73

Table G15 (Continued). Repeat Dose Pharmacokinetics in Beagle Dogs

Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	Mean Pharmacokinetic Parameters						
			AUC (h*µg/mL)†		C_{max} (µg/mL)		T_{max} (h)‡		GW433908X: Amprenavir ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
Report RD1998/02605/00 (continued)									
75 [50]	F	1	0.020	32.58	0.020	5.21	1.0	1.0	0.04
		28	0.0092	34.99	0.0092	5.55	1.0	1.0	0.02
194 [130]		1	0.365	67.72	0.147	10.7	1.0	1.3	0.83
		28	0.069	56.75	0.053	12.2	1.0	1.0	0.18
523 [350]		1	0.176	128.5	0.056	15.1	1.0	1.5	0.12
		28	2.638	238.8	0.700	24.5	1.2	2.0	0.93
747 [500]		1	1.366	123.0	0.542	16.5	1.0	1.7	0.76
		28	2.213	156.2	0.890	23.4	1.0	1.3	1.17
Report RD1998/02861/01									
75 [50]	M	1	0.038	24.1	0.012	4.55	1.0	1.0	0.07
		180	0.040	30.1	0.075	6.28	0.4	1.0	0.11
		273	0.028	22.9	0.058	3.98	0.3	0.8	0.13
195 [130]		1	0.062	64.2	0.029	11.7	1.0	1.0	0.10
		180	0.103	98.2	0.105	13.2	0.5	1.0	0.25
		273	0.127	113	0.114	14.4	0.4	1.3	0.15
525/337^ [350/225]		1	0.627	86.3	0.301	12.0	1.2	1.5	0.65
		180	0.417	139	0.317	17.3	0.7	1.5	0.30
		273	0.556	159	0.375	17.9	0.7	1.5	0.44
750 [500] ^		1	0.697	71.0	0.409	12.4	1.0	1.2	0.85

Table G15 (Continued). Repeat Dose Pharmacokinetics in Beagle Dogs

Dose (mg/kg/day) [amprenavir equivalents]	Sex	Day	Mean Pharmacokinetic Parameters						
			AUC (h*µg/mL)†		C_{max} (µg/mL)		T_{max} (h)‡		GW433908X: Amprenavir ratio (%)~
			GW433908X	Amprenavir	GW433908X	Amprenavir	GW433908X	Amprenavir	
Report RD1998/02861/01 (continued)									
75 [50]	F	1	0.039	30.0	0.013	5.86	2.0	1.0	0.11
		180	0.044	34.8	0.056	6.63	0.4	1.0	0.31
		273	0.048	30.6	0.041	4.55	0.3	0.8	0.22
195 [130]		1	0.060	62.6	0.019	8.22	1.0	1.3	0.15
		180	0.204	147	0.129	17.3	0.8	1.3	0.12
		273	0.381	143	0.292	20.5	1.0	1.3	0.21
525/337^ [350/225]		1	0.335	129	0.134	12.5	1.0	2.2	0.23
		180	1.045	214	0.838	21.4	1.1	1.7	0.41
		273	1.632	257	0.850	25.1	1.2	1.8	0.67
750 [500] ^		1	1.129	106	0.523	13.2	1.0	1.5	0.91

Key:

† = AUC_{∞} on Day 1 or AUC_{24} on succeeding days, except for the 2-week study when all as AUC_{∞}

‡ = time to C_{max} after first or second daily dose

~ = Calculated using individual animal AUC data except 14-day study where mean data used

^ = High dose groups 525 and 750 mg/kg/day (350 and 500 mg/kg/day APV-equivalents) discontinued dosing on Day 24 and resumed on Day 29 at 337 mg/kg/day (225 mg/kg/day APV-equivalents)

Table G16. Single Dose Pharmacokinetics in Male Beagle Dogs Following Intravenous Administration (Report WD1998/00588/01)

Dose (mg/kg) [Amprenavir equivalents]	Pharmacokinetic Parameters				
	AUC_{∞} (h*µg/mL)		C_{max} (µg/mL)		GW433908: Amprenavir Ratio (%) ~
	GW433908X	Amprenavir	GW433908X	Amprenavir	
30 [22.4]	44.8	33.2	147	17.2	140
100 [74.6]	289	190	545	55.0	163

Key:
~ = Calculated using individual animal AUC data

4. DISTRIBUTION

As in vivo whole body distribution studies were carried out with APV (see APPENDIX G3) and GW433908X is extensively converted to APV on oral dosing, these studies were not repeated with GW433908G. A protein binding study has been carried out to determine any interactions between GW433908X and APV. Additionally, a study to investigate protein binding interactions between APV and the two human plasma metabolites, GW549445X and GW549444X, has been carried out. The locations of these reports in this submission are listed in Table G17.

4.1. In Vitro Plasma Protein Binding Studies

The results from the protein binding studies are summarised in Table G18.

Correlation of unbound Amprenavir and α1-acid glycoprotein (Report RD1998/03075/00)

As APV was highly bound to α_1-acid glycoprotein (AAG, see Report TEIN/94/0053 in Appendix G3), a study was carried out to determine the correlation between the percent unbound APV and concentrations of AAG (Report RD1998/03075/00). Human serum samples were spiked with ^{14}C-APV to concentrations of 1.0 and 10 µg/mL and the % unbound APV determined by ultrafiltration. The levels of AAG in these samples were also determined.

Although there was a trend towards increases in percent unbound APV as AAG decreased, a statistically significant correlation was not observed (r = -.0132).

Protein binding interactions between Amprenavir and GW433908G (Report RD2001/01671/00)

In a study to determine protein binding interactions between APV and GW433908G, pooled human plasma was incubated with ^{14}C-GW433908G (0.1 to 5.0 µg/mL) in the absence or presence of APV (1.0 or 10 µg/mL). Unbound ^{14}C-GW433908 was separated from bound ^{14}C-GW433908 by ultrafiltration, and concentrations determined by liquid scintillation.

The specific activity of the radiolabelled compound (19.6 µCi/mg) limited the lower limit of quantification such that plasma protein binding could not be determined accurately at 0.1 µg/mL. Plasma protein binding of ^{14}C-GW433908G was approximately 96% at 0.2 µg/mL, approximately 92% between 0.5 and 2 µg/mL, and approximately 90% at 5 µg/mL. Since plasma concentrations of GW433908X in clinical studies are generally less than 0.1 µg/mL and generally no more than 1% of APV plasma concentrations, the influence of GW433908X plasma protein binding is likely negligible. Displacement at 1 µg/mL ^{14}C-GW433908 was not observed with 1 µg/mL APV, although 10 µg/mL APV caused a 1% decrease in ^{14}C-GW433908 plasma protein binding. This was considered unlikely to have a detectable effect on the clinical pharmacokinetics of APV or GW433908X.

Table G17. List of Distribution Studies Performed with GW433908G or Amprenavir

Species (strain)	Salt	Concentration (μg/mL)	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Human	APV	[1 and 10]	In vitro protein binding interaction study	No	GSK	RD1998/03075/00 (98AVT0021)	Vol 29 p328
Human	G	0.1 to 5.0	In vitro protein binding interaction study	No	GSK	RD2001/01671/00 (01AVT0028)	Vol 29 p340
Human	APV	[1 or 10]	In vitro protein binding interaction study	No	GSK	RD2001/00984/00 (01AVT0013)	Vol 29 p352

Key: [ ] = amprenavir dose **Salt:** G = Calcium salt APV = Amprenavir **Testing Facility:** GSK = GlaxoSmithKline Inc, RTP, NC, USA

Protein binding interactions between Amprenavir and its Metabolites (Report RD2001/00984/00)

Pooled human plasma was incubated with ^{14}C-APV (1 or 10 µg/mL), either alone or with the human plasma APV metabolites GW549445X (0.42 and 4.21 µg/mL) or GW549444A (0.22 or 2.21 µg/mL). APV concentrations used were based on typical therapeutic plasma concentrations (maximum steady state C_{max} 7.57 µg/mL). The low concentration of GW549445X was similar to that seen in humans in a comparative pharmacokinetic study, while the low concentration of GW549444X was approximately 5 to 10-fold higher than that seen in humans (see Subsection 6.1.4). Unbound ^{14}C-APV was separated from bound ^{14}C-APV by ultrafiltration, and concentrations determined by liquid scintillation counting.

At 1 µg/mL, APV was 92.4% protein bound, which decreased by approximately 2% in the presence of APV metabolites. At 10 µg/mL, APV was 90.9% protein bound, which decreased 3 to 4% in the presence of APV metabolites.

Table G18. Protein Binding Studies with ^{14}C-GW433908G or Amprenavir

Report RD1998/03075/00								
Clinical Study	PROA1010				PROA1011			
Ethnic Origin	White		Black		White		Black	
Analyte	unbound APV (%)	AAG (mg/dL)	unbound APV (%)	AAG (mg/dL)	unbound APV (%)	AAG (mg/dL)	unbound APV (%)	AAG (mg/dL)
APV 1.0 µg/mL	14.2	92.5	12.5	83.0	12.5	81.0	18.3	85.3
10 µg/mL (subset)	17.9	92.5	19.9	83.0	15.0	81.0	19.0	85.3
10 µg/mL (total)	17.9	92.1	19.0	82.3	16.2	102	20.9	83.0

Report RD2001/01671/00						
	% Bound in Plasma					
	[^{14}C]GW433908 (µg/mL)					
	0.1	0.2	0.5	1.0	2.0	5.0
Amprenavir 0 µg/mL	BQL	96.2	91.6	92.4	91.8	90.1
1.0 µg/mL	BQL	-	-	92.5	-	-
10 µg/mL	BQL	-	-	91.2	-	-

Report RD2001/00984/00		
	% Bound in Plasma	
Displacer (µg/mL)	[^{14}C]-amprenavir (1.0 µg/mL)	[^{14}C]-amprenavir (10 µg/mL)
Control (No Displacer)	92.4	90.9
GW549445X 4.2	90.0	86.8
0.42	90.3	87.1
GW549444A 2.2	90.6	87.4
0.22	90.7	87.6

Key: BQL = Below Limit of Quantification; - = Not determined

5. EXCRETION

A range of studies has been performed in which the excretion balance of APV and GW433908X was investigated following single oral administration to mice, rats or dogs. The locations of these reports in this submission are listed in Table G19.

5.1. Mouse

5.1.1. Excretion following oral administration

The results from the oral excretion studies in mice are summarised in Table G20.

To complete the pharmacokinetic profile of APV, an excretion balance study was carried out in male CD-1 mice following a single oral administration of 150 mg/kg ^{14}C-APV (Report RD2001/00558/00). Urine and feces were collected at intervals up to 144 hours post-dosing and the levels of radiocarbon determined.

The majority of the dose was excreted in the feces, with urinary excretion only accounting for 12.0% of the dose. Total recovery was 94% of the dose, with the majority recovered in excreta within 48 hours post-dose (73.9% and 11.0% for feces and urine, respectively). This pattern of excretion was similar to that reported for APV in other species, including humans (see APPENDIX G3).

A study was carried out to assess the rate and route of radiocarbon excretion following administration of a single oral gavage dose of ^{14}C-GW433908G at 150 mg/kg (105 mg/kg APV-equivalents) to male CD-1 mice (Report RD2001/00560/00). Urine and feces were collected at intervals up to 168 hours post-dosing.

The majority of the dose was excreted in the feces, with urinary excretion only accounting for 5.11% of the dose. Total recovery was 93% of the dose, with the majority recovered in excreta within 48 hours post-dose (83.2% and 4.33% for feces and urine, respectively). The pattern of excretion was similar to that seen following administration of APV to mice (see above).

5.2. Rat

5.2.1. Excretion following oral administration

A study was carried out to assess the rate and route of radiocarbon excretion following administration of a single oral gavage dose of ^{14}C-GW433908G at 110 mg/kg (approximately 78 mg/kg APV-equivalents, uncorrected for impurities) to male Han Wistar rats (Report RD2002/00725/00). Urine and feces were collected at intervals up to 168 hours post-dosing. The results from this study are summarised in Table G21.

