MODULE 2.6.4. PHARMACOKINETICS WRITTEN SUMMARY

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List of Abbreviations

$AUC_{(0-24)}$	Area under the plasma concentration verses time curve (from time 0 to 24 hours)
$AUC_{(0-t)}$	Area under the plasma concentration verses time curve (from time 0 to last time point)
CAB	Cabotegravir
C_{max}	Maximum observed plasma drug concentration
CL_b	Blood clearance
CL_p	Plasma clearance
CL_i	Intrinsic clearance
equiv	Equivalents
F	Absolute bioavailability
IM	Intramuscular
LAP	Long-acting parenteral
MRI	Magnetic resonance imaging
$PAIC_{90}$	Protein-adjusted IC_{90}
RPV	Rilpivirine
SC	Subcutaneous
t_{max}	Time at which C_{max} occurred
$t_{½}$	Half-life
V_{SS}	Volume of distribution at steady state

1. SUMMARY

Studies have been performed to characterise the absorption, pharmacokinetics, distribution, metabolism and excretion of cabotegravir (GSK1265744 or CAB). In vivo studies were primarily conducted by the oral route and by the subcutaneous / intramuscular routes, as these are the proposed therapeutic routes for CAB in humans. Also, the intravenous route was used experimentally to assess the pharmacokinetics and bioavailability. In vitro investigations have also been conducted to determine the binding of CAB to plasma proteins, its association with red blood cells, its metabolism by or interaction with cytochrome P450 isoenzymes, and the potential interaction with various transporters. These studies have been conducted in compliance with Company Divisional Standard Operating Procedures and Policies and in general accordance with the principles of Good Laboratory Practice (GLP). Analysis in support of the pivotal repeated dose toxicity studies, whole body autoradiography studies and excretion studies was performed in full compliance with GLP regulations and were conducted in an Organisation for Economic and Cooperation and Development (OECD) member country in accordance with the OECD Test Guidelines. Nothing occurred to affect adversely the quality or integrity of the experimental data.

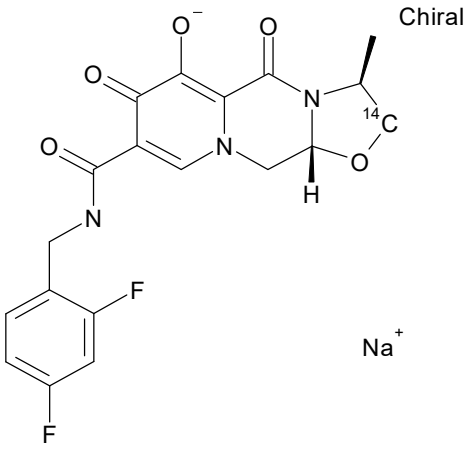
The species and strains used in the present studies reflected those employed in the toxicological testing of CAB, to enable meaningful assessment of the exposure levels in the toxicity studies and provide confidence in the conclusions drawn regarding the safety of CAB in humans. The species and strains used were CD-1 mice, Sprague Dawley rats, Dutch belted rabbits, beagle dogs and cynomolgus monkeys.

A summary of the important findings from the pharmacokinetic studies is provided below. In Sections 3 to 8, a discussion of the design and findings from these studies is presented. An overall assessment of the findings from these investigations is provided in Section 9, Discussion and Conclusions. Tabulations of these studies are provided in m2.6.5. A listing of the studies conducted, together with the location of the reports within Module 4 and their GLP status, is provided in Table 3.1,Table 4.1, Table 5.1, Table 6.1. A list of studies that were undertaken with CAB but are not included in this submission is presented in Appendix 2.

1.1. Test Substance

A selection of single dose pharmacokinetic studies and those investigating the distribution, metabolism and excretion of CAB were performed with stable [^{14}C]-CAB (see Figure 1.1 for structure). The desired specific activity of radioactivity was achieved by diluting radiolabelled drug with unlabelled CAB.

Figure 1.1 Structure of [^{14}C]-CAB



^{14}C denotes position of radiolabel

Repeat dose toxicokinetic analyses were carried out by measuring non-radiolabelled CAB in plasma samples taken during repeat dose toxicity studies, and a number of other studies were also performed using non-radiolabelled CAB.

All studies described in this section were performed using GSK1265744A (parent form/free acid), GSK1265744B (sodium salt) or GSK1265744F (^{14}C-labelled sodium salt). In study reports, the parent compound was designated as GSK1265744A or ERC-265744, and the sodium salt was designated as GSK1265744B or ERC-265744 sodium. All doses and concentrations quoted in this summary are expressed in terms of the parent compound (referred to simply as GSK1265744, cabotegravir, or CAB).

Summary of Findings

Absorption

- Following oral administration as a solution, the oral bioavailability of CAB was good (44 to 83%) and consistent with its high passive permeability. However, when administered as a suspension, or in solid dosage forms, the bioavailability appeared limited by dissolution rate or solubility which resulted in a less than proportional increase in systemic exposure of CAB relative to dose.
- In rats and monkeys given a single SC or IM injection, CAB was slowly released from the injection site with a mean apparent plasma half-life ranging from 12 to 29 days (SC) or from 8 to 12 days (IM).
- There was no observed PK interaction between CAB and rilpivirine (RPV) when co-administered IM to rats or monkeys.

Distribution

- The protein binding of CAB in rat, dog, monkey and human plasma and serum was high (>99%).
- CAB is a substrate for Pgp and BCRP, but due to its high permeability, no alteration in absorption would be expected by co-administration of either Pgp or BCRP inhibitors.
- Elimination of radioactivity was slow with most tissues containing low but quantifiable radioactivity at 28 days. Association of radioactivity to the melanin-containing tissues in the eye and skin was not observed.
- After oral administration of [14C]CAB to rats, radioactivity was slowly absorbed, widely distributed to tissues and minimally associated with blood cellular components.

Metabolism

- The metabolism of CAB in the nonclinical species reflects that observed in humans, with CAB being the principal component circulating in plasma.
- The major metabolite of CAB in all species was CAB glucuronide, which was eliminated by renal and biliary routes and formed primarily by UGT1A1 (with some involvement from UGT1A9).

Excretion

- Across all species, elimination of drug-related material occurred predominantly via the feces (58.5 to 94.5% of the dose).
- In rodents, most of the absorbed radioactivity was secreted into the bile and renal excretion was minimal. In monkeys, the absorbed radioactivity was eliminated via both the biliary and renal routes.

Drug interactions

- Nonclinical studies have not been performed in vivo to specifically investigate potential pharmacokinetic interactions with drugs that are likely to be co-administered with CAB. However, in vitro studies investigating enzyme inhibition / induction, protein binding and transporter interactions have been conducted and, except for strong UGT1A1 inducers (e.g. rifampin), CAB has a low propensity for drug interactions based on in vitro results and physiologically based pharmacokinetic (PBPK) modelling [see m2.7.2, Section 3.2.9].

2. METHODS OF ANALYSIS

In preliminary PK studies, CAB in diluted blood and plasma samples was analyzed by a high performance liquid chromatography tandem mass spectrometric (HPLC-MS/MS) method following protein precipitation (quantitation range from 1 to100 ng/mL). Subsequently, additional HPLC-MS/MS methods were developed and validated for the determination of CAB in mouse, rat, rabbit, monkey and human plasma to support single and repeat dose toxicity studies and studies in humans. For nonclinical species, the HPLC-MS/MS methods used for analysis were validated using an LLOQ of 100 ng/mL in rat, mouse, monkey and rabbit plasma. For clinical studies, the HPLC-MS/MS methods used for analysis were validated using an LLOQ of either 10 or 25 ng/mL, depending on the study requirements.

An assay to support the determination of the bound and free fractions of CAB was validated using an LLOQ of 1 ng/mL in human plasma ultrafiltrate: human plasma (90:10) matrix. In addition, assays were validated for the determination of CAB in human cerebrospinal fluid (LLOQ of 1 ng/mL) and in human breast milk / colostrum (LLOQ of 10 ng/mL). The methods and limits of quantification were suitable to support the kinetic analyses of CAB. Further methods were developed to assess the stability of the O-glucuronide metabolite (also M1 or GSK3388352) in various buffers used in the in vitro drug transporter assays and CAB glucuronide was shown to be stable under the in vitro conditions used.

Determination of the radioactivity in in vitro or in vivo biological samples following administration of [^{14}C]-CAB was carried out by either direct liquid scintillation counting (LSC) or by LSC following combustion of the sample. For radioactivity concentrations in tissues, quantitative whole body autoradiography was used. The metabolic profiling of CAB was conducted by using chromatographic separation with radiometric detection and identification of metabolites performed by using LC-MS^n; nuclear magnetic resonance (NMR) methods were used to confirm structures not confirmed by mass spectrometric methods.

Summaries of the assay methodologies and validation are provided in Appendix 1. The reports are provided in m4.2.2.1, Analytical Methods and Validation Reports.

Non-compartmental methods were used for pharmacokinetic and toxicokinetic data analysis.

3. ABSORPTION

Studies investigating the absorption and pharmacokinetics of CAB after single and repeated administration have been performed in the mouse, rat, rabbit, dog and monkey and are listed in Table 3.1. An inter-species comparison of CAB plasma concentrations following its oral administration in the definitive toxicity studies is presented in Table 9.1, Section 9, Discussion and Conclusions to Pharmacokinetics Studies. A tabular summary of the repeat dose toxicokinetic data derived from the toxicity studies is presented in m2.6.7, Table 3.

In the in vivo studies in rats, dogs and monkeys, CAB was administered intravenously (IV) as a bolus injection prepared as a solution in DMSO:Solutol:0.05 M N-methylglucamine (10:10:80 v:v:v).

Subcutaneous (SC) and intramuscular (IM) injection studies were conducted with CAB as a suspension in varying vehicles.

1. 1.7% polyvinylpyrrolidone (PVP), 0.2% Polysorbate 80 (Tween 80), 0.18% methylparaben, 0.02% propylparaben, 0.008M $NaH_2PO_4H_2O$, 0.006M Na_2HPO_4 and 0.81% NaCl, pH-adjusted to 6.8.
2. 2% (w/w) pluronic F127/0.2% (w/v) Tween 80/0.18% (w/v) methylparaben/0.02% (w/v) propylparaben/0.004 M $NaH2PO_4$ H_20/0.006 M Na_2HPO_4 with NaCl.
3. 2% w/v Tween 80, 2% w/v polyethylene glycol 3350, and 4.5% w/v mannitol in sterile water for injection.

Oral studies were conducted with CAB as a solution, suspension or powder in a capsule. For solution dosing, CAB was prepared in DMSO:Solutol:0.05 M N-methylglucamine (10:10:80 v:v:v) and for suspensions in aqueous 0.5% hydroxypropylmethylcellulose and 0.1% Tween 80. For capsule formulation using the crystalline form, CAB was formulated with Avicel PH 102 and sodium starch glycolate (SSG) as excipients at approximate ratios of 2:1:6, respectively, for rats and 2:1:3, respectively, for dogs and monkeys.

3.1. Mouse

3.1.1. Oral administration

3.1.1.1. Pharmacokinetics/toxicokinetics after single doses

CAB was given to male mice (15/group) by gavage as a single oral dose at dose levels of 10, 100, 1000, or 2000 mg/kg [Report RD2009/00691]. CAB (batch [redacted], micronized) was formulated as a solution (1 mg/mL) or suspension (≥10 mg/mL) in 0.5% hydroxypropyl methylcellulose/0.1% Tween 80 in reverse osmosis-treated water. The toxicokinetic data for CAB determined in this study are presented in m2.6.5, Table 3.4. A summary of the toxicology data is presented in m2.6.6, Section 2.2.

CAB was quantifiable through the 24 hour time point in each dose group. The systemic exposure (C_{max} and AUC_{0-24}) of CAB increased less than proportionally with the increase in dose. There was no difference in systemic exposure following the 2000 mg/kg dose compared to that after the 1000 mg/kg dose.

3.1.1.2. Pharmacokinetics/toxicokinetics after repeated administration

14 day study

In a 14 day study, CAB (sodium salt) was given to male and female mice (n=10/sex/group) at nominal doses of 10, 75 or 1000 mg/kg/day once daily for 14 days by oral gavage [Report RD2009/00692]. CAB (batch [redacted]) was formulated as a suspension in 0.5% hydroxypropyl methylcellulose/0.1% Tween 80 in reverse osmosis-treated water. The toxicokinetic values for CAB determined in this study are presented in m2.6.7, Table 6.2. A summary of the toxicology data is presented in m2.6.6, Section 3.2.1.1.

The systemic exposure (plasma C_{max} and AUC_{0-24} values) of CAB increased less than proportionally with the increase in dose in each sex on both Days 1 and 14. On Day 14 when the dose was increased 7.5- or 100-fold from 10 mg/kg/day, C_{max} increased 2.2- to 2.6-fold or 3.4- to 4.0-fold, respectively; AUC_{0-24} increased 2.1- to 2.3-fold or 3.5- to 4.0-fold, respectively. No marked (≥2-fold) differences were observed in the CAB plasma C_{max} or AUC_{0-24} values between the sexes on either Day 1 or Day 14. The systemic exposure was slightly higher (about 10% to 70%) on Day 14 compared to Day 1; however, the differences did not reach a notable threshold (>2-fold).

13 week study

The toxicokinetics of CAB were investigated in a 13 week repeat dose toxicity study in mice [Report 2012N142081]. Satellite groups of CD-1 mice (54/sex/group) were orally administered CAB (sodium salt) at dose levels of 10, 75 and 1000 mg/kg/day once daily for 13 weeks. CAB was formulated as a suspension in a vehicle of 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80 in Ultra Pure Water. Blood was collected (n = 3/sex/timepoint) at various time intervals on Day 1, Week 4 and Week 13 for plasma concentration analysis. The plasma toxicokinetic parameters were estimated based on mean plasma concentration-time profiles and are presented in m2.6.7, Table 7.1. A summary of the toxicology data is presented in m2.6.6, Section 3.2.1.2.

The systemic exposure values for CAB increased less than proportionally to the increase in dose in males and females on each toxicokinetic day. A 7.5- or 100-fold increase in dose from 10 mg/kg/day resulted in a 2- to 3-fold or a 3- to 5-fold increase, respectively, in systemic exposure.

The composite C_{max} and AUC_{0-t} values (systemic exposure) for CAB in females were similar (difference within 35%) to those in males at each dose level and on each toxicokinetic day (Day 1, Weeks 4 and 13).

The systemic exposure values for CAB on Week 13 were generally slightly greater (generally 19 to 96% higher) than those values on Day 1. The systemic exposure values for CAB on Week 13 were similar (difference within 26%) to those values on Week 4.

104 week study (carcinogenicity)

The toxicokinetics of CAB were investigated during a 104 week carcinogenicity study in mice [Report 2017N310750]. Satellite groups of 42 male and female CD-1 mice were orally administered CAB sodium salt at dose levels of 0 (vehicle), 2.5, 10 or 75 mg/kg/day (males) and at 0 (vehicle), 2.5, 5 or 35 mg/kg/day (females) once daily for a minimum of 104 weeks. CAB was formulated as a suspension in a vehicle of 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80 in Ultra Pure Water. Plasma was collected (n=3/sex/timepoint) at 0.5, 1, 2, 4, 8 and 24 hours after dosing during Week 4 and Week 26. The plasma toxicokinetic parameters were estimated based on mean plasma concentration-time profiles and are presented in Table 9.1. A summary of the toxicology data is presented in m2.6.6, Section 5.2.1.1.

The systemic exposure (composite C_{max} and AUC_{0-t}) increased with increasing dose. The increases in systemic exposure during Weeks 4 and 26 were approximately proportional with dose increases from 2.5 to 10 mg/kg/day in males and from 2.5 to 5 mg/kg/day in females; however, with further increase in doses, the systemic exposure increased less than dose-proportionally. For males, an increase in dose from 2.5 to 10 mg/kg/day, a 4-fold increase, resulted in 2.3- to 2.7-fold increase in C_{max} and AUC_{0-t} values. Additionally, for males, an increase in dose from 10 to 75 mg/kg/day, a 7.5-fold increase, resulted in 2.0- to 2.2-fold increase in C_{max} and AUC_{0-t} values. For females, an increase in dose from 2.5 to 5 mg/kg/day, a 2-fold increase, resulted in 1.7- to 1.8-fold increase in C_{max} and AUC_{0-t} values. Additionally, for females, an increase in dose from 5 to 35 mg/kg/day, a 7.0-fold increase, resulted in 2.0- to 2.5-fold increase in C_{max} and AUC_{0-t} values.

At 2.5 mg/kg/day, for both males and females, there was no marked (>2-fold) sex difference in the systemic exposure during both Weeks 4 and 26. The systemic exposure during Week 26 was similar to that during Week 4 at each dose level for both males and females.

3.2. Rat

3.2.1. Intravenous administration

3.2.1.1. Pharmacokinetics/toxicokinetics after single doses

A study was conducted to characterize the plasma pharmacokinetics of CAB in non-fasted male CD rats (n=3) following intravenous (IV; 1 mg/kg) administration of CAB (free acid) [Report RH2007/00168]. CAB was formulated in DMSO:Solutol:0.05 M N-methylglucamine (10:10:80). Blood samples were collected at various intervals up through 24 hours in two animals, and up through 72 hours in one animal; plasma was processed for analysis of concentrations of CAB. The sampling scheme was insufficient

to characterize the terminal elimination phase of CAB; therefore, certain derived pharmacokinetic parameters could not be determined.

3.2.2. Oral administration

3.2.2.1. Pharmacokinetics/toxicokinetics after single doses

A series of studies were conducted to characterize the plasma pharmacokinetics of CAB i) in fasted rats (n=4/group) following oral solution (5 mg/kg) administration of CAB (free acid); ii) in non-fasted rats (n=3/group) following suspension (5, 50, 150, and 500 mg/kg) administration of CAB (free acid); and iii) in fasted rats (n=6/group) following oral capsule (approximately 5 mg free acid/kg) administration of CAB as free acid or sodium salt [Report RH2007/00168]. To prepare solutions for dosing, CAB was formulated in DMSO:Solutol:0.05 M N-methylglucamine (10:10:80). To prepare suspensions for dosing, CAB was formulated in 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80. Capsules for dosing contained CAB blended with 0.7 mg of sodium starch glycolate (SSG) and 4.5 mg of Avicel PH 102. Due to insufficient characterization of the terminal elimination phase of CAB in these studies, only observed or measured parameters (C_{max}, T_{max} and AUC_{0-t}) are reported. Blood samples were collected at various time intervals and plasma was processed for analysis of CAB concentrations. The pharmacokinetic data for CAB derived from this study are presented in m2.6.5, Table 3.3.

Following a 5 mg/kg oral solution dose of CAB (free acid) in fasted rats, a similar mean $DNAUC_{0-24}$ (151 µg.h/mL/mg/kg) to that obtained following intravenous administration ($DNAUC_{0-24}$,181 µg.h/mL/mg/kg) was observed, suggesting high oral bioavailability. However, CAB exposure was approximately 3-fold lower following oral capsule administration of CAB as either free acid or sodium salt (at a comparable dose), with $DNAUC_{0-24}$ values averaging 48 or 50 µg.h/mL/mg/kg, respectively. Following oral administration of CAB (free acid) in a 0.5% hydroxypropyl methylcellulose / 0.1% Tween 80 suspension formulation to non-fasted rats, CAB exposures increased less than proportionally with increases in dose from 5 to 500 mg/kg. Overall, this 100-fold increase in dose resulted in a 4.1-fold increase in C_{max} and a 4.9-fold increase in AUC_{0-24}. Mean $DNAUC_{0-24}$ values following doses of 5, 50, 150, and 500 mg/kg were 47, 14, 7, and 2 µg.h/mL/mg/kg, respectively. Taken together, these data suggest that the oral absorption of CAB is limited by its solubility and/or dissolution rate.

CAB was given to groups of Sprague Dawley rats (3/sex/group) as a single dose of 3, 30, 300, or 1000 mg/kg by oral gavage [Report RD2008/00200]. CAB was formulated as a suspension in 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80. Analysis was performed on samples collected on Day 1. The exposure parameters of CAB from this study are presented in m2.6.5, Table 3.5.

The mean systemic exposure to CAB (as measured by AUC_{0-24} and C_{max}) increased in a less than dose-proportional manner in male and female rats after single dose administration. Median values for T_{max} ranged from 2 to 4 hours post-dose. No notable (greater than 2-fold) sex differences in mean AUC_{0-24} or C_{max} values were observed after oral gavage administration of CAB.

In another oral study, groups of Sprague Dawley rats (3/sex/group) were given a single dose of micronized or non-micronized CAB (sodium salt) at 1000 mg/kg by oral gavage [Report RD2008/01308]. CAB was formulated as a suspension in 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80. Serial blood samples were collected at various time intervals up to 24 hours post-dose and plasma was analysed for determination of CAB concentrations. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.10.

The mean plasma systemic exposure (AUC_{0-24} and C_{max}) to micronized CAB was not notably different (≥2-fold) than systemic exposure to non-micronized CAB in males (3068 μg.h/mL and 157 μg/mL, respectively, vs 2655 μg.h/mL and 132 μg/mL, respectively) and females (3407 μg.h/mL and 167 μg/mL, respectively, vs 3155 μg.h/mL and 148 μg/mL, respectively). No notable (≥2-fold) sex differences in mean AUC_{0-24} and C_{max} values were observed after oral gavage administration of micronized or non-micronized CAB (sodium salt).

3.2.2.2. Pharmacokinetics/toxicokinetics after repeated administration

14 day study

In a 14 day oral gavage toxicity study in Sprague Dawley rats (n=3/sex/group), CAB (free acid) was administered orally at doses of 30, 100 and 300 mg/kg/day [Report RD2006/01741]. CAB was formulated as a suspension in aqueous 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80. To evaluate toxicokinetics, plasma samples were taken on Day 1 and Day 14 at intervals up to 24 hours post-dose and plasma was analysed for concentrations of CAB. The toxicokinetic parameters of CAB derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.3.1.1.

CAB was quantifiable in the plasma of all animals up to at least 24 hours post-dose. Median T_{max} values occurred between 1 and 10 hours after dosing. The mean systemic exposure to CAB (AUC_{0-24} and C_{max}) increased in a less than dose-proportional manner in male and female rats on Days 1 and 14. No notable (>2-fold) differences in mean AUC_{0-24} and C_{max} values were observed between sexes. Exposures were approximately 3- to 6-fold greater on Day 14 compared to Day 1.

4 week study

In a 4 week oral toxicity study, male and female rats (n=3/sex) were administered CAB sodium salt by oral gavage at nominal doses of 1, 75 and 1000 mg/kg/day [Report RD2008/00448]. CAB was formulated as a suspension in 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80 in reverse osmosis-treated water. The plasma concentration of CAB was measured in 3 animals/sex/group at various intervals up through 24 hours post-dose on Days 1 and 28. The toxicokinetic data for CAB determined from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.3.1.2.

CAB was quantifiable in the plasma of all animals receiving CAB up to at least 24 hours post-dose. Median T_{max} values occurred between 0.5 and 4 hours after dosing. The mean systemic exposure to CAB (AUC_{0-24} and C_{max}) increased in a less than dose-proportional manner in male and female rats on Day 1 and Day 28. Overall, for a 1000-fold increase in dose from 1 to 1000 mg/kg/day, mean AUC_{0-24} and C_{max} values increased approximately 4.5-fold and 4.4-fold, respectively, on Day 28. No notable differences in mean AUC_{0-24} and C_{max} values were observed between the sexes. Notable (≥2-fold) differences in mean plasma systemic exposure (AUC_{0-24} and C_{max}) to CAB on Day 28 were observed compared to Day 1 in the 1 mg/kg/day dose group (8.1- to 11-fold) and in the 75 mg/kg/day dose group (2.2- to 2.6-fold) but not in the 1000 mg/kg/day dose group.

26 week study

In a 26 week oral gavage repeat dose toxicity study, male and female rats were administered CAB (sodium salt) once daily at doses of 0.5, 5 and 1000 mg/kg/day for at least 26 weeks [Report RD2009/00031]. CAB was formulated as a suspension in 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80 in deionized water. The plasma concentration of CAB was measured in 3 animals/sex/group at various times through 24 hours post-dose during Weeks 4, 13, and 26. The toxicokinetic parameters of CAB determined in this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.3.1.3.

The systemic exposure to CAB (as defined by AUC_{0-24} and C_{max} values) increased less than proportionately with the increase in dose on each sampling occasion (Weeks 4, 13 and 26) in both sexes. For a 2000-fold increase in dose from 0.5 to 1000 mg/kg/day, the mean AUC_{0-24} and C_{max} values increased 6- to 11-fold. Systemic exposure to CAB was not notably different (>2-fold) among sampling occasions at any dose level. No notable differences in mean systemic exposure to CAB were observed between males and females at any of the doses or sampling occasions.

104 week study (carcinogenicity)

The toxicokinetics of CAB were investigated during a 104 week carcinogenicity study in rats [Report 2017N310751]. Satellite groups of 6 male and female Sprague Dawley rats were dosed by oral gavage with CAB sodium salt once daily at dose levels of 0.25, 2.5 and 75 mg/kg/day. CAB was formulated as a suspension in 0.5% (w/v) hydroxypropyl methylcellulose / 0.1% (w/v) Tween 80 in Ultra Pure Water. Plasma was collected (n=3/sex/group at each timepoint) during Week 4 and Week 26 and composite plasma concentration profiles were determined. The toxicokinetic parameters of CAB determined in this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 5.3.1.1.

CAB was quantifiable in plasma during the entire 24 hour sampling period in Weeks 4 and 26 for all animals. In Weeks 4 and 26 (i.e. Days 28 and 182, respectively), the median T_{max} values ranged from 0.5 to 4 hours for males and from 0.25 to 2 hours for females.

The systemic exposure (gender-averaged C_{max} and AUC_{0-t}) increased with increasing dose. Systemic exposure (C_{max} and AUC_{0-t}) for males and females given CAB were similar (within 2-fold), and so, gender-averaged C_{max} and AUC_{0-t} values were used for interpretation. The increases in systemic exposure during Weeks 4 and 26 were less than dose-proportional. For a 10-fold increase in dose from 0.25 to 2.5 mg/kg/day, the C_{max} values increased 5.0- to 5.6-fold and the AUC_{0-t} values increased 4.9- to 5.8-fold. For a 30-fold increase in dose from 2.5 to 75 mg/kg/day, the C_{max} values increased 2.2- to 2.5-fold and the AUC_{0-t} values increased 2.2- to 2.6-fold. The systemic exposure was similar in Week 26 compared to Week 4.

3.2.3. Subcutaneous and intramuscular administration

3.2.3.1. Pharmacokinetics/toxicokinetics after single doses

CAB (free acid) was given to groups of male Sprague Dawley rats (n=3/group) as a single subcutaneous (SC) or intramuscular (IM) dose at 5 mg/kg. CAB was formulated as a suspension at a concentration of 2.47 mg/mL (SC) and 10.2 mg/mL (IM) in 1.7% polyvinylpyrrolidone (PVP), 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben, 0.004M NaH_2PO_4, 0.006M Na_2HPO_4 and 0.84% NaCl, pH-adjusted to 6.7. Pharmacokinetic evaluation (serial profiling) was performed on samples collected from rats from Day 1 to 36 after administration of CAB [Report RH2009/00012]. The pharmacokinetic data for CAB determined in this study are presented in m2.6.5, Table 3.6.

Overall, exposure (AUC_{0-t}) and C_{max} via the IM route was higher than via the SC route at a dose of 5 mg/kg. At approximately 864 hours post-dose (Day 36), blood concentrations of CAB were approximately 4.3 to 4.6-fold higher than the protein adjusted inhibitory concentration ($PAIC_{90}$) of 166 ng/mL in the SC and IM groups, respectively.

A second investigative study was conducted to characterize the effect of CAB particle size on the release profile. CAB (free acid) was given to groups of male Sprague Dawley rats (n=5/group) as a single subcutaneous (SC) or intramuscular (IM) dose at 5 mg/kg. CAB was formulated as a wet bead milled (WBM) suspension; particle size x90; 5.59 microns and as a homogenized suspension, particle size x90; 68.9 microns in 1.7% PVP, 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben, 0.004M NaH_2PHO_4, 0.006M Na_2HPO_4 and 0.85% NaCl, target pH 6.7, and administered to rats at a dose volumes of 2 mL/kg and 0.5 mL/kg. Blood samples were collected at various intervals up through Day 67 and analysed for CAB [Report RH2009/00013]. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.6.

Following a single SC or IM dose of CAB in a long acting parenteral formulation (two particle sizes, 5.59 and 68.9 microns) to rats at a dose of 5 mg/kg, blood concentrations were quantifiable up to 1608 hours (Day 67) post-dose. Overall, exposure (AUC_{0-t}) and C_{max} in the SC and IM arm of the smaller particle size treated groups were higher than those seen with the large particle size groups. A 20% decrease in the SC exposure (AUC_{0-t}) and a 24% decrease in IM exposure were seen in the larger particle size group compared to the rats treated with CAB as a smaller particle size formulation. Blood concentrations of CAB in the SC arm for both groups at Day 67 were approaching the

$PAIC_{90}$ of 166 ng/mL, whereas in the IM arm in both groups, the blood concentrations were 2 to 3-fold lower than the $PAIC_{90}$. The half-life in the IM arms of both the groups were similar (9 days), whereas in the SC arm the half-life in the larger particle size group was prolonged from 10 days to 14 days when compared to the smaller particle group.

In a toxicokinetics study, CAB (free acid) was given to groups of male Sprague Dawley rats (n=6/group) at a dose of 10 mg/kg as a single subcutaneous or intramuscular injection [Report RD2009/00865]. Toxicokinetic evaluation was conducted on whole blood samples collected on Days 1, 2, 3, 4, 6, 8, 15, 22, 29, 36, 43, 50, 57 and 64. CAB was formulated as wet bead-milled suspensions at concentrations of 0 and 20 mg/mL in aqueous 1.7% (w/v) polyvinylpyrrolidone (PVP)/0.2% (w/v) Tween 80/0.18% (w/v) methylparaben/ 0.02% (w/v) propylparaben/0.004 M NaH_2PO_4 H_20/0.006 M Na_2HPO_4 with NaCl (particle size of x50 at 0.72 microns and x90 at 1.26 microns) or in aqueous 2% (w/w) pluronic F127/0.2% (w/v) Tween 80/0.18% (w/v) methylparaben/0.02% (w/v) propylparaben/0.004 M NaH_2PO_4 H_20/0.006 M Na_2HPO_4 with NaCl (particle size of x50 at 0.27 microns and x90 at 1.05 microns). The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.7. A summary of the toxicology data is presented in m2.6.6, Section 2.3.

CAB was slowly released from the injection sites and gave sustained blood drug concentrations via both routes in the PVP group. The mean half-life of CAB was 23 days by the SC route and 12 days by the IM route. The release profile of CAB was much faster with the pluronic F formulation compared with the PVP formulation; the mean half-life was 8 days by the SC route and 9 days by the IM route. The C_{max} was greater and was achieved faster by both routes of administration with the pluronic F formulation as compared with the PVP formulation. No notable (≥2-fold) difference in systemic exposure (AUC_{0-t}) was observed between the pluronic F and PVP formulations.

On Day 64, the blood concentrations after administration of the PVP formulation were approximately 15-fold greater by the SC route and 7-fold greater by the IM route than the protein-adjusted concentration for 90% inhibition ($PAIC_{90}$) of the human immunodeficiency virus (0.166 μg/mL), whereas CAB concentrations with the pluronic F formulation after SC and IM administration were below or approaching the $PAIC_{90}$. On Day 64 in the PVP group, approximately 78% of the total dose administered SC was recovered, whereas when administered IM, approximately 100% of the dose was recovered. Similarly, in the pluronic F group, approximately 100% of the total dose administered was recovered via either route on Day 64.

In another toxicokinetics study, CAB (free acid) was given to groups of male Sprague Dawley rats (n=3/group) as single doses of 10, 30, or 50 mg/kg by the SC route, or as doses of 5, 20, or 35 mg/kg by the IM route [Report RD2009/00906]. Toxicokinetic evaluation was performed on samples collected on Days 1, 2, 3, 4, 6, 8, 15, 22, 29, 35, 42, 49, and 56. CAB was formulated as suspensions in 2% pluronic F127, 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben, 0.004 M sodium phosphate dibasic (NaH_2PO_4), 0.006M sodium phosphate monobasic (Na_2HPO_4) and 0.83% sodium chloride (NaCl) in sterile water for injections, USP, at a pH of 6.4. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.7. A summary of the toxicology data is presented in m2.6.6, Section 2.2.