Table G19. List of Excretion Studies Performed with GW433908G or Amprenavir

Species (strain)	Sex	Route	Salt Form	Dosage (mg/kg) †	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Mouse (CD-1)	M	Oral (gavage)	APV	[150]	Single dose	Mass Balance	No	/GSK	RD2001/00558/00 (01AVV0003)	Vol 29 p001
Mouse (CD-1)	M	Oral (gavage)	G	150	Single dose	Mass Balance	No	/GSK	RD2001/00560/00 (01AVV0004)	Vol 29 p074
Rat (Han Wistar)	M	Oral (gavage)	G	110	Single dose	Mass Balance	No	/GSK	RD2002/00725/00 (98AVV0018)	Vol 29 p141
Dog (Beagle)	M	Oral (gavage)	G	24	Single dose	Mass Balance	No	/GSK	RD2002/00724/00 (98AVV0017)	Vol 29 p216

Key:
† = In terms of the relevant salt
[ ] = APV dose

Salt:
APV = amprenavir
G = calcium salt

Testing Laboratory:
GSK = GlaxoSmithKline Inc, RTP, NC, USA

Table G20. Excretion of Radiocarbon following Oral Administration of ^{14}C-Amprenavir or ^{14}C-GW433908G to Male CD-1 Mice

Dose (mg/kg) [Amprenavir Equivalent]	Sample	% Dose Recovered*
Report RD2001/00558/00		
[150]	Urine	12.0
	Feces	77.8
	Cage wash/wipe	4.52
	Total	94.3
Report RD2001/00560/00		
150 [105]	Urine	5.11
	Feces	85.2
	Cage wash/wipe	2.47
	Total	92.8

Key: * = 144 hours for RD2001/00558/00 or 168 hours for RD2001/00560/00

The majority of the dose was excreted in the feces, with urinary excretion only accounting for 2.63% of the dose. Essentially the entire dose was accounted for, with the majority recovered in excreta within 48 hours post-dose (87.4% and 2.51% for feces and urine, respectively). The pattern of excretion was similar to that previously reported for APV in rats (see Report RD1998/00070/00 summary, Appendix G3).

Table G21. Excretion of Radiocarbon following Oral Administration of ^{14}C-GW433908G to Male Wistar Han Rats (Report RD2002/00725/00)

Dose (mg/kg) [Amprenavir Equivalent]	Sample	% Dose Excreted Over the 168 h Study Period	
		Mean	SD
110 [78]	Urine	2.63	1.37
	Feces	90.2	5.54
	Cage wash/wipe	0.08	0.07
	Total	92.9	5.58

5.3. Dog

5.3.1. Excretion following oral administration

A study was carried out to assess the rate and route of radiocarbon excretion following administration of a single oral gavage dose of ^{14}C-GW433908 calcium salt at 24 mg/kg (approximately 17 mg/kg APV-equivalents, uncorrected for impurities) to male beagle dogs (Report RD2002/00724/00). Urine and feces were collected at intervals up to 168 hours post-dosing. The results from this study are summarised in Table G22.

The majority of the dose was excreted in the feces, with urinary excretion only accounting for 13.0% of the dose. Essentially the entire dose was accounted for, with the majority recovered in excreta within 48 hours post-dose (77.5% and 12.5% for feces and urine, respectively). The pattern of excretion was similar to that previously reported for APV in dogs (see APPENDIX G3).

Table G22. Excretion of Radiocarbon following Oral Administration of ^{14}C-GW433908G to Male Beagle Dogs (Report RD2002/00724/00)

Dose (mg/kg) [Amprenavir Equivalent]	Sample	% Dose Excreted Over the 168 h Study Period	
		Mean	SD
24 [17]	Urine	13.0	14.4
	Feces	80.0	17.9
	Cage wash/wipe	2.06	1.24
	Total	95.1	2.45

6. METABOLISM

The metbolism of APV and GW433908G has been investigated in vivo and in vitro. Further studies investigated the potential for cytochrome P450 induction following oral administration of GW433908G. The locations of these reports are listed in Table G23.

6.1. In Vivo Studies

6.1.1. Mouse

Two metabolite-profiling studies were carried out in mice to compare profiles following administration of either APV or GW433908G. As the aim of these studies was to demonstrate qualitatively similar profiles following administration of either compound, full metabolite identification was not carried out.

6.1.1.1. Metabolic profile following oral administration

The in vivo metabolite profile of APV was studied in male CD-1 mice using excreta and plasma collected during an excretion balance study (see Subsection 5.1.1.), where mice received a single oral 150 mg/kg dose of ^{14}C-APV (Report RD2002/00504/00). The metabolite profile was determined by liquid chromatography with UV, radiochemical and MS detection, with reference to analytical standards for the major rat and human APV metabolites, GW549445X and GW549444X (see Subsection 6.1.2.1), and the minor APV metabolite 4228W94. Other metabolites were not identified beyond determination of molecular weight, where possible. Profiling in excreta used the 0 to 8 hour urine and feces samples which contained >60% of the administered dose (approximately 54% in feces and 7% in urine). Plasma profiling used pooled 0.5, 1 and 3 hour samples. Table G24 summarises the results from this study, and Figure G2 shows the structures of the metabolite reference standards.

Mouse feces contained APV and 5 metabolites. The major component was the metabolite GW549445X. APV was a secondary component, with two unidentified metabolites. Two further unidentified metabolites were minor components. Mouse urine contained APV and 17 metabolites, including GW549445X. In plasma, the major component was APV, with four metabolites present as minor components. One of the metabolites was identified as 4228W94, and three were unidentified. GW549444X was not detected in mouse excreta or plasma.

A similar metabolite profiling study was carried out using excreta and plasma from male CD-1 mice collected during an excretion balance study (see Subsection 5.1.1.), where mice received ^{14}C-GW433908G at a dose level of 150 mg/kg (Report RD2002/00505/00). The metabolite profile was determined with reference to the analytical standards for GW549445X, GW549444X and 4228W94. Other metabolites were not characterised beyond determination of molecular weight, where possible. The profile in excreta used the 0 to 8 hour samples only, with these samples containing >70% of the administered dose (approximately 70% in feces and 2% in urine). Plasma profiling used pooled 0.5, 1 and 2 hour samples. The results are summarised in Table G25.

Table G23. List of Metabolism Studies Performed With GW433908G or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage (mg/kg) †	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Mouse (CD-1)	M+F	Oral (gavage)	APV	[150]	Single dose	In vivo metabolite profiling	No	GSK	RD2002/00504/00 (02AVV0014)	Vol 29 p361
Mouse (CD-1)	M+F	Oral (gavage)	G	150	Single dose	In vivo metabolite profiling	No	GSK	RD2002/00505/00 (02AVV0015)	Vol 29 p380
Rat (Han Wistar) Human	M	Oral (gavage)	APV	[80] [12*]	Single dose	In vivo metabolism study	No	GSK	RD1998/00831/01 (99AVV0027)	Vol 29 p401
Rat (Han Wistar)	M+F	Oral (gavage)	G	110	Single dose	In vivo metabolite identification	No	GSK	RD2001/01618/01 (98AVV0018)	Vol 29 p432
Rat (Han Wistar)	M+F	Oral (gavage)	G	110	Single dose	In vivo metabolite profile	No	/GSK	RD2002/00725/00 (98AVV0018)	Vol 29 p141
Dog (Beagle)	M+F	Oral (gavage)	G	24	Single dose	In vivo metabolite identification	No	GSK	RD2000/02370/01 (98AVV0017)	Vol 29 p476
Dog (Beagle)	M+F	Oral (gavage)	G	24	Single dose	In vivo metabolite profile	No	/GSK	RD2002/00724/00 (98AVV0017)	Vol 29 p216

Key:
† = In terms of the relevant salt
* = assuming 50 kg human
[ ] = APV dose

Salt:
G = calcium salt
APV = amprenavir

Testing Facility:
GSK = GlaxoSmithKline Inc, RTP, NC, USA

Table G23 (Continued). List of Metabolism Studies Performed With GW433908G or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage (mg/kg) †	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Rat (Han Wistar)	M+F	Oral (gavage)	G	1493 2240	4 weeks	Comparative pharmacokinetics of metabolites.	No	GSK	RD2002/00576/00 (02APK0018)	Vol 29 p514
Dog (Beagle)			G	337	9 months					
Human			APV G	[24*], [36*] 24*	up to 40 weeks single dose					
Mouse (CD-1)	M+F	Oral (gavage)	APV	250 500 750 1000	13 weeks	Cytochrome P450 induction	No	GSK	RD1998/00926/00 (M40220) GSK Appendix 2	Vol 20 p562
Mouse (CD-1)	M+F	Oral (gavage)	G	3200	13 weeks	Cytochrome P450 induction	No		RD2002/00646/00 (M40725)	Vol 29 p547
Rat (Han Wistar)	M+F	Oral (gavage)	G	149 478 1493 2240	4 weeks	Cytochrome P450 induction	No	GSK	RD1998/02573/00 (R40427) Appendix 15	Vol 4 p857

Key:
† = In terms of the relevant salt
[ ] = APV dose

Salt:
G = calcium salt
APV = amprenavir

Testing Facility:
GSK = GlaxoSmithKline Inc, RTP, NC, USA

Table G23 (Continued). List of Metabolism Studies Performed With GW433908G or Amprenavir

Species (strain)	Sex	Route	Salt	Dosage (mg/kg) †	Duration of Dosing	Type of Study	GLP	Testing Facility	Report No. (Study No.)	Location
Rat (Han Wistar)	M+F	Oral (gavage)	APV G	[50] [160] [500] 149 478 1493 2240	4 weeks	CYP3A protein levels from repeat dose studies	No	GSK	RD1999/02460/02 (99AVV0024)	Vol 29 p560
Dog (Beagle)	M+F	Oral (gavage)	G	75 194 523 747	4 weeks	Cytochrome P450 induction	No	GSK	RD1998/02605/00 (D40436) Appendix 13	Vol 13 p547

Key:
† = In terms of the relevant salt
[ ] = APV dose

Salt:
G = calcium salt
APV = amprenavir

Testing Facility:
GSK = GlaxoSmithKline Inc, RTP, NC, USA

Figure G2. Metabolites of GW433908G and Amprenavir

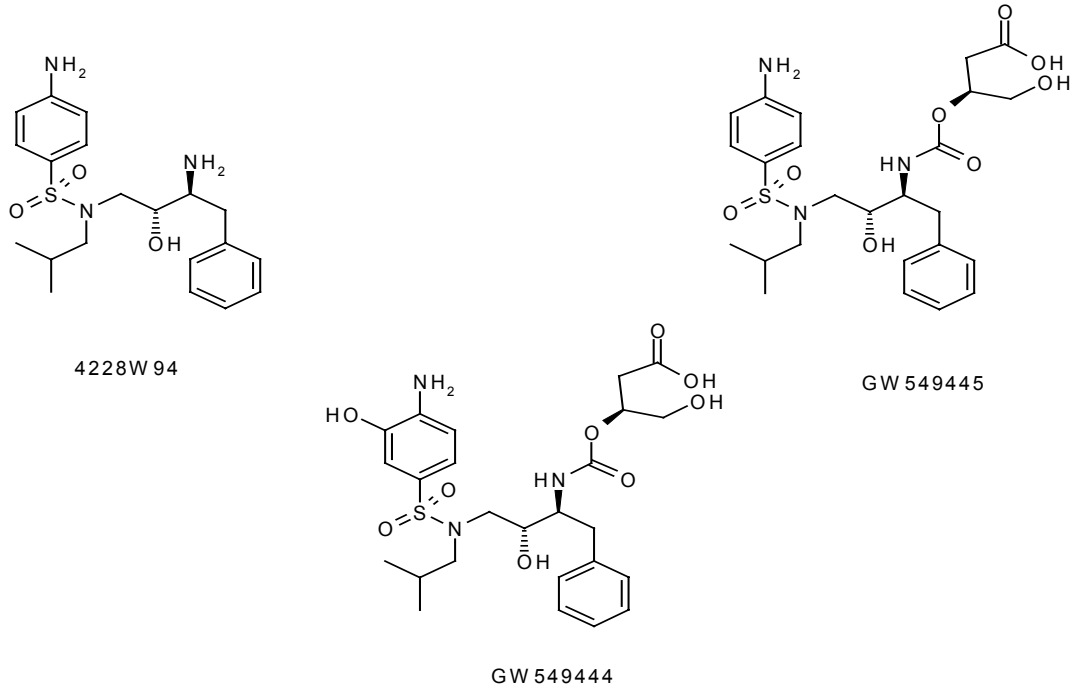


Table G24. Metabolite in Excreta and Plasma following Oral Administration of ^{14}C-APV to Male CD-1 Mice (Report RD2002/00504/00)

Proposed Identity	Molecular Weight indicated by LC/MS analysis	Relative % peak area (dpm)		
		Feces (0 to 8 hours)	Urine (0 to 8 hours)	Pooled Plasma (0.5 to 3 hours)
ND	583	-	9	-
4228W94	391	-	4	4
ND	695	-	4	-
ND	697	-	7	-
ND	503	-	-	6
ND	524	14	2	-
GW549445	537	37	1	-
ND	503	-	-	9
ND	504	-	2	-
ND	521	17	1	-
141W94	505	17	8	72
Unidentified metabolites*	ND	16 (n = 2)	58 (n = 9)†	2 (n = 1)

Key: ND = Not Determined; * = All metabolites where molecular weight could not be determined;
† = One unidentified metabolite accounted for 19% of the urinary relative % peak area

Table G25. Metabolite in Excreta and plasma Following Oral Administration of ^{14}C-GW433908G to Male CD-1 Mice (Report RD2002/00505/00)

Proposed Identity	Molecular Weight indicated by LC/MS analysis	Relative % peak area (dpm)		
		Feces (0 to 8 hours)	Urine (0 to 8 hours)	Plasma (0 to 2 hours)
ND	583	-	25	-
ND	407	-	9	-
ND	583	-	8	-
4228W94	391	-	4	5
ND	523	8	-	-
ND	697	-	4	-
ND	503	-	-	5
GW433908	585	24	-	-
GW549445	537	25	-	-
ND	503	-	-	7
ND	521	18	-	-
141W94	505	16	9	79
Unidentified metabolites*	ND	9 (n = 1)	42 (n = 3)†	4 (n = 1)

Key: ND = Not Determined; * = All metabolites where molecular weight could not be determined; † = One unidentified metabolite accounted for 35% of the urinary relative % peak area

In feces, the major components were GW433908X and GW549445X. The GW433908X was considered likely to have travelled through the gastrointestinal tract unabsorbed as pharmacokinetic analysis indicated extensive conversion to APV during absorption (see Subsection 3.1.1.). APV was also present feces with three other unidentified metabolites. In urine, APV and 8 metabolites were detected. The two major components of urine were unidentified metabolites accounting for 60% of the urinary-excreted radiocarbon (<1% of administered dose). The only identified urinary metabolite was 4228W94. The major circulating component in plasma was APV, with 4 minor components, one of which was 4228W94. GW549444X was not detected in mouse excreta or plasma.