Except for a single animal, the plasma concentrations of CAB remained above the protein-adjusted concentration of 0.16 μg/mL for 90% inhibition of the human immunodeficiency virus ($PAIC_{90}$) through the last time point (Day 56) following all SC or IM doses examined. The median T_{max} values ranged from 72 to 168 hours after the SC doses and from 12 to 168 hours following IM doses; this occurred earlier following the two lower IM doses compared to the two lower SC doses and tended to increase as the dose was increased.

When the SC dose was increased 3- and 5-fold from 10 mg/kg, the mean C_{max} values increased 1.8- and 2.6-fold, respectively. The mean AUC_{0-24} values were similar among the three doses. The mean $AUC_{0-\infty}$ values increased 2.5- and 3.9-fold when the dose was increased 3- and 5-fold, respectively, from 10 mg/kg.

When the IM dose was increased 4- and 7-fold from 5 mg/kg, the mean C_{max} values increased 2.2- and 2.7-fold, respectively. The mean AUC_{0-24} values increased 1.7- and 1.8-fold when the dose was increased 4- and 7-fold, respectively, from 5 mg/kg; the mean AUC_{0-24} values after the 20 and 35 mg/kg doses were similar. The mean $AUC_{0-\infty}$ values increased 2.7- and 4.5-fold when the dose was increased 4-and 7-fold, respectively, from 5 mg/kg.

The plasma AUC_{0-24} values for CAB following the 10, 30, and 50 mg/kg SC doses represent on average 4%, 2%, and 1%, respectively, of the $AUC_{0-\infty}$ values. The plasma AUC_{0-24} values for CAB following the 5, 20, and 35 mg/kg IM doses represent on average 8%, 5%, and 3%, respectively, of the $AUC_{0-\infty}$ values.

A further toxicokinetics study was conducted in which CAB (free acid) was given to groups of male Sprague Dawley rats (n=3/group) as single SC doses of 10, 30, or 50 mg/kg or as single IM doses of 5, 20, or 35 mg/kg [Report RD2009/01216]. Toxicokinetic evaluation was performed on samples collected on Days 1, 2, 3, 4, 6, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 85, 92, 99, and 106. CAB was formulated as a suspension in 2% Tween 20, 2% polyethylene glycol 3350, and 4.5% mannitol in water. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.8. A summary of the toxicology data is presented in m2.6.6, Section 2.2.

Following the SC dose, plasma concentrations of CAB remained above the $PAIC_{90}$ of 0.16 μg/mL through the last time point (Day 106) for every animal. Following the IM dose, plasma concentrations remained above 0.16 μg/mL through the last time point of Day 106 in all 3 animals that received 20 mg/kg, and in 2 of the 3 animals that received 35 mg/kg. Following 5 mg/kg, the plasma concentrations remained above 0.16 μg/mL to Day 71.

In a GLP toxicokinetics study, CAB (free acid) was given as a single dose to groups of male and female Sprague Dawley rats (n=3/sex/group) by SC injection at dose levels of 5, 30, or 100 mg/kg or by IM injection at dose levels of 2.5, 10, or 75 mg/kg [Report RD2009/01359]. Toxicokinetic evaluation was performed on samples collected at approximately 2, 4, 8, 12, 24, 48, 72, 120, 168, and 336 hours after dosing, and on Day 21 (480 hours), Day 31 (720 hours), Day 41 (960 hours), Day 51 (1200 hours), Day 61 (1440 hours), Day 75 (1776 hours), and Day 85 (2016 hours, SC only) after

dosing. CAB dosing formulation was prepared in a wet bead-milled suspension at a concentration of 208 mg/mL in 2% polysorbate 20 (Tween 20)/2% polyethylene glycol 3350/4.5% mannitol in sterile water for injection. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.8. A summary of the toxicology data is presented in m2.6.6, Section 2.2.

There were no marked sex differences (≥2-fold) in the mean C_{max} or AUC_{0-t} values following any of the SC or IM doses. The systemic exposure (mean C_{max} and AUC_{0-t} values) of CAB generally increased less than proportionally to the increase in dose following the SC or IM injections. However, sustained systemic exposure of CAB would be expected to continue past the day of the last timepoint (Day 85) following the SC injections at 30 or 100 mg/kg, but not following 5 mg/kg. The disproportionate increase of AUC_{0-t} with dose, particularly following 100 mg/kg SC, may be a result of systemic exposure that continues beyond Day 85. Generally, there was no extended systemic exposure beyond Day 74 following the IM injections at any dose.

The pharmacokinetics and relative bioavailability of CAB (free acid) were determined following a single IM administration of a long acting formulation of CAB (10 mg/kg) in combination with a nano-milled formulation of rilpivirine at 60 mg/kg. Groups of male Sprague Dawley rats (n=3/group) were administered a single IM dose of either CAB alone or rilpivirine alone, and another group of male rats (n=6) were administered both CAB and rilpivirine together to determine any effects of co-administration on the pharmacokinetics [Report 2011N127517]. Pharmacokinetic evaluation was performed on samples collected at various time intervals up through 60 days post-dose. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.12.

The systemic exposure (mean plasma C_{max} or AUC values) for CAB when dosed in combination with rilpivirine was not substantially different (within 22%) from when CAB was dosed alone. The apparent mean plasma $t_{1/2}$ for CAB following combination treatment with rilpivirine was 82% longer than when CAB was dosed alone. However, the difference may be due more to stopping collection of blood samples prior to achieving the terminal phase of elimination in a few animals than to an inherent difference in the rate of elimination. The median CAB plasma T_{max} was 14 days regardless of whether CAB was administered alone or with rilpivirine.

3.2.3.2. Pharmacokinetics/toxicokinetics after repeated administration

In a 13 week SC and IM injection toxicity study, groups of male and female Sprague Dawley rats were administered CAB (free acid) by SC injection at 5, 30, or 100 mg/kg/dose, or by IM injection at 2.5, 10, or 75 mg/kg/dose, on Days 1, 31, and 61 [Report 2010N104820]. A separate group of male and female rats received CAB by SC injection at 100 mg/kg/dose once a week for 13 weeks. Toxicokinetic evaluation was performed on samples collected at various time intervals throughout the study. CAB was formulated as a suspension in 2% (w/v) Tween 20, 2% (w/v) polyethylene glycol 3350, and 4.5% (w/v) mannitol in sterile water for injection. The C_{max} and AUC values along with the comparative animal to human exposure ratios are presented in Table 9.2, and a summary of the toxicology data is presented in m2.6.6, Section 3.3.2.1.

The systemic exposure during the third monthly SC dose interval increased less than proportionally to the increase in dose. For a 20-fold increase in dose systemic exposure increased 6- to 9-fold. The systemic exposure during the third monthly IM dose interval increased in approximate proportion from the low to the mid dose and less than proportionally at the high dose. For a 30-fold increase in dose, systemic exposure increased 7- to 19-fold. The mean AUC values during the third monthly SC dose intervals ranged from 2- to 3-fold higher than those during the first monthly dose intervals, whereas the increase in AUC values over this timeframe following monthly IM injections was minimal (1.1-to 1.4-fold). This suggests that the apparent half-life for disposition was shorter from the IM injection site than from the SC site.

Based on the general review of weekly pre-dose plasma concentration values, it appears that steady-state was reached by the 8th weekly SC dose. No notable (≥2-fold) sex differences were observed in the mean C_{max} or AUC values for CAB.

3.3. Rabbit

3.3.1. Oral administration

3.3.1.1. Pharmacokinetics/toxicokinetics after repeated administration

Pregnant rabbits

In a dose range toxicity study, pregnant Dutch Belted rabbits (n=4/group) were administered CAB (sodium salt) by oral gavage for 13 days (Days 7 to 19 post-coitum) [Report CD2008/01276]. Dose levels of 30, 250, 500, 1000, 2000 mg/kg/day (once daily) and 2000 mg/kg/day (at 1000 mg/kg twice daily) were tested in three rounds. Each round was composed of two test article-treatment groups of 4 rabbits each and a control group of 2 rabbits. Dose levels in Round 1 were 30 and 250 mg/kg/day. Round 2 dose levels were 500 and 1000 mg/kg/day and Round 3 explored 2000 mg/kg/day given once daily or as 1000 mg/kg twice daily (~6 hours apart). CAB was formulated as a suspension in 0.5% hydroxypropyl methylcellulose / 0.1% Tween 80 in purified water. Blood samples for toxicokinetic evaluation were collected from females dosed once daily at 0.5, 1, 2, 4, 8 and 24 hours post-dosing, and from females dosed twice daily at 0.5, 1, 2, 4, 6 (prior to second dose); 6.5, 7, 8, 10, 14 and 24 hours after the first daily dose. A summary of the toxicology data is presented in m2.6.6, Section 6.3.2.1.

At Day 11 post-coitum (dosing Day 5), the systemic exposure to CAB (expressed as mean AUC_{0-24} and C_{max} values) increased less than proportionately with the increase in dose from 30 to 1000 mg/kg/day. In the group administered 2000 mg/kg/day, no notable difference in mean systemic exposure was observed between Day 11 and Day 19 post-coitum. The Day 11 exposures at 2000 mg/kg/day were 1.9-fold greater than the 1000 mg/kg/day Group at Day 11, the only day for which exposure estimates were assessed. No notable difference in mean AUC_{0-24} or C_{max} values was observed between the 2000 mg/kg/day dosing regimens (given once daily or given as a divided dose of 1000 mg/kg twice daily approximately 6 hours apart) on either Day 11 or Day 19 pc.

In an embryofetal development study, pregnant Dutch Belted rabbits were administered CAB (sodium salt) by oral gavage once daily at doses of 30, 500 or 2000 mg/kg/day for 13 days (Days 7 to 19 post-coitum) [Report CD2009/00842]. CAB was formulated as a suspension in 0.5% hydroxypropyl methylcellulose / 0.1% Tween 80 in purified water. To construct a composite concentration-time profile for toxicokinetic evaluation, the following sampling schedule was used: on Day 5 of dosing (Day 11 post-coitum), 3 subgroups of 3 rabbits (total 9 rabbits) within each group were each bled approximately twice: the first subgroup 0.5 and 8 hours after dosing, the second subgroup at 1 and 24 hours after dosing and the third subgroup at 2 and 4 hours after dosing. The C_{max} and AUC values along with the comparative animal to human exposure ratios are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 6.3.2.1.

The systemic exposure (plasma C_{max} and AUC_{0-24}) of CAB increased less than proportionally with the increase in dose. A 17-fold increase in dose from 30 to 500 mg/kg/day resulted in a 3.6- or 4.5-fold increase in C_{max} or AUC_{0-24}, respectively, and a 67-fold increase in dose from 30 to 2000 mg/kg/day resulted in a 7.9- or 9.1-fold increase in C_{max} or AUC_{0-24}, respectively.

3.4. Dog

3.4.1. Intravenous administration

3.4.1.1. Pharmacokinetics/toxicokinetics after single doses

Studies were conducted to characterize the single-dose pharmacokinetics of CAB in fasted male beagle dogs following intravenous (1 mg free acid/kg) administration of CAB as the free acid (n=3/group) or as the sodium salt (n=1/group) [Report RH2007/00169]. CAB was formulated in DMSO:Solutol:0.05 M N-methylglucamine in water (10:10:80, v:v:v). Blood samples were collected for plasma analysis at 5, 15, and 30 min, and 1, 2, 4, 6, 8, 12, and 24 hours post-dose. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.1.

The results for intravenous administration of both forms of CAB were pooled together (n=4) and the mean plasma clearance of CAB in the dog averaged 0.34 mL/min/kg, or 1.1% of dog hepatic blood flow. Its mean terminal elimination half-life was 5.7 hours. Mean steady-state volume of distribution of CAB was 0.14 L/kg, approximately 23% of total body water volume in the dog.

3.4.2. Oral administration

3.4.2.1. Pharmacokinetics/toxicokinetics after single doses

Studies were conducted to characterize the single-dose pharmacokinetics of CAB in fasted or non-fasted male beagle dogs following oral solution (5 mg/kg; n=1 or 2) or in fasted male beagle dogs following oral capsule (approximately 5.1 mg/kg; n=3/group) administration of CAB as the free acid or the sodium salt form [Report RH2007/00169]. CAB was formulated as a solution in DMSO:Solutol:0.05 M N-methylglucamine, 10:10:80. Blood samples were collected for plasma analysis at 15 and 30 min, and 1, 2, 4, 6, 8, 12, and 24 hours post-dose. The pharmacokinetic data for CAB determined in this study are presented in m2.6.5, Table 3.2.

Following a 5 mg/kg oral solution dose in fasted dogs (n=2), oral bioavailability averaged 63%. While a 5 mg/kg oral solution dose administered to a single fed dog resulted in an oral bioavailability of 44%, the number of dogs receiving oral solution doses was too low to make conclusions regarding the effect of feeding on oral bioavailability.

CAB exposure in fasted dogs (n=3/group) was low following oral capsule administration of approximately 5.2 mg/kg of the form 1 crystalline CAB free acid, 5.1 mg/kg of the form 2 crystalline free acid, or 5 mg/kg of CAB sodium salt, with oral bioavailability averaging 8%, 3%, and 6%, respectively.

In another series of single dose pharmacokinetics studies in fasted and non-fasted male beagle dogs, CAB was administered as an oral suspension of the free acid (5 and 30 mg/kg) or the sodium salt (1, 5 and 30 mg/kg). CAB was formulated as a suspension in a 0.5% hydroxypropyl methylcellulose/0.1% Tween 80. Blood samples were collected for plasma analysis at various time points up to 48 hours post-dose [Report RH2007/00170]. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.3.

Following oral administration to non-fasted dogs, CAB exposure increased less than proportionally with a dose increase from 5 to 30 mg/kg; $AUC_{0-\infty}$ increased approximately 1.8-fold with a 6-fold increase in dose. In fasted dogs receiving 30 mg/kg CAB (free acid), $AUC_{0-\infty}$ was approximately 2.1-fold lower than in non-fasted dogs receiving an equivalent dose.

Following administration of single oral doses of CAB sodium salt in suspension (1, 5, and 30 mg/kg), increases in exposure were less than proportional to increases in dose. A 5-fold increase in dose from 1 to 5 mg/kg resulted in a 2.4-fold increase in AUC and a 2.1-fold increase in C_{max}; a 6-fold dose increase from 5 to 30 mg/kg resulted in an approximate 2.5-fold increase in AUC and 1.8-fold increase in C_{max}. In conclusion, administration of a 30 mg/kg dose of CAB as the sodium salt to fasted male dogs resulted in a 6- to 7-fold higher exposure (based on C_{max} and AUC values) than an equivalent dose of CAB as the free acid.

3.5. Monkey

3.5.1. Intravenous administration

3.5.1.1. Pharmacokinetics/toxicokinetics after single doses

The intravenous pharmacokinetic parameters of CAB were determined following a single intravenous dose of CAB sodium salt (1 mg/kg) to fasted male cynomolgus monkeys (n=2) [Report RH2007/00171]. The dosing solution was prepared in a formulation of DMSO:Solutol:0.05 M N-methylglucamine in water (10:10:80, v:v:v). Blood samples were collected at various time intervals through 24 hours post-dose for concentration analysis. The intravenous pharmacokinetic data for CAB determined in this study are presented in m2.6.5, Table 3.1.

After intravenous administration of CAB sodium salt (1 mg/kg), average plasma clearance was extremely low (0.3 mL/min/kg, or less than 1% of monkey hepatic blood flow); steady-state volume of distribution was 0.09 L/kg (13% of total body water volume in the monkey). The average terminal elimination half-life was 4 hours.

3.5.2. Oral administration

3.5.2.1. Pharmacokinetics/toxicokinetics after single doses

A series of studies were conducted to characterize the single dose pharmacokinetics of CAB in fasted male cynomolgus monkeys following oral solution administration of CAB sodium salt (5 mg/kg), oral capsule administration of CAB free acid (5 mg/kg), and oral suspension administration of CAB free acid (5 and 30 mg/kg) and CAB sodium salt (30 mg/kg) [Report RH2007/00171]. The solution was prepared in a formulation of DMSO:Solutol:0.05 M N-methylglucamine in water (10:10:80, v:v:v). CAB for oral capsule administration was prepared by milling using a mortar and pestle and then combining with sodium starch glycolate (SSG) and Avicel PH 102 (approximate ratios 2:1:3, drug:SSG:Avicel). The suspension formulations were prepared in 0.5% hydroxypropylmethylcellulose/0.1% Tween 80. Blood samples were collected at various time intervals through 24 hours post-dose for concentration analysis. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.2.

Following oral administration of CAB sodium salt in solution to male monkeys (n=2), CAB had an average terminal elimination half-life of 4.3 hours, C_{max} of 19150 ng/mL, T_{max} of 2 hours, and oral bioavailability of 56%. Oral bioavailability of CAB free acid in male monkeys (n=2) following capsule administration was substantially lower (averaging 6%), suggesting that oral bioavailability of CAB from a solid dose formulation may be limited by its solubility and/or dissolution rate.

Administration of CAB free acid in suspension to male monkeys (n=6) at 5 and 30 mg/kg resulted in a less than proportional increase in systemic exposure to CAB: a 6-fold increase in dose, from 5 to 30 mg/kg, resulted in a 2 to 3-fold increase in C_{max} and AUC. Following administration of CAB sodium salt (30 mg/kg) in suspension, systemic

exposure to CAB was 3 to 4-fold higher than that achieved from an equivalent dose of CAB free acid.

A toxicokinetics study was designed to determine the profile of CAB (sodium salt) when administered as single doses to groups of fasted male cynomolgus monkeys (n=2/group) by oral gavage at dose levels of 150, 300 and 1000 mg/kg [Report RD2007/01415]. The toxicokinetic profile was compared to that of a batch of CAB sodium synthesized by another company at 150 mg/kg. Toxicokinetic evaluation was performed on samples collected at various time intervals post-dose. All formulations of CAB were prepared in a vehicle of 0.5 w/w% hydroxypropylmethylcellulose with 0.1 w/w% Tween 80 aqueous solution. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.11. A summary of the toxicology data is presented in m2.6.6, Section 2.4.1.

The plasma concentration-time profiles, C_{max} and AUC_{0-48h}, were nearly comparable between both synthesized batches of CAB at 150 mg/kg, indicating no appreciable differences in the exposure levels to these compounds. Exposure (C_{max} and AUC_{0-48h}) values of CAB increased with escalating dose levels of 150, 300 and 1000 mg/kg; however, the increases were less than dose-proportional. T_{max} values were 2 hours at 150 and 300 mg/kg and 2 or 4 hours at 1000 mg/kg and plasma concentrations gradually decreased thereafter. However, CAB was still detected 48 hours after dosing at all dose levels.

Another study was performed to compare the toxicokinetics of micronized and unmicronized CAB (sodium salt) in a single dose study in male cynomolgus monkeys [Report CD2008/01223]. Four male monkeys were given single oral (gavage) doses of micronized and unmicronized formulations of CAB (500 mg/kg) using a crossover design with 1 week between doses. CAB was formulated as a suspension in 0.5% (w/w) hydroxypropyl methylcellulose (HPMC) with 0.1% (w/w) Tween 80 in purified water. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.11.

There were no marked differences (≥2-fold) in mean AUC_{0-24}, C_{max} or T_{max} between the micronized and unmicronized formulations.

3.5.2.2. Pharmacokinetics/toxicokinetics after repeated administration

7 day study

In a 7 day toxicity study in monkeys (n=1/sex/group), CAB (sodium salt) was administered orally by gavage once daily at 50, 150 and 1000 mg/kg/day [Report CD2007/00577]. CAB was formulated as a suspension in 0.5% (w/w) hydroxypropyl methylcellulose (HPMC) with 0.1% (w/w) Tween 80 in purified water. Toxicokinetic evaluation was performed on samples collected on Days 1 and 7. A summary of the toxicology data is presented in m2.6.6, Section 3.4.1.1.

Increases in systemic exposure to CAB (AUC_{0-24} and C_{max}) were generally less than dose-proportional in monkeys on Days 1 and 7. However, an approximate dose-proportional

increase in exposure was noted in females from 50 to 150 mg/kg/day on Day 1. Overall, for a 20-fold increase in dose of CAB from 50 to 1000 mg/kg/day, CAB AUC_{0-24} and C_{max} values increased approximately 3.7- and 2.5-fold, respectively, in males and 4.9- and 2.9-fold, respectively, in females on Day 7. T_{max} values ranged from 1 to 8 hours post-dose. No notable (>2-fold) differences in systemic exposure were observed for any dose between sexes. No notable difference in systemic exposure was noted on Day 7 compared to Day 1, except for the female monkey at 150 mg/kg/day where there was a ~70% decrease in systemic exposure (AUC_{0-24} and C_{max}) to CAB on Day 7.

14 day study

In a 14 day toxicity study in cynomolgus monkeys (n=3/sex/group), CAB (sodium salt) was administered by oral gavage once daily at 8, 25 and 1000 mg/kg/day [Report CD2007/00680]. Blood samples were collected for plasma analysis at timepoints of 0.25, 0.5, 1, 2, 4, 8 and 24 hours after dosing on Days 1 and 14 with the exception that males given 1000 mg/kg/day were euthanized prior to collection of the 8 and 24 hour samples on Day 14. CAB was formulated as a suspension in 0.5% (w/w) HPMC with 0.1% (w/w) Tween 80. The C_{max} and AUC values along with the comparative animal to human exposure ratios are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.4.1.2.

Increases in systemic exposure (AUC_{0-t} and C_{max}) were approximately dose-proportional (1.4- to 2.2-fold) from 8 to 25 mg/kg/day, and less than dose-proportional from 25 to 1000 mg/kg/day. There were no notable (>2-fold) sex-related differences at any of the doses examined. Systemic exposure to CAB was generally similar on Days 1 and 14. Median values for T_{max} ranged from 1 to 8 hours after administration of CAB, with T_{max} values generally increasing with increasing dose.

4 week study

In a 4 week oral toxicity study, groups of male and female monkeys (n= 3 to 5/group) were administered CAB (sodium salt) by oral gavage once daily at nominal doses of 5, 50 and 500 mg/kg/day [Report CD2008/00632]. CAB was formulated as a suspension in 0.5% HPMC with 0.1% Tween 80. Toxicokinetic evaluation was performed on samples collected on Days 1 and 25. The C_{max} and AUC values along with the comparative animal to human exposure ratios are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.4.1.3.

CAB was quantifiable in the plasma of all animals receiving CAB up to at least 24 hours post-dose. Median T_{max} values occurred between 1 and 4 hours after dosing. Mean plasma systemic exposure (AUC_{0-24} and C_{max}) increased in a less than proportional manner with dose as the dose was increased from 5 to 50 mg/kg/day and when the dose was increased from 50 to 500 mg/kg/day. For a 100-fold increase in dose from 5 to 500 mg/kg/day, mean AUC_{0-24} and C_{max} values increased from 5.8- to 11.2-fold. No notable (>2-fold) differences in mean systemic exposure (AUC_{0-24} and C_{max}) between males and females or between Day 1 and Day 28 were observed for any dose group.

39 week study

In a 39 week oral gavage repeat dose toxicity study, groups of male and female cynomolgus monkeys (n=4/sex/group except in the high dose group where n=6/sex/group) were administered CAB (sodium salt) once daily at doses of 5, 50 and 500 mg/kg/day for at least 39 weeks [Report RD2009/00027]. Toxicokinetic evaluation was performed on samples collected during Weeks 4, 26 and 39. Suspensions of CAB were prepared in formulations of 0.5% (w/v) HPMC with 0.1% (w/v) Tween 80 in deionized water. The C_{max} and AUC values along with the comparative animal to human exposure ratios are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.4.1.4.

For a 100-fold increase in dose from 5 to 500 mg/kg/day, the mean AUC_{0-24} and C_{max} values increased 3.6- to 14.4-fold. The systemic exposure to CAB (as defined by AUC_{0-24} and C_{max} values) increased less than proportionately with the increase in dose on each sampling occasion (Weeks 4, 26 and 39) in both sexes. No consistently different changes in systemic exposure to CAB among sampling occasions at any dose level were observed.

No notable (>2-fold) differences in mean systemic exposure to CAB were observed between males and females at any dose or sampling occasion.

3.5.3. Subcutaneous and intramuscular administration

3.5.3.1. Pharmacokinetics/toxicokinetics after single doses

In a study to investigate the toxicokinetics of CAB in monkeys, groups of male cynomolgus monkeys (n=4/group) were administered SC or IM doses of CAB (free acid; 1 mg/kg) on Day 1; each animal then received a second dose (5 mg/kg) of CAB on Day 43 [Report CD2009/00373]. Toxicokinetic evaluation was performed on samples collected at various time intervals post-dose. CAB was formulated as a suspension in 1.7% polyvinylpyrrolidone (PVP) (Plasdone K29/32), 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben, 0.004M monosodium phosphate, 0.006M disodium phosphate, and 0.84% sodium chloride in sterile water. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.9. A summary of the toxicology data is presented in m2.6.6, Section 2.4.2.

For a dose of 1 mg/kg (at least 90% of particles are in 5.11 microns size range with 10% in the less than 5.11 microns range; Day 1), plasma concentrations were quantifiable up to 816 hours (Day 34) in the SC and IM groups in general. The median T_{max} was observed at 96 hours in the SC group and 60 hours in the IM group.

At 1 mg/kg, CAB was slowly released from the injection sites and gave sustained plasma drug concentrations via both routes. The mean half-life of CAB was 5 days (111 h) by the SC route and 10 days (238 h) by the IM route. The mean C_{max} concentration was 0.26 μg/mL by the SC route and 0.17 μg/mL by the IM route. The C_{max} was achieved faster and subsequent plasma concentrations decreased faster via the SC route than the IM route. The overall systemic exposure by SC administration (AUC_{0-t} = 61 μg.h/mL)

was similar to the exposure observed following IM injection (AUC_{0-t} = 57 μg.h/mL). Plasma concentrations of CAB after SC administration were undetectable in one animal at 648 hours (Day 27) and in another after 816 hours (Day 34), whereas plasma concentrations of CAB after IM administration were still detectable up to 816 hours (Day 34) in all 4 animals. Collection of plasma samples was stopped after 816 hours (Day 34) as plasma concentrations in both groups had either reached or were approaching the LLOQ. Through 816 hours, plasma concentrations accounted for ~100% of the total SC dose administered and ~93% of the total IM dose.

For a dose of 5 mg/kg (at least 90% of particles are in 6.84 microns size range with 10% in the less than 6.84 size range; Day 43), plasma concentrations were quantifiable up to 1032 hours (Day 85) in the SC and IM groups in general. The median T_{max} was observed at 120 hours in the SC group and 108 hours in the IM group.

At 5 mg/kg, CAB was slowly released from the injection sites and gave sustained plasma drug concentrations via both routes. With a 5-fold increase in dose, the apparent mean half-life of CAB increased from 5 to 26 days (619 h) by the SC route and from 10 to 21 days (500 h) by the IM route. As the dose was increased 5-fold from 1 to 5 mg/kg, the increase in systemic exposure (AUC_{0-t}) was less than dose proportional for each route of administration. An approximate 3- and 2.8-fold increase in SC AUC (191 μg.h/mL) and IM AUC (157 μg.h/mL), respectively, was observed with a 5-fold increase in dose. Increases in mean SC and IM C_{max} concentrations with increasing dose were less than dose proportional (<2 fold). Plasma concentrations of CAB after SC and IM administration were still detectable in all animals up to 1032 hours. Sample collection was stopped after 1032 hours as plasma concentrations in both groups were below the protein adjusted inhibitory concentration ($PAIC_{90}$) of 0.17 μg/mL. Through 1032 hours, plasma concentrations accounted for ~70% of the total SC dose administered and ~100% of the total IM dose.

In another toxicokinetics study in monkeys, CAB (free acid; 5 mg/kg) was given to groups of male cynomolgus monkeys (n=4/group) as a single subcutaneous or intramuscular injection [Report CD2009/00513]. Toxicokinetic evaluation (serial profiling) was performed on samples collected at 0.25, 0.5, 1, 2, 4, 8 and 24 (Day 1), 168 (Day 7), 336 (Day 14), 504 (Day 21), 672 (Day 28), 840 (Day 35), 1008 (Day 42), 1152 (day 48) and 1344 (Day 56) hours after dosing. CAB was formulated as a suspension (particle size = 67.7 microns) in 1.7% polyvinylpyrrolidone (PVP) (Plasdone K29/32), 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben, 0.004M monosodium phosphate, 0.006M disodium phosphate, and 0.84% sodium chloride in sterile water for injection, with an approximate final pH 6.71. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.9. A summary of the toxicology data is presented in m2.6.6, Section 2.4.2.

Following a single SC or IM dose of CAB in this long acting parenteral formulation, plasma concentrations in monkeys were quantifiable up to 1344 hours (Day 56) in the SC and IM groups in general. The median T_{max} was observed at 180 hours (8 days) in the SC group and 420 hours (18 days) in the IM group.

CAB was slowly released from the injection sites and gave sustained plasma drug concentrations via both routes. The mean half-life of CAB was 20 days (478 h) by the SC route and 9 days (219 h) by the IM route. The mean C_{max} concentration of CAB was 0.16 μg/mL by the SC route and 0.46 μg/mL by the IM route.

Plasma concentrations of CAB after SC administration were undetectable in one animal at 1344 hours (Day 56), whereas plasma concentrations after IM administration were still detectable up to 1344 hours in all 4 animals. Sample collection was stopped after 1344 hours as plasma concentrations in both groups were below the protein adjusted inhibitory concentration ($PAIC_{90}$) of 0.17 μg/mL. At 1344 hours, ~43% of the total SC dose and ~95% of the IM dose administered was recovered.

A further toxicokinetics study was conducted in which CAB (free acid; 10 mg/kg) was administered to groups of male cynomolgus monkeys (n=4/group) as either single SC or IM injections [Report CD2009/00656]. Toxicokinetic evaluation (serial profiling) was performed on samples collected at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 120, 168, and 336 hours after dosing, with additional single samples obtained on Days 21, 28, 35, 42 and 49. CAB was formulated as a suspension in 2% Pluronic F-127, 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben, 0.008M NaH2PO4H2O, 0.006M Na2HPO4 and 0.81% NaCl, pH-adjusted to 6.6. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.9. A summary of the toxicology data is presented in m2.6.6, Section 2.4.2.

Plasma concentrations were quantifiable through Day 49 in the SC and IM groups in general. The median T_{max} was observed on Day 7 in the SC group and on Day 6 in the IM group.

CAB was slowly released from the injection sites, yielding sustained plasma drug concentrations over time via both routes. The mean half-life of CAB was approximately 32 days (759 hours) by the SC route and approximately 7 days (171 hours) by the IM route. The plasma concentration-time profiles indicated the presence of multiple maxima (particularly after IM administration), some separated by more than 4 days. The reported C_{max} values represent the greatest concentration in the profile over the entire sampling course. The mean C_{max} of CAB given via the IM route was 2.6X higher than the mean C_{max} via the SC route.

On Day 49, plasma concentrations of CAB were approaching the protein-adjusted 90% inhibitory concentration ($PAIC_{90}$) of 166 ng/mL in the SC group and were below the $PAIC_{90}$ in the IM group.

A combination toxicokinetics study was conducted to assess the co-administration of CAB and rilpivirine to cynomolgus monkeys [Report 2010n105579]. CAB (free acid; 10 mg/kg) and rilpivirine (60 mg/kg) were administered as single IM injections alone or in combination to groups of male cynomolgus monkeys (n=2/group when given alone, or 4/group in combination). Blood samples were collected from each animal for plasma analysis at the following approximate times: 2, 4, 8, 12 and 24 hours (Day 2) post-dose; 48 h (Day 3), 72 h (Day 4), 120 h (Day 6), 168 h (Day 8), 336 h (Day 15), 480 h (Day 21), 648 h (Day 28) post-dose; and on Days 41, 51, and 61 post-dose at the approximate

time of dosing on Day 1. The pharmacokinetic parameters of CAB from this study are presented in m2.6.5, Table 3.12. A summary of the toxicology data is presented in m2.6.6, Section 2.4.2.

The systemic exposure to CAB or rilpivirine (mean plasma C_{max} or AUC values) when dosed in combination or alone was similar, suggesting there is no PK interaction between CAB and rilpivirine in monkeys.