The metabolite profiles in mice following administration of either APV or GW433908G were quantitatively similar, indicating administration of the prodrug did not alter the metabolism of APV in mice. The major metabolite was GW549445X, similar to that seen in rats and humans. Metabolite GW549444X was not detected in mice.

6.1.2. Rat

6.1.2.1. Metabolic profile following oral administration

As part of the development program for APV, the structures of the two principal human metabolites were tentatively characterised as BD/8064/120/2, a product of di-oxidation of the APV tetrahydrofuran (THF) ring, and BD9064/104/2, a product of both di-oxidation of the THF ring and oxidation of the 4-amino sulfonamide phenyl ring. As both of these metabolites could exist in two possible isomeric forms, a study was carried out to further characterise the structure of these two metabolites (Report RD1998/00831/01). In this study, rat feces were collected from males administered a single 80 mg/kg oral dose of ^{14}C-APV. Fecal extracts were partially purified by solid-phase extraction and metabolites separated by HPLC. Following identification of the analytes of interest by LC/MS, the relevant fractions were collected and solvent removed by evaporation. The purified metabolites were then characterised by a variety of spectroscopic techniques including ^{1}H-NMR, 2-dimensional (2D) COSY (vicinal H-H correlations), 2D-TOCSY (all H-H correlations in a spin network) and 2D-HSQC (^{1}H-^{13}C NMR). These techniques were also used to identify metabolites isolated from human liver S9-mix following incubation with 100 µM APV (0.5 µg/mL), and from human feces following oral administration of 600 mg ^{14}C-APV.

The results from this study identified definitively the structures of BD/8064/120/2 and BD/8064/104/2, the two principal human metabolites of APV. The metabolites were named GW549445X and GW549444X, respectively, and were present in both rat and human feces. The structures of these metabolites are shown in Figure G2. Using the structures determined in this study, GW549445X (Batch R5574/143/7) and GW549444X (Batch R6755/20/12) were synthesised by Chemical Development Division, GlaxoSmithKline, and used as analytical standards during the nonclinical pharmacokinetics development programme for GW433908G.

The in vivo metabolism of GW433908G has been investigated in Han Wistar rats using urine and feces obtained from an excretion balance study. In this study, male rats received a single 110 mg/kg oral dose of ^{14}C-GW433908G (see Subsection 5.2.1.). The metabolites in excreta were profiled and identified by LC/MS and LC/MS/MS and by reference to analytical standards (Report RD2001/01618/01). As 87.4% of the dose was eliminated in feces by 48 hours post-dose, only the 0 to 24 and 24 to 48 hour samples were used for fecal profiling. As urinary excretion was very low (2.63% of the dose over 168 hours), only the 0 to 24 hour sample was used for urinary profiling. The resulting metabolite profile is discussed in Report RD2002/00725/00, and summary of these results is given in Table G26.

In feces, the principal drug-related components were APV, GW549444X and GW549445X, a result in agreement with previous metabolism studies with APV. In this study, GW549444X was more abundant than GW549445X (23.9% versus 20.4% of the dose, respectively). In a previous study with ^{14}C-APV (see Report RD1998/00070/00 summary, Appendix G3) and during a metabolite pharmacokinetic study with GW433908G and APV (see Subsection 6.1.4), GW549445X was present in greater abundance than GW549444X. The reason for the difference between the studies is

unclear, but it is not outside the scope of biological variation. All other identified metabolites in feces were present at levels ≤8% of the dose. There were 12 unidentified metabolites in feces that in total accounted for 2.16% of the dose, with no one metabolite making up more than 0.87% of the dose. All identified metabolites had been seen following oral administration of ^{14}C-APV to rats.

In urine, 7 identified metabolites and APV were seen, with no identified component accounting for more than 0.33% of the dose. There were 6 unidentified metabolites (3 unique to urine) that in total accounted for 1.32% of the dose, with no one unidentified metabolite accounting for more that 0.67% of the dose. All identified metabolites had been seen in urine following oral administration of ^{14}C-APV to rats.

Table G26. Metabolite Profile following Oral Administration of ^{14}C-GW433908G to Male Han Wistar Rats (Reports RD2001/01618/01 and RD2002/00725/00)

		Mean % dose in feces (n = 3*)			Mean % dose in urine (n = 3*)
Metabolite	m/z	0 to 24 hours	24 to 48 hours	0 to 48 hours	0 to 24 hours
BD/8064/125/1 or BD/8064/125/2	408	ND	0.26	0.26	0.04
4228W94	392	1.25	0.27	1.52	0.11
BD/8064/104/4	522	ND	ND	ND	0.07
GW549444X	554	22.7	1.23	23.9	ND
BD/8064/106/1	522	3.67	0.83	4.50	0.33
GW549445X	538	17.8	2.63	20.4	ND
BD/8064/123/1 or BD/8064/123/2	504	1.30 ND	0.35 0.19	1.65 0.19	0.02
BD/8064/121/1	522	2.31	0.66	2.97	ND
BD/8064/104/3	564	3.71	0.16	3.87	0.01
GW291272	548	ND	ND	ND	0.06
Amprenavir	506	15.1	2.01	17.1	0.22
Unidentified †	-	0.87 (n = 1)	1.29 (n = 12)	2.16 (n = 12)	1.32 (n = 6)

Key: ND = Not Determined; † = All metabolites where molecular weight could not be determined; * = 3 groups of 3 males

6.1.3. Dog

6.1.3.1. Metabolic profile following oral administration

The in vivo metabolism of GW433908G has been investigated in beagle dogs by using urine and feces obtained from the excretion balance study. In this study, male dogs received a single 24 mg/kg oral dose of ^{14}C-GW433908G (see Subsection 5.3.1.). The metabolites in excreta were profiled and identified by LC/MS and LC/MS/MS and

reference to analytical standards (Report RD2000/02370/01). As approximately 85% of the dose had been eliminated by 24 hours post-dose, only the 0 to 24 hours samples were used for identification and profiling. The resulting metabolite profile is discussed in Report RD2002/00724/00, and summary of these results is given in Table G27.

The major component of dog feces was APV, which accounted for 27.3% of the administered dose. Five metabolites were identified in dog feces, with one metabolite, BD/8064/106/1, accounting for 22.7% of the administered dose. This metabolite is the product of mono-oxidation of APV, most likely at the aliphatic region of the molecule. It was the predominant component in feces from one dog (50% of the administered dose) which consequently increased the overall mean value. However, the high value in this one animal was considered likely due to microbial contamination of the sample leading to microbial metabolism of fecal APV. BD/8064/106/1 was not seen in urine or feces of dogs administered ^{14}C-APV (see Report RD1996/00289/00 summary, Appendix G3). All other fecal metabolites were consistent with the APV study.

Table G27. Metabolite Profile following Oral Administration of ^{14}C-GW433908G to Male Beagle Dogs (Reports RD2000/02370/01 and RD2002/00724/00)

Metabolite	m/z	Mean % dose administered (n = 3)	
		Feces (0 to 24 hours)	Urine (0 to 24 hours)
BD/8064/125/1 or BD/8064/125/2	408	ND	0.26
4228W94	392	2.44	1.06
GW433908X	586	ND	9.98
BD/8064/106/1	522	22.7	0.77
BD/8064/121/2	524	ND	0.20
BD/8064/121/2 or BD/8064/122/1 or BD/8064/122/2	524 or 520	1.26	ND
GW549445X or BD/8064/122/1 or BD/8064/122/2	538 or 520	1.19	ND
BD/8064/123/1 or BD/8064/123/2	504	ND	0.33
BD/8064/121/1	522	7.38	ND
Amprenavir	506	27.3	0.31
Unknown	-	ND (n = 0)	0.32 (n = 2)

Key: ND = Not Detected

GW433908X, APV and 7 metabolites were seen in dog urine. Although GW433908X was the most prominent component, accounting for 9.98% of the dose, it was considered present due to contamination by vomitus, and not due to urinary excretion. This is supported by the observation that GW433908X was only seen in the urine of one out of three dogs, and that pharmacokinetic analysis indicated extensive conversion of GW433908X to APV in this study (see Subsection 3.4.1.1). Of the 5 identified metabolites, only BD/8064/106/1 (0.77% of the dose) had not been seen in urine following oral administration of APV to dogs. Two unidentified metabolites were found in urine that in total accounted for 0.32% of the dose.

6.1.4. Comparative Pharmacokinetics of Amprenavir, GW549445X and GW549444X

A study was carried out in rats, dogs and humans to compare the plasma pharmacokinetics of APV with the two principal metabolites found in human plasma, GW549445X and GW549444X (Report RD2002/00576/00). Rat plasma samples were obtained on Days 1 and 23 of the 4-week repeat dose toxicity study where male and female Han Wistar rats were administered GW433908G by oral gavage at dose levels of 1493 or 2240 mg/kg/day (1000 or 1500 mg/kg/day APV-equivalents). Dog plasma samples were obtained on Day 95 of a 40-week repeat dose toxicity study where male and female beagle dogs were administered GW433908G by oral gavage at 337 mg/kg/day (225 mg/kg/day APV-equivalents). Human plasma samples were obtained following oral administration of either APV at 600 or 900 mg BID (24 or 36 mg/kg/day based on 50 kg human) with 100 mg BID ritonavir (samples taken on Weeks 24, 32 or 40, Protocol ESS40006), or a single 1400 mg GW433908G dose (24 mg/kg APV-equivalents, Protocol APV10008).

At each sampling occasion, samples were taken from 0 to 24 hours post-dosing and pharmacokinetic parameters were determined following analysis for APV, GW549445X and GW549444X. Due to limited sample availability, rat samples were pooled, whereas samples from individual dogs and humans were analysed. Concentrations of APV, GW549445X and GW549444X in plasma were determined by LC/MS/MS methods with validated calibration ranges of 10 to 5000 ng/mL (rat and dog) or 5 to 5000 ng/mL (human) for amprenavir, and 10 to 1000 ng/mL (rat and dog) or 5 to 1000 ng/mL (human) for GW549445X and GW549444X. The results from this study are summarised in Table G28.

Exposure to APV and GW549445X was seen in all species. In humans, the ratio of GW549445X AUC to APV AUC was 3 to 4%, regardless of whether sampling was after single dosing (i.e. Protocol APV10008) or at steady-state (i.e. Protocol ESS40006). In rats, although the absolute exposure to GW549445X decreased slightly on repeat-dosing (Day 1 to Day 23), the ratio of GW549445X to APV on Day 23 had increased approximately 2-fold compared to Day 1, and was then similar to that seen in humans. These findings are consistent with induction of drug metabolism enzymes in rats following repeat-dosing. In dogs, the ratio of GW549445X to APV at steady state was approximately 10-fold lower than that seen in rats at steady state.

Table G28. Comparative Pharmacokinetics of Amprenavir, GW549445X and GW549444X in Rats, Dogs and Humans (Report RD2002/00576/00)

Species	Dose (mg/kg/day)	Day (Week)	Sex	Mean Pharmacokinetic Parameters			
Amprenavir							
				C_{max} (µg/mL)	T_{max} (h)	AUC_{24h} (µg*h/mL)	
Rat	1493 [1000]	1	M	9.60	2.0	224	
			F	11.1	1.0	133	
		23	M	5.38	1.0	47.2	
			F	4.89	4.0	68.1	
	2240 [1500]	1	M	11.3	2.0	184	
			F	10.4	6.0	234	
		23	M	4.54	6.0	53.1	
			F	5.38	1.0	80.8	
Dog	337 [225]	95	M	21.8 ± 5.79	1.6 ± 0.55	240 ± 112	
			F	22.0 ± 4.87	1.4 ± 0.55	222 ± 45.4	
Human	24 or 36 †	(24, 32, or 40)	M/F	13.3 ± 2.87	0.75 ± 0.18	62.2 ± 25.1	
	28 [24] †	1	M/F	7.28 ± 2.88	1.25 ± 0.50	23.3 ± 10.6	
GW549445X							
				C_{max} (µg/mL)	T_{max} (h)	AUC_{24h} (µg*h/mL)	AUC Ratio (%)‡
Rat	1493 [1000]	1	M	0.198	2	3.161	1.41
			F	0.079	4	1.359	1.02
		23	M	0.118	1	1.362	2.89
			F	0.107	4	1.498	2.20
	2240 [1500]	1	M	0.176	2	2.990	1.62
			F	0.165	18	2.211	0.94
		23	M	0.104	2	1.453	2.74
			F	0.109	0 (18)	2.012	2.49
Dog	337 [225]	95	M	0.054 ± 0.038	2	0.605 ± 0.416	0.24 ± 0.06^
			F	0.043 ± 0.010	2.20 ± 1.10	0.449 ± 0.149	0.21 ± 0.07^
Human	24 or 36 †	(24, 32, or 40)	M/F	0.582 ± 0.677	1.25 ± 0.50	2.673 ± 4.237	3.22 ± 3.71^
	28 [24] †	1	M/F	0.282 ± 0.088	1.50 ± 0.50	0.750 ± 0.181	4.03 ± 2.27^

Key: [ ] = amprenavir-equivalent dose of GW433908 (conversion factor = 1.493)
† = Based on 50 kg human; ‡ = Comparison to amprenavir AUC; ^ = calculated for individual samples, not using mean values.

Table G28 (Continued). Comparative Pharmacokinetics of Amprenavir, GW549445X and GW549444X in Rats, Dogs and Humans (Report RD2002/00576/00)

Species	Dose (mg/kg/day)	Day (Week)	Sex	Mean Pharmacokinetic Parameters			
GW549444X							
				C_{max} (µg/mL)	T_{max}(h)	AUC_{24h} (µg*h/mL)	AUC Ratio(%)
Rat	1493 [1000]	1	M	0.013	18	ND	ND
			F	BQL	ND	ND	ND
		23	M	BQL	ND	ND	ND
			F	10.8	1	ND	ND
	2240 [1500]	1	M	0.012	18	ND	ND
			F	0.016	18	ND	ND
		23	M	BQL	ND	ND	ND
			F	BQL	ND	ND	ND
Dog	337 [225]	95	M	BQL	ND	ND	ND
			F	BQL	ND	ND	ND
Human	24 or 36 †	(24, 32, or 40)	M/F	0.022 ± 0.019	0.75 ± 0.25	0.173 ± 0.210	0.18 ± 0.18^
	28 [24] †	1	M/F	0.043 ± 0.016	1.40 ± 0.42	0.189 ± 0.061	1.03 ± 0.62^

Key: [ ] = amprenavir equivalent dose of GW433908 (conversion factor = 1.493)
† = Based on 50 kg human; ‡ = Comparison to amprenavir AUC; ^ = calculated for individual samples, not with mean values; BQL = Below the lower limit of quantitation (10 ng/mL for rat and dog plasma); ND = Not determined

In humans, plasma concentrations of GW549444X were 4- to 15-fold lower than the concentrations of GW549445X. In rats, calculation of AUC values, and thus exposure ratios, was precluded as GW549444X was only detected at single time-points in some animals, predominantly on Day 1, at concentrations that were at the limit of quantification. GW549444X was below the limit of quantification in dog plasma.

These results were consistent with the findings from the in vivo metabolism studies in rats and dogs where GW549445X and GW549444X were the principal metabolites in rats, but only GW549445X was detected in dogs and at low levels. Therefore, the comparative pharmacokinetic results were consistent with human metabolism being similar to rats, rather than dogs.

6.1.5. Cytochrome P450 induction in animals

6.1.5.1. Mouse

The effects of repeated oral administration of APV or GW433908G on CD-1 mouse cytochrome P450 were assessed as part of the 13-week repeat-dose toxicity studies following administration of either APV (Report RD1998/00926/00, see Subsection 3.1.1) or GW433908G (Report RD2002/00646/00, see Subsection 3.1.1). In the APV study, mice received 250, 500, 750 or 1000 mg/kg/day, while in the GW433908G study mice received 3200 mg/kg/day (2250 mg/kg/day APV-equivalents). Liver microsomes were prepared following terminal necropsy, and total cytochrome P450 (CYP450) content, and ethoxyresorufin O-deethylase (EROD), pentoxyresorufin O-dealkylase (PROD) and testosterone 6β-hydroxylase (6βT) activities were determined. In the APV study, activities of NADPH cytochrome P450 reductase (REDC) and uridine glucuronosyltransferase (UGT) were also assessed. The results are summarised in Table G29.

Table G29. Mean CYP450 Content and CYP450 Isozyme Activities Relative to Control Following 13-Weeks Oral Administration of Amprenavir or GW433908G to CD-1 Mice

GW433908G [Amprenavir Equivalents] (mg/kg/day)	Sex	CYP450 Concentration	EROD Activity	PROD Activity	REDC Activity	6βT Activity	UGT Activity
Amprenavir Study (Report RD1998/00926/00)							
[250]	M	0.64	0.36*	2.5*	1.5*	1.2	1.2
	F	0.74	0.45*	1.8*	1.5	1.2	1.7*
[500]	M	0.62	0.41*	3.1*	1.7*	1.6	1.2
	F	0.73	0.60	1.8*	1.7	1.5	1.1
[750]	M	0.67	0.54	3.1*	1.9*	1.8	1.5
	F	0.69	0.55	1.7*	1.6*	1.3	1.2
[1000]	M	0.55*	0.54	3.0*	1.7*	1.9	1.2
	F	0.59*	0.54	1.4	1.7*	1.3	1.2
GW433908G Study (Report RD2002/00646/00)							
3200 [2250]	M	1.0	0.90	0.59	ND	2.9	ND
	F	1.4	0.70	1.5	ND	4.6	ND

Key: * = Statistically significant from control ($p < 0.05$) (mean values shown, statistical analysis performed on individual data); CYP450 = total cytochrome P450 content; EROD = ethoxyresofufin O-deethylase; PROD = pentoxyresorufin O-dealkylase; 6βT = testosterone 6β-hydroxylase; ND = Not Determined

In the APV study, CYP2B (PROD) and cytochrome P450 reductase activity increased in both males and females by 2 to 3 fold. Increases in these enzymes are typical of phenobarbital type induction, but the response was weak (<20%) in comparison to the response to phenobartital, and this induction was not replicated in the GW433908G

study. CYP3A activity (6βT) showed a small increase compared to controls, but the response was not statistically significant. The only statistically significant change in CYP1A (EROD) or UGT activities were at the low doses only and considered not biologically significant.

In the GW433908G study, induction of CYP3A activity (6βT) was seen in this study, with activity increased 2.9-fold in treated male mice and 4.6-fold in treated female mice. No biologically significant effect on CYP450 content or CYP 1A- or CYP 2B-mediated activities (EROD and PROD, respectively) was seen. Although these results different to those seen following administration of APV to mice, they are consistent with those following repeat administration of GW433908G to rats (see Subsection 6.1.5.2).

6.1.5.2. Rat

The effects of repeated oral administration of GW433908G on rat cytochrome P450 activities were assessed as part of the 4-week repeat dose toxicity study (Report RD1998/02573/00, see Subsection 3.2.1.1). In this study, rats received GW433908G at 149, 478, 1493 or 2240 mg/kg/day (100, 320, 1000 or 1500 mg/kg/day APV-equivalents). Liver microsomes were prepared following terminal necropsy, and CYP450 content, and REDC, EROD, PROD and 6βT activities were determined. The results are summarised in Table G30.

Table G30. Mean CYP450 Content and CYP450 Isozyme Activities Relative to Control Following 4-Weeks Oral Administration of GW433908G to Rats (Report RD1998/02573/00)

GW433908G [Amprenavir Equivalents] (mg/kg/day)	CYP450 Concentration	EROD Activity	PROD Activity	REDC Activity	6βT Activity
Male					
149 [100]	0.73*	0.89	0.99	1.1	1.2
478 [320]	0.82	0.88	0.87	1.4*	1.7
1493 [1000]	0.74*	0.72	0.61	1.5*	2.2*
2240 [1500]	0.67*	0.51*	0.58	1.7*	1.4
Female					
149 [100]	1.0	1.0	1.3	1.3	4.6*
478 [320]	0.87	1.1	0.79	1.8*	12.5*
1493 [1000]	0.99	0.81	0.64	1.9*	11.7*
2240 [1500]	0.93	0.68	0.48*	2.0*	12.2*

Key: * = Statistically significant from control ($p < 0.05$) (mean values shown, statistical analysis performed on individual data); P450 = total cytochrome P450 content; EROD = ethoxyresofufin O-deethylase; PROD = pentoxyresorufin O-dealkylase; REDC = NADPH cytochrome P450 reductase; 6βT = testosterone 6β-hydroxylase

A statistically significant increase in REDC activity was seen males and females at ≥478 mg/kg/day, with the increase up to 2.0-fold in females. Statistically significant increases in 6βT activity were seen only at 1493 mg/kg/day in males (2.2-fold), but at all doses in females, with the increase in activity approximately 12-fold at ≥478 mg/kg/day. EROD and PROD activities were decreased in both males and females, but the decrease was only statistically significant at 2240 mg/kg/day in males for EROD and females for PROD. CYP450 content was also decreased in males and females, but only statistically significantly in males. A dose that yielded no effect on the hepatic cytochrome P450 enzymes was not achieved in this study.

The results indicate induction of CYP3A, the enzyme that mediates not only 6βT, but also the metabolism of APV. Induction of CYP3A was qualitatively consistent with toxicokinetic and toxicity data from repeat-dose toxicity studies in rats with GW433908G (see Subsection 3.2.1.1). Previously submitted cytochrome P450 activity studies with APV showed a 2- to 3-fold inductive effect on CYP3A4 following 13-weeks administration to rats (see Report RD1998/00348/00 summary, Appendix G3).

To further investigate the CYP3A inductive effect in rats, the amount of recombinant human CYP3A4-like immunoreactivity (rHuCYP3A4-LI) was determined by fluorescence immunoassay with microsomal preparations from a 4-week repeat-dose toxicity study with GW433908G, and a 13-week repeat dose toxicity study with APV (Report RD1999/02460/02). The results from this study are summarised in Table G31.

Table G31. Concentrations of rHuCYP3A4-LI in Rat Liver Microsomes from Repeat Dose Studies with Amprenavir or GW433908G (Report RD1999/02460/02)

Test Material	Dose (mg/kg/day) [amprenavir equivalents]	µg rHuCYP3A4-LI/mg microsomal protein ± standard deviation	
		Male	Female
Amprenavir	Vehicle control	BQL	BQL
	Water control	BQL	BQL
	[50]	0.47	0.35
	[160]	3.67 ± 0.77	4.78 ± 1.46
	[500]	7.97 ± 1.39	9.55 ± 2.52
GW433908G	0 [0]	BQL	BQL
	149 [100]	1.35 ± 0.36	BQL
	478 [320]	5.20 ± 2.09	5.47 ± 1.01
	1493 [1000]	11.2 ± 2.7	12.4 ± 4.9
	2240 [1500]	11.8 ± 6.5	12.5 ± 4.4

Key:
BQL = Below the lower limit of quantitation for the assay.

In general, rHuCYP3A4-LI concentrations increased with increasing APV or GW433908G dose, with levels similar following administration of APV or GW433908G at APV-equivalent doses. Levels reached a plateau at 1493 or 2240 mg/kg/day GW433908G (1000 or 1500 mg/kg/day APV-equivalents). The levels were slightly greater in females compared to males. These results were consistent with APV autoinduction of CYP3A in rats. The 11-fold difference between the low and high dose animals in content of CYP3A protein was consistent with induction of clearance that would account for decreases in exposure (AUC) of up to 80% after repeated dosing to rats (see Subsection 3.2.1). The disparity between the induction of CYP3A content (>10-fold) and CYP3A activity (<5-fold) may be due to concomitant inhibition of CYP3A by APV.