3.6. In Vitro Absorption

3.6.1. Metal ion chelation

HIV INSTIs are known to chelate with polyvalent cations, resulting in decreased absorption. In an in vitro absorption study, the affinity of 10 multivalent metal cations for the free-acid of CAB was determined at pH 5.0, 7.5 and 9.0 [Report 2019N401615]. For comparative purposes, the affinity of these ligands for the free-acid of dolutegravir (DTG or GSK1349572B) were measured in parallel. Both CAB and DTG were tested at 500nM with 5 minutes of mixing on a microplate mixer at room temperature, and each plate was read with a fluorescence detector. All conditions employed an excitation wavelength of 270 nm with a 2.5 nm bandwidth. Fluorescence emission was measured by an 18 point scan from 330 to 500 nm, with 10 nm increments and a 20 nm bandwidth. The selection of this range brackets the maximum fluorescence emission wavelength for CAB free acid (400 nm) and GSK1349572B (410 nm). The equilibrium dissociation constant (K_d) for each of these ligands was determined by measuring the dose-dependent modulation of the intrinsic fluorescence of either CAB or GSK1349572B upon complex formation with metal cation ligands. A tabulated summary of this study is in m2.6.5, Table 3.13.

K_d measures ranged from just under the target concentration (500 nM) and up to beyond 0.1 M (the top concentration tested). For some ligands, no saturating binding was observed under some pH conditions. Overall, K_d measures of each ligand for CAB and GSK1349572B were similar [see m2.7.2, Section 3.2.9 for discussion of the clinical significance].

Table 3.1 List of Absorption Studies Performed with CAB

Type of Study	Species (Strain)/ Test System	No./Sex/Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Mouse	15M	Oral (gavage)	B	10, 100, 1000, 2000	Single	No	GSK	RD2009/00691 (M42451)	m4.2.3.1
Pharmacokinetics	Rat (Sprague Dawley) Rat (Sprague Dawley) Rat (Sprague Dawley) Rat (Sprague Dawley)	3M 4M 6M 6M	IV (bolus) Oral	A A A B	1[a] 5[a] 5.1[b] 4.9[b]	Single	No	GSK	RH2007/00168 (07APK043)	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	3M	Oral (gavage)	A	5, 50, 150, 500[c]	Single	No	GSK	RH2007/00168 (07APK043)	m4.2.2.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	B	3, 30, 300, 1000	Single	No	GSK	RD2008/00200 (R42257)	m4.2.2.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	B	1000	Single	No	GSK	RD2008/01308 (R42353)	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	3M	SC IM	A	5	Single	No	GSK	RH2009/00012 (N11105-5)	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	5M	SC IM	A	5	Single	No	GSK	RH2009/00013 (N11105-12)	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	3 to 6M	IM	A	10[k]	Single	No	JAN	2011N127517 (FK7565)	m4.2.2.2

List of Absorption Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rat (Sprague Dawley)	6M	SC IM	A	10	Single	No	GSK	RD2009/00865 (R42469)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M	SC IM	A	10, 30, 50 5, 20, 35	Single	No	GSK	RD2009/00906 (R42473)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M	SC IM	A	10, 30, 50 5, 20, 35	Single	No	GSK	RD2009/01216 (R42506)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M/ 3F	SC IM	A	5, 30, 100 2.5, 10, 75	Single	Yes	GSK	RD2009/01359 (R42516)	m4.2.3.1
Pharmacokinetics	Dog (beagle)	4M 1[d] or 2M[e] 3M[e] 3M[e] 3M[e]	IV (bolus) Oral (gavage) Oral (capsule)	A or B B A A B	1[a] 5[a] ~5.2[b,f] ~5.1[b,g] ~5.0[b]	Single	No	GSK	RH2007/00169 (07APK044)	m4.2.2.2
Pharmacokinetics	Dog (beagle)	2[d] or 3M[d,e] 2M[e]	Oral (gavage)	A B	5, 30[c] 1, 5, 30[c]	Single	No	GSK	RH2007/00170 (07APK045)	m4.2.2.2

List of Absorption Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Pharmacokinetics	Monkey (cynomolgus)	2M 2M 2M 2M 2M	IV (bolus) Oral (gavage) Oral (capsule) Oral (gavage) Oral (gavage)	B B A A B	1[a] 5[a] ~5.1[b] 5, 30[c] 30[c]	Single	No	[redacted]	**In-life:** RH2007/00202 (06RCD7689) RH2007/00201 (06RCD7572) RH2007/00203 (06RCD8045) RH2007/00205 (06RCD8533) **Data analysis:** RH2007/00171 (07APK046)	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	2M	Oral (gavage)	B	150, 300, 1000[c]	Single	No	[redacted]	RD2007/01415 (E-265744-TF-006-R)	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus)	4M	Oral (gavage)	B	500	Single	No	GSK	CD2008/01223 (D08226)	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	4M 4M	SC IM	A	1, 5 1, 5	Single	No	GSK	CD2009/00373 (D09052)	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus)	4M 4M	SC IM	A	5 5	Single	No	GSK	CD2009/00513 (D09084)	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus)	4M 4M	SC IM	A	10 10	Single	No	GSK	CD2009/00656 (D09112)	m4.2.3.1

List of Absorption Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Monkey (cynomolgus)	2M	IM	A	10[k]	Single	No	[redacted]	2010N105579 ([redacted])	m4.2.3.1
Toxicokinetics	Mouse (CD-1)	36M/ 36F	Oral (gavage)	B	10, 75, 1000	14 days	Yes	GSK	RD2009/00692 (M42452)	m4.2.3.2
Toxicokinetics	Mouse (CD-1)	54M/ 54F	Oral (gavage)	B	10, 75, 1000	13 weeks	Yes	GSK	2012N142081 (M42936)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	A	30, 100, 300[c]	14 days	Yes	GSK	RD2006/01741 (R41937)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	B	1, 75, 1000	4 weeks	Yes	GSK	RD2008/00448 (R42288)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	12M/12F	SC (monthly) IM (monthly) SC (weekly)	A	5, 30, 100 2.5, 10, 75 100	13 weeks	Yes	[redacted]	2010N104820 (R42698)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	B	0.5, 5, 1000	26 weeks	Yes	GSK	RD2009/00031 (R42404)	m4.2.3.2
Toxicokinetics	Rabbit (Dutch Belted)	4F	Oral (gavage)	B	30, 250, 500, 1000, 2000 [i,j]	13 days (Day 5)	No	GSK	CD2008/01276 D08251	m4.2.3.5.2

List of Absorption Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rabbit (Dutch Belted)	9F	Oral (gavage)	B	30, 500, 2000	13 days (Day 5)	Yes	GSK	CD2009/00842 (G08307)	m4.2.3.5.2
Toxicokinetics	Monkey (cynomolgus)	1M/1F	Oral (gavage)	B	50, 150, 1000[c]	7 days	No	GSK	CD2007/00577 (D07170)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	3M/3F	Oral (gavage)	B	8, 25, 1000[c]	14 days	Yes	GSK	CD2007/00680 (G07171)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	3 to 5 M/F	Oral (gavage)	B	5, 50, 500	4 weeks	Yes	GSK	CD2008/00632 (G08079)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	4M/4F	Oral (gavage)	B	5, 50, 500	39 weeks	Yes	GSK	RD2009/00027 (P42405)	m4.2.3.2
Metal chelation	In vitro	NA	In vitro	A	500 nM	NA	No	GSK	2019N401615	m4.2.2.2

Key:
A = Parent. B = Sodium salt. LA = Long-acting formulation derived from parent.
PrEP = Pre-exposure prophylaxis.
a = GSK1265744 in solution.
b = GSK1265744 as a capsule.
c = GSK1265744 in suspension.
d = Non-fasted dogs.
e = Fasted dogs.
f = GSK1265744 as crystalline Form 1 was dosed.
g = GSK1265744 as crystalline Form 2 was dosed.
h = The 2000 mg/kg/day dose was given as a single daily dose and a BID (twice daily) dose of 1000 mg/kg/dose,6 hours apart.
i = Toxicokinetic measurements were also taken on Day 13 of dosing at the 2000 mg/kg/day doses (single and BID).
j = A wet bead milled suspension (particle size = 5.89 μm) and a homogenized suspension (particle size = 68.9 μm) were used for each route.
k = GSK1265744 was administered with and without Rilpivirine.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]
JAN = Janssen Research & Development.
[redacted] = [redacted]

4. DISTRIBUTION

A range of in vitro studies have been performed to investigate the binding of CAB to serum and plasma proteins and to investigate CAB interactions with a range of cellular transporters. In addition, in vivo studies have been performed to investigate CAB blood to plasma ratio, liver to blood ratio, lacteal secretion, placental transfer, and tissue distribution of radioactivity following administration of [^{14}C]-CAB. These studies are listed in Table 4.1.

4.1. In Vitro Distribution Studies

4.1.1. Serum and plasma protein binding studies

In a preliminary study, the in vitro plasma protein binding of CAB was assessed in the male rat, dog, monkey and human by using equilibrium dialysis at 2 μg/mL (5 μM) [Report RH2007/00193]. Equilibrium dialysis was conducted for 6 hours at 37°C. Plasma and phosphate buffered saline were analyzed for CAB by an HPLC-MS/MS method. A tabulated summary of this study is presented in m2.6.5, Table 6.1.

The percentage of CAB bound to plasma proteins was >99.9% in rats, 99.3% in dogs, 99.7% in monkeys and 99.6% in humans.

In another preliminary study, the in vitro serum protein binding of CAB was determined in the male rat, dog, monkey and human using equilibrium dialysis at 4 μg/mL (10 μM) [Report RH2007/00222]. Equilibrium dialysis was conducted for 24 hours at 37°C. Serum and phosphate buffered saline were analyzed for CAB by an HPLC-MS/MS method. A tabulated summary of this study is presented in m2.6.5, Table 6.1.

The percentage of CAB bound to serum proteins was >99.9% in rats, 99.6% in dogs, 99.8% in monkeys and 99.9% in humans.

The extent of binding of CAB to plasma proteins in human was investigated in vitro at concentrations of 500, 1,000, 5,000, 10,000 and 20,000 ng/mL using equilibrium dialysis at 37ºC and an experimentally established equilibration time of 5 hours [Report 2015N235936]. Frozen human pooled plasma from at least three male volunteers was used in this study. A fully validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitation of CAB in a matrix of 90% plasma ultrafiltrate and 10% plasma was used for sample analysis. The dialysate matrix used in the dialysis devices was plasma ultrafiltrate and all donor and receiver cell samples were mixed to 90% plasma ultrafiltrate and 10% plasma prior to LC-MS/MS analysis. A tabulated summary of this study is presented in m2.6.5, Table 6.1.

CAB was highly bound to plasma proteins in human in vitro. Plasma protein binding was concentration dependent between the lowest and highest concentrations tested, with unbound CAB ranging from 0.47% at 500 ng/mL to 0.62% at 20,000 ng/mL. However, there was no significant statistical difference when comparing concentration dependence between 1,000 ng/mL and 20,000 ng/mL.

CAB glucuronide was tested in a study designed to assess the in vitro plasma protein binding in male human and male rat [Report 2017N333514]. Plasma protein binding was assessed in either fresh plasma, or plasma which had been subjected to one freeze thaw cycle, across the concentration range 0.2 to 2.0 μg/mL. Equilibrium dialysis was conducted for 8 hours at 37°C. Plasma and phosphate buffered saline were analyzed for CAB glucuronide by an HPLC-MS/MS method. A tabulated summary of this study is presented in m2.6.5, Table 6.1.

The plasma protein binding (PPB) was approximately 16% and 33% in the human and rat, respectively. There was generally no concentration dependence in plasma protein binding with increasing CAB glucuronide concentration across the species. Co-incubation of CAB glucuronide in the presence of varying concentrations of CAB showed no effect on PPB in the rat; however, in human, binding of CAB glucuronide to plasma proteins increased.

4.1.2. P-glycoprotein transport and membrane permeability

The apparent passive permeability of CAB (3 μM) was determined in stably transfected human multidrug resistance 1-Madin Darby canine kidney (hMDR1-MDCK) cells heterologously expressing human Pgp [Report RH2007/00199]. The transport studies were conducted at 37°C in a humidified incubator with shaking for 60 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.5.

The apparent passive permeability of CAB was high (424 nm/s) in the presence of a potent inhibitor of Pgp, but was attenuated by approximately 30% in the absence of the inhibitor, suggesting that CAB may be a substrate for an efflux transporter.

[^{14}C]-CAB was evaluated as a substrate of the human P-glycoprotein (Pgp) transporter in vitro [Report 2012N146040]. The bi-directional transport of [^{14}C]-CAB (3 μM) was investigated using MDCKII-MDR1 cells transfected with the human MDR1 gene, which produces the Pgp protein. Cell monolayers were pre-incubated at 37°C in transport medium, with and without 2 μM GF120918, for 15 to 30 minutes prior to addition of CAB. After adding test article, incubation continued for 90 minutes. Also, the passive membrane permeability of [^{14}C]-CAB was determined at pH 7.4, and the absorptive membrane permeability of [^{14}C]-CAB was determined at pH 5.5 and 7.4, using a biorelevant buffer (FaSSIF, fasted state simulated intestinal fluid) to simulate conditions in the gastro-intestinal tract. Permeabilities were measured using MDCKII-MDR1 cell monolayers in the presence of the potent Pgp inhibitor, GF120918. A tabulated summary of the results is presented in m2.6.5, Table 8.3.

The data demonstrate that [^{14}C]-CAB was a substrate for human Pgp with a moderate efflux ratio of 3.0 at a concentration of 3 μM.

[^{14}C]-CAB was determined to have high passive membrane permeability of 256 nm/s at pH 7.4. The absorptive membrane permeabilities of [^{14}C]-CAB in the presence of FaSSIF were both high at pH 7.4 and pH 5.5 with a P7.4[abs] value of 1088 nm/s and a P5.5[abs] value of 1374 nm/s.

4.1.3. Inhibition of P-glycoprotein

CAB was evaluated as an inhibitor of the human P-glycoprotein (Pgp) in vitro [Report 2012N146041]. Inhibition of Pgp-mediated transport of digoxin (30 nM) by CAB (concentration range 0.03 to 30 μM) was investigated by determining the transport of [^{3}H]-digoxin by MDCKII-MDR1 cells transfected with the human MDR1gene, which produces the Pgp protein. After pre-incubation, the plates were incubated at 37°C with shaking for a further 90 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

CAB did not inhibit transport of digoxin via human Pgp in vitro at the concentration range of 0.03 to 30 μM.

4.1.4. Human breast cancer resistance protein-mediated transport

[^{14}C]-CAB was evaluated as a substrate of the human Breast Cancer Resistance Protein (BCRP) transporter in vitro [Report 2012N155942]. The bi-directional transport of [^{14}C]-CAB (3 μM) was investigated using MDCKII-BCRP cells transfected with the human BCRP gene, which produces the BCRP protein. [^{14}C]-cimetidine (3 μM) was used as a positive control. Plates were incubated at 37°C with shaking for 90 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.3.

The data demonstrate that [^{14}C]-CAB was a substrate for human BCRP with an efflux ratio of 2.6 at a concentration of 3 μM.

4.1.5. Inhibition of BCRP transport

CAB was evaluated as an inhibitor of the human Breast Cancer Resistance Protein (BCRP) in vitro [Report 2012N150360]. Inhibition of BCRP-mediated transport of cimetidine (200 nM) by CAB (concentration range 0.03 to 30 μM) was investigated by determining the potential to inhibit the transport of [^{14}C]-cimetidine in MDCKII-BCRP cells. Plates were incubated at 37°C with shaking for 90 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

CAB inhibited human BCRP-mediated the transport of cimetidine in vitro by 22% at 30 μM. However, the degree of inhibition was insufficient to allow the calculation of an IC_{50} value under the assay conditions.

4.1.6. Inhibition of human bile salt export pump transporter and multidrug resistance associated protein-2 transporter

CAB was evaluated as an inhibitor of the human multidrug resistance associated protein-2 transporter (MRP2) and human bile salt export pump (BSEP) in vitro [Report 2013N174589]. Inhibition of uptake of the probe substrates [^{3}H]-estradiol 17-β-D-glucuronide (EG) and [^{3}H]-taurocholic acid (TC) by CAB (concentration range 0.03 to 30 μM) was investigated using membrane vesicles prepared from recombinant baculovirus infected Sf9 cells expressing the human MRP2 or human BSEP transporter. In separate

experiments, the MRP2 or BSEP membrane vesicles were pre-incubated for 10-15 minutes at 37°C with each concentration of CAB. Following preincubation, reactions were initiated by the addition of 50 μM [^{3}H]-EG or 2 μM [^{3}H]-TC, for MRP2 or BSEP, respectively. Additional incubations were performed in the absence of inhibitor and in the presence of 10 mM MgAMP solution containing either 50 μM [^{3}H]-EG or 2 μM [^{3}H]-TC for passive transport. After a 5 minute incubation, reactions were terminated. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

CAB did not inhibit MRP2 or BSEP, at concentrations up to 30 μM, under these in vitro assay conditions.

4.1.7. Inhibition of OAT1, OAT3, MATE1, MATE2-K and MRP4

A study was conducted to determine the potential for CAB to act as an inhibitor of the renal transporters organic anion transporter 1 and 3 (OAT1 and OAT3), multidrug and toxin extrusion transporters 1 and 2-K (MATE1 and MATE2-K), and multidrug resistance associated protein 4 (MRP4) [Report 2013N174474]. The in vitro inhibitory potential of CAB (concentrations up to 30 μM) was investigated in S2 cells expressing OAT1 or OAT3, HEK293 cells expressing MATE1 or MATE2-K, and in membrane vesicles expressing MRP4. The probe substrates used for each transporter were [^{3}H]-p-aminohippuric acid (PAH) for OAT1, [^{3}H]-estrone sulfate (ES) for OAT3, [^{14}C]-metformin for MATE1 and MATE2-K, and [^{3}H]-estradiol 17-β-D-glucuronide (EG) for MRP4. All reaction mixtures were pre-incubated with CAB at each concentration, followed by addition of probe substrates and an additional incubation at 37°C for 2 minutes (except for HEK293 cells, which were incubated for 5 minutes). A tabulated summary of this study is presented in m2.6.5, Table 8.1.

CAB inhibited the OAT1-mediated uptake of [^{3}H]-PAH with an IC_{50} value of 0.812 μM. The OAT3-mediated transport of [^{3}H]-ES was inhibited by CAB with an IC_{50} value of 0.411 μM. CAB inhibited the MATE1-mediated uptake of [^{14}C]-metformin with an IC_{50} value of 18.2 μM and inhibited the MATE2-K-mediated uptake of [^{14}C]-metformin with an IC_{50} value of 14.2 μM.

CAB did not inhibit the MRP4-mediated transport of [^{3}H]-EG.

4.1.8. Inhibition of OATP1B1 and OATP1B3

CAB was evaluated for the potential to inhibit the human uptake transporters OATP1B1 and OATP1B3 in vitro [Report 2013N164529]. Inhibition of uptake of the probe substrate [^{3}H]-EG for OATP1B1 and OATP1B3 by CAB (concentration range 0.1 to 30 μM) was investigated using cell lines which over express OATP1B1 and OATP1B3. HEK MSRII cells which overexpressed OATP1B1 and OATP1B3 were incubated at 37°C with the varying concentrations of CAB. Cells were first pre-incubated for 15-30 minutes with CAB and then the reaction was initiated by addition of [^{3}H]-EG followed by additional incubation for 3 minutes for OATP1B1 and 10 minutes for OATP1B3. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

CAB did not inhibit human OATP1B1 and OATP1B3 in vitro up to 30 μM.

4.1.9. Inhibition of OCT1 and OCT2 mediated transport

A study was conducted to evaluate the potential of CAB to inhibit the renal transporters organic cation transporter 1 (OCT1) and organic cation transporter 2 (OCT2). The inhibitory potencies of CAB were determined in HEK293 cells expressing OCT1 and OCT2 [Report 2012N146057]. The transporter-expressing cells were pre-incubated with CAB (concentrations ranging from 0.01 to 30 μM) at 37°C for 15 minutes. After pre-incubation, the substrate [^{14}C]-metformin (10 μM) was added to the mixtures followed by further incubation for 5 minutes for OCT1 and 2 minutes for OCT2. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

CAB inhibited the OCT1 and OCT2-mediated uptake of metformin. However, the degree of inhibition was insufficient to allow the calculation of an IC_{50} value under these assay conditions.

4.1.10. Hepatic uptake of CAB

An in vitro study was performed in cryopreserved pooled human hepatocytes to determine the potential hepatic uptake of CAB [Report 2018N391028]. Hepatocyte monolayers were pre-incubated at 37°C with buffer alone or buffer containing a cocktail of transporter inhibitors (100 μM rifamycin and 100 μM imipramine). After pre-incubation, cells were incubated in triplicate for the appropriate amount of time with 0.7 or 9 μM CAB. [^{3}H]-estradiol glucuronide([^{3}H]-EG) (0.02 μM) was used as positive control substrate in a separate set of incubations under the conditions stated. A tabulated summary of this study is presented in m2.6.5, Table 8.4.

The in vitro hepatic uptake of CAB was not mediated by the transporters OATP1B1, OATP1B3, OATP2B1 or OCT (which are all inhibited by the addition of the rifamycin and imipramine cocktail) in cryo-preserved pooled human hepatocytes.

4.1.11. Uptake by macrophages

A study was conducted to assess the potential uptake of CAB long-acting parenteral (LAP) formulations (200 nm, 1 μm, and 5 μm particle sizes) by macrophages [Report 2015N237779]. Macrophage cultures were prepared and CAB LAP was added for incubation at concentrations of 0.2 and 10 μg/mL. The cells were further incubated for 24 hours or 48 hours before collection for analysis. Each treatment (particle size + concentration + incubation time) was performed in triplicate. A tabulated summary of this study is presented in m2.6.5, Table 8.7.

The results showed the CAB LAP formulation undergoes uptake by human macrophages. The formulation with 1 μm particle size was preferable for macrophage uptake compared to 200 nm and 5 μm at the concentration of 10 μg/mL. Based on the LSC and TEM observations it is difficult to conclude, with certainty, that the material within these cells is CAB LAP.

4.1.12. Glucuronide metabolite as a substrate of transporters

CAB glucuronide was evaluated as a potential substrate of various hepatic and renal transporters, specifically human organic anion transporting polypeptide 1B1 (OATP1B1) and 1B3 (OATP1B3), multidrug resistance associated proteins (MRP2, 3 and 4) and organic anion transporter 1 (OAT1), OAT3 and OAT4 in vitro [Report 2016N298553]. The human transporters OATP1B1, OATP1B3, OAT1, OAT3 and OAT4 were each singly expressed in Human Embryonic Kidney MSRII (HEK293-MSRII) cell lines, and membrane vesicles were used to test human MRP2, 3 and 4. Various methodologies were used for each of the different transporters, generally including a pre-incubation period at 37°C followed by addition of the test substrates to initiate the reaction and then a further period of incubation. A tabulated summary of this study is presented in m2.6.5, Table 8.6.

Studies in cells singly overexpressing OATP1B1, OATP1B3, OAT1, OAT3, OAT4 or vesicles overexpressing MRP2, MRP3 or MRP4 indicated that CAB glucuronide is a substrate of OATP1B1, OATP1B3, OAT3, MRP2, MRP3, MRP4, but was not transported by OAT1 and OAT4.

4.1.13. Glucuronide metabolite as an inhibitor of transporters

A study was conducted to evaluate the potential of CAB glucuronide to inhibit the following transporters: OATP1B1, OATP1B3, OAT1, OAT3, OCT2, Pgp, BCRP, BSEP, MRP2, MRP4, MATE1 and MATE2-K [Report 2015N253914]. The in vitro inhibitory potential was determined by measuring the activity of each transporter in the absence or presence of CAB glucuronide (0.01 to 300 μM). A variety of materials and methods were used to test the potential inhibition of each of the different transporters. A tabulated summary of this study is presented in m2.6.5, Table 8.2.

CAB glucuronide was not an inhibitor of MDR1, BCRP, BSEP, MRP2, OATP1B1, OATP1B3, OCT2 and MATE2-K up to 300 μM. CAB glucuronide inhibited human MRP4 and MATE1 by 39.7% and 55.7%, respectively, at the highest concentration tested, 300 μM. However, the degree of inhibition was insufficient to allow the calculation of an IC_{50} value under these assay conditions. CAB glucuronide inhibited the OAT1 and OAT3 mediated transport of each probe substrate with calculated IC_{50} values of 73.4 μM and 36.5 μM, respectively.

Following completion of the report, it was noted that the batch of CAB glucuronide ([redacted]) used contained ca. 2.5% of CAB (GSK1265744, parent molecule of CAB glucuronide) as an impurity. CAB has previously been shown to be an inhibitor of MATE1, MATE2K, OAT1 and OAT3 with IC_{50} values of 18.2, 14.2, 0.81 and 0.42 μM, respectively [2013N174474]. Consequently, this concentration of CAB will impact the accuracy of the MATE1, MATE2-K, OAT1 and OAT3 inhibition profiles currently attributed to CAB glucuronide. A 1.1% presence of CAB in the inhibition experiments would account for the reported IC_{50} values, which is in line with the CAB impurity quantity reported in the CAB glucuronide CoA (2.5%). Therefore, CAB glucuronide is unlikely to be an inhibitor of MATE1, MATE2-K, OAT1 and OAT3.

4.1.14. Glucuronide metabolite as a substrate of Pgp and BCRP

A study was conducted to determine whether CAB glucuronide was a substrate of Pgp and BCRP [Report 2016N288197]. The in vitro substrate potential was determined by measuring the uptake of CAB glucuronide in transporter expressing vesicles. The reaction mixture to assess the potential for CAB glucuronide as a substrate of Pgp used verapamil (100 μM) as a Pgp inhibitor, and to assess BCRP, benzbromarone (30 μM) was used as a BCRP inhibitor. The assay mixtures were pre-incubated at 37°C for 5 minutes and, after the reactions were initiated, the reaction tubes were incubated for a further 10 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.6.

CAB glucuronide was not a substrate of Pgp or BCRP under these assay conditions.

4.1.15. Hepatic uptake clearance of CAB glucuronide

The hepatic uptake clearance of CAB glucuronide was determined using cryopreserved pooled human hepatocytes [Report 2017N320631]. The hepatocytes were incubated with CAB glucuronide (50 μM) at 37°C for periods of 0.5, 1, 2.5, 5, 10 and 15 minutes. Samples were analyzed by LC-MS/MS. A [^{3}H]-EG positive control substrate was run simultaneously to demonstrate functional activity of transporters. A tabulated summary of this study is presented in m2.6.5, Table 8.8.

The predicted hepatic uptake clearance of CAB glucuronide was 0.52 mL/min/kg. The hepatic extraction ratio was found to be approximately 0.025, which suggests that hepatic uptake clearance is not the major mechanism in the systemic removal of CAB glucuronide.

4.1.16. Potential for CAB inhibition of human folate transporters and folate receptor

Three independent in vitro experiments were conducted in which CAB (0.03 to 100 μM) was tested for the potential to inhibit human proton-coupled folate transporter (PCFT), reduced folate carrier (RFC) or folate receptor α (FRα) in MDCK-II cells transfected with each of the respective targets [Report 2019N396076]. In each experiment, CAB was pre-incubated with cells for 30 minutes, followed by addition of substrate and co-incubation for 5 minutes for the PCFT and RFC assays, or co-incubation for 2 hours for the FRα assay. A tabulated summary of this study is presented in m2.6.5, Table 8.9.

In the PCFT assay, CAB did not inhibit folic acid transport up to the highest concentration tested. In the RFC assay, CAB did not inhibit RFC activity. In the FRα assay, CAB demonstrated 36.7% inhibition at 25.8 μM; this observed in vitro inhibition was not projected to be clinically relevant. In all 3 assays, the positive control methotrexate demonstrated expected results.

4.2. In Vivo Distribution Studies

4.2.1. Mouse

4.2.1.1. Blood to Plasma and Liver Ratios

As part of an excretion study (see Section 6), the concentrations of radioactivity in blood, plasma, and liver were determined in samples taken at 1, 4, 8, 24 and 168 hours after a single oral dose of [^{14}C]-CAB (30 mg/kg) to intact male CD-1 mice (n=10/timepoint, except at 168 hours where n=4) [Report 2012N145873]. A tabulated summary of this study is presented in m2.6.5, Table 8.10.

In intact male mice, the elimination of [^{14}C]-related radioactivity from blood and plasma over 168 hours was slow, with pooled concentrations approximately equal at 1, 4, and 8 hours post-dose. Blood to plasma ratios of radioactivity were relatively constant up to 168 hours, with values of 0.52 to 0.54, and indicated that radioactivity was minimally associated with the cellular components of blood. Mean liver to blood concentration ratios of radioactivity were also similar over time, with values of 0.20 to 0.23, through 24 hours (with no observed detection of [^{14}C]-related radioactivity in liver at 168 hours) and indicated no accumulation of radioactivity in the liver.

4.2.2. Rat

4.2.2.1. Whole body autoradiography

The tissue distribution of CAB-related radioactivity was investigated after a single oral administration of [^{14}C]-CAB at 30 mg/kg to fasted partially pigmented (Lister-Hooded) male rats (n=7; 1/timepoint) by using quantitative whole body autoradiography at 1, 2, 4 and 8 hours and 1, 7 and 28 days post-dose [Report RD2008/00840]. A tabulated summary of this study is presented in m2.6.5, Table 5.1.

Radioactivity was widely distributed and slowly absorbed with most tissues exhibiting their highest concentrations at 1 day post-dose. Excluding the GI tract, tissues with high concentrations of radioactivity at this timepoint included blood, lung, bulbourethral gland, renal medulla, adrenal medulla and pigmented skin. By 7 days post-dose, concentrations of radioactivity had declined in all tissues. Elimination of radioactivity was slow with over 50% of the tissues still containing low but quantifiable radioactivity at 28 days. Following peak blood concentrations at 1 day post-dose, radioactivity declined in blood and was still measurable at 28 days. Except for the GI tract, concentrations of radioactivity in blood were higher relative to other tissues at all sampling times after dosing. Concentrations of radioactivity measured in the brain were low but quantifiable up to and including 7 days.

The highest concentrations of radioactivity in the uveal tract, pigmented and non-pigmented skin were measured 1 day after dose administration. Radioactivity then declined by 28 days. Highest concentrations of radioactivity in the meninges were observed at 8 hours and decreased thereafter. These results, relative to the long-term

retention in many other tissues, suggest no selective association of radioactivity with melanin.

4.2.2.2. Blood to Plasma and Liver Ratios

In conjunction with a preliminary excretion study (see Section 6), a single oral dose of [^{14}C]-CAB (sodium salt, 30 mg/kg) was administered to an intact and a bile duct cannulated (BDC) male Sprague Dawley rat [Report RD2008/00021]. Blood, plasma and liver were collected from the BDC male rat at 6 hours post-dose and from the intact male rat at 24 hours post-dose.

In the BDC and intact male rat, plasma concentrations of total radioactivity at 6 and 24 hours were 46733 and 35041 ng-equivalents/g, respectively. Blood to plasma ratios of total radioactivity were 0.55 and 0.53 at 6 and 24 hours, respectively, indicating minimal association with the cellular components of blood. Liver concentrations of total radioactivity were 4290 and 3646 ng-equivalents/g at 6 and 24 hours, respectively. Liver to blood concentration ratios of total radioactivity at 6 and 24 hours were 0.17 and 0.20, respectively. CAB was identified as the principal component in plasma and liver at 6 and 24 hours post-dose.

As part of a comprehensive excretion study (see Section 6), liver concentrations of radioactivity and blood and plasma concentrations of [^{14}C]-related material were determined following a single oral administration of [^{14}C]-CAB (30 mg/kg) to intact male Sprague Dawley rats (n=3/time point) [Report 2012N143605]. Concentrations of radioactivity in blood, plasma, and liver were determined in samples taken at 1, 4, 8, 24, 96 and 168 hours after dosing. A tabulated summary of this study is presented in m2.6.5, Table 8.10.

In intact male rats, the elimination of [^{14}C]-related radioactivity from blood and plasma over 168 hours was slow, with the highest mean concentrations observed at 4 hours post-dose. Blood to plasma ratios of radioactivity ranged from 0.54 to 0.56, indicating that radioactivity was minimally associated with the cellular components of blood. Mean liver to blood concentration ratios of radioactivity ranged from 0.20 to 0.25, through 168 hours, indicating no accumulation of radioactivity in the liver.