6.1.5.3. Dog

The effects of repeated oral administration of GW433908G on dog cytochrome P450 activities were assessed as part of the 4-week repeat-dose toxicity study (Report RD1998/02605/00, see Subsection 3.4.1). In this study, dogs received GW433908G at 75, 194, 523 or 747 mg/kg/day (50, 130, 350 or 500 mg/kg/day APV-equivalents). Liver microsomes were prepared following terminal necropsy, and CYP450 content, and REDC, EROD, PROD and 6βT activities were determined. The results are summarised in Table G32.

Table G32. Mean CYP450 Content and CYP450 Isozyme Activities Relative to Control Following 4-Weeks Oral Administration of GW433908G to Dogs (Report RD1998/02605/00)

GW433908G [Amprenavir Equivalents] (mg/kg/day)	CYP450 Concentration	EROD Activity	PROD Activity	REDC Activity	6βT Activity
Male					
75 [50]	1.4	0.91	2.4*	1.3	0.70
194 [130]	0.94	0.85	2.3*	1.3*	0.83
523 [350]	1.2	0.59	2.6*	1.6*	1.5
747 [500]	1.1	0.67	2.7*	1.7*	1.4
Female					
75 [50]	1.4	0.87	2.8*	1.5*	0.69
194 [130]	1.3	0.82	2.7*	1.6*	0.86
523 [350]	1.1	0.59	2.0	1.9*	1.0
747 [500]	1.1	0.89	1.7	1.8*	0.90

Key: * = Statistically significant from control ($p < 0.05$) (mean values shown, statistical analysis performed on individual data); P450 = total cytochrome P450 content; EROD = ethoxyresofufin O-deethylase; PROD = pentoxyresorufin O-dealkylase; REDC = NADPH cytochrome P450 reductase; 6βT = testosterone 6β-hydroxylase

There were no statistically significant effects on CYP450 content, or EROD or 6βT activities in either males or females. PROD activity increased at all doses in males and at 75 and 194 mg/kg/day in females by 2.4- to 2.8-fold. REDC activity increased at all dose levels in females and at ≥194 mg/kg/day in males, consistent with induction of CYP450-mediated clearance in the dog.

The increase in PROD and REDC activity indicates induction of the CYP2B family. However, compared to a 18- to 19-fold increases in PROD activities seen in dogs treated with phenobarbital (the classical CYP2B inducer) during this study, the induction was poorly reflected by the activity measurements in this study. In a previously submitted study where APV was administered orally for 13-weeks, PROD activity was unaffected and 6βT activity decreased (see Report RD1996/00213/00 summary, Appendix G3).

7. DRUG INTERACTION STUDIES

A range of studies was performed investigating potential pharmacokinetic interactions as part of the AGENERASE application (see APPENDIX G3). However, further studies have been carried out with APV and are included in this submission. An in vitro study assessed protein-binding interactions between APV and other anti-HIV drugs, while an in vivo study evaluated interactions of ^{14}C-APV with P-glycoprotein in mice. The details of the location of these studies within the submission are listed in Table G33.

7.1. In Vitro Protein Binding Studies

A study was carried out to determine any protein binding interactions between amprenavir and other antiviral compounds (Report RD2001/00527/01). Human plasma was incubated with [^{14}C]-APV (1 or 10 µg/mL) either alone or with the antiviral compounds ritonavir (0.1 or 10 µg/mL), delavirdine (2.0 or 30 µg/mL) or efavirenz (1.0 or 20 µg/mL). In addition, plasma was incubated with [^{3}H]-ritonavir (0.1 or 10 µg/mL) either alone or with APV (10 or 0.1 µg/mL). Concentrations used were based on typical therapeutic plasma concentrations for each drug. The results are summarised in Table G34.

At 1 µg/mL, APV alone was 92.4% bound. Co-incubation with ritonavir (10 µg/mL) decreased APV binding to 86.6%, a 5.8% decrease. The other antivirals decreased APV binding by 2 to 3%. At 10 µg/mL, APV alone was 90.9% bound. Co-incubation with ritonavir (10 µg/mL) decreased APV binding to 85.3%, a 5.6% decrease. The other antivirals decreased APV binding by 4 to 5%. APV did not displace ritonavir from human plasma proteins at either low or high concentrations.

7.2. In Vivo P-glycoprotein Interaction Study

As part of the AGENERASE™ submission, in vitro and in vivo studies indicated APV appeared to be a substrate for P-glycoprotein (P-gp), a transmembrane efflux pump that contributes to multiple drug resistance (see Report RD1998/00321/00 summary, Appendix G3). Subsequent to the AGENERASE™ submission, an in vivo study was carried out to further investigate the interactions between APV and P-gp in FVB and 1a/1b P-gp knockout mice (Report RD1998/02179/00). Fed male mice from each strain were administered 50 mg/kg ^{14}C-APV by oral gavage. Two hours after dosing, mice were processed for whole body autoradiography by using ^{14}C-sensitive phophor-imaging plates and computerised digital imaging for quantitation. The results are summarised in Table G35.

Radiocarbon was higher in tissues from the P-gp knockout mice compared to the FVB mice, with the exception of the CSF. This difference was greatest in brain tissue, where concentrations in P-gp knockout mice were >26-times the FVB concentrations. This data is consistent with APV being a P-gp substrate, and suggest P-gp contributes to the low tissue concentrations of APV.

8. OVERALL CONCLUSIONS

Single-dose pharmacokinetic studies in mice, rats and dogs with GW433908G indicated rapid absorption and extensive conversion to APV. On repeat oral administration of GW433908A or GW433908G to mice, rats or dogs, systemic exposure to APV increased in a generally dose-related, but not dose-proportional, manner. In rats and mice, exposure decreased on repeat dosing indicating autoinduction. In dogs, exposure increased on repeat dosing. In carcinogenicity studies with APV in mice and rats, similar pharmacokinetics were seen.

In rats, exposure to APV following single and repeat administration of GW433908G was up to 50% lower than dose equivalent exposure following administration of APV. Reduced APV exposure was also seen in dogs, but the difference was variable and less pronounced than in rats. The reasons for the reduced exposure to APV at following dosing with GW433908G, particularly in rats, is unclear, but it has not been replicated in humans.

Systemic exposure to GW433908X in mice, rats and dogs after oral administration of GW433908A or GW433908G generally increased with increasing dose, but not dose-proportionally. Plasma concentrations of GW433908X were highly variable; no consistent pattern of decrease or increase in systemic exposure to GW433908X was observed over time. Exposure ratios of GW433908X to APV were generally <3% in mice, <2% in rats and <1% in dogs, indicating extensive conversion during absorption.

There was no effect of pregnancy on exposure to either APV or GW433908X in rats. Exposure to APV in pregnant rabbits was dose-related, and increased in a greater than dose-proportional manner. The GW433908X:APV exposure ratio was variable and generally >3%, indicating that the conversion of GW433908X to APV was not as efficient in rabbits as other nonclinical species.

Table G36 and Table G37 compare toxicokinetic parameters for APV and GW433908X (arithmetic mean values), respectively, in nonclinical species and humans. Exposure to APV at the high dose levels in rats and dogs were similar to those seen in humans at the proposed GW433908G therapeutic dose when administered with ritonavir. Exposure to APV in the rabbit, however, was lower than that seen in humans, and higher doses were not tolerated.

During the toxicokinetic studies, APV was detected in control samples during the APV carcinogenicity studies, and APV or GW433908X were detected in control samples in 8 out of 18 toxicology studies with GW433908A or GW433908G (see APPENDIX G2 for individual study details). In the mouse APV carcinogenicity study, the findings were generally confined to one sampling time (Week 52). In the rat study, control samples with detectable APV were seen at all sampling times. However, although it was not possible to discern whether the source of the APV was analytical contamination or misdosing, the APV concentrations were generally low compared to the treated groups and were not considered to have effected the validity or conclusions of this study.

In 5 of the GW433908G studies, relatively low concentrations of GW433908X were detected in the absence of measurable APV in a small number of samples from control animals (n=1 in 3 studies and n=3 or 6 in the remaining studies). Detection of GW433908X in the absence of APV suggests sample contamination during handling or analysis.

In the 15-day juvenile toxicity study in rats, low levels of APV were detected in the absence of measurable GW433908X in a single sample, which may indicate either mis-dosing or sample contamination during handling or analysis. In the 40-week dog study, low levels of APV or GW433908X were detected in the absence of the other compound in 4 unrelated samples, suggestive of contamination. High concentrations of APV and GW433908X were detected a single control animal sample which was suggestive of mis-dosing, but sample contamination during handling or analysis cannot be ruled out.

In the rat fertility study, GW433908X was detected in the majority of control animal samples, and APV was detected in 3 samples. The generally random pattern of these findings and the absence of measurable APV concentrations in all but 3 samples suggests sample contamination with GW433908X during handling or analysis. In addition, the presence of GW433908X concentrations and the absence of paired APV concentrations in 7 out of 24 Day 1 pre-dose (0 hour) samples also was consistent with sample contamination.

The generally low levels of GW433908X or APV in a small number of control samples were not considered to have affected the toxicokinetic evaluation of the GW433908G studies. With respect to the fertility study, it was possible that the plasma levels of GW433908X in the GW433908G dose groups were overestimated, however human plasma levels are still likely to be very much lower than those seen in rats at the high dose, where no effects on fertility were seen. Contamination with APV was very low and not considered to have affected APV toxicokinetic parameter evaluation. Therefore, the presence of APV or GW433908X in control samples from these studies was not considered to have negative impact on toxicokinetic data or conclusions based on this data.

GW433908X was >96% protein bound at clinically relevant concentrations. There were no clinically relevant protein binding interactions between APV and GW433908X, or between APV and its metabolites.

Fecal elimination was the predominant route of excretion in nonclinical species following administration of GW433908G. Urinary excretion accounted for 5.11, 2.63 or 13.0% of the administered dose in mice, rats or dogs, respectively.

A similar qualitative metabolic profile was seen in mice following oral administration of either APV of GW433908G, with GW549445X the major mouse metabolite. GW433908X was present in feces of mice administered GW433908G, probably the result of unabsorbed material passing through the gastrointestinal tract. In rats, the principal metabolites were GW549445X and GW549444X, consistent with findings following administration of APV. In dogs, APV was the principal component of feces, consistent with previous APV studies. Unlike the APV studies, however, was the presence of the metabolite BD/8064/106/1, a mono-oxidation product of APV. High levels of this

metabolite in the feces of one dog may have been the result of sample microbial contamination, but the reason for the presence of this metabolite in the feces of other dogs following administration of GW433908G is unclear. Table G38 compares the metabolite profile of animals and humans. The results from the new studies with GW433908G are consistent with those previously reported with APV, with human metabolism most similar to rats. All metabolites seen in humans were seen at least one of the animals studies used for toxicity studies.

A study to investigate the pharmacokinetics of the APV metabolites GW549445X and GW549444X in rats, dogs and humans noted GW549445X as the principal circulating metabolite in all species. GW549444X was present at 4 to 15-fold lower levels in human plasma, and this metabolite was only detected in some rats at low levels. GW549444X was not detected in dog plasma. These results are consistent with GW433908G and APV metabolism in humans being similar to rats rather than dogs.

Studies in rats and mice showed administration of GW433908G induces CYP3A, similar to findings with APV in rats. This is consistent with toxicokinetic findings in these species, where APV exposure decreased on repeat dosing. In dogs, no effect on CYP3A was noted following repeated administration of GW433908G, although a weak inductive effect on CYP2B was seen.

Human plasma protein-binding displacement interactions between APV and the antiviral compounds ritonavir, delavirdine, and efavirenz were greatest in the presence of ritonavir (up to 6%), but APV displacement was seen with the other antivirals at between 2% and 5%. APV did not displace ritonavir from plasma proteins. Decreases of 4% to 6% in APV protein binding at high concentrations of APV might be expected to have detectable effects on clinical pharmacokinetic parameter estimates.

An in vivo study in P-glycoprotein knockout mice confirmed that APV is a substrate for this transporter. This finding suggests P-glycoprotien contributes to the low tissue concentrations of APV after oral dosing.

Studies of the absorption, distribution, metabolism and excretion of GW433908X and APV after administration of GW433908G (or GW433908A) in the species used in the toxicological evaluation of this compound have demonstrated these to be good models for characterising the fate of APV in humans. The disposition of GW433908X and APV was similar in mice, rats, dogs and humans with animal species and humans exposed to qualitatively similar profiles of metabolites, and in rats and humans the profiles of major metabolites were quantitatively similar. The contribution of faecal excretion to the total excretion of an APV dose in rats, mice and dogs was similar to that seen in humans.

Table G36. Exposure Ratio of Amprenavir in Toxicology Test Species Following Repeat Dose Administration of GW433908G and in Humans

Study Type Report No. (Study/Protocol)	GW433908G [APV-equivalents] (mg/kg/day)	Sex	C_{max}[a] (µg/mL)	AUC_{24h}[a] (µg h/mL)	Ratio of Animal to Human AUC_{24SS}[e]
Mouse 13 week RD2000/02408/00 (M40725)	400 [281]	M	12.7	57.5	0.7
		F	9.02	55.5	0.7
	800 [562]	M	12.7	72.5	0.9
		F	11.6	78.7	0.9
	1600 [1125]	M	13.4	143	1.7
		F	16.3	187	2.2
	3200 [2250]	M	29.2	359	4.3
		F	29.1	352	4.2
Rat 6 month RD1998/02858/01 (R40417)	149 [100]	M	2.90	19.8	0.2
		F	2.31	22.7	0.3
	478 [320]	M	5.09	46.2	0.6
		F	5.23	54.3	0.7
	1493 [1000]	M	5.33	57.0	0.7
		F	7.28	62.3	0.7
	2240 [1500]	M	5.28	54.9	0.7
		F	7.68	107	1.3
Rabbit embryofetal RD1999/01035/00 (L40461)	74.8 [50]	F	0.22	1.81	0.02
	224.3 [150]	F	0.57	3.88	0.05
	672.8 [450]	F	3.33	25.8	0.3
Dog 9 month RD1998/02861/01 (D40418)	75 [50]	M	3.98	22.9	0.3
		F	4.55	30.6	0.4
	195 [130]	M	14.4	113	1.4
		F	20.5	143	1.7
	337 [225] [b]	M	17.9	159	1.9
		F	25.1	257	3.1
Human[d] (APV10010 or APV10009)	28 [24] [c]	M+F	7.57	83.2	N/A

Key:
a = Steady state arithmetic mean values.
b = reduced dose level; c = based on 50 kg human
d = 1400 mg GW433908G: 200 mg ritonavir QD or 700 mg GW433908G:100 mg ritonavir BID
e = most stringent comparison made i.e. QD data

Table G37. Exposure Ratio of GW433908X in Toxicology Test Species and Humans Following Repeat Dose Administration of GW433908G

Study Type Report No. (Study/Protocol)	GW433908G [APV-equivalents] (mg/kg/day)	Sex	C_{max}[a] (µg/mL)	AUC_{24h}[a] (µg h/mL)	Ratio of Animal to Human AUC_{24SS}[f]
Mouse 13 week RD2000/02408/00 (M40725)	400 [281]	M	0.027	0.095	4.0
		F	0.031	0.194	8.0
	800 [562]	M	0.061	0.240	10
		F	0.103	0.440	18
	1600 [1125]	M	0.444	2.738	114
		F	1.597	14.19	591
	3200 [2250]	M	1.056	11.90	495
		F	0.954	10.11	505
Rat 6 month RD1998/02858/01 (R40417)	149 [100]	M	0.015	0.030	1.3
		F	0.102	0.271	11
	478 [320]	M	0.032	0.229	9.5
		F	0.381	3.267	136
	1493 [1000]	M	0.102	0.930	39
		F	0.184	1.001	42
	2240 [1500]	M	0.117	1.079	45
		F	0.143	1.815	76
Rabbit embryofetal RD1999/01035/00 (L40461)	74.8 [50]	F	0.013	0.043	0.7
	224.3 [150]	F	0.034	0.156	2.5
	672.8 [450]	F	0.190	0.881	14
Dog 9 month RD1998/02861/01 (D40418)	75 [50]	M	0.058	0.028	1.2
		F	0.041	0.048	2
	195 [130]	M	0.114	0.127	5.3
		F	0.292	0.381	16
	337 [225][b]	M	0.375	0.556	23
		F	0.850	1.632	68
Human[e] (APV10010 or APV 10009)	28 [24][d]	M + F	0.015	0.024	N/A

Key:
a = Steady state arithmetic mean values.
b = reduced dose level; d = based on 50 kg human.
e = 1400 mg GW433908G: 200 mg ritonavir QD or 700 mg GW433908G:100 mg ritonavir BID
f = most stringent comparison made i.e. QD data

Table G38. Comparative metabolism of GW433908G or Amprenavir following Single Oral Dosing

	Percent of Dose Recovered						
Species	Human	Mouse		Rat		Dog	
Dose	600 mg ^{14}C-APV[a]	150 mg/kg ^{14}C-APV[b]	150 mg/kg ^{14}C-GW433908[c] (105 mg/kg APV)	80 mg/kg ^{14}C-APV[d]	110 mg/kg ^{14}C-GW433908[e] (78 mg/kg APV)	25 mg/kg ^{14}C-APV[f]	24 mg/kg ^{14}C-GW433908[g] (17mg/kg APV)
Feces							
Total Recovery	75%	78%	85%	104%	90%	84%	80%
GW549445X	46%	28%	20%	48%	20%	5.0%	1.2%
GW549444X	24%	ND	ND	6.0%	24%	ND	ND
APV	BLQ	13%	13%	13%	17%	52%	27%
4228W94	ND	ND	ND	ND	1.5%	2.0%	2.4%
Other metabolites	5.0%	37%	29%	37%	16%	25%	35%[h]
GW433908	NA	NA	23%	NA	BLQ	NA	BLQ
Urine							
Total Recovery	14%	12%	5.0%	3.0	3.0%	11%	13%
GW549445X	BLQ	0.1	ND	BLQ	ND	0.3%	ND
GW549444X	BLQ	ND	ND	BLQ	ND	ND	ND
APV	BLQ	1.0	0.4	0.2	0.2%	0.2%	0.3%
4228W94	ND	0.5	0.2	ND	0.1%	0.6%	1.1%
Other metabolites	BLQ	10%	4.4%	0.8%	1.9%	3.0	2.0%
GW433908	NA	NA	BQL	NA	BLQ	NA	10%[i]

Key: NA = Not applicable; ND = not detectable; a = Reference Protocol PROA1007; b = RD/2001/00558/00 and RD2002/00504/00;
c = RD2001/00560/00 and RD2002/00505/00; d = RD1998/00070/00; e = RD2002/00725/00; f = RD1996/00289/00; g = RD2002/00724/00
h = APV metabolite BD/8064/106/1 accounted for 23% of dose recovered in feces; high value in 1 out 3 males (50%) increased the overall mean value. High value in this male was considered likely due to microbial contamination of the sample leading to microbial metabolism of fecal APV.
i = GW433908 presence in urine likely due to vomit rather than excretion.

9. REFERENCES

Reference Reports

Report RD1998/01974/00
The validation of an automated LC/MS/MS method RBMT-MET-068.00 for the determination of 141W94 in mouse plasma.

Report R1998/02888/00
The validation of an automated LC/MS/MS method RBMT-MET-069.00 for the determination of 141E94 in rat plasma.

Report RD2002/00233/00
Validation of a method for the determination of GW433908 and amprenavir in mouse plasma using 96-well technology and LC/MS/MS.

Report RD1999/02681/00
Validation of a method for the determination of GW433908 and amprenavir in rat plasma using 96-well technology and LC/MS/MS.

Report RD1999/02498/00
Validation of a method for the determination of GW433908 and amprenavir in rabbit plasma using 96-well technology and LC/MS/MS.

Report RD1999/00101/00
The validation of a semi-automated LC/MS/MS method (RBMT-MET-093.01) for the determination of GW433908 and amprenavir in dog plasma.

Report RD2002/00232/00
Validation of a method for the determination of GW433908 and amprenavir in dog plasma using 96-well technology and LC/MS/MS.

Report RD1999/02148/00
The validation of a time-resolved immunofluorometric assay (TR-IFA) method RBMT-MET-134.00 for the determination of rHuCYP3A4-like immunoreactivity in rat liver microsomes.

Report RD1996/00211/00
Validation of a High-Performance Liquid Chromatographic Method for the Measurement of 141W94 in Plasma

APPENDIX G1.
Analytical Methods used During the Preclinical Development of GW433908G

1. INTRODUCTION

The concentrations of GW433908X and amprenavir in plasma from animals were determined during pharmacokinetic and toxicokinetic studies with GW433908A, GW433908G or amprenavir. A summary of the analytical methods used for safety studies is shown in Table G39. Details of the methods are included in the validation reports, and these are summarised in the following section.

A time-resolved immunofluorometric assay was developed and validated to determine levels of recombinant human CYP3A4-like immunoreactivity (rHuCYP3A4-LI) in rat liver microsomes. A summary of the analytical method used is shown in Table G40. Details of the methods are given in a validation report and further details are given in the following section.

The concentrations of GW433908X and amprenavir in plasma, or recombinant human CYP3A4-like immunoreactivity (rHuCYP3A4-LI) in rat liver microsomes were determined from calibration curves constructed from analysis of spiked samples with linear regression. The suitability of the method was confirmed by determining the specificity, linearity, limits of quantitation, accuracy and precision. In addition, quality control samples were analysed in parallel to ensure acceptable quality at the time of analysis. The acceptability was confirmed when quality control standards were within +/- 15% of the nominal concentration.

2. ANALYTICAL METHODS

2.1. Analysis of GW433908X or Amprenavir in Plasma

Validation of a method for the determination of GW433908 and amprenavir in mouse plasma using 96-well technology and LC/MS/MS (Report RD2002/00233/00)

Summary: Solid-phase extraction, HPLC with tandem mass spectrometry.

Range: 5 to 1000 ng/mL (GW433908X) and 10 to 5000 ng/mL (amprenavir)

(Method Sheet Reference: RBMT-MET-093.01)

Concentrations of GW433908X and amprenavir were determined in citrated plasma by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS-MS) by using the multiple reaction monitoring (MRM) technique. Unknown samples, QC samples or standards (250 µL) were mixed with a 250-µL portion of internal standard solution ([$^{13}C_6$]-amprenavir and [$^{13}C_6$]-GW433908), and extracted with acetonitrile by solid phase extraction with a Waters OASIS™ HLB Extraction Plate. The extract was diluted with acetonitrile/20 g/L ammonium acetate (20:80 v/v), analysed by reversed-phase HPLC with a C_{18} column, and detected by tandem mass spectrometry with the turbo ionspray source in the positive ion mode. The mobile phase for determination of GW433908X was 0.02 g/L ammonium acetate in acetonitrile:water (15:85, v/v). The mobile phase for determination of amprenavir was 0.02 g/L ammonium acetate in

acetonitrile:water (55:45, v/v). The chromatographic run time for each analyte was 3 minutes.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. Linearity was confirmed over a range of 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir). Quality control samples of 700, 240 and 16 ng/mL (GW433908X) and 4000, 1000, and 36 ng/mL (amprenavir) in plasma were interspersed throughout the analytical run to monitor assay performance. The lower limits of quantitation were 5 and 10 ng/mL, respectively, for GW433908X and amprenavir.

The validation of an automated LC/MS/MS method RBMT-MET-068.00 for the determination of amprenavir in mouse plasma (Report RD1998/1974/00)

Summary: Solid-phase extraction, HPLC with tandem mass spectrometry.

Range: 10 – 5000 ng/mL (amprenavir)

(Method Sheet Reference: RBMT-MET-068.00)

Concentrations of amprenavir were determined in plasma by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS-MS) by using the multiple reaction monitoring (MRM) technique. Unknown samples, QC samples or standards (50 µL) were mixed with a 50-µL portion of internal standard solution ([$^{13}C_6$]-amprenavir, and centrifuged. A 50-µL portion of the supernatant was combined with 0.1% formic acid in water (50:50 v:v), analysed by reversed-phase HPLC with a C_{18} column, and detected by tandem mass spectrometry with the turbo ionspray source in the positive ion mode. The mobile phase for determination of amprenavir was 0.1% formic acid in 55% acetonitrile and 45% water:55% acetonitrile and 45% water (55:45, v:v) delivered at a flow rate of 0.35 mL/min. The chromatographic run time was 4 minutes.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. Linearity was confirmed over a range of 10 – 5000 ng/mL. Quality control samples of 4000, 2000, and 30 ng/mL in plasma were interspersed throughout the analytical run to monitor assay performance. The lower limit of quantitation was 10 ng/mL amprenavir.

Validation of a method for the determination of GW433908 and amprenavir in rat plasma using 96-well technology and LC/MS/MS (Report RD1999/02681/00)

Summary: Solid-phase extraction, HPLC with tandem mass spectrometry.