4.2.2.3. Distribution following intramuscular and subcutaneous administration

In a study to assess the tissue distribution of CAB in the gastrocnemius muscle and subcutaneous abdominal injection sites, male Sprague Dawley rats (n=5) were administered a single injection of CAB (40 mg/kg) and the injection depots were examined 14 days after injection [Report 2014N221244]. Tissue sections were collected from the region of the injection depots and analyzed by matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS). The injection depots in both the intramuscular (IM) and subcutaneous (SC) tissues were visible in pre-analysis optical scans and were targeted for several high spatial resolution (5 μm) MALDI IMS experiments. These were collected in addition to several low resolution (100 μm) survey scans of the entire tissue sections, which included both the depot and surrounding tissue. A tabulated summary of this study is presented in m2.6.5, Table 8.11.

In the low resolution MALDI IMS analyses of the whole issue sections, the CAB distribution was highly localized to the injection depots. The highest signal intensity was detected from the core of the depots with a decrease in relative signal intensity at the borders of the depots in both IM and SC. Very low relative signal intensity was observed in the surrounding tissue. No CAB metabolites were observed in the tissues.

In the high spatial resolution MALDI IMS experiments, extracellular and intracellular CAB were distinguished by the ion adducts formed during the MALDI process. In conjunction with immunohistochemical and histology staining, images displaying intracellular CAB in macrophages and multinucleated giant cells were observed primarily on the periphery of the injection depots.

Groups of male Sprague Dawley rats (n=5/group) received a single administration of either an IM (gastrocnemius) or SC (abdomen) injection of CAB LAP (40 mg/kg) [Report 2015N231295]. A separate group of rats (n=3) received an IM injection of CAB LAP in the right gastrocnemius together with vehicle (Tween 20, polyethylene glycol 3350, mannitol and water) injected in the left gastrocnemius. Magnetic Resonance Imaging (MRI) was performed immediately post-dose, and again at Day 1, 2, 3, 4, 7, and 14. In another group of rats (n=2) an MRI contrast agent, Feraheme™ (USPIO), was administered 2 days post-dose to investigate the potential involvement of macrophage trafficking to the CAB LAP and vehicle depot sites. A tabulated summary of this study is presented in m2.6.5, Table 8.13.

The drug depot volume as assessed by MRI showed a rapid ~3 to 7-fold increase in volume by Day 2 post-dose in the CAB LAP IM injected rats compared with an ~1-fold increase at Day 2 in the SC injected rats. Conversely, the vehicle depot did not increase in size with time and decreased below baseline by Day 2. In addition, the USPIO contrast agent labelled macrophages were shown to be present in the depot region of the CAB LAP injected gastrocnemius with potentially less USPIO than that which was observed in the vehicle injected gastrocnemius.

In conclusion, MRI can be used to non-invasively identify the drug depot location and volumetric changes in both IM and SC locations following CAB LAP administration. The IM depot volume increased rapidly to a maximum volume at 2 days after CAB LAP administration, while the vehicle depot did not suggest that the active drug substance and/or particle was a key driver for drug depot evolution. The depot expansion was associated with an increase in macrophage infiltration and edema in and around the depot region and was related to plasma drug concentration at early time points (0 to 4 days).

4.2.2.4. Potential for lymphatic exposure

The concentration of CAB was determined in lymph fluid, plasma and lymph nodes following a single IM administration of CAB (40 mg/kg) to cannulated (thoracic lymphatic duct and jugular vein; n=1) and intact (n=2/time point) male Sprague Dawley rats [Report 2014N222547]. Blood and lymph were collected from the cannulated rat at 0.5, 1, 2, 4, 8 and 24 hours post-dose. Blood, lymph, and lymph nodes were collected from 2 intact rats per time point at 24, 48, 72, 96 and 168 hours post-dose. A tabulated summary of this study is presented in m2.6.5, Table 8.12.

Based on the result from a single cannulated rat, the concentration of CAB in the lymph fluid was approximately 5% to 18% of that in plasma at the collection time points during the first 24 hours after dosing. Following administration to intact rats, the concentration of CAB in the lymph nodes was approximately 27% to 37% of that in plasma from 24 hours to 168 hours post-dose. CAB concentrations in both plasma and lymph nodes increased with time for the duration of the study (168 hours), suggesting that CAB was still being absorbed in blood and lymph nodes during the study period after a single IM administration.

4.2.3. Monkey

4.2.3.1. Blood to plasma

As a part of an excretion study (see Section 6), the concentrations of radioactivity in blood and plasma were determined following a single oral administration of [^{14}C]-CAB (10 mg/kg) to three intact male cynomolgus monkeys [Report 2013N174861]. Concentrations of radioactivity in blood and plasma were determined in samples taken at pre-dose, 1, 4, 8, 24, 96 and 168 hours after dosing. Due to blood analysis issues, only the plasma values were reported.

The highest mean plasma (~10.970 µg equiv/g) concentration was observed at the 1 hour time point. By 8 hours post-dose, the mean levels in plasma had declined to 6.337 µg equiv/g, with further decrease to 0.007 µg equiv/g by 96 hours. The blood to plasma ratios could not be calculated for this study due to the blood analysis issue.

Table 4.1 List of Distribution Studies Performed with CAB

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Plasma protein binding	Rat, Dog, Monkey, Human	NA	In vitro	B	5 μM	NA	No	GSK	RH2007/00193 (06RCD7725)	m4.2.2.3
Plasma protein binding	Human	NA	In vitro	A	500 to 20000 ng/mL	NA	No	[redacted]	2015N235936 (8309868)	m4.2.2.3
Plasma protein binding with CAB glucuronide	Rat, Human	NA	In vitro	N/A	200 to 20000 ng/mL	NA	No	GSK	2017N333514 (17DMW004)	m4.2.2.3
Serum protein binding	Rat, Dog, Monkey, Human	NA	In vitro	A	10 μM	NA	No	GSK	RH2007/00222	m4.2.2.3
BCRP inhibition	Human	NA	In vitro	B	0.03 to 30 μM	NA	No	GSK	2012N150360 (12DMR027)	m4.2.2.3
BCRP transport	Human	NA	In vitro	E	3 μM	NA	No	GSK	2012N155942 (12DMR032)	m4.2.2.3
Pgp inhibition	Human	NA	In vitro	B	0.03 to 30 μM	NA	No	GSK	2012N146041 (12DMR022)	m4.2.2.3
Pgp transport and membrane permeability	Human	NA	In vitro	E	3 μM	NA	No	GSK	2012N146040 (12DMR021)	m4.2.2.3
Folate receptor transporter inhibition	Human	NA	In vitro	B	0.03 to 100 μM	NA	No	[redacted]	2019N396076	m4.2.2.3

List of Distribution Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
OCT1, OCT2 inhibition	Human	NA	In vitro	B	0.01 to 30 μM	NA	No	[redacted]	2012N146057 (XS-0376)	m4.2.2.3
OATP1B1, OATP1B3 inhibition	Human	NA	In vitro	B	0.1 to 30 μM	NA	No	GSK	2013N164529 (12DMM039)	m4.2.2.3
MRP2, BSEP inhibition	Human	NA	In vitro	B	0.03 to 30 μM	NA	No	GSK	2013N174589 (13DMR019)	m4.2.2.3
Hepatic uptake	Human	NA	In vitro	B	0.7, 9 μM	NA	No	GSK	2018N391028 (18DMW024)	m4.2.2.3
OAT1, OAT3, MATE1, MATE2-K, MRP4 inhibition	Mouse (S_2), Human (HEK293), vesicles	NA	In vitro	B	0.03 to 30 μM	NA	No	[redacted]	2013N174474 (XS-0446)	m4.2.2.3
Blood to plasma partitioning	Rat (Sprague Dawley)	1M 1M (BDC)	Oral	F	30	Single	No	GSK	RD2008/00021 (08DMR002)	m4.2.2.3
Blood to liver partitioning	Rat (Sprague Dawley)	1M 1M (BDC)	Oral	F	30	Single	No	GSK	RD2008/00021 (08DMR002)	m4.2.2.3
Membrane permeability and transport by Pgp	Human	NA	In vitro	A	3 μM	NA	No	GSK	RH2007/00199 (07APK053)	m4.2.2.3

List of Distribution Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Uptake by macrophages	Human (differentiated macrophages)	NA	In vitro	A	0.2, 10 μg/mL	24 or 48 hours	No	GSK	2015N237779 (V43296N)	m4.2.2.3
Assessment of injection site depots	Rat (Sprague Dawley)	5M	IM or SC	A	40	Single (Day 14)	No	GSK	2014N221244 (14DMR029)	m4.2.2.3
IM and SC distribution	Rat (Sprague Dawley)	5M 2 or 3M	IM or SC IM	A	40 40	Single (Days 1, 2, 3, 4, 7 and 14)	No	GSK	2015N231295	m4.2.2.3
Lymphatic exposure	Rat (Sprague Dawley)	2M (LDC) 10M (intact)	IM	A	40	Single (Days 1, 2, 3, 4, 7)	No	GSK	2014N222547 (14DMR031)	m4.2.2.3
Whole body autoradiography	Rat (Lister Hooded)	7M	Oral (gavage)	F	30	Single	Yes	[redacted]	RD2008/00840 (2990/254)	m4.2.2.3

Key:
A = Parent. B = Sodium salt.
E = [^{14}C]-GSK1265744A (parent). F = [^{14}C]-GSK1265744B (sodium salt).
IM = Intramuscular. SC = Subcutaneous.
MATE = Multidrug and Toxin Extrusion Transporters.
MRP = Multidrug Resistance Associated Protein.
HEK293 = Human Embryonic Kidney cell line.
S_2 = Mouse cell line derived from interstitial renal proximal tubules.
BCRP = Breast Cancer Resistant Protein.
BDC = Bile Duct Cannulated.
BSEP = Bile Salt Export Pump.
LDC = Lymph Duct Cannulated.
NA = Not applicable.
OAT = Organic Anion Transporter.
OCT = Organic Cation Transporter.
Pgp = P-glycoprotein.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]

5. METABOLISM

In vitro and in vivo studies have been performed to investigate the metabolic fate of CAB. These studies are listed in Table 5.1. The metabolic flow diagram for CAB is in m2.6.5 Section 11.

5.1. In Vitro Studies

5.1.1. Metabolism studies with microsomes, hepatocytes or recombinant enzymes derived from animals and humans

5.1.1.1. Metabolic stability in liver S9 and in hepatocytes

A preliminary study was conducted to evaluate the metabolic stability of CAB (1 μM) in rat, dog, monkey and human liver S9 preparations and at 0.5 μM in rat and human fresh and cryopreserved hepatocytes [Report RH2007/00183]. Incubations were performed at 37°C at intervals up to 60 or 120 minutes for S9 or hepatocytes, respectively. The supernatant from each reaction was analyzed using LC-MS/MS analysis. A tabulated summary of this study is provided in m2.6.5, Table 10.1.

CAB was stable in rat, dog, monkey and human S9 ($t_{1/2}$ >180 minutes). CAB was stable in rat and human cryopreserved hepatocytes ($t_{1/2}$ >360 minutes). The intrinsic clearance rate and half-life of CAB in fresh rat hepatocytes was 29 mL/min/kg body weight and 229 minutes, respectively, and was 14 mL/min/kg body weight and 311 minutes, respectively, in fresh human hepatocytes.

5.1.1.2. Metabolic activation in liver microsomes

A study was performed to evaluate the potential for metabolic activation when [^{14}C]-CAB (10 μM) was incubated with pooled rat, monkey and human liver microsomes, using acetaminophen as a positive control [Report RD2007/01629]. Incubations were performed at 37°C for 30 or 60 minutes and samples were analyzed using liquid scintillation counting. A tabulated summary of this study is provided in m2.6.5, Table 10.2.

The total non-extracted binding of [^{14}C]-CAB to liver microsomes, following 5-solvent washing, was moderate in human (180 pmol eq/mg protein/hour), and relatively high in rat and monkey (984 and 794 pmol eq/mg protein/hour, respectively). The non-extracted radioactivity observed was predominantly dependent on NADPH co-factor. The co-factor dependent binding, co-factor independent binding and total non-extracted binding values for [^{14}C]-acetaminophen in human liver microsomes were consistent with previously reported data.

5.1.1.3. Metabolism by hepatocytes

In cryopreserved rat, dog, monkey and human hepatocytes, the potential formation of metabolites of CAB (10 μM) was assessed following incubation at 37°C for up to 24 hours [Report RH2007/00188]. Samples were analyzed by using LC-MS/MS under positive or negative ionization modes. A tabulated summary of this study is provided in m2.6.5, Table 10.3.

No metabolites of CAB were detected under the conditions of this assay.

In a follow up study, the metabolism of [^{14}C]-CAB (50 μM) was investigated in male rat, monkey and pooled (mixed gender) human cryopreserved hepatocytes with incubation at 37°C up to 24 hours [Report RD2008/00073]. Samples were analyzed using liquid scintillation counting. A tabulated summary of this study is provided in m2.6.5, Table 10.4.

The metabolic turnover of [^{14}C]-CAB in rat and monkey hepatocytes was similar to that observed in human hepatocytes. The route of metabolism for [^{14}C]-CAB in rat, monkey and human hepatocytes was by glucuronidation of CAB.

5.1.1.4. Potential metabolic formation of stereoisomers

CAB has two chiral centers, and the potential for metabolism of CAB (10 μM) to its respective enantiomer (gsk001*) and one of the two diastereomers (gsk003*) was investigated in incubations with cryopreserved rat, dog, monkey and human hepatocytes [Report RH2007/00207]. The other diastereomer of CAB was not available as a synthetic standard at the time of this study and its formation could not be monitored. Incubations were performed at 37°C for 0, 1, 4, 6 and 24 hours and samples were analyzed using LC/MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 10.5.

The enantiomer (gsk001*) and diastereomer (gsk003*) were not chromatographically resolved from one another. A peak corresponding to the retention time of these two unresolved stereoisomers represented ~6.7% of the peak area of CAB in control incubations without hepatocytes. This peak did not increase in size in hepatocyte incubations for up to 24 hours, suggesting the peak was initially present as an impurity in the early synthetic batch of CAB used for this study. These data indicate that no significant metabolic conversion of CAB to its enantiomer or one of two possible diastereomers occurs in rat, dog, monkey or human hepatocytes.

5.1.1.5. Formation of glucuronide metabolite

The potential of CAB (0.5, 5 and 50 μM) to undergo glucuronidation by UDP glucuronosyltransferase 1A1 (UGT1A1) was investigated in incubations with alamethacin-treated pooled human liver microsomes (PHLM) and in UGT1A1 supersomes (recombinantly expressed human UGT1A1 in baculovirus infected insect cells) [Report RH2007/00166]. Incubations were performed at 37°C for up to

120 minutes and samples were analyzed using LC/MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 10.7.

While there was no significant loss of CAB at 5 or 50 μM in PHLM and UGT1A1 supersomes in incubations up to 60 minutes, formation of a glucuronide metabolite could be detected by LC/MS/MS in both incubations. Under optimized study conditions, 73% of original CAB concentrations remained at the end of a 120 minute incubation of recombinant UGT1A1 supersomes with CAB (0.5 μM).

In a follow up study, the potential formation of CAB glucuronide in human liver, kidney and intestinal microsomal fractions was determined [Report 2014N222268]. Microsomes were activated by the addition of alamethacin and then pre-incubated with [^{14}C]-CAB (5 μM) for 5 minutes. The reactions were initiated by the addition of cofactor and incubations proceeded, in duplicate, for 120 minutes (liver and kidney) and 60 minutes (intestinal) at 37°C with shaking. A tabulated summary of this study is provided in m2.6.5, Table 10.9.

In human liver, kidney and intestinal microsomal incubations, [^{14}C]-CAB (5 μM) was metabolized to a single UDPGA-dependent metabolite, CAB glucuronide, comprising approximately 17%, 8.4% and 4.0% of the total radioactivity, respectively. The estimated mean rates of metabolite formation (pmol/min/mg) in human liver, kidney and intestinal microsomes were 14, 7.0 and 3.3 pmol/min/mg, respectively.

Another study was conducted in which the human UDP glucuronosyltransferase (UGT) enzymology of CAB was investigated in vitro using pooled human liver microsomes and recombinant human UGT enzymes [Report 2012N145430]. Incubations were performed with [^{14}C]-CAB (5 μM) at 37°C, in duplicate, for up to 120 minutes with shaking. Select samples were analyzed by liquid scintillation counting to determine the recovery of radiolabeled material, and supernatant samples from reaction mixtures were analyzed by radio-HPLC. The enzyme kinetics, V_{max} and K_m, were also determined for [^{14}C]-CAB glucuronide formation by testing [^{14}C]-CAB (2 to 100 μM) in human liver microsomes and recombinant UGT1A1 and 1A9 enzymes. A tabulated summary of this study is provided in m2.6.5, Table 10.8.

The results showed that [^{14}C]-CAB (5 μM) was metabolized in human liver microsomes to a single UDPGA-dependent metabolite, CAB glucuronide. The metabolite CAB glucuronide was also formed in recombinant UGT1A1 and 1A9 incubations. Glucuronidation was not observed in UGT1A3, 1A4, 1A6, 2B4, 2B7, 2B15 or control UGT incubations. The enzyme kinetic parameters, K_m, V_{max}, and intrinsic clearance (V_{max}/K_m), for CAB glucuronide formation were determined in human liver microsomes, UGT1A1 and 1A9 incubations. Intrinsic clearances were 2.6, 4.5, and 2.2 μL/min/mg, respectively. The fraction of total clearance (f_{CL}) of CAB mediated by UGT1A1 and 1A9 in vitro was 0.67 and 0.33, respectively.

The data from human liver microsomes and recombinant UGT enzymes suggest that UGT1A1 is the primary UGT enzyme involved in the glucuronidation of CAB in vitro with contribution from UGT1A9.

5.1.1.6. Formation of glutathione adducts

A study was conducted to determine the potential for CAB (100 μM) to form electrophilic metabolites following incubation with glutathione (5 mM) in dog, monkey and pooled human liver microsomes [Report RH2007/00164]. CAB was incubated with the mixture at 37°C for 30 minutes and samples were analyzed by LC-MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 10.6.

A glutathione adduct of CAB was detected in rat, monkey and human liver microsomes, but not dog liver microsomes. The observed protonated molecular ion was consistent with addition of glutathione through oxidative defluorination.

5.1.2. Ex vivo metabolism study

5.1.2.1. CAB glucuronide in the isolated perfused rat liver model

The formation and hepatic disposition of CAB glucuronide was investigated following perfusion of CAB through isolated perfused male Sprague Dawley rat livers [Report 2017N320633]. Livers (n=4) were perfused with a buffer containing CAB (10 μM) with a single pass for 1 hour. Bile and perfusate samples were collected at approximately 5 minute intervals, and liver extract was also prepared for analysis. Samples were analyzed using LC-MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 10.10.

CAB glucuronide is formed in the liver and is excreted in the bile as well as in perfusion media. The canalicular and basolateral clearance of CAB glucuronide were comparable and found to be 0.16 and 0.21 mL/min/kg, respectively.

5.1.3. In vitro inhibition and induction potential in animals and humans

5.1.3.1. Potential PXR induction in the rat

To determine the potential for Pregnane X receptor (PXR) induction, CAB (0.2 nM to 10 μM) was assessed in vitro in HepG2 cells transfected with rat PXR [Report RR2007/00043]. Transcription of the reporter gene (luciferase), due to PXR activation, was measured by a luminescence assay. A tabulated summary of this study is provided in m2.6.5, Table 12.4.

Treatment with CAB resulted in weak PXR activation with a maximum response of 2.2 to 5.2% of that observed with the positive control rat PXR activator PCN (5-Pregnan-3β-OL-20-ONE-16α carbonitrile).

5.1.3.2. Potential PXR induction in humans

An in vitro study was performed to determine the potential for CAB to activate human PXR [Report RR2007/00046]. CAB (0.2 nM to 10 μM) was tested in HepG2 cells transfected with human PXR and rifampicin was used as a positive control. Transcription

of the reporter gene (luciferase), due to PXR activation, was measured by a luminescence assay. A tabulated summary of this study is provided in m2.6.5, Table 12.4.

Treatment with CAB resulted in PXR activation with a maximum response of 0.5 to 12.3% of that observed with the human PXR activator, rifampicin. CAB was a weak activator of human PXR in vitro and therefore not expected to cause induction of PXR target genes, including CYP3A4, in humans at the proposed therapeutic dose.

5.1.3.3. Potential for cytochrome P450 inhibition

In preliminary studies, the potential for CAB (free acid, 0.033 to 33 μM) to inhibit human cytochrome P450 (CYP) enzymes was assessed with recombinant enzymes and pooled human liver microsomes (PHLM) [Report RH2007/00181]. Initial screening was performed on two separate occasions for CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 using recombinant enzymes and higher throughput fluorescence methods. Inhibition was also examined for the same CYP isoforms using pooled human liver microsomes (PHLM) with LC-MS/MS analysis. Time-dependent inhibition (TDI) for CYP3A4 was also assessed in PHLM. A tabulated summary of this study is provided in m2.6.5, Table 12.1.

Using recombinant human CYP enzymes, low inhibition was observed for CYP1A2 (IC_{50} = 12.5 or >33 μM, in two separate studies) and no inhibition (IC_{50} >33 μM) was observed for 2C9, 2C19, 2D6 and 3A4. Using PHLM, no inhibition (IC_{50} >33 μM) was observed for 1A2, 2D6 and 3A4, and low inhibition was observed for 2C9 and 2C19 (IC_{50} = 15 and 13 μM, respectively). No time-dependent inhibition of CYP3A4 by CAB was observed in PHLM.

An additional study was conducted to determine the potential of CAB (sodium salt) to act as a direct, time-dependent and metabolism-dependent inhibitor of CYP activity [Report 2012N151766]. The effects of CAB (0.1 to 100 μM) on CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 was determined in human liver microsomes using three different marker substrate reactions: atorvastatin ortho-hydroxylation, midazolam 1′-hydroxylation and nifedipine oxidation. To distinguish between time-dependent and metabolism-dependent inhibition, CAB was preincubated with human liver microsomes for 30 minutes without and with an NADPH-generating system, respectively, prior to the incubation with the marker substrate. Known direct and metabolism-dependent inhibitors of CYP enzymes were included as positive controls in all experiments. A tabulated summary of this study is provided in m2.6.5, Table 12.3.

CAB directly inhibited CYP3A4/5 (atorvastatin ortho-hydroxylation) with an IC_{50} value of 84 μM. In addition, there was evidence of direct inhibition of CYP2B6 and CYP3A4/5 (nifedipine oxidation) by CAB, as approximately 20% and 28% inhibition, respectively, was observed at the highest concentration of CAB evaluated (100 μM), and the IC_{50} values were reported as greater than 100 μM. There was little or no evidence of direct inhibition of CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6 or 3A4/5 (midazolam 1′-hydroxylation) by CAB. The IC_{50} values for these enzymes were reported as greater than 100 μM CAB.

After a 30-minute preincubation with pooled human liver microsomes in the presence of NADPH, a greater than 20% increase in inhibition and/or a greater than 1.5-fold shift decrease in IC_{50} values was observed indicating that CAB is a metabolism-dependent inhibitor of CYP3A4/5 (atorvastatin ortho-hydroxylation, midazolam 1′-hydroxylation and nifedipine oxidation). There was little or no evidence of time-dependent or metabolism-dependent inhibition of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19 or 2D6 by CAB.

5.1.3.4. Inductive potential in human hepatocytes

A study was conducted to examine the potential for induction of select nuclear receptor target genes by CAB when tested in vitro at concentrations of 0.03 to 30 μM [Report 2013N166279]. After an acclimatization period, cultured human hepatocytes (n=3 donors) were treated with CAB at 37°C for 24 hours and quantitative real time PCR was used to determine the mRNA levels of CYP1A2, 2B6 and 3A4. Prototypical inducers were included as positive controls. A tabulated summary of this study is provided in m2.6.5, Table 12.5.

No notable increases in the mean mRNA levels of CYP1A2, CYP2B6 or CYP3A4 were observed.

5.1.3.5. Potential inhibition of UDP-glucuronosyltransferase

An in vitro study was performed to determine whether CAB (0.1 to 100 μM) inhibits human uridine 5'-diphosphoglucuronosyl transferase (UGT) catalytic activity using human recombinant UGTs or human liver microsomes (UGT1A1 only) [Report 2013N159049]. The enzymes examined were UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B15, and 2B17. The reaction mixtures were incubated at 37°C for periods ranging from 10 to 30 minutes. The reaction mixtures included relevant substrates for each of the UGT enzymes and the potential for CAB to inhibit the glucuronidation was determined. The glucuronide metabolites were detected by HPLC separation followed with absorbance detection. Appropriate positive control inhibitors were used for each UGT enzyme when available. A tabulated summary of this study is provided in m2.6.5, Table 12.2.

CAB inhibited UGT1A3 and UGT1A9, with IC_{50} values of 12 μM and 46 μM, respectively. At the highest concentration tested (100 μM), CAB inhibited UGT1A1 by 15% (human liver microsomes) to 33% (recombinant UGT1A1) and UGT2B17 by 24%. The IC_{50} values for these enzymes were >100 μM. There was little (<20% at the highest concentration tested) to no inhibition of UGT1A4, 1A6, 2B4, 2B7, or 2B15. The IC_{50} values for these enzymes were >100 μM.

Table 5.1 List of Metabolism Studies Performed with CAB

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Metabolic stability in liver S9	Rat, Dog, Monkey, Human	NA	In vitro	A	1 μM	NA	No	GSK	RH2007/00183 (07APK038)	m4.2.2.4
Metabolic stability in hepatocytes	Rat, Human	NA	In vitro	A	0.5 μM	NA	No	GSK	RH2007/00183 (07APK038)	m4.2.2.4
Metabolism in hepatocytes	Rat, Dog, Monkey, Human	NA	In vitro	A	10 μM	NA	No	GSK	RH2007/00188 (07APK040)	m4.2.2.4
Metabolism in hepatocytes	Rat, Monkey, Human	NA	In vitro	E	50 μM	NA	No	GSK	RD2008/00073 (08DMR007)	m4.2.2.4
Metabolic activation in microsomes	Rat, Monkey, Human	NA	In vitro	E	10 μM	NA	No	GSK	RD2007/01629 (07DMR126)	m4.2.2.4
Potential metabolic formation of stereoisomers	Rat, Dog, Monkey, Human	NA	In vitro	A	10 μM	NA	No	GSK	RH2007/00207 (07APK056)	m4.2.2.4
Formation of glucuronide metabolite	Human	NA	In vitro	A	0.5, 5, 50 μM	NA	No	GSK	RH2007/00166 (07APK033)	m4.2.2.4

List of Metabolism Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Hepatic uptake clearance of CAB glucuronide	Human (cryopreserved hepatocytes)	NA	In vitro	NA	50 μM	NA	No	GSK	2017N320631	m4.2.2.4
Hepatic canalicular and basolateral excretory clearances	Rat (Sprague Dawley)	4M	Ex vivo	B	10 μM	NA	No	GSK	2017N320633	m4.2.2.4
Formation of glutathione adducts in microsomes	Rat, Dog, Monkey, Human	NA	In vitro	A	100 μM	NA	No	GSK	RH2007/00164 (07APK031)	m4.2.2.4
PXR activation assays	Rat Human	NA	In vitro	A	0.2 nM to 10 μM	NA	No	GSK	RR2007/00043 RR2007/00046	m4.2.2.4
CYP genes mRNA expression	Human (cultured hepatocytes)	NA	In vitro	B	0.03 to 30 μM	NA	No	GSK	2013N166279 (12DMM040)	m4.2.2.4
CYP inhibition in recombinant enzymes and liver microsomes	Human	NA	In vitro	B	0.1 to 100 μM	NA	No	[redacted]	2012N151766 ([redacted])	m4.2.2.4

List of Metabolism Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
CYP inhibition in recombinant enzymes and liver microsomes	Human	NA	In vitro	A	0.033 to 33 μM	NA	No	GSK	RH2007/00181 (07APK036)	m4.2.2.4
Potential in vivo epimerization in plasma	Human	8M[a]	Oral	B	25 mg	14 days	No	GSK	RD2008/01340 (08DMR068)	m4.2.2.4
In vivo metabolism	Mouse (CD-1)	4M 3M (BDC)	Oral	F	30	Single	No	GSK	2012N146480 (12DMR024)	m4.2.2.4
In vivo metabolism	Rat (Sprague Dawley)	3M 3M (BDC)	Oral	F	30	Single	No	GSK	2012N146427 (12DMR023)	m4.2.2.4
In vivo metabolism	Rat (Sprague Dawley)	1M 1M (BDC)	Oral	F	30	Single	No	GSK	RD2008/00021 (08DMR002)	m4.2.2.4
Potential induction of CYP mRNA	Rat (Sprague Dawley)	3M/3F	In vivo	A	30 100, 300[b]	NA	No	GSK	RD2006/01999 (06DMR115)[c]	m4.2.2.4
Glucuronidation in recombinant enzymes and liver microsomes	Human	NA	In vitro	E	2 to 100 μM	NA	No	GSK	2012N145430 (12DMR018)	m4.2.2.4

List of Metabolism Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
UGT inhibition	Human	NA	In vitro	B	0.1 to 100 μM	NA	No	[redacted]	2013N159049	m4.2.2.4
Evaluation of CAB glucuronide as a transporter inhibitor	Human	NA	In vitro	NA	0.01 to 300 μM	NA	No	[redacted]	2015N253914 (XS-0681)	m4.2.2.4
Evaluation of CAB glucuronide as a Pgp and BCRP substrate	Human	NA	In vitro	NA	10 μM	NA	No	[redacted]	2016N288197 (XS-0848)	m4.2.2.4
Evaluation of CAB glucuronide as a substrate for hepatic and renal transporters	Human	NA	In vitro	NA	10 or 50 μM	NA	No	GSK	2016N298553 (15DMM040)	m4.2.2.4
Profiling, quantification and structural characterizations	Monkey (cynomolgus)/ Urine, bile, feces and plasma	3M 3M (BDC)	In vivo	F	10	Single	No	GSK	2013N171159 (13DMR016)	m4.2.2.4

List of Metabolism Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Glucuronidation assessments	Human/ Liver, kidney and intestinal microsomes	NA	In vitro	E	5 μM	NA	No	GSK	2014N222268 (14DMR030)	m4.2.2.4

Key:
a = Plasma samples were collected from the Clinical Study ID ITZ111451 following a repeat dose of 25 mg in healthy male subjects.
b = GSK1265744 in suspension.
c = Liver samples for this study were obtained from the 14 day rat toxicity study [Report RD2006/01741].
A = Parent. B = Sodium salt. E = [^{14}C]GSK1265744A (parent). F = [^{14}C]GSK1265744B (sodium salt).
BDC = Bile duct cannulated. CYP = Cytochrome P450. NA = Not applicable. PXR = Pregnane X receptor.
UGT = Uridine 5'-diphosphoglucuronosyl transferase.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]
[redacted] = [redacted]

5.2. In Vivo Studies

5.2.1. Mouse

5.2.1.1. Metabolic profile following oral administration

The in vivo metabolism of [^{14}C]-CAB was investigated in intact and bile duct cannulated (BDC) male CD-1 mice following a single oral administration of 30 mg/kg [Report 2012N146480]. Samples of plasma and liver homogenates (10 animals per time point) were pooled using equal weights to produce a single representative sample per time point (1, 4, 8 and 24 hours post-dose). Bile (0 to 72 hours), feces (0 to 48 hours), and urine (0 to 96 hours) were pooled proportionally by sample weight to obtain pools containing ≥90% of the radioactivity excreted via that route. All samples were obtained from an excretion study in mice [Report 2012N145873], with the elimination results discussed in Section 6.1.1. A tabulated summary of the data from this study is provided in m2.6.5, Table 9.1.

[^{14}C]-CAB was the principal component in plasma and liver extracts through 24 hours post-dose, representing up to 99.4% and 91.4% of the radioactivity, respectively. The plasma concentrations of [^{14}C]-CAB were similar between 1 and 8 hours post-dose ranging from 79.3 to 82.3 nmol/g and decreased to 40.5 nmol/g at 24 hours post-dose. The extraction efficiency of total radioactivity from plasma and homogenates of liver were greater than 90%.

Unchanged [^{14}C]-CAB was the principal component in feces and represented a mean of 78.5 and 77.4% of the dose in intact and BDC mice, respectively.

The absorbed dose (approximately 2.4%) was eliminated as metabolites in bile (1.8% of dose) and urine (0.76% of dose). The two major metabolites observed in bile resulted from glucuronidation (CAB glucuronide) and a combination of oxidation, loss of fluorine and glutathione conjugation (M5). CAB glucuronide was the major metabolite in urine along with a minor oxidative metabolite (M6).

[^{14}C]-CAB was detected in urine, but below the lower limit of quantification (LLQ) and it was not detected in bile.

5.2.2. Rat

5.2.2.1. Metabolic profile following oral administration

In a preliminary study, the metabolism of CAB was investigated following a single oral dose of [^{14}C]-CAB (sodium salt, 30 mg/kg) to an intact male and a bile duct cannulated (BDC) male Sprague Dawley rat [Report RD2008/00021].

CAB glucuronide was the principal component in bile (0 to 6 hours). CAB was the principal component in plasma and liver at 6 and 24 hours post-dose, and in the feces (intact male rat) at 24 hours post-dose. The amount of non-extractable radiocarbon in the

plasma and liver from the intact and BDC male rats was low (≤1.4% or 7.8 pmol eq./mg protein) at 6 and 24 hours, respectively.

A further study was conducted to investigate the in vivo metabolism of CAB in male Sprague Dawley rats following a single oral dose of [^{14}C]-CAB at a dose level of 30 mg/kg [Report 2012N146427]. Plasma and liver homogenates (3 animals per time point) were pooled using equal weights to produce a single representative sample per time point (1, 4, 8, 24, 96 and 168 hours post-dose). Urine (0-120 hours), bile (0 to 96 hours) and feces (0 to 48 hours) were pooled proportionally by sample weight to obtain pools containing ≥92% of the radioactivity excreted via that route. Urine samples from BDC rats were not analyzed. Samples were obtained from an excretion study in rats [Section 6.2.1, Report 2012N143605]. A tabulated summary of the data from this study is provided in m2.6.5, Table 9.1.

[^{14}C]-CAB was the principal component in plasma and liver extracts through 168 hours post-dose representing up to 92.3% and 88.2% of the radioactivity, respectively. [^{14}C]-CAB was slowly eliminated from the systemic circulation, with similar plasma concentrations between 1 and 8 hours post-dose, ranging from 77.3 to 93.1 nmol/g and decreased to 22.1 nmol/g by 168 hours post-dose. The extraction efficiencies of total radioactivity from plasma and homogenates of liver were greater than 90%.

[^{14}C]-CAB was eliminated primarily unchanged in feces, accounting for a mean of 75.7% and 71.9% of the dose in the intact and BDC rats, respectively.

The absorbed dose (approximately 1.9%) was eliminated primarily through metabolism and excreted in bile and urine, accounting for 1.6% and 0.33% of the dose, respectively. The major route of metabolism observed was glucuronidation, accounting for a mean of 1.4% of the dose in bile and 0.13% in urine. Other metabolites observed in urine were products of glucose conjugation and oxidation. [^{14}C]-CAB was a very minor component in urine, accounting for a mean of 0.02% of the dose.

5.2.2.2. Cytochrome P450 induction

The effect of CAB on the mRNA levels of hepatic CYP genes was examined in rat liver samples after 14 days of oral administration of CAB [Report RD2006/01999]. The liver samples were obtained from male and female Sprague Dawley rats (n=3/sex/group) administered CAB (free acid) orally at 30, 100, 300 mg/kg/day in a 14 day oral toxicity study. The levels of mRNA expression for each specific cytochrome P450 were determined using quantitative real-time polymerase chain reaction technology. A tabulated summary of this study is provided in m2.6.5, Table 12.6 and a summary of the toxicology data is presented in m2.6.6, Section 3.3.

No notable increases in mRNA levels of CYP 1A2, 2B1, 2B2, 2E1, 3A2 (male-specific), 3A23 and 4A1 were observed. CYP1A1 expression was below the level of quantification in vehicle control and in CAB-treated groups.

5.2.3. Monkey

5.2.3.1. Metabolic profile following oral administration

The metabolism of [^{14}C]-CAB was investigated in intact and cannulated male cynomolgus monkeys following a single oral administration at a dose level of 10 mg/kg [Report 2013N171159]. Plasma (3 animals per time point) was pooled using equal volumes to produce a single representative sample per time point (1, 4, 8 and 24 hours post-dose). Plasma samples collected at 96 and 168 hours post-dose were not analyzed due to low concentrations of radioactivity. Urine (0 to 48 hours), bile (0 to 48 hours) and feces (0 to 48 hours) were pooled proportionally by sample weight to obtain pools containing ≥90% of the radioactivity excreted via that route. All samples were obtained from an excretion study in monkeys [Section 6.3.1, Report 2013N174861]. A tabulated summary of the data from this study is provided in m2.6.5, Table 9.1.

[^{14}C]-CAB was the principal component in plasma extracts through 24 hours post-dose representing up to 95.9% of the total plasma radioactivity. The plasma concentrations of [^{14}C]-CAB were similar between 1 and 8 hours post-dose ranging from 9.62 to 5.75 μmol/g and decreased to 1.93 μmol/g by 24 hours post-dose. The extraction efficiency of total radioactivity from plasma was greater than 95%.

[^{14}C]-CAB was eliminated primarily unchanged in feces, accounting for 58.5% and 44.5% of the dose in the intact and cannulated monkeys, respectively, over 48 hours.

The absorbed dose (approximately 26%), which accounted for 11.1% and 14.7% of dose based on radioactivity excreted in urine and bile, was eliminated through metabolism. The major route of metabolism observed was glucuronidation, accounting for 8.55% and 9.76% of the administered dose in urine and bile, respectively. Two minor metabolites observed in both urine and bile were the product of glucose conjugation and a combination of oxidative deflourination and cysteine or glutathione conjugation followed by degradation. [^{14}C]-CAB was not detected in the urine and bile.

5.2.4. Human

5.2.4.1. Potential for the in vivo epimerization of CAB in human plasma

The potential for epimerization of CAB to occur in vivo was assessed by the analysis of human plasma obtained from healthy subjects following repeat oral administration of CAB (sodium salt, 25 mg) for 14 days [Report RD2008/01340]. Plasma samples were collected from healthy human subjects under protocol ITZ111451 [RM2008/00009] and transferred to the present study. Plasma was collected from subjects (n=8) at 1, 4 and 24 hours post-dose following 14 days dosing. A tabulated summary of this study is provided in m2.6.5, Table 9.2.

There was no evidence for the in vivo epimerization of CAB to any of its stereoisomers, gsk001*, gsk003* or gsk016*, in human plasma samples following repeat oral administration of CAB for 14 days.

*新薬承認情報提供時に置き換え

6. EXCRETION

A range of studies has been performed in which the excretion balance of [^{14}C]-CAB was investigated. These studies are listed in Table 6.1.

6.1. Mouse

6.1.1. Excretion following oral administration

The elimination of radioactivity was investigated following a single oral administration of [^{14}C]-CAB (sodium salt, 30 mg/kg) to intact and bile duct cannulated (BDC) male CD-1 mice (n=4 intact; n=3 BDC) [Report 2012N145873]. [^{14}C]-CAB was formulated as a suspension in 0.5% hydroxypropyl methylcellulose / 0.1% Tween 80 in water. All samples were analyzed by liquid scintillation counting (LSC) for total radioactivity. A tabulated summary of this study is presented in m2.6.5, Table 13.1.

The major route of elimination of drug-related material from intact and male BDC mice was via the feces with approximately 94% of the dose eliminated via this route. At least 2% of the radioactivity dosed was absorbed, as judged by the amount of drug-related material recovered in bile and urine in the BDC animals. Initial elimination of radioactivity was rapid, with approximately 88% of the radioactivity recovered in the first 24 hours, and approximately 5% further recovered at 48 hours. The percentage of dose recovered in each subsequent 24 hour period was then halved at each timepoint, such that the mean total recovery (including cage wash) of the dose in intact and BDC mice was 95% (0 to 168 hours) and 96% (0 to 72 hours), respectively.

6.2. Rat

6.2.1. Excretion following oral administration

In a preliminary study, a single oral dose of [^{14}C]-CAB (sodium salt, 30 mg/kg) was administered to an intact male and a bile duct cannulated (BDC) male Sprague Dawley rat [Report RD2008/00021]. [^{14}C]-CAB was formulated as a suspension in 0.5% hydroxypropyl methylcellulose / 0.1% Tween 80 in water. All samples were analyzed by LSC for total radioactivity.

In the intact male rat, the major route of elimination of [^{14}C]-related material during the 0 to 24 hour collection period was via feces with 80.0% of the dose recovered, while urinary elimination accounted for 0.36% of the dose. In the BDC male rat, 1.89% and 0.18% of the dose was eliminated via bile and urine, respectively, during the 0 to 6 hour collection period. No radioactivity was found in feces at 6 hours post-dose.

In another excretion study, the elimination of radioactivity was investigated following a single oral administration of [^{14}C]-CAB (sodium salt, 30 mg/kg) to intact and bile duct cannulated male Sprague Dawley rats (n=3/group) [Report 2012N143605]. [^{14}C]-CAB was formulated as a suspension in 0.5% hydroxypropyl methylcellulose / 0.1% Tween 80

in water. All samples were analyzed by LSC for total radioactivity. A tabulated summary of this study is presented in m2.6.5, Table 13.1.

The major route of elimination of drug-related material from intact and male BDC rats was via the feces with greater than 88% of the dose recovered in the first 48 hours. At least 2% of the dosed radioactivity was absorbed, as judged by the amount of drug-related material recovered in bile and urine in the BDC animals. Elimination after 48 hours was slow with approximately 1% of the dose excreted for each subsequent collection interval through the 168 hour collection. The mean total recovery (including cage wash) of the dose in intact and BDC rats was 95% (0 to 168 hours) and 91% (0 to 96 hours), respectively.

6.3. Monkey

6.3.1. Excretion following oral administration

The elimination of radioactivity was investigated in intact and cannulated male cynomolgus monkeys (n=3/group) following a single oral dose of [^{14}C]-CAB (sodium salt, 10 mg/kg) [Report 2013N174861]. [^{14}C]-CAB was formulated as a suspension in 0.5% hydroxypropyl methylcellulose / 0.1% Tween 80 in water. All samples were analyzed by LSC for total radioactivity. A tabulated summary of this study is presented in m2.6.5, Table 13.1.

Biliary excretion of drug-related material in a single cannulated male monkey accounted for recovery of 14.5% of the dose; fecal and renal excretion in the cannulated animal accounted for 54.6% and 14.7% of the dose, respectively. Total recovery of radioactivity from all matrices at 48 hours post-dose was 84.1% of the dose. Due to bile flow issues, only data from one BDC animal were reported, as two of the cannulated monkeys on study did not produce sufficient bile samples to provide reliable data, and thus are not included in the study summary.

Overall, following a single oral dose of [^{14}C]-CAB to intact monkeys, 90.1% of the administered dose was eliminated after 168 hours. The major route of elimination of drug-related radioactivity was via the feces with moderate amounts of radioactivity excreted via the renal and biliary routes.

Table 6.1 List of Excretion Studies Performed with CAB

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Elimination	Mouse (CD-1)	4M 4M (BDC)	Oral	F	30	Single	No	GSK	2012N145873 (12DMR019)	m4.2.2.5
Preliminary elimination	Rat (Sprague Dawley)	1M 1M (BDC)	Oral	F	30	Single	No	GSK	RD2008/00021 (08DMR002)	m4.2.2.5
Elimination	Rat (Sprague Dawley)	3M 3M (BDC)	Oral	F	30	Single (1, 4, 8, 24, 48 hours)	No	GSK	2012N143605 (12DMR017)	m4.2.2.5
Elimination	Monkey (cynomolgus)	3M 3M (BDC)	Oral	F	10	Single (1, 4, 8, 24, 96, 168 hours)	No	[redacted]	2013N174861 (10875)	m4.2.2.5

Key:
F = [^{14}C]GSK1265744B (sodium salt).
BDC = Bile duct cannulated.

Testing Facility:
GSK = GlaxoSmithKline.
[redacted] = [redacted]

7. PHARMACOKINETIC DRUG INTERACTIONS

Nonclinical studies have not been performed in vivo to specifically investigate potential pharmacokinetic interactions with drugs that are likely to be co-administered with CAB. However, relevant data from cytochrome P450 inhibition/induction and transporter studies are available.

In vitro, the metabolism of CAB is primarily mediated by UGT1A1, suggesting a potential for UGT1A1 inducers to alter the pharmacokinetics of CAB. A subsequent clinical study confirmed the potential for an interaction with strong UGT inducers, e.g. rifampin [m2.7.2, Section 3.2.9]. Therefore, co-administration of CAB with strong inducers of UGT1A1 is contraindicated.

RPV is metabolized by CYP3A4 and, although CAB is a weak inhibitor of CYP3A4, there was not a drug interaction between CAB and RPV at therapeutic doses in a clinical drug interaction study [m2.7.2, Section 2.5.6.1].

8. OTHER PHARMACOKINETIC STUDIES

No studies appropriate to this category have been performed with CAB.

9. DISCUSSION AND CONCLUSIONS TO PHARMACOKINETICS STUDIES

Pharmacokinetic, absorption, distribution, metabolism and elimination studies with CAB have been performed in mice, rats, rabbits, dogs and monkeys. A comparison of the systemic exposure of CAB achieved in the nonclinical species during toxicity studies and in humans at the planned therapeutic dose is presented below in Table 9.1.

Following single intravenous administration of CAB to dogs and monkeys, the plasma clearance (<2% of hepatic plasma flow) and steady-state volume of distribution (<0.35 L/kg) were low, with half-life values of 4 to 6 hours. Following oral administration as a solution, the oral bioavailability of CAB was good (44 to 83%) and consistent with its high passive permeability. However, when administered as a suspension, or in solid dosage forms, the bioavailability appeared limited by dissolution rate or solubility which resulted in a less than proportional increase in systemic exposure of CAB relative to dose. In mice, rats and monkeys, no consistent notable (>2-fold) difference in oral systemic exposure between sexes was observed.

In rats and monkeys given a single subcutaneous (SC) or intramuscular (IM) injection, CAB was slowly released from the injection site with a mean apparent plasma half-life ranging from 12 to 29 days (SC) or from 8 to 12 days (IM).

The protein binding of CAB in rat, dog, monkey and human plasma and serum was high (>99%). CAB is a substrate for Pgp and BCRP, but due to its high permeability, no alteration in absorption would be expected by co-administration of either Pgp or BCRP inhibitors. After oral administration of [^{14}C]-CAB to rats, radioactivity was slowly absorbed, widely distributed to tissues and minimally associated with blood cellular components. Elimination of radioactivity was slow with most tissues containing low but quantifiable radioactivity at 28 days. Association of radioactivity to the melanin-containing tissues in the eye and skin was not observed.

In general, the metabolism of CAB in the nonclinical species reflects that observed in humans, with CAB being the principal component circulating in plasma. The major metabolite of CAB in all species was CAB glucuronide, formed primarily by UGT1A1 (with some involvement from UGT1A9), which was eliminated by renal and biliary routes. Additional studies in human (IM, SC and PO) confirmed that the metabolism and excretion of CAB is independent of route of administration [see m2.7.2, Section 1.2, Table 2]. Metabolic conversion of CAB to its stereoisomers was not detected in rat, dog, monkey or human hepatocytes, or in human plasma following repeat oral administration for 14 days.

Across all species, elimination of drug-related material occurred predominantly via the feces (58.5 to 94.5% of the dose). In rodents, most of the absorbed radioactivity was secreted into the bile and renal excretion was minimal. In monkeys, the absorbed radioactivity was eliminated via both the biliary and renal routes.

In vitro studies have been performed to assess the risk of perpetrator PK drug interactions with CAB. No clinical drug interaction risk was identified for CAB when co-

administered with substrates of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 3A4, UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B15, and 2B17, Pgp, BCRP, BSEP, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE 2-K, MRP2 or MRP4. In vitro studies investigating CAB as a victim of PK drug interactions highlighted metabolism by UGT1A1 and a clinical study has confirmed a drug interaction with the UGT inducer rifampin. Therefore, co-administration of CAB with strong inducers of UGT is contraindicated.

These data, taken together, support the selection of the toxicology species for the evaluation of the safe use of CAB in the proposed patient population when prescribed according to the proposed dosing regimen.

Table 9.1 A Table of the Exposure Ratio of CAB in Toxicology Test Species and Humans Following Oral Administration

Species (Duration)	Dose (mg/kg/day)	Sex	C_{max} (μg/mL)		AUC_{0-24} (μg.h/mL)		Animal to human ratio for AUC[a]
			Day 1	End of Study	Day 1	End of Study	
Mouse[h] (102 weeks)	2.5	M	13.5	12.3	250	230	1.58
	2.5	F	18.8	17.1	306	296	2.03
	10	M	33.3	32.6	583	574	3.93
	5	F	31.8	28.8	538	532	3.64
	75	M	67.5	65.4	1290	1140	7.81
	35	F	70.9	70.2	1370	1060	7.26
Rat[b] (14 days)	30	M	14.4	82.1	294	1849	12.66
		F	14.4	73.8	308	1605	10.99
	100	M	22.9	98.4	490	2223	15.23
		F	24.0	111	472	2435	16.68
	300 (NOAEL)	**M**	**35.8 [33.9 to 37.9]**	**114 [112 to 115]**	**708 [668 to 761]**	**2510 [2422 to 2640]**	**17.19**
		F	**41.0 [39.6 to 43.6]**	**147 [131 to 171]**	**799 [760 to 842]**	**3277 [2952 to 3828]**	**22.45**
Rat[b] (4 weeks)	1	M	4.23	34.3	70.7	739	5.06
		F	4.48	38.8	78.3	852	5.84
	75	M	66.1	143	1309	3000	20.55
		F	72.8	176	1484	3832	26.25
	1000 (NOAEL)	**M**	**161 [150 to 180]**	**150 [149 to 151]**	**3368 [3266 to 2519]**	**3345 [3214 to 3432]**	**22.91**
		F	**181 [161 to 200]**	**193 [172 to 214]**	**3732 [3451 to 3993]**	**4125 [3659 to 4229]**	**28.25**
Rat[b] (26 weeks)	0.5	M	16.2	23.1	329	451	3.09
		F	19.4	31.6	404	675	4.62
	5	M	93.2	91.4	1961	1861	12.75
		F	85.1	102	1672	2083	14.27
	1000 (NOAEL)	**M**	**174 [167 to 179]**	**148 [146 to 150]**	**3753 [3656 to 3896]**	**3203 [3005 to 3313]**	**21.94**
		F	**210 [200 to 220]**	**224 [215 to 233]**	**4403 [4101 to 4627]**	**4781 [4711 to 4907]**	**32.75**
Rat[h] (102 weeks)	0.25	M	9.97	13.1	206	277	1.90
		F	14.2	19.7	295	428	2.93
	2.5	M	59.6	73.1	1260	1470	10.07
		F	76.3	92.0	1620	1980	13.56
	75	M	160	137	3360	2840	19.45
		F	181	230	4010	4810	32.95
Monkey[b] (14 days)	8	M	12.2	14.6	128	144[d]	0.99
		F	11.8	15.5	104	124	0.85
	25 (NOAEL)	**M**	**20.8 [20.4 to 21.5]**	**22.7 [20.1 to 24.6]**	**233 [190 to 287]**	**233 [198 to 257]**	**1.60**
		F	**23.6 [19.2 to 30.9]**	**22.2 [15.0 to 29.9]**	**234[d] [159 to 321]**	**231 [192 to 286]**	**1.58**
	1000	M	67.0	61.9	1051	224[cd]	1.53
		F	59.2	65.9	961[d]	946	6.48

A Table of the Exposure Ratio of CAB in Toxicology Test Species and Humans Following Oral Administration (Continued)

Species (Duration)	Dose (mg/kg/day)	Sex	C_{max} (μg/mL)		AUC_{0-24} (μg.h/mL)		Animal to human ratio for AUC[a]
Monkey[b] (4 weeks)	5	M	12.6	10.0	115	100	0.68
		F	10.4	9.07	85.3	80.9	0.55
	50	M	20.8	17.4	311	276	1.89
		F	23.9	20.0	311	279	1.91
	500 (NOAEL)	**M**	**38.2 [33.1 to 43.3]**	**58.1 [50.3 to 63.3]**	**697 [616 to 773]**	**901 [860 to 945]**	**6.17**
		F	**39.1 [33.3 to 43.1]**	**65.0 [53.7 to 79.1]**	**664 [577 to 732]**	**904 [792 to 1114]**	**6.19**
Monkey (39 weeks)	5	M	7.15	3.37	62.1	37.7	0.26
		F	8.77	6.33	69.6	67.8	0.46
	50	M	22.1	21.1	251	229	1.57
		F	27.3	17.4	303	254	1.74
	500 (NOAEL)	**M**	**45.3 [37.4 to 61.7]**	**36.8 [23.9 to 57.2]**	**644 [505 to 826]**	**542 [359 to 781]**	**3.71**
		F	**56.4 [43.5 to 66.7]**	**32.4 [29.8 to 35.1]**	**807 [557 to 1008]**	**552 [447to 623]**	**3.78**
Rabbit (Embryofetal development)[ef]	30	F	NA	0.95	NA	10.5	0.072
	500	F	NA	3.4	NA	47.4	0.32
	2000 (NOAEL)	**F**	**NA**	**1.0**	**NA**	**96.1**	**0.66**
Human[g]	30 mg	M/F	-	8.1	-	146	NA

Notes:
NOAELs are in **bold text**. Values in brackets represent the range.

a. Calculated for AUC based on end of treatment values.
b. Values are the mean of n=3 to 5.
c. AUC_{0-4}. All males given 1000 mg/kg/day were euthanized in moribund condition on Day 14 following the 4 hour toxicokinetic time point. Therefore, the AUC_{0-24} could not be calculated for males on Day 14.
d. Emesis was observed in one animal in this dose group.
e. Composite TK results from n=3/group.
f. Values in end of study column were from Day 11 postcoitum or dosing Day 5.
g. Mean exposure (C_{max} and AUC_{0-24}) following CAB 30 mg PO once daily (POPPK analysis), report 2018N384611.
h. AUC and C_{max} values given for Week 4 and Week 26.

Table 9.2 Estimated Margins of CAB Relative to Clinical Exposure Following Administration of CAB injectable suspension

Species	Dose (mg/kg)	Sex	Route of administration	Cmax (μg/mL)[a,b]	AUC (μg.h/mL)[a,c]	Animal to human ratio for AUC
Rat (Single dose)	5	M	SC	8.36	4346	1.77
		F	SC	9,04	5367	2.18
	30	M	SC	38.7	19978	8.12
		F	SC	36.9	19218	7.81
	100[d] (NOAEL)	**M**	**SC**	**98.3 [78.4 to 117]**	**47912 [38207 to 56466]**	**19.47**
		F	**SC**	**104 [80.9 to 133]**	**51104 [40209 to 64474]**	**20.77**
	2.5	M	IM	12.6	4321	1.76
		F	IM	14.2	4525	1.84
	10	M	IM	32.4	15926	6.47
		F	IM	40.6	16464	6.69
	75 (NOAEL)	M	IM	**105 [95.3 to 119]**	**60071 [53582 to 64941]**	**24.41**
		F	IM	**124 [107 to 141]**	**64765 [60919 to 70666]**	**26.32**
Rat (3-month)	5	M	SC monthly	19.2	11204	4.55
		F	SC monthly	26.8	15238	6.19
	30	M	SC monthly	84.8	48082	19.54
		F	SC monthly	96.8	55956	22.74
	100 (NOAEL)	**M**	**SC monthly**	**137 [107 to 166]**	**70494 [59895 to 85122]**	**28.64**
		F	**SC monthly**	**195 [172 to 208]**	**116602 [105082 to 124604]**	**47.38**
	2.5	M	IM monthly	16.9	7031	2.86
		F	IM monthly	15.9	5500	2.23
	10	M	IM monthly	49.6	26001	10.57
		F	IM monthly	55.2	24934	10.13
	75 (NOAEL)	**M**	**IM monthly**	**135 [129 to 142]**	**78051 [74734 to 80570]**	**31.72**
		F	**IM monthly**	**181 [179 to 183]**	**107080 [92466 to 115252]**	**43.51**
	100 (NOAEL)	**M**	**SC weekly**	**166 [154 to 184]**	**22291 [21631 to 22907]**	**NA**
		F	**SC weekly**	**226 [221 to 235]**	**34315 [32842 to 35565]**	**NA**
Human[e]	400 mg monthly	M/F	IM	4.2	2461	NA

Notes:
n=3 animals/sex/dose, except where noted. NOAELs are in **bold text**

a. Results are reported as mean and [range].
b. For rat 3-month, C_{max} reported as overall study C_{max}
c. For rat single dose, AUC reported as $AUC_{0\text{-}720}$, AUC through 30 days (morning of Day 31). For rat 3-month, AUC for SC or IM monthly groups reported for the 3rd monthly interval, $AUC_{1440\text{-}2160h}$. For rat 3-month, AUC for SC weekly groups is reported as AUC during Week 13 (from predose on Day 85 to Day 92), $AUC_{2016\text{-}2184}$.
d. n = 2 males for AUC0-t in the 100 mg/kg dose group due to unscheduled death on Day 39.
e. Mean exposure (C_{max} and $AUC_{0\text{-}t}$) month 3 onward following a 400 mg IM monthly dose (POPPK analysis), report 2018N384611.

APPENDIX 1 ANALYTICAL METHODS USED FOR THE DETERMINATION OF CAB IN BIOLOGICAL FLUIDS

Mouse Plasma Assays

Two methods for the determination of CAB in mouse plasma have been validated using HPLC-MS/MS. CAB was extracted from mouse plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard ([$^{13}C^{2}H_3$]-GSK364735 or for the latter method [$^{13}C^{2}H_2{}^{15}N$]-CAB). Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The methods are selective, and summaries of the validation data are tabulated below.

	Report RD2009/00828	**Reports 2014N205858 & 2015N229621**
Calibration Model	Linear weighted $1/x^2$	Linear weighted $1/x^2$
MS/MS Interface	TurboIonSpray	TurboIonSpray
Validated Range	100 to 50000 ng/mL	100 to 100000 ng/mL
Precision (%CV) within-run	≤2.7%	≤6.2%
Precision (%CV) between-run	Not assessed	≤5.5%
Accuracy (% bias)	-7.9%≤%Bias≤3.0%	-7.3%≤%Bias≤9.0%
Stability in Mouse Blood or Plasma	24 hours in plasma at ambient temperature	2 hours in whole blood at room temperature or on wet ice. 24 hours in plasma at ambient temperature 181 days in plasma at -10 to -30°C 181 days in plasma at -60 to -80°C
Freeze-thaw Stability	3 cycles at -30°C to ambient temperature	5 cycles at -10 to -30°C to ambient temperature. 5 cycles at -60 to -80°C to ambient temperature.
Processed Extract Stability	24 hours at ambient temperature	139 hours at 2 to 8ºC

Rat Plasma Assays

Two methods for the determination of CAB in rat plasma have been validated using HPLC-MS/MS. CAB was extracted from rat plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard ([$^{13}C^2H_3$]-GSK364735 or for the latter method, [$^{13}C^2H_2{}^{15}N$]-CAB). Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The methods are selective, and summaries of the validation data are tabulated below.

	Report RD2006/01725[1] & 2014N208172[1]	**Report 2014N205573[2] & 2015N237610**
Calibration Model	Linear weighted $1/x^2$	Linear weighted $1/x^2$
MS/MS Interface	TurboIonSpray	TurboIonSpray
Validated Range	100 to 100000 ng/mL	100 to 100000 ng/mL
Precision (%CV) within-run	≤7.4%	≤6.5%
Precision (%CV) between-run	≤6.0%	≤7.8
Accuracy (% bias)	-11.1%≤%Bias≤4.0%	4.3%≤%Bias≤10.0%
Stability in Rat Blood or Plasma	24 hours in plasma at ambient temperature 6 months in plasma at -30ºC	2 hours in whole blood at ambient temperature or on wet ice. 24 hours in plasma at ambient temperature. 182 days in plasma at -10ºC to -30ºC and -60ºC to -80ºC
Freeze-thaw Stability	3 cycles at -30°C to ambient temperature	5 cycles from -10 to -30°C to ambient temperature. 5 cycles from -60 to -80°C to ambient temperature.
Processed Extract Stability	24 hours at ambient temperature	96 hours at 2 to 8ºC

Notes:
1. [$^{13}C^2H_3$]-GSK364735 was used as the isotopically labeled internal
2. [$^{13}C^2H_2{}^{15}N$]-CAB was used as the isotopically labeled internal standard

Rabbit Plasma Assay

A method for the determination of CAB in rabbit plasma has been validated using HPLC-MS/MS. CAB was extracted from rabbit plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard ([$^{13}C^{2}H_3$]-GSK364735). Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The method is selective, and a summary of the validation data are tabulated below.

	Report RD2009/00190
Calibration Model	Linear weighted $1/x^2$
MS/MS Interface	TurboIonSpray
Validated Range	100 to 50000 ng/mL
Precision (%CV) within-run	≤12.0%
Precision (%CV) between-run	Not assessed
Accuracy (% bias)	-6.4%≤%Bias≤0.0%
Stability in Rabbit Plasma	24 hours at ambient temperature
Freeze-thaw Stability	3 cycles from -30°C to ambient temperature
Processed Extract Stability	24 hours at ambient temperature

Monkey Plasma Assay

A method for the determination of CAB in monkey plasma has been validated using HPLC-MS/MS. CAB was extracted from monkey plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard ([$^{13}C^{2}H_3$]-GSK364735). Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The method is selective, and a summary of the validation data are tabulated below.

	Report RD2007/01020
Calibration Model	Linear weighted $1/x^2$
MS/MS Interface	TurboIonSpray
Validated Range	100 to 50000 ng/mL
Precision (%CV) within-run	≤8.9%
Precision (%CV) between-run	≤7.4%
Accuracy (% bias)	-6.3%≤%Bias≤9.0%
Stability in Monkey Plasma	24 hours at ambient temperature
Freeze-thaw Stability	3 cycles from -30°C to ambient temperature
Processed Extract Stability	24 hours at ambient temperature

APPENDIX 2 ADDITIONAL INFORMATION

The following reports have been reviewed within GlaxoSmithKline (GSK) and the information is considered to have no bearing on safety. These reports are not included in the study listing tables for the following reasons:

Report No. (Study No.)	Title	Reason for Exclusion from Study Listing Tables
2015N239662	Cabotegravir (CAB): Single-Dose Intramuscular Pharmacokinetic Study in Male Rats: Comparison of FA-CAB and CAB-LAP	The formulation used is not relevant to the current marketing application.
2020N432961	Evaluation of the Systemic Exposure of Potentially Long Acting Parenteral Formulations of GSK1265744 (Cabotegravir) Following Either Intramuscular or Subcutaneous Administration at a Target Dosage of 10 mg/kg in the Male Sprague-Dawley Rat	The formulation used is not relevant to the current marketing application.
2017N324036	Pilot Pharmacokinetic Study Reporting and Analysis for GSK1265744: A pharmacokinetic study to explore the viability of stable isotope approach when administering through intramuscular injection	Study concluded that administration of CAB via an IM injection in two distinct locations did not result in high correlation between the non-enriched and enriched compound, implying that SIL approach may not be viable approach for future human bioequivalence studies.
2017N324878	Pilot Pharmacokinetic Study of GSK1265744 following Low-Volume Intramuscular Administration to Male Sprague-Dawley Rats	In-life report for study 2017N324036
2014N202915 (14DMR016)	Evaluation of the stability of the GSK1265744 glucuronide metabolite (GSK3388352) in various buffers by LC-MS/UV	Supportive documentation for study experiments.
2014N202915 (14DMR016)	Evaluation of the stability of the GSK1265744 glucuronide metabolite (GSK3388352) in various buffers by LC-MS/UV.	Supportive documentation for study experiments.

Report No. (Study No.)	Title	Reason for Exclusion from Study Listing Tables
2016N270354	Non-GLP Determination of GSK1265744 in Monkey Plasma Samples by LC/MS/MS in Support of Rhesus Macaque IV Challenge II	Sample analysis report in support of an external study.
2016N270355	Non-GLP Determination of GSK1265744 in Monkey Plasma Samples by LC/MS/MS in Support of Rhesus Macaque Resistance Study	Sample analysis report in support of an external study.
2019N409906	Determination of GSK1265744 in Monkey Plasma, Rectal and Vaginal Secretion Samples by LC/MS/MS.	Sample Analysis Report only in support of an external study

Studies listed in Appendix 2 comprise those conducted during the formal research and development of the compound. Early screening studies completed on multiple compounds during the candidate selection phase have not been included.