Range: 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir)

(Method Sheet Reference: RBMT-MET-093.01)

Concentrations of GW433908X and amprenavir were determined in citrated plasma by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS-MS) by using the multiple reaction monitoring (MRM) technique. Unknown samples, QC samples or standards (250 µL) were mixed with a 250-µL portion of internal standard solution ([$^{13}C_6$]-amprenavir and [$^{13}C_6$]-GW433908), and extracted with acetonitrile by solid phase extraction with a Waters OASIS™ HLB Extraction Plate. The extract was diluted with acetonitrile/20 g/L ammonium acetate (20:80 v/v), analysed by reversed-phase HPLC with a C_{18} column, and detected by tandem mass spectrometry with the turbo ionspray source in the positive ion mode. The mobile phase for determination of GW433908X was 0.02 g/L ammonium acetate in acetonitrile:water (15:85, v/v). The mobile phase for determination of amprenavir was 0.02 g/L ammonium acetate in acetonitrile:water (55:45, v/v). The chromatographic run time for each analyte was 3 minutes.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. Linearity was confirmed over a range of 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir). Quality control samples of 848, 16 and 8 ng/mL (GW433908X) and 4400, 1000, and 36 ng/mL (amprenavir) in plasma were interspersed throughout the analytical run to monitor assay performance. The lower limits of quantitation were 5 and 10 ng/mL, respectively, for GW433908X and amprenavir.

The validation of an automated LC/MS/MS method RBMT-MET-069.00 for the determination of amprenavir in rat plasma (Report R1998/02888/00)

Summary: Solid-phase extraction, HPLC with tandem mass spectrometry.

Range: 10 – 5000 ng/mL (amprenavir)

(Method Sheet Reference: RBMT-MET-069.00)

Concentrations of amprenavir were determined in plasma by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS-MS) by using the multiple reaction monitoring (MRM) technique. Unknown samples, QC samples or standards (50 µL) were mixed with a 50- or 100-µL portion of internal standard solution ([$^{13}C_6$]-amprenavir, and centrifuged. A portion (50 or 75 µL) of the supernatant was combined with 0.1% formic acid in water (50:50 v:v), analysed by reversed-phase HPLC with a C_{18} column, and detected by tandem mass spectrometry with the turbo ionspray source in the positive ion mode. The mobile phase for determination of amprenavir was 0.1% formic acid in 55% acetonitrile and 45% water:55% acetonitrile and 45% water (55:45, v:v) delivered at a flow rate of 0.35 mL/min. The chromatographic run time was 4 minutes.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. Linearity was confirmed over a range of 10 – 5000 ng/mL. Quality control samples of 4000, 2000, and

30 ng/mL in plasma were interspersed throughout the analytical run to monitor assay performance. The lower limit of quantitation was 10 ng/mL amprenavir.

Validation of a method for the determination of GW433908 and amprenavir in rabbit plasma using 96-well technology and LC/MS/MS (Report RD1999/02498/00)

Summary: Solid-phase extraction, HPLC with tandem mass spectrometry.

Range: 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir)

(Method Sheet Reference: RBMT-MET-093.01)

Concentrations of GW433908X and amprenavir were determined in citrated plasma by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS-MS) by using the multiple reaction monitoring (MRM) technique. Unknown samples, QC samples or standards (250 µL) were mixed with a 250-µL portion of internal standard solution ([$^{13}C_6$]-amprenavir and [$^{13}C_6$]-GW433908), and extracted with acetonitrile by solid phase extraction with a Waters OASIS™ HLB Extraction Plate. The extract was diluted with acetonitrile/20 g/L ammonium acetate (20:80 v/v), analysed by reversed-phase HPLC with a C_{18} column, and detected by tandem mass spectrometry with the turbo ionspray source in the positive ion mode. The mobile phase for determination of GW433908X was 0.02 g/L ammonium acetate in acetonitrile:water (15:85, v/v). The mobile phase for determination of amprenavir was 0.02 g/L ammonium acetate in acetonitrile:water (55:45, v/v). The chromatographic run time for each analyte was 3 minutes.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. Linearity was confirmed over a range of 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir). Quality control samples of 700, 240 and 16 ng/mL (GW433908X) and 4000, 1000, and 36 ng/mL (amprenavir) in plasma were interspersed throughout the analytical run to monitor assay performance. The lower limits of quantitation were 5 and 10 ng/mL, respectively, for GW433908X and amprenavir.

Validation of a semi-automated LC/MS/MS method for the determination of GW433908 and amprenavir in dog plasma (Report RD1999/00101/00)

Summary: Solid-phase extraction, HPLC with tandem mass spectrometry.

Range: 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir)

(Method Sheet Reference: RBMT-MET-093.01)

Concentrations of GW433908X and amprenavir were determined in citrated plasma by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS-MS) by using the multiple reaction monitoring (MRM) technique. Unknown samples, QC samples or standards (250 µL) were mixed with a 250-µL portion of internal standard solution ([$^{13}C_6$]-amprenavir and [$^{13}C_6$]-GW433908), and extracted with acetonitrile by

solid phase extraction with a Waters OASIS™ HLB Extraction Plate. The extract was diluted with acetonitrile/20 g/L ammonium acetate (20:80 v/v), analysed by reversed-phase HPLC with a C_{18} column, and detected by tandem mass spectrometry with the turbo ionspray source in the positive ion mode. The mobile phase for determination of GW433908X was 0.02 g/L ammonium acetate in acetonitrile:water (15:85, v/v). The mobile phase for determination of amprenavir was 0.02 g/L ammonium acetate in acetonitrile:water (55:45, v/v). The chromatographic run time for each analyte was 3 minutes.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. Linearity was confirmed over a range of 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir). Quality control samples of 848, 16 and 8 ng/mL (GW433908X) and 4400, 1000, and 36 ng/mL (amprenavir) in plasma were interspersed throughout the analytical run to monitor assay performance. The lower limits of quantitation were 5 and 10 ng/mL, respectively, for GW433908X and amprenavir.

Validation of a method for the determination of GW433908 and amprenavir in dog plasma using 96-well technology and LC/MS/MS (Report RD2002/00232/00)

Summary: Solid-phase extraction, HPLC with tandem mass spectrometry.

Range: 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir)

(Method Sheet Reference: RBMT-MET-093.01)

Concentrations of GW433908X and amprenavir were determined in citrated plasma by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS-MS) by using the multiple reaction monitoring (MRM) technique. Unknown samples, QC samples or standards (250 µL) were mixed with a 250-µL portion of internal standard solution ([$^{13}C_6$]-amprenavir and [$^{13}C_6$]-GW433908), and extracted with acetonitrile by solid phase extraction with a Waters OASIS™ HLB Extraction Plate. The extract was diluted with acetonitrile/20 g/L ammonium acetate (20:80 v/v), analysed by reversed-phase HPLC with a C_{18} column, and detected by tandem mass spectrometry with the turbo ionspray source in the positive ion mode. The mobile phase for determination of GW433908X was 0.02 g/L ammonium acetate in acetonitrile:water (15:85, v/v). The mobile phase for determination of amprenavir was 0.02 g/L ammonium acetate in acetonitrile:water (55:45, v/v). The chromatographic run time for each analyte was 3 minutes.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. Linearity was confirmed over a range of 5 – 1000 ng/mL (GW433908X) and 10 – 5000 ng/mL (amprenavir). Quality control samples of 700, 240 and 16 ng/mL (GW433908X) and 4000, 1000, and 36 ng/mL (amprenavir) in plasma were interspersed throughout the

analytical run to monitor assay performance. The lower limits of quantitation were 5 and 10 ng/mL, respectively, for GW433908X and amprenavir.

Validation of a high-performance liquid chromatographic method for the measurement of amprenavir in plasma (Report RD1996/00211/00)

Summary: Solid-phase extraction, HPLC with fluorescence detection.

Range: 50 – 5000 ng/mL (rat and dog) and 10 – 1000 ng/mL (human)

(Method Sheet Reference: RBMT-MET-007.00)

Concentrations of amprenavir were determined in plasma by high performance liquid chromatography (HPLC) with fluorescence detection. Unknown samples, QC samples or standards (500 µL) were mixed with a 500-µL portion of internal standard solution (VB 11599, Vertex Pharmaceuticals, Inc.), and extracted with acetonitrile by solid phase extraction with Sep Pak C_{18} cartridges. Extracts were reduced in volume and analysed by reversed-phase HPLC with a C_{18} column and fluorescence detection. The mobile phase for determination of amprenavir was acetonitrile:water (43:57, v:v) with a flow rate of 0.8 mL/minute. Excitation and emission wavelengths were set at 245 nm and 340 nm, respectively. The chromatographic run time for each analyte was 15 minutes.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. Linearity was confirmed over a range of 50 – 5000 ng/mL (rat and dog) and 10 – 1000 ng/mL (human). Quality control samples of 3000, 600 and 80 ng/mL in dog plasma were interspersed throughout the analytical run to monitor assay performance. The lower limit of quantitation was 50 ng/mL for amprenavir.

2.2. Analysis of Recombinant Human CYP3A4-like Immunoreactivity in Rat Liver Microsomes

The validation of a time-resolved immunofluorometric assay for the determination of rHuCYP3A4-like immunoreactivity in rat liver microsomes (Report RD1999/02148/00)

Summary: Extraction, time-resolved immunoassay.

Range: 3.4 to 250 ng/mL

(Method Sheet Reference: RBMT-MET-134.00)

Unknown rat liver microsome samples were diluted to approximately 5 mg/mL protein with extraction buffer and shaken for approximately 2 hours at room temperature. Extraction supernatant was removed after centrifugation and diluted 1000-fold with diluent/blocking buffer. Diluted extraction supernatant, QC samples or standards were added to a 96-well plate coated with Xenotech #A3103 mouse monoclonal antibody against rat CYP3A1. After refrigeration overnight, the plate was treated with Xenotech #A3005 polyclonal rabbit antibody against rat CYP3A1/2. After incubation for 1 hour at

room temperature, the plate was treated with Europium-labelled sheep anti-rabbit IgG. After further incubation for 1 hour at room temperature, plates were treated with DELFIA enhancement solution, mixed for 10 minutes, and read on a time-resolved fluorometer at approximately 1 hour after enhancement solution application.

The method performance was demonstrated and validated for specificity, linearity, limits of quantitation, accuracy and precision. Quantitation was achieved by comparison of drug/internal standard peak area ratios of samples versus standards. The assay had acceptable precision and accuracy over a range of 3.4 to 250 ng/mL, with rHuCYP3A4-LI stable in rat microsomes for 1 freeze-thaw cycle. The lower limit of quantitation was 3.4 ng/mL.

Table G39. Summary of Bioanalytical Methods and Validation for the Determination of GW433908X or Amprenavir in Plasma During Safety Studies with GW433908G or Amprenavir

Matrix Plasma	Method Report (Validation Report)	Preclinical Study Number (Report Number)	Method Type	Validation Summary
Mouse	RBMT-MET-093.01 (RD2002/00233/00)	M40725 (RD2000/02408/00)	LC/MS/MS	Range: 5 – 1000 ng/mL (GW433908X) 10 – 5000 ng/mL (amprenavir) LLOQ: 5 and 10 ng/mL Intra-assay precision (%CV): <2.1 – 2.7% (GW433908X) <0.5 – 1.5% (amprenavir) Accuracy: <±7.1% (GW433908X) <±5.0% (amprenavir)
Mouse	RBMT-MET-068.00 (RD1998/01974/00)	M40221 (RD1998/02066/01)	LC/MS/MS	Range: 10 – 5000 ng/mL (amprenavir) LLOQ: 10 ng/mL (amprenavir) Intra-assay precision (%CV): ≤11.2% (amprenavir) Accuracy: ≤±11.3% (amprenavir)
Rat	RBMT-MET-093.01 (RD1999/02681/00)	S22240 (WD1999/00154/00) R40357 (RD1998/00541/00) 98APK0135 (RD1998/02935/00) R40427 (RD1998/02573/00) R40417 (RD1998/02858/01) R40877 (RD2000/02506/00) R40576 (RD1999/02344/00) R40476 (RD1999/00412/00) R40458 (RD1999/01281/00) R40470 (RD1999/02690/00) R40860 (RD2002/00045/00) R40857 (RD2001/01884/00) R40917 (RD2001/00212/00)	LC/MS/MS	Range: 5 – 1000 ng/mL (GW433908X) 10 – 5000 ng/mL (amprenavir) LLOQ: 5 and 10 ng/mL Intra-assay precision (relative standard deviation): 0.8 – 12.5% (GW433908X) 1.4 – 8.1% (amprenavir) Accuracy: ±8.8% (GW433908X) ±11.2% (amprenavir)

Key: LLOQ = Lower limit of quantitation

Table G39 (Continued). Summary of Bioanalytical Methods and Validation for the Determination of GW433908X and Amprenavir in Plasma during Safety Studies with GW433908G or Amprenavir