MODULE 2.6.5. PHARMACOKINETICS TABULATED SUMMARY

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1. PHARMACOKINETICS: OVERVIEW FOR CAB (ALSO CABOTEGRAVIR OR GSK1265744)

Table 1.1 Listing of Absorption Studies with CAB

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Mouse	15M	Oral (gavage)	B	10, 100, 1000, 2000	Single	No	GSK	RD2009/00691 (M42451)	m4.2.3.1
Pharmacokinetics	Rat (Sprague Dawley)	3M 4M 6M 6M	IV (bolus) Oral	A A A B	1[a] 5[a] 5.1[b] 4.9[b]	Single	No	GSK	RH2007/00168 (07APK043)	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	3M	Oral (gavage)	A	5, 50, 150, 500[c]	Single	No	GSK	RH2007/00168 (07APK043)	m4.2.2.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	B	3, 30, 300, 1000	Single	No	GSK	RD2008/00200 (R42257)	m4.2.2.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	B	1000	Single	No	GSK	RD2008/01308 (R42353)	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	3M	SC IM	A	5	Single	No	GSK	RH2009/00012 (N11105-5)	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	5M	SC IM	A	5	Single	No	GSK	RH2009/00013 (N11105-12)	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	3 to 6M	IM	A	10[k]	Single	No	JAN	2011N127517 (FK7565)	m4.2.2.2

List of Absorption Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rat (Sprague Dawley)	6M	SC IM	A	10	Single	No	GSK	RD2009/00865 (R42469)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M	SC IM	A	10, 30, 50 5, 20, 35	Single	No	GSK	RD2009/00906 (R42473)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M	SC IM	A	10, 30, 50 5, 20, 35	Single	No	GSK	RD2009/01216 (R42506)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M/ 3F	SC IM	A	5, 30, 100 2.5, 10, 75	Single	Yes	GSK	RD2009/01359 (R42516)	m4.2.3.1
Pharmacokinetics	Dog (beagle)	4M 1[d] or 2M[e] 3M[e] 3M[e] 3M[e]	IV (bolus) Oral (gavage) Oral (capsule)	A or B B A A B	1[a] 5[a] ~5.2[b,f] ~5.1[b,g] ~5.0[b]	Single	No	GSK	RH2007/00169 (07APK044)	m4.2.2.2
Pharmacokinetics	Dog (beagle)	2[d] or 3M[d,e] 2M[e]	Oral (gavage)	A B	5, 30[c] 1, 5, 30[c]	Single	No	GSK	RH2007/00170 (07APK045)	m4.2.2.2

List of Absorption Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Pharmacokinetics	Monkey (cynomolgus)	2M 2M 2M 2M 2M	IV (bolus) Oral (gavage) Oral (capsule) Oral (gavage) Oral (gavage)	B B A A B	1[a] 5[a] ~5.1[b] 5, 30[c] 30[c]	Single	No	[redacted]	In-life: RH2007/00202 (06RCD7689) RH2007/00201 (06RCD7572) RH2007/00203 (06RCD8045) RH2007/00205 (06RCD8533) Data analysis: RH2007/00171 (07APK046)	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	2M	Oral (gavage)	B	150, 300, 1000[c]	Single	No	[redacted], [redacted]	RD2007/01415 (E-265744-TF-006-R)	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus)	4M	Oral (gavage)	B	500	Single	No	GSK	CD2008/01223 (D08226)	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus) Monkey (cynomolgus)	4M 4M	SC IM	A	1, 5 1, 5	Single	No	GSK	CD2009/00373 (D09052)	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus) Monkey (cynomolgus)	4M 4M	SC IM	A	5 5	Single	No	GSK	CD2009/00513 (D09084)	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus) Monkey (cynomolgus)	4M 4M	SC IM	A	10 10	Single	No	GSK	CD2009/00656 (D09112)	m4.2.3.1

List of Absorption Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Monkey (cynomolgus)	2M	IM	A	10[k]	Single	No	[redacted]	2010N105579 ([redacted])	m4.2.3.1
Toxicokinetics	Mouse (CD-1)	36M/ 36F	Oral (gavage)	B	10, 75, 1000	14 days	Yes	GSK	RD2009/00692 (M42452)	m4.2.3.2
Toxicokinetics	Mouse (CD-1)	54M/ 54F	Oral (gavage)	B	10, 75, 1000	13 weeks	Yes	GSK	2012N142081 (M42936)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	A	30, 100, 300[c]	14 days	Yes	GSK	RD2006/01741 (R41937)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	B	1, 75, 1000	4 weeks	Yes	GSK	RD2008/00448 (R42288)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	12M/12F	SC (monthly) IM (monthly) SC (weekly)	A	5, 30, 100 2.5, 10, 75 100	13 weeks	Yes	[redacted]	2010N104820 (R42698)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	3M/3F	Oral (gavage)	B	0.5, 5, 1000	26 weeks	Yes	GSK	RD2009/00031 (R42404)	m4.2.3.2
Toxicokinetics	Rabbit (Dutch Belted)	4F	Oral (gavage)	B	30, 250, 500, 1000, 2000 [i,j]	13 days (Day 5)	No	GSK	CD2008/01276 D08251	m4.2.3.5.2
Toxicokinetics	Rabbit (Dutch Belted)	9F	Oral (gavage)	B	30, 500, 2000	13 days (Day 5)	Yes	GSK	CD2009/00842 (G08307)	m4.2.3.5.2

List of Absorption Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Monkey (cynomolgus)	1M/1F	Oral (gavage)	B	50, 150, 1000[c]	7 days	No	GSK	CD2007/00577 (D07170)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	3M/3F	Oral (gavage)	B	8, 25, 1000[c]	14 days	Yes	GSK	CD2007/00680 (G07171)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	3 to 5 M/F	Oral (gavage)	B	5, 50, 500	4 weeks	Yes	GSK	CD2008/00632 (G08079)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	4M/4F	Oral (gavage)	B	5, 50, 500	39 weeks	Yes	GSK	RD2009/00027 (P42405)	m4.2.3.2
Metal chelation	In vitro	NA	In vitro	A	500 nM	NA	No	GSK	2019N401615	m4.2.2.2

Key:
A = Parent. B = Sodium salt. LA = Long-acting formulation derived from parent.
a = GSK1265744 in solution.
b = GSK1265744 as a capsule.
c = GSK1265744 in suspension.
d = Non-fasted dogs.
e = Fasted dogs.
f = GSK1265744 as crystalline Form 1 was dosed.
g = GSK1265744 as crystalline Form 2 was dosed.
h = The 2000 mg/kg/day dose was given as a single daily dose and a BID (twice daily) dose of 1000 mg/kg/dose,6 hours apart.
i = Toxicokinetic measurements were also taken on Day 13 of dosing at the 2000 mg/kg/day doses (single and BID).
j = A wet bead milled suspension (particle size = 5.89 μm) and a homogenized suspension (particle size = 68.9 μm) were used for each route.
k = GSK1265744 was administered with and without Rilpivirine.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]
JAN = Janssen Research & Development.
[redacted] = [redacted]

Table 1.2 Listing of Distribution Studies with CAB

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Plasma protein binding	Rat, Dog, Monkey, Human	NA	In vitro	B	5 μM	NA	No	GSK	RH2007/00193 (06RCD7725)	m4.2.2.3
Plasma protein binding	Human	NA	In vitro	A	500 to 20000 ng/mL	NA	No	[redacted]	2015N235936 (8309868)	m4.2.2.3
Plasma protein binding with GSK3388352[a]	Rat, Human	NA	In vitro	N/A	200 to 20000 ng/mL	NA	No	GSK	2017N333514 (17DMW004)	m4.2.2.3
Serum protein binding	Rat, Dog, Monkey, Human	NA	In vitro	A	10 μM	NA	No	GSK	RH2007/00222	m4.2.2.3
BCRP inhibition	Human	NA	In vitro	B	0.03 to 30 μM	NA	No	GSK	2012N150360 (12DMR027)	m4.2.2.3
BCRP transport	Human	NA	In vitro	E	3 μM	NA	No	GSK	2012N155942 (12DMR032)	m4.2.2.3
Pgp inhibition	Human	NA	In vitro	B	0.03 to 30 μM	NA	No	GSK	2012N146041 (12DMR022)	m4.2.2.3
Pgp transport and membrane permeability	Human	NA	In vitro	E	3 μM	NA	No	GSK	2012N146040 (12DMR021)	m4.2.2.3
Folate receptor & transporters	Human	NA	In vitro	B	0.03 to 100 μM	NA	No	[redacted]	2019N396076	m4.2.2.3

List of Distribution Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
OCT1, OCT2 inhibition	Human	NA	In vitro	B	0.01 to 30 μM	NA	No	[redacted]	2012N146057 (XS-0376)	m4.2.2.3
OATP1B1, OATP1B3 inhibition	Human	NA	In vitro	B	0.1 to 30 μM	NA	No	GSK	2013N164529 (12DMM039)	m4.2.2.3
MRP2, BSEP inhibition	Human	NA	In vitro	B	0.03 to 30 μM	NA	No	GSK	2013N174589 (13DMR019)	m4.2.2.3
Hepatic uptake	Human	NA	In vitro	B	0.7, 9 μM	NA	No	GSK	2018N391028 (18DMW024)	m4.2.2.3
OAT1, OAT3, MATE1, MATE2-K, MRP4 inhibition	Mouse (S_2), Human (HEK293), vesicles	NA	In vitro	B	0.03 to 30 μM	NA	No	[redacted]	2013N174474 (XS-0446)	m4.2.2.3
Blood to plasma partitioning	Rat (Sprague Dawley)	1M 1M (BDC)	Oral	F	30	Single	No	GSK	RD2008/00021 (08DMR002)	m4.2.2.3
Blood to liver partitioning	Rat (Sprague Dawley)	1M 1M (BDC)	Oral	F	30	Single	No	GSK	RD2008/00021 (08DMR002)	m4.2.2.3
Membrane permeability and transport by Pgp	Human	NA	In vitro	A	3 μM	NA	No	GSK	RH2007/00199 (07APK053)	m4.2.2.3

List of Distribution Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Uptake by macrophages	Human (differentiated macrophages)	NA	In vitro	A	0.2, 10 μg/mL	24 or 48 hours	No	GSK	2015N237779 (V43296N)	m4.2.2.3
Assessment of injection site depots	Rat (Sprague Dawley)	5M	IM or SC	A	40	Single (Day 14)	No	GSK	2014N221244 (14DMR029)	m4.2.2.3
IM and SC distribution	Rat (Sprague Dawley)	5M 2 or 3M	IM or SC IM	A	40 40	Single (Days 1, 2, 3, 4, 7 and 14)	No	GSK	2015N231295	m4.2.2.3
Lymphatic exposure	Rat (Sprague Dawley)	2M (LDC) 10M (intact)	IM	A	40	Single (Days 1, 2, 3, 4, 7)	No	GSK	2014N222547 (14DMR031)	m4.2.2.3
Whole body autoradiography	Rat (Lister Hooded)	7M	Oral (gavage)	F	30	Single	Yes	[redacted]	RD2008/00840 (2990/254)	m4.2.2.3

Key:
a = GSK3388352 is the ether glucuronide metabolite of CAB.
A = Parent. B = Sodium salt.
E = [^{14}C]-GSK1265744A (parent). F = [^{14}C]-GSK1265744B (sodium salt).
IM = Intramuscular. SC = Subcutaneous.
MATE = Multidrug and Toxin Extrusion Transporters.
MRP = Multidrug Resistance Associated Protein.
HEK293 = Human Embryonic Kidney cell line.
S_2 = Mouse cell line derived from interstitial renal proximal tubules.
BCRP = Breast Cancer Resistant Protein.
BDC = Bile Duct Cannulated.
BSEP = Bile Salt Export Pump.
LDC = Lymph Duct Cannulated.
NA = Not applicable.
OAT = Organic Anion Transporter.
OCT = Organic Cation Transporter.
Pgp = P-glycoprotein.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]

Table 1.3 Listing of Metabolism Studies with CAB

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Metabolic stability in liver S9	Rat, Dog, Monkey, Human	NA	In vitro	A	1 μM	NA	No	GSK	RH2007/00183 (07APK038)	m4.2.2.4
Metabolic stability in hepatocytes	Rat, Human	NA	In vitro	A	0.5 μM	NA	No	GSK	RH2007/00183 (07APK038)	m4.2.2.4
Metabolism in hepatocytes	Rat, Dog, Monkey, Human	NA	In vitro	A	10 μM	NA	No	GSK	RH2007/00188 (07APK040)	m4.2.2.4
Metabolism in hepatocytes	Rat, Monkey, Human	NA	In vitro	E	50 μM	NA	No	GSK	RD2008/00073 (08DMR007)	m4.2.2.4
Metabolic activation in microsomes	Rat, Monkey, Human	NA	In vitro	E	10 μM	NA	No	GSK	RD2007/01629 (07DMR126)	m4.2.2.4
Potential metabolic formation of stereoisomers	Rat, Dog, Monkey, Human	NA	In vitro	A	10 μM	NA	No	GSK	RH2007/00207 (07APK056)	m4.2.2.4
Formation of glucuronide metabolite	Human	NA	In vitro	A	0.5, 5, 50 μM	NA	No	GSK	RH2007/00166 (07APK033)	m4.2.2.4

List of Metabolism Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Hepatic uptake clearance of GSK3388352[d]	Human (cryopreserved hepatocytes)	NA	In vitro	NA	50 μM	NA	No	GSK	2017N320631	m4.2.2.4
Hepatic canalicular and basolateral excretory clearances	Rat (Sprague Dawley)	4M	Ex vivo	B	10 μM	NA	No	GSK	2017N320633	m4.2.2.4
Formation of glutathione adducts in microsomes	Rat, Dog, Monkey, Human	NA	In vitro	A	100 μM	NA	No	GSK	RH2007/00164 (07APK031)	m4.2.2.4
PXR activation assays	Rat Human	NA	In vitro	A	0.2 nM to 10 μM	NA	No	GSK	RR2007/00043 RR2007/00046	m4.2.2.4
CYP genes mRNA expression	Human (cultured hepatocytes)	NA	In vitro	B	0.03 to 30 μM	NA	No	GSK	2013N166279 (12DMM040)	m4.2.2.4
CYP inhibition in recombinant enzymes and liver microsomes	Human	NA	In vitro	B	0.1 to 100 μM	NA	No	[redacted]	2012N151766 ([redacted])	m4.2.2.4

List of Metabolism Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
CYP inhibition in recombinant enzymes and liver microsomes	Human	NA	In vitro	A	0.033 to 33 μM	NA	No	GSK	RH2007/00181 (07APK036)	m4.2.2.4
Potential in vivo epimerization in plasma	Human	8M[a]	Oral	B	25 mg	14 days	No	GSK	RD2008/01340 (08DMR068)	m4.2.2.4
In vivo metabolism	Mouse (CD-1)	4M 3M (BDC)	Oral	F	30	Single	No	GSK	2012N146480 (12DMR024)	m4.2.2.4
In vivo metabolism	Rat (Sprague Dawley)	3M 3M (BDC)	Oral	F	30	Single	No	GSK	2012N146427 (12DMR023)	m4.2.2.4
In vivo metabolism	Rat (Sprague Dawley)	1M 1M (BDC)	Oral	F	30	Single	No	GSK	RD2008/00021 (08DMR002)	m4.2.2.4
Potential induction of CYP mRNA	Rat (Sprague Dawley)	3M/3F	In vivo	A	30 100, 300[b]	NA	No	GSK	RD2006/01999 (06DMR115)[c]	m4.2.2.4
Glucuronidation in recombinant enzymes and liver microsomes	Human	NA	In vitro	E	2 to 100 μM	NA	No	GSK	2012N145430 (12DMR018)	m4.2.2.4

List of Metabolism Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
UGT inhibition	Human	NA	In vitro	B	0.1 to 100 μM	NA	No	[redacted]	2013N159049	m4.2.2.4
Evaluation of GSK3388352 as a transporter inhibitor[d]	Human	NA	In vitro	NA	0.01 to 300 μM	NA	No	[redacted]	2015N253914 (XS-0681)	m4.2.2.4
Evaluation of GSK3388352 as a Pgp and BCRP substrate[d]	Human	NA	In vitro	NA	10 μM	NA	No	[redacted]	2016N288197 (XS-0848)	m4.2.2.4
Evaluation of GSK3388352 as a substrate for hepatic and renal transporters[d]	Human	NA	In vitro	NA	10 or 50 μM	NA	No	GSK	2016N298553 (15DMM040)	m4.2.2.4
Profiling, quantification and structural characterizations	Monkey (cynomolgus)/ Urine, bile, feces and plasma	3M 3M (BDC)	In vivo	F	10	Single	No	GSK	2013N171159 (13DMR016)	m4.2.2.4

List of Metabolism Studies Performed with CAB (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Glucuronidation assessments	Human/ Liver, kidney and intestinal microsomes	NA	In vitro	E	5 μM	NA	No	GSK	2014N222268 (14DMR030)	m4.2.2.4

Key:
a = Plasma samples were collected from the Clinical Study ID ITZ111451 following a repeat dose of 25 mg in healthy male subjects.
b = GSK1265744 in suspension.
c = Liver samples for this study were obtained from the 14 day rat toxicity study [Report RD2006/01741].
d = GSK3388352 is the glucuronide metabolite.
A = Parent. B = Sodium salt. E = [^{14}C]GSK1265744A (parent). F = [^{14}C]GSK1265744B (sodium salt).
BDC = Bile duct cannulated. CYP = Cytochrome P450. NA = Not applicable. PXR = Pregnane X receptor.
UGT = Uridine 5'-diphosphoglucuronosyl transferase.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]
[redacted] = [redacted]

Table 1.4 Listing of Excretion Studies with CAB

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Elimination	Mouse (CD-1)	4M 4M (BDC)	Oral	F	30	Single	No	GSK	2012N145873 (12DMR019)	m4.2.2.5
Preliminary elimination	Rat (Sprague Dawley)	1M 1M (BDC)	Oral	F	30	Single	No	GSK	RD2008/00021 (08DMR002)	m4.2.2.5
Elimination	Rat (Sprague Dawley)	3M 3M (BDC)	Oral	F	30	Single (1, 4, 8, 24, 48 hours)	No	GSK	2012N143605 (12DMR017)	m4.2.2.5
Elimination	Monkey (cynomolgus)	3M 3M (BDC)	Oral	F	10	Single (1, 4, 8, 24, 96, 168 hours)	No	[redacted]	2013N174861 (10875)	m4.2.2.5

Key:
F = [^{14}C]GSK1265744B (sodium salt).
BDC = Bile duct cannulated.

Testing Facility:
GSK = GlaxoSmithKline.
[redacted] = [redacted]

2. ANALYTICAL METHODS AND VALIDATION REPORTS

Table 2.1 Pharmacokinetics: Analytical Methods and Validation Reports for CAB

Type of Study	Species	Quantification Limits	Report No. (Method Reference)	Location in CTD
The abbreviated validation of a method for the determination of GSK1265744 in mouse plasma using HPLC-MS/MS.	Mouse	100 to 5000 ng/mL	RD2009/00828 (GSK1265744MOPVALB)	m4.2.2.1
Validation of a method for the determination of GSK1265744 in mouse plasma by HPLC with MS/MS detection.	Mouse	100 to 100000 ng/mL	2014N205858 (126MPP)	m4.2.2.1
Validation of a method for the determination of GSK1265744 in mouse plasma by HPLC with MS/MS detection (Addendum No. 1).	Mouse	100 to 100000 ng/mL	2015N229621 (126MPP)	m4.2.2.1
Validation of a method for the determination of GSK1265744 in rat plasma using HPLC-MS/MS.	Rat	100 to 100000 ng/mL	RD2006/01725 (GSK1265744RTPLVALA)	m4.2.2.1
Supplemental validation data to "The validation of a method for the determination of GSK1265744 in rat plasma (range 100 to 100000 ng/mL) using HPLC-MS/MS" (Method Reference Number: GSK1265744RTPVALA).	Rat	100 to 100000 ng/mL	2014N208172 (GSK1265744RTPVALA)	m4.2.2.1
Validation of a method for the determination of GSK1265744 in rat plasma by HPLC with MS/MS detection.	Rat	100 to 100000 ng/mL	2014N205573 (126RPP)	m4.2.2.1
Validation of a method for the determination of GSK1265744 in rat plasma by HPLC with MS/MS detection - Addendum No. 1.	Rat	100 to 100000 ng/mL	2015N237610 (126RPP)	m4.2.2.1
The abbreviated validation of a method for the determination of GSK1265744 in rabbit plasma using HPLC-MS/MS.	Rabbit	100 to 50000 ng/mL	RD2009/00190 (GSK1265744RBPLVALB)	m4.2.2.1
An abbreviated validation of a method for the determination of GSK1265744 in monkey plasma using HPLC-MS/MS.	Monkey	100 to 50000 ng/mL	RD2007/01020 (GSK1265744CYPLVALB)	m4.2.2.1

3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

Table 3.1 Plasma Pharmacokinetic Parameters for CAB Following Intravenous Administration

Test Article: CAB **Location in CTD:** m4.2.2.2

Species (Strain):	**Rat (CD)**	**Dog (beagle)**	**Monkey (cynomolgus)**
Report No.	RH2007/00168	RH2007/00169	RH2007/00171
Gender (M/F)/Number of Animals:	M/3	M/4	M/2
Feeding Condition:	Non-fasted	Fasted	Fasted
Vehicle/Formulation:[a]	[a]	[a]	[a]
Method of Administration:	Intravenous	Intravenous	Intravenous
Dose (mg/kg):	1	1	1
Sample:	Plasma	Plasma	Plasma
Analyte:	GSK1265744	GSK1265744	GSK1265744
Assay:	HPLC/MS/MS	HPLC/MS/MS	HPLC/MS/MS
PK Parameters:			
V_{ss} (L/kg)	[b]	0.14	0.09
CLp (mL/min/kg)	[b]	0.34	0.32
$t_{½}$ (h)	[b]	5.7	4.0
$AUC_{0-\infty}$ (ng.h/mL)	181000 [c]	49145	54802

1. **Additional Information:**
   a = CAB was dissolved in DMSO:Solutol:0.05 M N-methylglucamine in water (10:10:80, v:v:v).
   b = The sampling scheme was insufficient to characterize the PK parameters in rats.
   c = AUC_{0-24} (ng.h/mL).
   CLp = Plasma clearance.
   $t_{½}$ = Half-life.
   V_{ss} = Steady state volume of distribution.

Table 3.2 Estimates of the Bioavailability in Male Dogs and Monkeys After CAB is Administered as a Single Oral Dose in a Solution, Suspension or Capsule Formulation

Test Article: CAB

Location in CTD: m4.2.2.2

Analyte: GSK1265744

Assay: HPLC-MS/MS

Sample: Plasma

Vehicle/Formulation:[a]

Dog (Male) Report RH2007/00169			Monkey (Male, n=2) Report RH2007/00171		
Dose (mg/kg)	**Formulation (Fasting State)**	**%F**	**Dose (mg/kg)**	**Formulation (Fasting State)**	**%F**
5	Solution (fasted; n=2)	63	5	Solution (fasted)	56
5	Solution (non-fasted; n=1)	44	5	Suspension (fasted)	21
5.2 [b]	Capsule (fasted; n=3)	8	5.1	Capsule (fasted)	6
5.1 [c]	Capsule (fasted; n=3)	3			
5 [d]	Capsule (fasted; n=3)	6			

Additional Information:

a = Solutions of CAB were formulated in DMSO:Solutol:0.05 M N-methylglucamine in water (10:10:80, v:v:v). Suspensions of CAB were formulated in 0.5% HPMC and 0.1% Tween 80. CAB for capsules was prepared by milling using a mortar and pestle and combining with sodium starch glycolate (SSG) and Avicel PH 102 (2:1:3 drug:SSG:Avicel).

b = Form 1 crystalline free acid

c = Form 2 crystalline free acid

d = Sodium salt (5 mg free acid/kg)

%F = Oral bioavailability

Table 3.3 Exposure of Male Rats, Dogs, and Monkeys to Single Oral Escalating Doses of CAB

Test Article: CAB

Location in CTD: m4.2.2.2

Analyte: GSK1265744

Vehicle/Formulation: 0.5% HPMC with 0.1% Tween 80

Sample: Plasma

Sample Collection Intervals: Rat - 15 and 30 min, 1, 2, 4, 8, 12, 24, 36, 48, 60 and 72 hours
Dog - 15 and 30 min, 1, 2, 4, 8, 12, 24 hours (additional samples at 36 and 48 hours for dogs given free acid at 30 mg/kg)
Monkey - 5, 15 and 30 min, 1, 2, 4, 6, 8, 12 and 24 hours

Assay: HPLC-MS/MS

Species:	**Rat (Male, n = 3)**				**Dog (Male, n = 2 or 3)**						**Monkey (Male, n = 2)**		
Report No.:	RH2007/00168				RH2007/00170						RH2007/00171		
Test Article Form:	**Free Acid**				**Free Acid**			**Sodium Salt**			**Free Acid**		**Sodium Salt**
Dose Level (mg/kg):	**5**	**50**	**150**	**500**	**5**	**30**	**30 ***	**1 ***	**5 ***	**30 ***	**5 ***	**30 ***	**30 ***
PK Parameters													
AUC_{0-24} (μg.h/mL)	234	703	982	1156	21.846	40.204	19.888	21.266	51.163	125.359	52.433	146.176	485.199
C_{max} (μg/mL)	12.8	37.9	49.2	52.8	2.235	3.627	2.000	2.925	6.285	11.050	5.360	10.650	45.400
T_{max} (h)	4	8	12	4	[2 - 2]	2	2	[1 - 1]	[1 - 1]	[1 - 2]	[2 - 2]	[2 - 2]	[2 - 4]

Additional Information:
* indicates fasted feeding condition, other dose groups are non-fasted.
Data presented are mean values (T_{max} reported as median or [range]).

Table 3.4 Exposure of Male Mice to Single Oral Escalating Doses of CAB

Test Article: CAB sodium salt

Location in CTD: m4.2.2.2

Analyte: GSK1265744 **Report No.:** RD2009/00691

Vehicle/Formulation: 0.5% HPMC with 0.1% Tween 80

Sample: Plasma

Sample Collection Intervals: 1, 2, 4, 8, and 24 hours

Assay: HPLC-MS/MS

	Composite Plasma Toxicokinetic Parameters (n=3 male mice/timepoint)			
Dose Level (mg/kg):	**10**	**100**	**1000**	**2000**
PK Parameters				
AUC_{0-24} (μg.h/mL)	383	1214	1834	1796
C_{max} (μg/mL)	29.4	72.4	116	130
T_{max} (h)	2.0	2.0	1.0	1.0

Table 3.5 Exposure of Rats to CAB Following Single Oral Escalating Doses

Test Article (Form): CAB (sodium salt) **Report No.:** RD2008/00200

Species (Strain): Rat (Sprague Dawley) **Location in CTD:** m4.2.2.2

Gender (M/F): M & F

Number of animals: n = 3/sex/group

Method of Administration: Oral gavage

Analyte: GSK1265744

Vehicle/Formulation: 0.5% HPMC with 0.1% Tween 80

Sample: Plasma

Sample Collection Intervals: 1, 2, 4, 8, 10 and 24 hours post-dose

Assay: HPLC-MS/MS

Dose Level (mg/kg):	**3**		**30**		**300**		**1000**	
Sex:	M	F	M	F	M	F	M	F
PK Parameters								
AUC_{0-24} (μg.h/mL)	168	271	853	912	2119	2176	3063	3487
Cmax (μg/mL)	9.27	17.1	45.2	48.4	106	117	146	176
Tmax (h)	2	2	2	4	2	4	2	2

Additional Information:
Data presented are mean values (T_{max} reported as median or [range]).

Table 3.6 Plasma Pharmacokinetic Parameters for CAB Following Single Intramuscular or Subcutaneous Administration to Rats

Test Article: CAB
Species (Strain): Rat (Sprague Dawley) **Location in CTD:** m4.2.2.2

Report No.:	RH2009/00012		RH2009/00013			
Gender (M/F)/Number of Animals:	M/3		M/5			
Feeding Condition:	Non-fasted		Non-fasted			
Vehicle/Formulation:	[a]		[d]			
Analyte:	GSK1265744		GSK1265744			
Assay:	HPLC-MS/MS		HPLC-MS/MS			
Sample:	Plasma		Plasma			
Sample Collection Intervals (Day):	1 [b], 3, 6, 7, 9, 13, 14, 16, 20, 22, 24, 27, 29, 36 and 106		1 [b], 2, 3, 7, 8, 10, 23, 30, 39, 45, 53, 60 and 67			
Method of Administration:	Subcutaneous	Intramuscular	Subcutaneous		Intramuscular	
Dose (mg/kg):	5	5	5	5	5	5
Test Material Particle Size (microns):	NA	NA	5.59	68.9	5.59	68.9
PK Parameters:						
AUC_{0-t} (μg.h/mL)	2606 [c]	3115 [c]	4320 [e]	3322 [e]	5042 [e]	3811 [e]
AUC_{0-24} (μg.h/mL)	63.3	60.3	61	42	87	51
C_{max} (μg/mL)	6.5	10.3	7.1	5	12.2	7.5
Median T_{max}	6 Days	7 Days	8 Days	8 Days	7 Days	7 Days

Additional Information:
NA = Not applicable.
a = Wet bead milled suspension in 1.7% polyvinylpyrrolidone (PVP), 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben, 0.004M NaH_2PO_4, 0.006M Na_2HPO_4 and 0.84% NaCl, pH-adjusted to 6.7.
b = Samples collected at multiple times on Day 1.
c = AUC through the last sampling time on Day 36.
d = 1.7% PVP, 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben, 0.004M NaH_2PHO_4, 0.006M Na_2HPO_4 and 0.85% NaCl, target pH 6.7.
e = AUC through the last sampling time on Day 67.

Table 3.7 Plasma Toxicokinetic Parameters for CAB Following Single Intramuscular or Subcutaneous Administration to Rats

Test Article: CAB (free acid)

Species (Strain): Rat (Sprague Dawley) **Location in CTD:** m4.2.3.1

Report No.:	RD2009/00865				RD2009/00906					
Gender (M/F):	M				M					
Number of Animals:	6				3					
Feeding Condition:	Non-fasted				Non-fasted					
Analyte:	GSK1265744				GSK1265744					
Assay:	HPLC-MS/MS				HPLC-MS/MS					
Sample:	Whole blood				Plasma					
Sample Collection:	Up to Day 64				Up to Day 56					
Route of Administration:	**SC**		**IM**			**SC**			**IM**	
Dose (mg/kg):	**10**		**10**		**10**	**30**	**50**	**5**	**20**	**35**
Vehicle/Formulation:	**PVP**	**P F**	**PVP**	**P F**		**P F**			**P F**	
PK Parameters (mean):										
AUC_{0-t} (μg.h/mL)	8969.1	9367.5	9984.0	8824.9	15821	38288	60945	9656	26211	43479
AUC_{0-24} (μg.h/mL)	49.87	297.68	171.97	481.85	632	667	704	785	1364	1447
C_{max} (μg/mL)	10.86	22.46	21.80	28.59	37.6	68.6	96.9	37.9	82.7	103
Median T_{max} (h)	228	72	120	48	72	168	168	12	72	168

Additional Information:

PVP = 1.7% (w/v) polyvinylpyrrolidone PVP/0.2% (w/v) polysorbate 80/0.18% (w/v) methylparaben/ 0.02% (w/v) propylparaben/0.004 M NaH_2PO_4 H_20/0.006 M Na_2HPO_4 with NaCl

P F = 2% Pluronic F127/0.2% (w/v) polysorbate 80/0.18% (w/v) methylparaben/0.02% (w/v) propylparaben/0.004 M NaH_2PO_4 H_20/0.006 M Na_2HPO_4 with NaCl

SC = Subcutaneous

IM = Intramuscular

Table 3.8 Plasma Toxicokinetic Parameters for CAB Following Single Intramuscular or Subcutaneous Administration to Rats

Test Article: CAB (free acid)
Species (Strain): Rat (Sprague Dawley) **Location in CTD:** m4.2.3.1

Report No.:	RD2009/01216						RD2009/01359					
Gender (M/F):	M						M & F					
Number of Animals:	3						3					
Feeding Condition:	Non-fasted						Non-fasted					
Analyte:	GSK1265744						GSK1265744					
Assay:	HPLC-MS/MS						HPLC-MS/MS					
Sample:	Plasma						Plasma					
Sample Collection:	Up to Day 106						Up to Day 85					
Route of Administration:	**SC**			**IM**			**SC**			**IM**		
Vehicle/Formulation:	[a]			[a]			[a]			[a]		
Dose (mg/kg):	**10**	**30**	**50**	**5**	**20**	**35**	**5**	**30**	**100** [b]	**2.5**	**10**	**75**
PK Parameters (mean):												
AUC_{0-t} (μg.h/mL)	15637	49065	73049	10770	33709	42172	M: 8607	M: 41284	M: 117641	M: 4617	M: 19110	M: 92117
	-	-	-	-	-	-	F: 9400	F: 47662	F: 126959	F: 4762	F: 18663	F: 100857
AUC_{0-24} (μg.h/mL)	33.4	78.1	119	87.6	125	111	M: 34.9	M: 79.5	M: 147	M: 86.9	M: 164	M: 278
	-	-	-	-	-	-	F: 49.2	F: 137	F: 177	F: 94.8	F: 189	F: 293
C_{max} (μg/mL)	14.0	53.9	69.5	21.5	40.8	46.3	M: 8.36	M: 38.7	M: 98.3	M: 12.6	M: 32.4	M: 105
	-	-	-	-	-	-	F: 9.04	F: 36.9	F: 104	F: 14.2	F: 40.6	F: 124
Median T_{max} (h)	504	504	336	168	336	336	M: 720	M: 336	M: 336	M: 168	M: 168	M: 336
	-	-	-	-	-	-	F: 168	F: 960	F: 336	F: 120	F: 168	F: 336

Additional Information:
a = Suspension in 2% Tween 20, 2% polyethylene glycol 3350, and 4.5% mannitol in water
b = n=2 males for AUC_{0-t} in the 100 mg/kg group due to unscheduled death on Day 39
SC = Subcutaneous
IM = Intramuscular

Table 3.9 Plasma Toxicokinetic Parameters for CAB Following Single Intramuscular or Subcutaneous Administration to Male Monkeys

Test Article: CAB (free acid)
Species (Strain): Monkey (cynomolgus) **Location in CTD:** m4.2.3.1

Report No.:	CD2009/00373				CD2009/00513		CD2009/00656	
Gender (M/F)/Number of Animals:	M / 4				M / 4		M / 4	
Feeding Condition:	Non-fasted				Non-fasted		Non-fasted	
Vehicle/Formulation:	[a]				[a]		[b]	
Analyte:	GSK1265744				GSK1265744		GSK1265744	
Assay:	HPLC-MS/MS				HPLC-MS/MS		HPLC-MS/MS	
Sample:	Plasma				Plasma		Plasma	
Sample Collection Intervals (Day):	Up to 43 days after dosing				Up to 56 days after dosing		Up to 49 days after dosing	
Method of Administration:	**Subcutaneous**		**Intramuscular**		**Subcutaneous**	**Intramuscular**	**Subcutaneous**	**Intramuscular**
Dose (mg/kg):	**1** [c]	**5** [d]	**1** [c]	**5** [d]	**5** [e]	**5** [e]	**10**	**10**
PK Parameters (mean):								
AUC_{0-t} (μg.h/mL)	61	191.1	57	156.7	119	310	505.3	682.1
AUC_{0-24} (μg.h/mL)	3.3	3.7	3.1	4.6	2.3	3.2	14.2	35.0
C_{max} (μg/mL)	0.26	0.42	0.17	0.33	0.16	0.46	1.0	2.6
Median T_{max} (h)	4	5	3	5	8	18	6	5

Additional Information:
a = 1.7% PVP, 0.2% Polysorbate 80, 0.18% methylparaben, 0.02% propylparaben, 0.004M monosodium phosphate, 0.006M disodium phosphate, and 0.84% sodium chloride in sterile water.
b = 2% Pluronic F-127, 0.2% Polysorbate 80, 0.18% methylparaben, 0.02% propylparaben, 0.008M $NaH_2PO_4H_2O$, 0.006M Na_2HPO_4 and 0.81% NaCl.
c = Particle size: x90 - 5.11 microns.
d = Particle size: x90 – 6.84 microns.
e = Particle size: x90 – 67.7 microns.

Table 3.10 Exposure to CAB Following Single Oral Administration of Micronized and Non-Micronized Forms

Test Article: CAB **Report No.:** RD2008/01308
Species (Strain): Rat (Sprague Dawley) **Location in CTD:** m4.2.2.2

Gender (M/F):	M & F
Method of Administration:	Oral gavage
Feeding Condition:	Non-fasted
Analyte:	GSK1265744
Dose (mg/kg):	1000
Sample:	Plasma
Assay:	HPLC-MS/MS
Vehicle/Formulation:	0.5% (w/w) hydroxypropyl methylcellulose and 0.1% (w/w) Tween 80
Serial Sample Collections:	1, 2, 4, 8, 10 and 24 hours post-dose

Test Article Form:	**Micronized GSK1265744 sodium salt**		**Non-micronized GSK1265744 sodium salt**	
Sex:	**M (n=3)**	**F (n=3)**	**M (n=3)**	**F (n=3)**
PK Parameters:				
AUC_{0-24} (μg.h/mL)	3068	3407	2655	3155
C_{max} (μg/mL)	157	167	132	148
Median T_{max} (h)	2	4	8	2

Additional Information:
Lower limit of quantitation (LLQ) for GSK1265744 was 100 ng/mL for a 25 μL portion of rat plasma.

Table 3.11 Exposure of Male Monkeys to CAB Following Single Oral Doses of Micronized and Non-Micronized Forms and Comparison of Exposure Using Test Material Produced at Different Facilities

Test Article (Form): CAB (sodium salt)
Species (Strain): Monkey (cynomolgus)
Method of Administration: Oral gavage
Analyte: GSK1265744
Vehicle/Formulation: 0.5% HPMC with 0.1% Tween 80
Sample: Plasma
Assay: HPLC-MS/MS

Report No.:	RD2007/01415				CD2008/01223	
Location in CTD:	m4.2.3.1				m4.2.2.2	
Sex:	Male				Male	
Number of animals:	n=2				n=4	
Dose Level (mg/kg):	**150 [a] (non-micronized)**	**150 [b] (non-micronized)**	**300 [b] (non-micronized)**	**1000 [b] (non-micronized)**	**500 (micronized)**	**500 (non-micronized)**
PK Parameters						
AUC_{0-t} (μg.h/mL)	381 / 460	393 / 380	568 / 639	654 / 852	592	458
C_{max} (μg/mL)	44.4 / 42.9	54.3 / 32.2	62.4 / 53.3	67.0 / 67.7	36.3	28.8
T_{max} (h)	2 / 2	2 / 2	2 / 2	4 / 2	4	4

Key:
Data presented are mean values (T_{max} reported as median) for report CD2008/01223, whereas individual animal data are presented for report RD2007/01415.
a = Test material manufactured at facility other than Shionogi.
b = Test material manufactured at Shionogi.

Table 3.12 Exposure to CAB in Male Rats and Monkeys when Co-administered with or without Rilpivirine

Test Articles: CAB (free acid) & Rilpivirine (TMC278)
Method of Administration: Intramuscular injection
Analyte: GSK1265744A
Formulation: Long-acting parenteral (LAP) injectable suspension
Sample: Plasma
Assay: HPLC-MS/MS

Species (Strain):	Rat (Sprague Dawley)				Monkey (cynomolgus)			
Gender (M/F):	Male				Male			
Number of animals:	n = 3 to 6				n = 2 to 4			
Sample Collection:	Up to 60 days post-dose				Up to 61 days post-dose			
Report No.:	2011N127517				2010N105579			
Location in CTD:	m4.2.2.2				m4.2.3.1			
Results:	**GSK1265744 Plasma Pharmacokinetic Parameters**							
Test Article:	**GSK1265744A**	**TMC278**	**GSK1265744A**	**TMC278**	**GSK1265744A**	**TMC278**	**GSK1265744A**	**TMC278**
Dose Level (mg/kg):	**10**	**0**	**10**	**60**	**10**	**0**	**10**	**60**
PK Parameters								
AUC_{0-t} (μg.h/mL)	10315		11126		316		420	
Cmax (μg/mL)	14.2		14.2		0.40		0.78	
Tmax (h)	14		14		NR		18	

Additional Information:
Data presented are mean values (T_{max} reported as median).
NR = Not reported since n=2.

Table 3.13 Metal Ion Chelation

Test Articles: CAB (free acid) & DTG (dolutegravir, free acid) Report No.: 2019N401615

Method of Administration: In vitro **Location in CTD:** m4.2.2.2

Analytes: GSK1265744A, GSK1349572B

Formulation: DMSO

Concentration of test articles: 500 nM

Assay: Fluorescence detection

Method: Solutions of multivalent metal cations were prepared in water immediately prior to use (<2 hours). The concentration of metal cations in these solutions was calculated from the mass added to a known volume of solvent and the formula weight provided by the supplier.

Dissociation constants of multivalent metal cations

Metal	GSK1265744A pH 5.0, μM	GSK1265744A pH 7.5, μM	GSK1265744A pH 9.0, μM	GSK1349572B pH 5.0, μM	GSK1349572B pH 7.5, μM	GSK1349572B pH 9.0, μM
Ca+2	>100,000	59,000	40,000	>100,000	>100,000	78,666
Mg+2	93,333	980	522	>100,000	2,000	666
Mn+2	19,667	90.5	18.4	60,667	162	4.1
Zn+2	636	33.2	199	>1,000	49.3	906
Al+3	6.82	369	5,000	18.6	671	3,000
Co+2	>1,000	8.18	1.99	>1,000	19.3	0.598
Fe+2	11.0	0.076	247	59.6	0.222	543
Fe+3	4.22	8.03	129	8.04	18.7	156
Ni+2	>100	6.04	2.04	>100	16.8	1.51
Cu+2	0.203	0.138	0.582	0.724	0.272	0.498

4. PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSES

The toxicokinetic parameters for CAB, determined following repeated administration during the toxicology studies, are summarized in m2.6.7, Table 3, Overview of Toxicokinetic Data, and in the individual report tables in m2.6.7.

5. PHARMACOKINETICS: ORGAN DISTRIBUTION

Table 5.1 Organ Distribution

Test Article: CAB sodium salt

Location in CTD: m4.2.2.3
Report No.: RD2008/00840 **Study No.:** 2990/254

Species:	Rat (Lister Hooded) - partially pigmented
Gender (M/F)/Number of Animals:	Male
Feeding Condition:	Fasted
Vehicle/Formulation:	0.5% HPMC with 0.1% Tween 80
Method of Administration:	Oral gavage
Dose (mg/kg):	30
Radionuclide:	^{14}C
Specific Activity:	0.266 MBq/mg
Sampling Times (h):	1, 2, 4 and 8 hours and Days 1, 7 and 28

Concentrations of Test Substance-Related Radioactivity in the Tissues of Male Partially Pigmented Rats After a Single Oral Administration of [^{14}C]-CAB at 30 mg/kg (μg equivalents of CAB/g of tissue)

		Animal Number and Sex	353M	354M	355M	356M	357M	358M	359M
Tissue Type	Tissue	Sampling Time	1 Hour	2 Hours	4 Hours	8 Hours	1 Day	7 Days	28 Days
Vascular/	Blood (cardiac)		13.0	15.1	20.2	21.1	23.0	10.4	0.546
Lymphatic	Aorta		2.67	3.76	7.46	8.73	8.36	3.23	BLQ
	Bone marrow		2.83	2.14	3.13	6.56	6.99	1.77	BLQ
	Mandibular lymph nodes		1.51	2.24	2.46	2.45	4.15	2.01	BLQ
	Spleen		2.61	1.73	2.25	2.70	2.88	1.06	0.100
	Thymus		0.370	0.620	1.16	1.20	2.26	0.722	BLQ
Metabolic/	Liver		6.92	7.51	9.60	10.6	7.25	3.94	0.210
Excretory	Renal cortex		3.92	3.94	6.61	8.35	8.09	4.49	0.191
	Renal medulla		5.36	5.12	7.71	11.8	12.5	5.17	0.218

Organ Distribution (Continued)

		μg equivalents of CAB/g of tissue						
	Animal Number and Sex	353M	354M	355M	356M	357M	358M	359M
Tissue Type	Tissue Sampling Time	1 Hour	2 Hours	4 Hours	8 Hours	1 Day	7 Days	28 Days
Central	Brain	0.217	0.220	0.412	0.359	0.444	0.183	BLQ
Nervous	Choroid plexus	0.711	1.81	4.50	3.51	5.56	0.482	BLQ
System	Meninges	0.680	1.86	1.94	4.30	3.43	0.870	0.196
	Pineal body	1.62	2.14	4.39	4.37	4.57	1.74	0.135
Endocrine	Adrenal cortex	4.19	2.99	5.70	6.42	7.88	3.50	0.258
	Adrenal medulla	7.11	5.29	11.2	10.2	12.0	5.87	BLQ
	Pituitary	1.80	2.76	4.80	5.99	4.94	2.19	0.097
	Thyroid	3.91	3.19	5.09	4.34	7.37	3.29	0.123
Secretory	Exorbital lachrymal gland	0.665	0.908	2.26	2.70	4.89	1.88	0.126
	Harderian gland	0.500	0.947	2.59	2.83	3.22	1.12	0.100
	Intra-orbital lachrymal gland	0.489	1.07	2.58	3.40	5.90	2.10	0.380
	Pancreas	1.71	1.95	4.17	5.43	5.92	1.56	0.119
	Salivary glands	1.39	2.30	3.70	4.42	5.42	2.36	BLQ
Fatty	Brown fat	1.20	1.18	3.03	2.67	4.79	1.52	BLQ
	White fat	0.342	0.340	0.401	1.19	1.11	0.351	BLQ
Reproductive	Bulbo-urethral gland	2.31	6.50	NS	9.16	14.6	6.05	0.186
	Epididymis	0.715	1.41	3.98	7.29	3.01	1.17	0.152
	Preputial gland	0.634	0.585	2.76	2.25	NS	1.13	BLQ
	Prostate	0.298	0.796	3.30	3.40	5.45	1.56	BLQ
	Seminal vesicles	0.104	0.254	1.15	0.979	2.52	0.366	BLQ
	Testis	0.771	1.40	2.65	3.87	4.21	1.49	0.114
Muscular	Muscle (Skeletal)	0.237	0.350	0.835	1.88	2.61	0.824	0.089
	Myocardium (heart)	4.42	4.87	6.48	7.28	7.69	3.21	0.140
Dermal	Non-pigmented skin	0.430	0.468	1.16	2.33	8.08	3.21	0.103
	Pigmented skin	0.725	0.644	1.55	3.48	10.7	4.07	0.273

Organ Distribution (Continued)

		µg equivalents of CAB/g of tissue						
	Animal Number and Sex	353M	354M	355M	356M	357M	358M	359M
Tissue Type	Tissue Sampling Time	1 Hour	2 Hours	4 Hours	8 Hours	1 Day	7 Days	28 Days
Ocular	Lens of the eye	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Uveal tract	0.933	1.45	4.46	3.83	5.38	2.36	0.095
Respiratory	Lung	11.9	14.3	16.7	18.9	19.0	8.97	0.423
Tract	Nasal turbinates	0.542	1.22	1.72	2.68	2.07	0.711	BLQ
Alimentary	Esophagus	1.83	0.592	3.57	4.16	7.04	2.78	0.237
Canal	Stomach mucosa	19.0	13.2	5.18	5.27	4.78	1.66	0.141
	Small intestine contents	17.4	799*	110	10.7	0.873	0.613	BLQ
	Small intestine mucosa	3.50	19.1[a]	4.03	4.20	3.11	1.64	BLQ
	Caecum mucosa	1.11	1.94	17.4	10.6	3.11	0.648	BLQ
	Large intestine contents	BLQ	NS	409	482*	7.80	4.33	0.133
	Large intestine mucosa	0.873	1.62	12.3[a]	18.2[a]	4.50	1.87	BLQ
	Rectum mucosa	0.412	1.11	1.80	14.6	2.65	1.63	BLQ

Upper Limit of Quantification	482	for all measurements
Lower Limit of Quantification	0.081	for all measurements

Additional Information:

BLQ = Tissue radioactivity concentration below the lower limit of quantification.

NS = Tissue not sectioned.

* = Tissue concentration above the upper limit of quantification.

a = Measurement affected by high levels of radioactivity in associated contents.

6. PHARMACOKINETICS: PROTEIN BINDING

Table 6.1 Protein Binding

Test Article: CAB

Study System: In vitro

Sex: Male

Method: Equilibrium dialysis followed by LC-MS/MS analysis

Species	Test System	Conc. Tested (μM)	% Bound	Report No.	Location in CTD
Rat (n=3)	Plasma	5	>99.9	RH2007/00193	m4.2.2.3
Dog (n=3)	Plasma	5	>99.3		
Monkey (n=3)	Plasma	5	>99.7		
Human (n=3)	Plasma	5	>99.6		
Rat (n=2)	Frozen pooled serum	10	>99.9	RH2007/00222	m4.2.2.3
Dog (n=3)	Frozen pooled serum	10	>99.6		
Monkey (n=5)	Frozen pooled serum	10	>99.8		
Human (n=5)	Frozen pooled serum	10	>99.9		
Human (n=3)	Frozen pooled plasma	Up to 20 μg/mL	>99[b]	2015N235936	m4.2.2.3

Additional Information:
Stock solutions of CAB were prepared in DMSO for all studies except report 2015n235936, where CAB was prepared in acetonitrile:water (v/v, 1:1).
b = The % bound value is an average of all test values. There was no evidence on this study of concentration dependence of CAB.

Plasma Protein Binding (Continued)

Test Article: CAB glucuronide (GSK3388352)

Study System: In vitro

Sex: Male

Method: Equilibrium dialysis followed by LC-MS/MS analysis

Species	Test System	Conc. Tested (μg/mL)	% Bound	Report No. (Study No.)	Location in CTD
Rat (n ≥ 3)	Pooled plasma	0.2, 1.0 and 2.0	~33	2017N333514	m4.2.2.3
Human (n=3)	Plasma	0.2, 1.0 and 2.0	~16 [a]		

Additional Information:
Stock solutions of CAB were prepared in DMSO.
a = The % bound value is an average of all test values. There was no evidence from this study of concentration dependence in either species.

7. PHARMACOKINETICS: STUDY IN PREGNANT OR NURSING ANIMALS

No studies appropriate to this category have been performed with CAB.

8. PHARMACOKINETICS: OTHER DISTRIBUTION STUDIES

Table 8.1 Summary of the In Vitro Inhibition of Human Transporters by CAB

Test Article: CAB

Target	Test System	Inhibition IC_{50} (μM)	Report No.	Location in CTD
Pgp	MDCKII-MDR1 cells	>30	2012N146041	m4.2.2.3
BCRP	MDCKII-BCRP cells	>30	2012N150360	m4.2.2.3
BSEP	membrane vesicles prepared from recombinant baculovirus infected Sf9 cells expressing BSEP	>30	2013N174589	m4.2.2.3
MRP2	membrane vesicles prepared from recombinant baculovirus infected Sf9 cells expressing MRP2	>30		
MRP4	Membrane vesicles expressing MRP4	>30		
MATE1	HEK 293 cells expressing MATE1	18.2	2013N174474	m4.2.2.3
MATE2-K	HEK 293 cells expressing MATE2-K	14.2		
OAT1	S_2 cells expressing OAT1	0.812		
OAT3	S_2 cells expressing OAT3	0.411		
OATP1B1	HEK MSRII cells expressing OAT1B1	>30	2013N164529	m4.2.2.3
OATP1B3	HEK MSRII cells expressing OAT1B3	>30		
OCT1	HEK 293 cells expressing OCT1	>30	2012N146057	m4.2.2.3
OCT2	HEK 293 cells expressing OCT2	>30		

Additional Information:
Pgp = P-glycoprotein.
BCRP = Breast Cancer Resistance Protein.
BSEP = Bile salt extrusion pump transporter.
MRP = Multidrug resistance associated protein.
OAT = Organic anion transporter.
MATE = Multidrug and toxin extrusion transporters.
OATP = Organic anion-transporting polypeptide.
OCT = Organic cation transporter.
S_2 cells derived from an interstitial renal proximal tubule of transgenic mice.

Table 8.2 Summary of the In Vitro Inhibition of Human Transporters by the Metabolite CAB Glucuronide

Test Article: GSK3388352, CAB glucuronide

Target	Test System	Inhibition IC_{50} (μM)	Report No.	Location in CTD
Pgp	Membrane vesicles	>300	2015N253914	m4.2.2.3
BCRP	Membrane vesicles	>300		
BSEP	Membrane vesicles	>300		
MRP2	Membrane vesicles	>300		
MRP4	Membrane vesicles	>300		
OAT1	S_2 cells stably expressing the transporter	73.4 *		
OAT3	S_2 cells stably expressing the transporter	36.5 *		
MATE1	HEK293 cells stably expressing the transporter	>300 *		
MATE2-K	HEK293 cells transiently expressing the transporter	>300 *		
OATP1B1	HEK293 cells stably expressing the transporter	>300		
OATP1B3	HEK293 cells stably expressing the transporter	>300		
OCT2	HEK293 cells stably expressing the transporter	>300		

Key:
GSK3388352 inhibited human MRP4 and MATE1 by 39.7% and 55.7%, respectively, at the highest concentration tested, 300 μM.
* = Following completion of the study, it was noted that the batch of GSK3388352 used contained approximately 2.5% of CAB as an impurity. CAB has been shown to be an inhibitor of MATE1, MATE2K, OAT1 and OAT3. Consequently, this concentration of CAB will impact the accuracy of the MATE1, MATE2-K, OAT1 and OAT3 inhibition profiles attributed to GSK3388352. A 1.1% presence of CAB in the inhibition experiments would account for the reported IC_{50} values. Therefore, GSK3388352 is unlikely to be an inhibitor of MATE1, MATE2-K, OAT1 and OAT3.

Table 8.3 In Vitro Human Transporter Substrate Studies for CAB

Test Article: CAB

Transporter	Concentration (μM)	Efflux Ratio	Report No.	Location in CTD
Pgp	3	3.0	2012N146040	m4.2.2.3
BCRP	3	2.6	2012N155942	m4.2.2.3

Additional Information:
[^{14}C]-CAB was the test material.
The results of these studies indicate that CAB is a substrate for these transporters in vitro.

Table 8.4 Hepatic Uptake of CAB

Test Article: CAB (sodium salt)

Location in CTD: m4.2.2.3

Report No.: 2018N391028 **Study No.:** 18DMW024

Study System: Plateable cryopreserved human(pooled) hepatocytes were used for the in vitro hepatocyte uptake assays.

Concentration: 0.7 and 9 μM

Method: Hepatocyte monolayers were pre-incubated at 37°C with buffer alone or buffer containing a cocktail of transporter inhibitors. After pre-incubation, cells were incubated in triplicate for the appropriate amount of time with CAB. [^{3}H]-estradiol glucuronide([^{3}H]-EG) (0.02 μM) was used as positive control substrate in a separate set of incubations under the conditions stated.

Analysis: UPLC MS/MS

Uptake in Cryopreserved Hepatocytes						
Time (min)	**0.7 μM[a] GSK1265744B (Average pmol/well)**			**9 μM[a] GSK1265744B (Average pmol/well)**		
	No inhibitor (Mean)	**Positive inhibitor cocktail[b] (Mean)**	**S:N[c]**	**No inhibitor (Mean)**	**Positive inhibitor cocktail[b] (Mean)**	**S:N[c]**
0.5	4.4	4.2	1.0	52	48	1.1
1	7.9	6.5	1.2	82	93	0.88
2	8.7	8.4	1.1	73	120	0.60
5	12	12	1.0	200	210	0.93
10	15	14	1.1	210	230	0.93
Time (min)	**0.02 μM [^{3}H]-EG [d]**			**0.02 μM [^{3}H]-EG [d]**		
4	**No inhibitor (Mean)**	**Positive inhibitor cocktail[b] (Mean)**	**S:N[c]**	**No inhibitor (Mean)**	**Positive inhibitor cocktail[b] (Mean)**	**S:N[c]**
	0.30	0.03	9.0	0.29	0.05	6.3

Key:
Data is rounded up to 2 significant figures.
a = Concentrations listed represent actual measured concentrations.
b = Inhibitor cocktail consists of 100 μM rifamycin SV, and 100 μM imipramine.
c = S:N = Signal:Noise
d = Control substrate

Table 8.5 In Vitro Human Cell Membrane Permeability for CAB

Test Article: CAB

P-glycoprotein (Pgp) Permeability					
Transporter	**Cells**	**Concentration (μM)**	**Permeabilities (nm/s)**	**Report No.**	**Location in CTD**
Pgp	MDCKII-hMDR1	3	Passive (Papp) = 424	RH2007/00199	m4.2.2.3
Pgp *	MDCKII-hMDR1	3	Passive (Papp) = 256	2012N146040	m4.2.2.3
Pgp *	MDCKII-hMDR1	3	$P_{7.4[abs]}$ and $P_{5.5[abs]}$ = 1088 and 1374	2012N146040	m4.2.2.3

Additional Information:
* = [^{14}C]-CAB was the test material.
Papp = Apparent passive permeability.
$P_{7.4[abs]}$ and $P_{5.5[abs]}$ = The absorptive membrane permeabilities at pH 7.4 and pH 5.5.
These studies were conducted using 3 μM amprenavir as a positive control. The results of these studies indicate that CAB has high membrane permeability.

Table 8.6 In Vitro Human Transporter Substrate Studies for the Metabolite CAB Glucuronide

Test Article: CAB Glucuronide (GSK3388352)

Method: GSK3388352 was incubated at 37°C in culture with cells / vesicles containing the respective transporters (with or without relevant inhibitors added) and the effect on GSK3388352 uptake was determined by LC-MS/MS.

Transporter	Concentration (μM)	Substrate (Yes/No)	Report No.	Location in CTD
Pgp	10	No	2016N288197	m4.2.2.3
BCRP	10	No		
OATP1B1	50	Yes	2016N298553	m2.2.2.3
OATP1B3	50	Yes		
MRP2	10	Yes		
MRP3	10	Yes		
MRP4	10	Yes		
OAT1	50	No		
OAT3	50	Yes		
OAT4	50	No		

Note: it was determined that the batch of GSK3388352 ([redacted]) used, contained ca. 2.5% of GSK1265744 (parent molecule of GSK3388352) as an impurity. However, this level of GSK1265744 is not expected to impact the overall conclusions of the study.

Additional Information:
Pgp = P-glycoprotein.
BCRP = Breast Cancer Resistance Protein.
MRP = Multidrug resistance associated protein.
OAT = Organic anion transporter.
OATP = Organic anion-transporting polypeptide.

Table 8.7 Assessment of Uptake of CAB by Human Macrophages

Test Article:	CAB	**Report No.:** 2015N237779	**Location in CTD:** m4.2.2.3
Concentrations tested:	0.2 and 10 μg/mL		
CAB Particle Sizes:	200 nm, 1 and 5 μm		
Formulation:	Long-acting parenteral (LAP) formulations		
Treatment Period:	24 or 48 hours at 37°C		
Methods of Analysis:	LC-MS/MS, Laser Scanning Cytometry (LSC) and Transmission Electron Microscopy (TEM)		
Macrophage Cultures:	Macrophages were differentiated from human peripheral blood mononuclear cells during a 6 day procedure. Each macrophage culture treatment (particle size + concentration + incubation time) was performed in triplicate and prepared for analysis.		

Results: At the concentration of 0.2 μg/mL, the uptake of CAB LAP formulations was below the quantitation limit. At 10 μg/mL, the percentage of uptake is approximately between 1.8% and 7.8%, and the 1 μm particle size is preferable for uptake by macrophages compared to the 200 nm and 5 μm particle sizes. Based on the LSC and TEM results, it is difficult to conclude with certainty that the material observed within the macrophage cells is CAB.

Table 8.8 Hepatic Uptake Clearance of CAB Glucuronide

Test Article:	CAB glucuronide (GSK3388352)	**Report No.:** 2017N320631	**Location in CTD:** m4.2.2.3
Concentration tested:	50 μM		
Vehicle:	DMSO		
Inhibitor Cocktail:	100 μM each of Rifamycin SV and imipramine		
Positive Control:	[^{3}H]-estradiol 17-β-D-glucuronide (EG)		
Test System:	Cryopreserved pooled human hepatocytes		
Treatment Period:	0.5, 1, 2.5, 5, 10 and 15 minutes at 37°C		
Methods of Analysis:	LC-MS/MS		
Procedure:	Wells (n=3) of human hepatocyte cultures were pre-incubated with or without inhibitor cocktail for 15 to 20 minutes. GSK3388352 working solution was then added, following the removal of the pre-incubation medium, and the hepatocyte cultures were incubated for the designated treatment periods. The multi-well plates were then prepared for analysis by LC-MS/MS.		

Results: The positive control demonstrated the functional activity of transporters in the cultured human hepatocytes. The predicted hepatic uptake clearance of GSK3388352 was 0.52 mL/min/kg. The hepatic extraction ratio was found to be approximately 0.025, which suggests that hepatic uptake clearance is not the major mechanism in the systemic removal of GSK3388352.

Table 8.9 Potential for CAB Inhibition of Human Folate Transporters and Folate Receptor

Test Article:	CAB sodium salt	**Report No.:** 2019N396076_01	**Location in CTD:** m4.2.2.3
Concentration tested:	0.03 to 100 μM		
Vehicle:	DMSO		
Controls:	Vector control cells, methotrexate		
Test System:	MDCK-II cells		
Treatment Period:	Pre-incubation for 30 minutes, followed by 5 minutes or 2 hours co-incubation		
Methods of Analysis:	LC-MS/MS		
Procedure:	Three independent experiments were conducted in which CAB was tested for the potential to inhibit human PCFT, RFC or FRα in MDCK-II cells transfected with each of the respective targets. In each experiment, CAB was pre-incubated with cells for 30 minutes, followed by addition of substrate and co-incubation for 5 minutes for the PCFT and RFC assays, or co-incubation for 2 hours for the FRα assay.		

Results: In the PCFT assay, CAB did not inhibit folic acid transport up to the highest concentration tested. In the RFC assay, CAB did not inhibit RFC activity. In the FRα assay, CAB demonstrated 36.7% inhibition at 25.8 μM; this observed in vitro inhibition was not projected to be clinically relevant. In all 3 assays, the positive control methotrexate demonstrated expected results.

Additional information:
PCFT = Proton-coupled folate transporter
RFC = Reduced folate carrier
FRα = Folate receptor α

Table 8.10 Blood:Plasma and Liver:Blood Ratios

Test Article: CAB
Gender (M/F): Male
Vehicle/Formulation: 0.5% HPMC and 0.1% Tween 80
Route of Administration: Oral
Radionuclide: [^{14}C]
Specific Activity: 5.13 μCi/mg in mouse and rat

Species: Mouse (n=10, except at 168 hrs n=5)) **Dose (mg/kg):** 30 **Report No.:** 2012N145873 **Location in CTD:** m4.2.2.5
Species: Rat (n=3) **Dose (mg/kg):** 30 **Report No.:** 2012N143605 **Location in CTD:** m4.2.2.5

Species	Feeding Condition	Time (Hour)	Blood : Plasma Ratio	Liver : Blood Ratio
Mouse	Non-fasted	1	0.53	0.22
		4	0.52	0.23
		8	0.52	0.23
		24	0.54	0.20
		168	0.53	NA
Rat	Non-fasted	1	0.55	0.24
		4	0.56	0.25
		8	0.55	0.23
		24	0.56	0.20
		96	0.54	0.20
		168	0.54	0.20

Additional Information:
NA = Not applicable

Table 8.11 Distribution of CAB Following Subcutaneous and Intramuscular Injections

Test Article: CAB **Report No.:** 2014N221244 **Location in CTD:** m4.2.2.3

Gender (M/F):	Male
Species:	Rat (n=5/group)
Dose (mg/kg):	40
Formulation:	Long acting parenteral formulation
Route of Administration:	Subcutaneous and Intramuscular
Tissue Samples:	Injection depot sites (Tissues collected from study report 2015N231295)
Methods of Analysis:	Matrix-assisted laser desorption/ionization (MALDI), imaging mass spectrometry (IMS), optical microscopy, and immunohistochemical (IHC) staining.

Sample preparation: At Day 14 after the injection, tissues were collected and frozen for sectioning and injection site analysis. Injection depots, for both intramuscular and subcutaneous routes of administration, were visible in pre-analysis optical scans and were targeted for high spatial resolution (5 μM) analysis. In addition, low resolution (100 μM) survey scans were collected of the entire tissue section which included both the depot and surrounding tissue.