Matrix Plasma	Method Report (Validation Report)	Preclinical Study Number (Report Number)	Method Type	Validation Summary
Rat	RBMT-MET-069.00 (RD1998/02888/00)	R40222 (RD1998/01521/01)	LC/MS/MS	Range: 10 – 5000 ng/mL (amprenavir) LLOQ: 10 ng/mL (amprenavir) Intra-assay precision (%CV): <12.9% (amprenavir) Accuracy: <±13.7% (amprenavir)
Rabbit	RBMT-MET-093.01 (RD1999/02681/00)	L40459 (RD1999/00465/00) L40460(RD1999/00716/00) L40461(RD1999/01035/00)	LC/MS/MS	Range: 5 – 1000 ng/mL (GW433908X) 10 – 5000 ng/mL (amprenavir) LLOQ: 5 and 10 ng/mL Intra-assay precision (relative standard deviation): 1.6 – 6.8% (GW433908X) 0.8 – 1.2% (amprenavir) Accuracy: ±5.0% (GW433908X) ±3.7% (amprenavir)
Dog	RBMT-MET-093.01 (RD1999/00101/00)	S22241 (WD1998/00543/00) S22321 (WD1999/00155/00) 98APK0135 (RD1998/02935/00) D40436 (RD1998/02605/00)	LC/MS/MS	Range: 5 – 1000 ng/mL (GW433908X) 10 – 5000 ng/mL (amprenavir) LLOQ: 5 and 10 ng/mL Intra-assay precision (%CV): ≤11.3% (GW433908X) ≤4.5% (amprenavir) Accuracy: -1.2 – 5.4% (GW433908X) -0.3 – 3.0% (amprenavir)

Key:
LLOQ = Lower limit of quantitation

Table G39 (Continued). Summary of Bioanalytical Methods and Validation for the Determination of GW433908X and Amprenavir in Plasma during Safety Studies with GW433908G or Amprenavir

Matrix Plasma	Method Report (Validation Report)	Preclinical Study Number (Report Number)	Method Type	Validation Summary	
Dog	RBMT-MET-093.01 (RD2002/00232/00)	D40418 (RD1998/02861/01)	LC/MS/MS	Range: LLOQ: Intra-assay precision (%CV): Accuracy: 	5 – 1000 ng/mL (GW433908X) 10 – 5000 ng/mL (amprenavir) 5 and 10 ng/mL <0.91 – 3.7% (GW433908X) <1.3 – 5.0% (amprenavir) <±4.3% (GW433908X) <±2.6% (amprenavir)
Dog	RBMT-MET-007.00 (RD1996/00211/00	S22365 (WD1998/00588/01)	LC/MS/MS	Range: LLOQ: Intra-assay precision (%CV): Accuracy:	50 - 5000 ng/mL (amprenavir) 50 ng/mL (amprenavir) 1.4 – 5.6% (amprenavir) <±10.0% (amprenavir)

Key:
LLOQ = Lower limit of quantitation

Table G40. Summary of the Method and Validation for Determination of Human CYP3A4-like Immunoreactivity in Rat Liver Microsomes

Matrix	Method Report (Validation Report)	Preclinical Study Number (Report Number)	Method Type	Validation Summary	
Rat liver microsomes	RBMT-MET-134.00 (RD1999/02148/00)	99AVV0024 (RD1999/02360/02)	Time-resolved immunofluorometric assay	Range: Intra-assay precision (%CV): Inter-assay precision (%CV) Accuracy:	3.4 - 250 ng/mL <12.8% <4.5% <3.5%

APPENDIX G2.
Control Sample Data from Toxicokinetic Studies with GW433908G

Introduction

The following tabulations present the control sample data from the safety studies submitted as part of this application. Table G41 and Table G42 present the control plasma amprenavir concentrations from the mouse and rat amprenavir carcinogenicity studies, respectively. Table G43 summarises control plasma amprenavir and GW433908X concentrations from safety studies with GW433908A or GW433908G. Table G44 details the control sample data for GW433908X from the GW433908G rat fertility study, while Table G45 presents the derived control sample GW433908X pharmacokinetic parameters from the fertility study.

Table G41. Control Sample Data from Mouse Carcinogenicity Study with Amprenavir (Report RD1998/02066/01, Study M40221)

Sampling Week	Control group	Sex	Amprenavir (µg/mL)†			
			Individual Animal Data			Mean ± SD
Week 1	Amprenavir was not detected in any control samples in Week 1					
26	Water	M	BQL	BQL	BQL	BQL
		F	BQL	BQL	0.02	0.01 ±0.01
	Vehicle	M	BQL	BQL	BQL	BQL
		F	BQL	BQL	BQL	BQL
52	Water	M	0.07	0.02	BQL	0.03 ± 0.03
		F	BQL	NR	0.02	0.01 ± 0.02
	Vehicle	M	0.04	0.02	0.05	0.04 ± 0.02
		F	0.03	0.02	0.05	0.03 ± 0.01
Week 78	Amprenavir was not detected in any control samples in Week 78					

Key: BQL = Below Quantifiable Limits (0.01 µg/mL), NR = No Result
† = Samples taken 7 hours after first daily dose (ie 1 hour after second daily dose)

Table G43. Control Sample Data in GW433908G Studies

[NB: On instances where GW433908X or APV was detected, only the detected concentrations only are shown; other samples at these timepoints were BQL. Control groups were made up of 3 to 4 (rats and mice) or 4 to 6 (dogs) animals/sex**]**.

Study Type Report No. (Study No.)	Sex	Day	Amprenavir (µg/mL)	Day	GW433908X (ng/mL)
Mouse 13 week RD2000/02408/00 (M40725)	M F	NA NA	BQL BQL	1 90 1	6.01 12.2 13.4
Rat micronucleus study RD1999/00412/00 (R40476)	M	NA	BQL	NA	BQL
Rat 14 day RD1998/00711/00 (R40364)	M	NA	BQL	NA	BQL
Rat 4 weeks RD1998/02573/00 (R40427)	M F	NA NA	BQL BQL	1 1 23	7.17, 104, 17.8 325, 89.3 8.00
Rat 26 weeks RD1998/02858/00 (R40417)	M F	NA NA	BQL BQL	NA 92	BQL 5.06
Rat 14 day impurity study RD2000/01884/00 (R40857)	M F	NA NA	BQL BQL	13 NA	179 BQL
Rat 14 day impurity study RD2001/00212/00 (R40917)	M F	NA NA	BQL BQL	NA NA	BQL BQL
Rat 15 day juvenile study RD2000/02506/00 (R40877)	M F	18 NA	0.324 BQL	NA NA	BQL BQL
Rat 4 week juvenile study RD1999/02344/00 (R40576)	M F	NA NA	BQL BQL	6 NA	28.5 BQL
Rat 13 week juvenile study RD2002/00045/00 (R40860)	M F	NA NA	BQL BQL	NA NA	BQL BQL

Key:
NA = Not applicable – GW4339089X or APV not detected in any control sample on any day
BLQ = Below the lower limit of quantitation (0.01 µg/mL amprenavir, 5.0 ng/mL GW433908X)

Table G43 (Continued). Control Sample Data in GW433908G Studies

Study Type Report No. (Study No.)	Sex	Day	Amprenavir (µg/mL)	Day	GW433908X (ng/mL)
Rat fertility study RD1999/01281/00 (R40458)	M F	42 NA	0.02, 0.03, 0.02 BQL	1, 27, 42 1, 27, 42	See Tables 2 + 3 See Tables 2 + 3
Rat embryofetal study RD1999/02690/00 (R40470)	F	NA	BQL	NA	BQL
Rabbit non-pregnant MRD RD1999/00465/00 (L40459)	F	NA	BQL	NA	BQL
Rabbit pregnant MRD RD1999/00716/00 (L40460)	F	NA	BQL	NA	BQL
Rabbit embryofetal study RD1999/01035/00 (L40461)	F	NA	BQL	NA	BQL
Dog 14 day study RD1998/00487/01 (D40350)	M	NA	BQL	NA	BQL
Dog 4 week study RD1998/02605/00 (D40436)	M F	NA NA	BQL BQL	NA NA	BQL BQL
Dog 40 week study RD1998/02861/01 (D40418)	M F	180 273	0.01 0.03, 9.45	273 1 273	7.60 13.6 251

Key:
NA = Not applicable – GW4339089X or APV not detected in any control samples on any day
BLQ = Below the lower limit of quantitation (0.01 µg/mL amprenavir, 5.0 ng/mL GW433908X)

Table G44. Concentration-time Profiles for GW433908X in Control Rats Following Oral Administration of GW433908G to During the Fertility Study (Report RD1999/01281/00)

A) MALES

Time (h)	GW433908X (ng/mL)					
Day 1						
Animal	1	2	3	4	AVG	SD
0	BQL	BQL	BQL	BQL	BQL	-
1	BQL	340	16.0	13.6	92.40	165.22
2	BQL	BQL	BQL	BQL	BQL	-
6	BQL	BQL	BQL	BQL	BQL	-
7	1220	601	45.1	20.2	471.58	566.42
8	BQL	21.4	72.6	34.4	32.10	30.50
10	17.8	15.8	18.1	16.0	16.93	1.19
24	BQL	BQL	BQL	BQL	BQL	-
Day 27						
Animal	5	6	7	8	AVG	SD
0	17.1	BQL	59.1	21.7	24.48	24.90
1	6.32	8.44	5.69	9.31	7.44	1.71
2	161	86.8	466	91.9	201.43	179.60
6	BQL	25.2	50.1	17.2	23.13	20.83
7	BQL	14.6	BQL	134	37.15	64.93
8	20.9	13.7	19.9	BQL	13.63	9.63
10	BQL	69.9	24.8	BQL	23.68	32.96
24	BQL	BQL	BQL	BQL	BQL	-
Day 42						
Animal	9	10	11	12	AVG	SD
0	19.5	36.0	13.6	64.5	33.40	22.80
1	13.5	18.0	10.7	44.0	21.55	15.27
2	145	104	151	887	321.75	377.41
6	10.7	47.4	41.4	BQL	24.88	23.10
7	152	15.8	153	21.2	85.50	77.40
8	29.2	51.8	5.58	9.46	24.01	21.22
10	BQL	5.22	BQL	BQL	1.31	2.61
24	8.14	26.8	20.9	27.8	21	9

Key: BQL = Below the lower limit of quantitation (5.0 ng/mL)

Table G44 (Continued). Concentration-time Profiles for GW433908X in Control Rats following Oral Administration of GW433908G to During the Fertility Study (Report RD1999/01281/00)

B) FEMALES

Time (h)	GW433908X (ng/mL)					
Day 1						
Animal	1	2	3	4	AVG	SD
0	22.9	113	7.24	BQL	35.79	52.36
1	2800	19.5	38.4	13.6	717.88	1388.12
2	19.3	21.8	62.1	215	79.55	92.41
6	46.4	9.59	501	8.88	141.47	240.33
7	29.0	17.6	28.8	7.08	20.62	10.48
8	NS	47.9	20.3	33.6	33.93	13.80
10	54.0	21.7	16.5	26.5	29.68	16.72
24	BQL	BQL	75.7	9.53	21.31	36.54
Day 13						
Animal	5	6	7	8	AVG	SD
0	5.60	53.4	55.0	30.1	36.03	23.26
1	22.6	BQL	12.0	27.9	15.63	12.34
2	BQL	BQL	11.2	BQL	2.80	5.60
6	BQL	BQL	BQL	88.6	22.15	44.30
7	52.6	15.0	17.5	11.8	24.23	19.06
8	6.75	BQL	BQL	BQL	1.69	3.38
10	27.0	7.26	BQL	BQL	8.57	12.76
24	BQL	BQL	BQL	BQL	BQL	-
Day 28						
Animal	9	10	11	12	AVG	SD
0	BQL	15.0	BQL	BQL	3.75	7.50
1	100	BQL	25.7	13.7	34.85	44.68
2	15.8	38.0	12.8	11.9	19.63	12.36
6	BQL	BQL	BQL	13.9	3.48	6.95
7	305	163	IS	36.1	168.03	134.52
8	73.3	86.9	33.8	40.9	58.73	25.46
10	BQL	BQL	8.70	BQL	2.18	4.35
24	13.1	23.4	22.1	32.5	22.78	7.94

Key: NS = No Sample; BQL = Below the lower limit of quantitation (5.0 ng/mL); IS = Insufficient sample

Table G45. Summary GW433908X Toxicokinetic Data in Control Rats from the Fertility Study (Report RD1999/01281/00)

Day	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC (ng*h/mL)	GW433908X:amprenavir AUC Ratio (%)
Males					
1	472	1.0	0.7	646	ND
27	201	2.0	ND	828	ND
42	322	2.0	ND	1184	4439
Females					
1	718	1.0	26	2533	ND
13	36.0	0 (24)	ND	191	ND
28	168	1.0	ND	528	ND

Key:
ND = Not determined