Results: In tissue cross-sections, IMS analysis revealed that the most intense signal intensity for CAB was in the injection depots with lower more diffuse signal in the surrounding areas. No CAB metabolites were observed in the tissues. In the high spatial resolution MALDI IMS experiments, extracellular and intracellular CAB were distinguished by the ion adducts formed during the MALDI process. After immunohistochemical and histology staining, images displaying intracellular CAB in macrophages and multinucleated giant cells were observed, primarily on the periphery of the injection depots.

Table 8.12 Lymphatic Exposure of CAB Following Intramuscular Injection

Test Article: CAB **Report No.:** 2014N222547 **Location in CTD:** m4.2.2.3

Gender (M/F): Male
Species: Rat (Sprague Dawley)
Dose (mg/kg): 40
Formulation: Long acting parenteral formulation
Route of Administration: Intramuscular
Tissue Samples: Lymph fluid, plasma and lymph nodes
Method of Analysis: LC-MS/MS
Sample preparation: Lymph node tissue was homogenized prior to analysis

Results:		**Concentration of CAB (%)**	
	Sample Collection Time (h)	**Lymph Fluid / Plasma Concentration**	**Lymph Node / Plasma Concentration**
Rat (n=1; thoracic lymphatic duct and jugular vein cannulated)	0.5	NA	NA
	1	NA	NA
	2	5.4	NA
	4	11.9	NA
	8	18.3	NA
	24	13.9	NA
Rat (n=2/time point; intact)	24	NA	37.5
	48	NA	35.4
	72	NA	27.8
	96	NA	27.1
	168	NA	27.1

Additional Information:
NA = Not applicable

Table 8.13 MRI Assessment of CAB Following Subcutaneous and Intramuscular Injections

Test Article: CAB **Report No.:** 2015N231295 **Location in CTD:** m4.2.2.3

Gender (M/F):	Male
Species (strain):	Rat (Sprague Dawley; n=5/group)
Dose (mg/kg):	40
Route of Administration:	Subcutaneous (SC; 1 group) and Intramuscular (IM; 2 groups)
Site of Injection:	IM (gastrocnemius) and SC (abdomen)
Vehicle:	Polysorbate 20 (20 mg/mL), Polyethylene Glycol 3350 (20 mg/mL), Mannitol (45 mg/mL) and water
MRI Time Intervals:	Immediately after dosing and at Day 1, 2, 3, 4, 7 and 14 post-dose
MRI Contrast Agent:	Feraheme (ferumoxytol; Ultrasmall Superparamagnetic Iron Oxide or USPIO)
Macrophage trafficking protocol:	Two rats in one of the IM injection groups were given USPIO contrast agent intravenously on Day 2 (to identify macrophage localization); these two rats were subsequently imaged on Days 3 and 4 then euthanized and tissues collected for histological examination.

Results: The drug depot volume as assessed by MRI showed a rapid ~3 to 7 fold increase to a maximum volume by Day 2 post-dose in the IM injected rats compared with an ~1 fold increase at Day 2 in the SC injected rats. Conversely, the vehicle depot did not increase in size with time and decreased below baseline by Day 2. There was a significant uptake of the USPIO contrast agent in the CAB depot region and minimal contrast agent uptake observed in the vehicle depot site. These MRI findings are consistent with the histological findings reflecting increased inflammatory cell infiltrate observed in the CAB depots.

Additional information:
MRI = Magnetic Resonance Imaging

9. PHARMACOKINETICS: METABOLISM IN VIVO

Table 9.1 Metabolism In Vivo After Single Oral Administration in Males

Test Article: CAB **Location in CTD:** m4.2.2.4 (and m5.3 for human study)
Gender (M/F): M
Feeding Condition: Mouse and rat = Non-fasted; monkey = fasted; human = fasted
Vehicle/Formulation: 0.5% HPMC / 0.1% Tween 80
Route of Administration: Oral
Dose Level: Mouse and rat = 30 mg/kg; monkey = 10 mg/kg; human = 30 mg/subject
Radionuclide: [^{14}C]
Specific Activity (μCi/mg): Mouse and rat = 5.13; monkey = 4.88; human = 2.49

				% of Compound in Sample (% of Administered Dose)					
Species (Report/Study No.)	**Sample**	**Sampling Time or Period (hr)**	**% of Dose in Total Sample**	**Parent**	**M1 (GSK3388352)**	**M2**	**M3**	**M5**	**M6**
Mouse	Plasma	0 to 24	NA	99.4	---	---	---	---	---
2012N146480	Urine	0 to 96	0.81	NQ	33.5 (0.31)	---	---	---	4.44 (0.02)
	Bile [a]	0 to 72	1.79	---	45.6 (0.81)	---	---	25.3 (0.45)	---
	Feces	0 to 48	94	85.9 (78.5)	---	---	---	---	---
Rat	Plasma	0 to 168	NA	92.3	---	---	---	---	---
2012N146427	Urine	0 to 120	0.31	8.41 (0.02)	43.01 (0.13)	5.01 (0.01)	---	---	1.63 (0.00)
	Bile [a]	0 to 96	1.60	---	87.5 (1.40)	---	---	---	---
	Feces	0 to 48	94.5	83.4 (75.7)	---	---	---	---	---
Monkey	Plasma	0 to 24	NA	95.9	---	---	---	---	---
2013N171159	Urine	0 to 48	11.1	---	80.4 (8.55)	5.2 (0.56)	5.3 (0.56)	---	---
	Bile [a,b]	0 to 48	14.5	---	68.3 (9.76)	8.3 (1.19)	14.0 (2.00)	---	---
	Feces	0 to 48	78.7	80.0 (58.5)	---	---	---	---	---

Metabolism In Vivo (Continued)

				% of Compound in Sample (% of Administered Dose)					
Species (Report/Study No.)	**Sample**	**Sampling Time or Period (hr)**	**% of Dose in Total Sample**	**Parent**	**M1 (GSK3388352)**	**M2**	**M3**	**M5**	**M6**
Human	Plasma	0 to 24	NA	94.3	---	---	---	---	---
2013N167401	Urine	0 to 144	26.8	---	75.0 (20.0)	NQ	---	---	---
	Bile [c]	6 [d]	NA	[c]	[c]	---	---	---	---
	Feces	0 to 216	58.5	83.4 (46.8)	---	---	---	---	---

Additional Information:
a = Samples from BDC animals (Bile duct-cannulated).
b = BDC monkey was n=1 due to insufficient samples from other animals.
c = Qualitatively assessed for metabolites in duodenal bile sample collected using the Entero-Test™. Parent and GSK3388352 were observed.
d = In humans this is a spot sample, which was 6 hours post-dose.
NA = Not applicable.
NQ = Not quantifiable.
--- = Not detected.

Table 9.2 Potential for In Vivo Epimerization of CAB in Human Plasma

Test Article: CAB

Species: Human

Number of Subjects: 8

Dosing Duration: 14 days

Sample Occasions: 1, 4 and 24 hours post-dose on Day 14

Analytical Method: LC-MS/MS

Source of Plasma Samples: Study conducted under report RM2008/00009

Route of Administration: Oral **Dose:** 25 mg/day **Report No.:** RD2008/01340 **Location in CTD:** m4.2.2.4

Tabulated Results:

Plasma samples were collected from healthy human subjects at 1, 4 and 24 hours post-dose following 14 days of repeat oral administration of GSK1265744B at 25 mg/day. The potential for GSK1265744 to undergo epimerization in plasma was assessed.

There was no evidence for the in vivo epimerization of GSK1265744 to any of its stereoisomers, gsk001*, gsk003* or gsk016*, in human plasma samples following repeat oral administration of CAB.

Additional Information:
There was no evidence for the in vivo epimerization of GSK1265744 to any of its stereoisomers in human plasma.

*新薬承認情報提供時に置き換え

10. PHARMACOKINETICS: METABOLISM IN VITRO

Table 10.1 Metabolic Stability in S9 and Hepatocytes In Vitro

Test Article: CAB

Location in CTD: m4.2.2.4

Study System: S9 and hepatocytes

Report No.: RH2007/00183

Study No.: 07APK038

Percent Parent Drug Remaining (GSK1265744)								
	S9				Rat Hepatocytes		Human Hepatocytes	
Time (min)	**Rat**	**Dog**	**Monkey**	**Human**	**Fresh**	**Cryopreserved**	**Fresh**	**Cryopreserved**
0	100	100	100	100	100	100	100	100
15	112	97	105	100	99	94	94	99
30	112	94	96	102	94	91	95	95
60	112	84	95	104	87	88	90	98
90	NT	NT	NT	NT	80	91	87	92
120	NT	NT	NT	NT	69	77	73	90
$t_{½}$ (min)	>180	>180	>180	>180	229	>360	311	>360
Cl'int (mL/min/kg body weight)	ND	ND	ND	ND	29	NSL	14	NSL

Additional Information:
NSL = No significant loss of parent.
ND = Not determined.

Table 10.2 In Vitro Metabolic Activation in Liver Microsomes

Test Article: CAB

Location in CTD: m4.2.2.4

Report No.: RD2007/01629

Study System: Pooled liver microsomes

Species: Rat, monkey, human

Method: The non-extracted binding of [^{14}C]-CAB (10 μM) and [^{14}C]-acetaminophen (10 μM) was determined in the presence and the absence of NADPH co-factor in rat, monkey and human liver microsomes. After termination of the reactions, samples were filtered and washed and analyzed with liquid scintillation counting.

Results:	**Non-Extracted Radioactivity (pmol eq/mg protein/hour)**	
Species	**[^{14}C]-GSK1265744**	**[^{14}C]-Acetaminophen**
Rat	984	119
Monkey	794	145
Human	180	221

Additional Information:
The non-extracted radioactivity observed was predominantly co-factor-dependent.

Table 10.3 In Vitro Metabolite Characterization Using Cryopreserved Hepatocytes

Test Article: CAB

Location in CTD: m4.2.2.4

Report No.: RH2007/00188

Study System: Hepatocytes

Species: Rat, dog, monkey, human

Method: Cryopreserved hepatocytes were pre-incubated at 37°C for 10 minutes prior to addition of GSK1265744 (10 μM). At 0, 4 and 24 hours, an organic mixture was added to quench the incubation reaction. The samples were analyzed by positive and negative ion LC-MS/MS.

Results:
After 24 hours of incubation, GSK1265744 was seen to be very stable in all species, and no metabolites were detected.

Table 10.4 In Vitro Metabolic Turnover in Hepatocytes

Test Article: CAB

Location in CTD: m4.2.2.4

Report No.: RD2008/00073

Study System: Hepatocytes

Species: Male rat and monkey, and pooled (mixed gender) human

Method: Hepatocytes were incubated at 37°C with [^{14}C]-GSK1265744 (50 μM). At 4 and 24 hours, the incubation reaction mixtures were quenched by addition of 1.0 mL of acetonitrile. The samples were analyzed by using liquid scintillation counting. Metabolic viability of the cells was evaluated with the probe substrate, 7-ethoxycoumarin.

Results:
The metabolic turnover of [^{14}C]-GSK1265744 in rat and monkey hepatocytes (approximately 9.6% and 6.0% turnover, respectively) was similar to that observed in human hepatocytes (approximately 6.0% turnover). The route of metabolism for [^{14}C]-GSK1265744 in rat, monkey and human hepatocytes was glucuronidation. The human metabolite of [^{14}C]-GSK1265744 was observed in monkey and rat hepatocyte incubations.

Table 10.5 In Vitro Metabolism of CAB to its Enantiomer and Diastereomers

Test Article: CAB

Location in CTD: m4.2.2.4

Report No.: RH2007/00207

Study System: Cryopreserved hepatocytes

Species: Rat, dog, monkey, human

Method: Cryopreserved hepatocytes were pre-incubated at 37°C for 10 minutes prior to addition of GSK1265744. At 0, 1, 4, 6 and 24 hours, an organic mixture was added to quench the incubation reaction. The samples were analyzed using a chiral LC-MS/MS assay.

Results:
Analytical standards for the enantiomer (gsk001*) and one diastereomer (gsk003*) were used to develop the chiral LC-MS/MS assay and to verify the elution times of the stereoisomers. The two stereoisomer standards available (gsk001* and gsk003*) could not be separated chromatographically. Chromatograms showed peaks (with peak areas of approximately 6.7 % of the peak area of GSK1265744) corresponding to the retention time of gsk001* and gsk003* at all incubation time points. The peak areas did not increase with time and were present in the no-cell-control-incubation, as well as spiked matrix, suggesting the presence of a trace impurity in the GSK1265744 incubation as opposed to a metabolically generated stereoisomer. Because an authentic standard of the second diastereomer of GSK1265744 was not synthesized, production of this compound in the incubation could not be monitored. The data indicate that no significant metabolic conversion of GSK1265744 to its enantiomer gsk001* or diastereomer gsk003* was observed after incubation with rat, dog, cynomolgus monkey, or human hepatocytes.

*新薬承認情報提供時に置き換え

Table 10.6 In Vitro Potential to Form Glutathione Adducts

Test Article: CAB

Location in CTD: m4.2.2.4

Report No.: RH2007/00164

Study System: Liver microsomes

Species: Rat, dog, monkey and human (pooled)

Method: Microsomes were incubated with GSK1265744 (5 μL of a 10 mM solution per incubation mix) at 37°C for 30 minutes and then the incubation mixture was quenched. Samples were analyzed by LC-MS/MS.

Results:
GSK1265744 showed evidence for formation of a common electrophilic metabolite in rat, monkey, and human microsomes, as assessed by formation of a common glutathione adduct. There was no evidence of an electrophilic metabolite in dog liver microsomes.

Table 10.7 In Vitro Metabolic Enzymology

Test Article: CAB

Study System: Pooled human liver microsomes (alamethacin activated) and recombinant enzymes **Location in CTD:** m4.2.2.4

Species: Human **Report No.:** RH2007/00166

Method: Incubation at 37°C for 20, 60 or 120 minutes

Metabolic Stability with Recombinant Human UGT1A1 and PHLM (GSK1265744 5.0 μM; UGT1A1 0.25 mg/mL and PHLM 1.0 mg/mL)				Metabolic Stability with and without Recombinant Human UGT1A1 (GSK1265744 0.5 μM; UGT1A1 2.5 mg/mL)			
		% Parent Remaining		With UGT1A1		Without UGT1A1	
Compound	Time (min)	UGT1A1	PHLM	% Parent Remaining	St Dev	% Parent Remaining	St Dev
GSK1265744	0	100	100	100	NA	100	NA
	20	89	97	NT	NA	NT	NA
	60	96	94	NT	NA	NT	NA
	120	NT	NT	73	5.9	108	29.6
β-estradiol[a]	0	100	100	NT	NA	NT	NA
	20	88	96	NT	NA	NT	NA
	60	99	63	NT	NA	NT	NA
7hydroxy4TFMC[b]	0	100	100	100	NA	100	NA
	20	96	1	NT	NA	NT	NA
	60	95	0	NT	NA	NT	NA
	120	NT	NT	25	1.0	107	7.6

Additional Information:

a = Metabolic stability of β-estradiol was determined for 50 μM incubations instead of 5 μM incubations because of poor MS sensitivity for this compound.

b = 7hydroxy4TFMC = 7-hydroxy-4-trifluoromethylcoumarin.

PHLM = pooled human liver microsomes.

NA = Not applicable.

NT = Not tested.

Table 10.8 In Vitro Metabolic Enzymology

Test Article: CAB

Study System: Pooled human liver microsomes (HLM) and recombinant UGT enzymes **Location in CTD:** m4.2.2.4
Species: Human **Report No.:** 2012N145430
Radionuclide: [^{14}C]
Specific Activity: 126 μCi/mg

Method: Incubations to determine glucuronide formation were performed at 37°C in duplicate for up to 2 hours. Each sample contained 5 μM [^{14}C]-GSK1265744 and 0.5 mg/mL protein.

UGT Enzyme	Summary of [^{14}C]-GSK1265744 Glucuronide Formation [a]		Enzyme Kinetic Constants for GSK1265744 Glucuronide Formation			
	Mean % Total Radioactivity	Mean Rate of Metabolite Formation (pmol/min/mg)	Km (μM)	V_{max} (pmol/min/mg)	Cl_{int} (μL/min/mg)	fCL_{UGT} [b]
HLM	22	18	496	1298	2.6	NA
UGT1A1	18	15	148	660	4.5	0.67
UGT1A3	0	0	NA	NA	NA	NA
UGT1A4	0	0	NA	NA	NA	NA
UGT1A6	0	0	NA	NA	NA	NA
UGT1A9	12	10	90	200	2.2	0.33
UGT2B4	0	0	NA	NA	NA	NA
UGT2B7	0	0	NA	NA	NA	NA
UGT2B15	0	0	NA	NA	NA	NA
Control UGT	0	0	NA	NA	NA	NA

Additional Information:
Metabolite was not detected in the no UDPGA controls.
NA = Not applicable.
a = Values indicate % total radioactivity or metabolite rates from 2 hour incubations with 5 μM [^{14}C]-GSK1265744.
b = Fraction of GSK1265744 total clearance mediated by UGT enzyme in vitro.

Table 10.9 In Vitro Metabolism

Test Article: CAB

Study System: Human liver, kidney and intestinal microsomes (alamethicin activated) **Location in CTD:** m4.2.2.4
Species: Human **Report No.:** 2014N222268
Radionuclide: [^{14}C]
Specific Activity: 126 μCi/mg

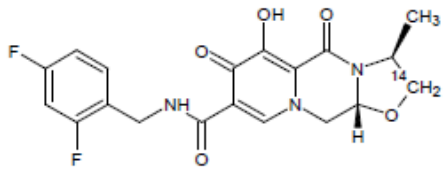
Method: Incubations were performed, in duplicate at 37°C with shaking, for 2 hours (liver and kidney) or 1 hour (intestinal). Microsomes were pre-incubated with [^{14}C]-GSK1265744 (5 μM) for 5 minutes then reactions were initiated by addition of cofactor (5 mM UDPGA final concentration).

Summary of [^{14}C]-GSK1265744 Glucuronide Metabolite (GSK3388352) Formation in Human Microsomes (mean of n=2)

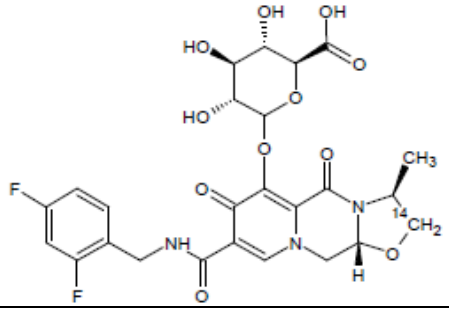
Test System	Mean % Total Radioactivity	Mean Rate of Metabolite Formation (pmol/min/mg)
Human Liver Microsomes	16.9	14.1
Human Kidney Microsomes	8.39	6.99
Human Intestinal Microsomes	3.97	3.31
Control UGT [a]	0	0

Structures:

[^{14}C]-GSK1265744



GSK3388352



Additional Information:
Structural confirmation of the glucuronide structural assignment was based on the comparison of HPLC radioprofiles and metabolite identification from human microsomal samples from a previous biotransformation study [2012N145430]. Structural confirmation of the glucuronide in this study was by LC/MS^n analysis.
a = GSK3388352 was not detected in the no UDPGA controls.

Table 10.10 Metabolism in Isolated Perfused Rat Liver

Test Article: CAB

Location in CTD: m4.2.2.4
Report No.: 2017N320633

Study System: Isolated perfused rat liver (IPRL)
Vehicle: DMSO
Method: Livers were prepared then perfused with 10 μM GSK1265744 for approximately 1 hour and samples of bile, perfusate and livers were collected. Samples were analyzed by LC-MS/MS.

Hepatic Disposition of Glucuronide Metabolite Following Perfusion of GSK1265744 Through Rat Livers (n=3)

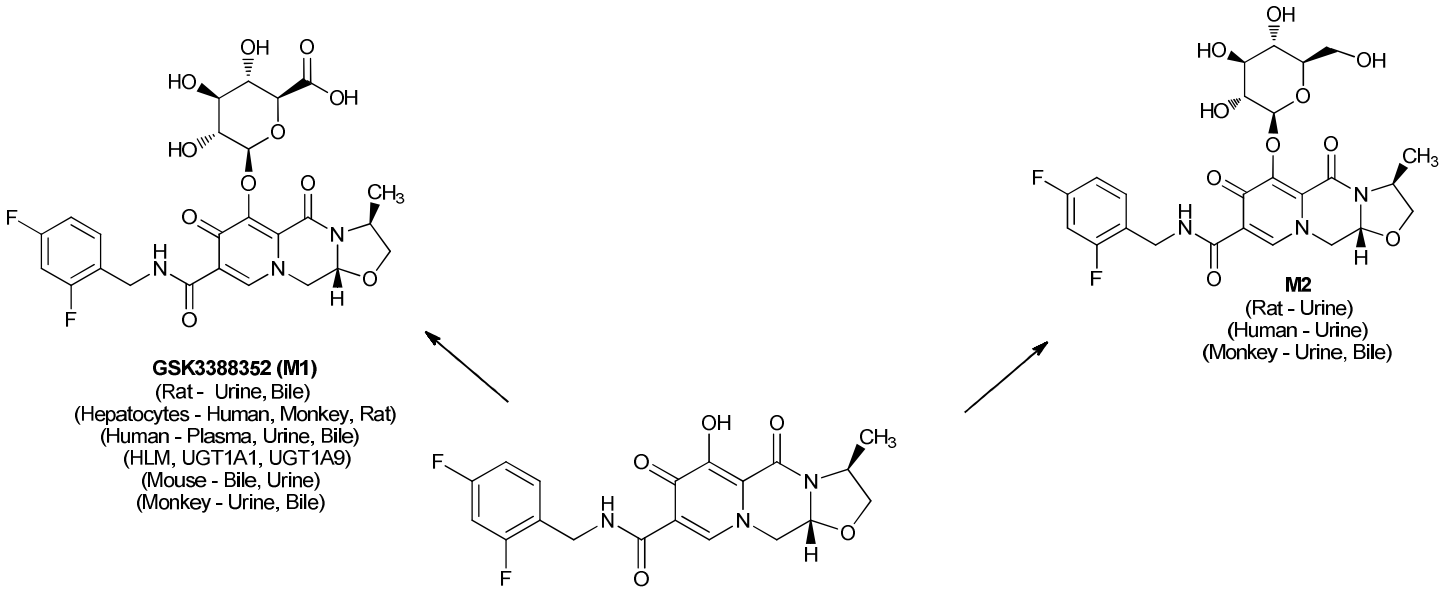
Analyte	Glucuronide metabolite (GSK3388352)	GSK1265744
Parameter:		
Cumulative biliary excretion (nmol)	79	64
Biliary excretion rate (nmol/min)	1.9	1.0
Perfusate concentration (nmol/mL)	0.13	NA
Cumulative perfusate excretion (nmol)	89.0	NA
Perfusate excretion rate (nmol/min)	2.3	NA
Liver homogenate concentration (nmol/mL) [a]	41.0	220
Canalicular clearance (mL/min/kg)	0.16	0.014
Basolateral clearance (mL/min/kg)	0.21	NA

Additional Information:
NA = not applicable.
a = Concentration measured after 60 minute perfusion.

11. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS

Table 11.1 Pharmacokinetics: Predominant Metabolic Pathways

Test Article: CAB



M2 is the preferred structure based on metabolic precedence and comparison with M1.
M3 (+cysteine, +O, -F) and M4 (+pentose) were also detected in human urine (<1% dose).
Two other minor metabolites: M5 (+glutathione, +O, -F) and M6 (+ O) were observed that were specific to the nonclinical species.
HLM = Human liver microsomes.

12. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLIZING ENZYMES

Table 12.1 Inhibition of Drug Metabolizing Enzymes

Test Article: CAB

Test System: Recombinant Enzymes and PHLM

Method of Analysis: Fluorescence detection for recombinant enzymes and LC-MS/MS for PHLM

Type of Study: Preliminary Investigation of the In Vitro Inhibition of Human Drug Metabolizing Enzymes by GSK1265744

Target	Inhibition IC_{50} (μM)			Time-Dependent Inhibition - Fold Shifts Using PHLM	Report No.	Location in CTD
	Recombinant enzymes		PHLM			
	1st run	2nd run				
CYP1A2	>33	12.5	>33	NA		
CYP2C9	>33	>33	15	NA		
CYP2C19	>33	>33	13	NA		
CYP2D6	>33	>33	>33	NA	RH2007/00181	m4.2.2.4
CYP3A4	>33 (DEF)	NA	NR (midazolam)	No fold shift		
CYP3A4	>33 (7BQ)	>33 (7BQ)	>33 (atorvastatin)	No fold shift		
CYP3A4	NA	NA	>33 (nifedipine)	No fold shift		

Additional Information:
CYP3A4 was tested with various probe substrates, which are specified in parentheses.
NR = Not reportable due to apparent activation.
NA = Not applicable.
PHLM = Pooled human liver microsomes.

Table 12.2 Inhibition of UGT Catalytic Activity

Test Article: CAB

Test System: Recombinant Enzymes and PHLM

Method of Analysis: HPLC and Absorbance Detection

Type of Study: Potential In Vitro Inhibition of Human UGT Catalytic Activity by GSK1265744B (CAB Sodium Salt), 0.1 to 100 μM.

	Inhibition IC_{50} (μM) [mean of n=2]			
Target	**Recombinant enzymes**	**PHLM**	**Report No.**	**Location in CTD**
UGT1A1	(33%)	(15%)		
UGT1A3	12	NA		
UGT1A4	>100	NA		
UGT1A6	>100	NA		
UGT1A9	46	NA	2013N159049	m4.2.2.4
UGT2B4	>100	NA		
UGT2B7	>100	NA		
UGT2B15	>100	NA		
UGT2B17	(24%)	NA		

Additional Information:
Values in parentheses are the % inhibition at maximum concentration tested, 100 μM.
NA = Not applicable.
PHLM = Pooled human liver microsomes.
UGT = Uridine 5'-diphosphoglucuronosyl transferase.

Table 12.3 Inhibition of Drug Metabolizing Enzymes

Test Article: CAB

Test System: Pooled Human Liver Microsomes

Method of Analysis: LC-MS/MS

Type of Study: Potential In Vitro Inhibition of Human Drug Metabolizing Enzymes by GSK1265744B (CAB Sodium Salt)

Target	Direct Inhibition IC_{50} (μM)	Time-Dependent Inhibition IC_{50} (μM) [a]	Metabolism-Dependent Inhibition IC_{50} (μM) [b]	Potential for Metabolism Dependent Inhibition	Report No.	Location in CTD
CYP1A2	>100	>100	>100	Little or no	2012N151766	m4.2.2.4
CYP2A6	>100	>100	>100	Little or no		
CYP2B6	>100	>100	>100	Little or no		
CYP2C8	>100	>100	>100	Little or no		
CYP2C9	>100	>100	>100	Little or no		
CYP2C19	>100	>100	>100	Little or no		
CYP2D6	>100	>100	>100	Little or no		
CYP3A4 /5 (atorvastatin)	84	>100	41	Yes		
CYP3A4 /5 (midazolam)	>100	>100	82	Yes		
CYP3A4 /5 (nifedipine)	>100	>100	57	Yes		

Additional Information:
IC_{50} values are the mean of duplicate samples (n=2).
CYP3A4 was tested with various probe substrates, which are specified in parentheses.
a = 30 minute pre-incubation without NADPH
b = 30 minute pre-incubation with NADPH

Table 12.4 Induction of Pregnane X Receptor (PXR) Target Genes (e.g., CYP3A4)

Test Article: CAB

Type of Study: **Potential In Vitro Transactivation of PXR**

Test System: HepG2 cells transfected with rat or human PXR

Number of Replicates: n=4 for rat and n=3 for human

Target	Maximum Response [a]	pEC_{50}	Maximum Response Concentration of GSK1265744A	Response	CYP3A4 Induction Potential	Report No. (Location in CTD)
Rat PXR	4.3	<5	13.7 nM	Weak	Unlikely	RR2007/00043 (m4.2.2.4)
Rat PXR	2.7	<5	13.7 nM	Weak	Unlikely	
Rat PXR	2.2	<5	1.52 nM	Weak	Unlikely	
Rat PXR	5.2	<5	10 μM	Weak	Unlikely	
Human PXR	0.5	<5	13.7 nM	Weak	Unlikely	RR2007/00046 (m4.2.2.4)
Human PXR	12.3	<5	1.11 μM	Weak	Unlikely	
Human PXR	8.6	<5	1.52 nM	Weak	Unlikely	

Additional Information:
a = Maximum response compared to positive control.
Positive controls for PXR activation were rifampicin for human PXR and 5-Pregnan-3β−OL-20-ONE-16α carbonitrile for rat PXR.

Table 12.5 Induction of Drug Metabolizing Enzymes

Test Article: CAB

Type of Study: Potential In Vitro Induction of Human Drug Metabolising Enzymes (CYP1A2, CYP2B6 and CYP3A4)

Report No.: 2013N166279 **Location in CTD:** m4.2.2.4

Study System:	Human hepatocytes were obtained from Celsis™ as cultured monolayers on a collagen substratum with a Matrigel overlay.
Method:	Pre-warmed duplicate culture medium solutions containing GSK1265744 (0.03, 0.1, 0.3, 1.0, 3, 10 and 30 μM), omeprazole (50 μM), phenytoin (50 μM) or rifampicin (10 μM) were added to human hepatocytes. All treatments were incubated at 37°C for 24 hours. At the end of the incubation period the culture medium was removed, cells were lysed, and total RNA was extracted. RNA was converted to double stranded cDNA and then PCR amplified. The specific mRNA level was quantitatively detected for the following genes: CYP1A2, 2B6, 3A4 and the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Effects of GSK1265744 and Prototypical CYP Inducers on the mRNA Levels of Cytochrome P450s (Ratio of Treated Over Control*)

Gene	CYP1A2	CYP2B6	CYP3A4
Concentration of GSK1265744 (μM)			
0.03	1.8	2.1	1.9
0.1	0.92	0.88	1.1
0.3	1.0	1.0	1.2
1.0	1.4	1.5	1.6
3	1.0	1.3	1.4
10	1.2	1.2	1.1
30	1.5	1.5	1.5
Prototypical Inducer	88	15	11

Additional Information:

* Controls are defined as 0.1% v/v DMSO.

Prototypical CYP inducers: 50 μM Omeprazole (CYP1A2), 50 μM Phenytoin (CYP2B6), 10 μM Rifampicin (CYP3A4).

Table 12.6 Induction of Drug Metabolizing Enzymes

Test Article: CAB

Type of Study: **Effects of CAB on mRNA Levels of Hepatic Cytochrome P450 Genes in a 14 Day Oral Toxicity Study in Rats**

Species/Strain: Rat / CD-IGS
Source of Liver Samples: Study conducted under report RD2006/01741
Vehicle/Formulation: Aqueous 0.5% HPMC with 0.1% Tween 80
Analytical Method: qRT-PCR
GLP Compliance: No **Report No.:** RD2006/01999 **Location in CTD:** m4.2.2.4

	Ratio of mRNA Expression in Treated Over Control Group							
Daily Dose (mg/kg)	**0 (Control)**		**30**		**100**		**300**	
(M/F):Number of Animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
Cytochrome P450								
CYP1A1	NC	NC	NC	NC	NC	NC	NC	NC
CYP1A2	1.0	1.0	1.4	1.3	1.3	0.87	1.4	1.0
CYP2B1	1.0	1.0	1.1	0.62	0.69	0.87	0.82	0.52
CYP2B2	1.0	1.0	0.73	2.8	0.61	0.64	1.4	2.0
CYP2E1	1.0	1.0	1.2	1.3	1.1	0.99	1.1	1.3
CYP3A2 *	1.0	*	1.3	*	1.2	*	0.83	*
CYP3A23	1.0	1.0	0.92	0.50	0.96	0.48	0.72	0.45
CYP4A1	1.0	1.0	1.3	0.92	1.4	0.77	1.6	0.77

Additional Information:
Group means are shown.
* = CYP3A2 is a male specific gene and therefore was not analyzed in the female livers.
NC = Not calculated; below limit of quantitation.

13. PHARMACOKINETICS: EXCRETION

Table 13.1 Excretion

Test Article: CAB

Species:	Mouse		Rat		Monkey		Human
Feeding Condition:	Non-fasted		Non-fasted		Fasted		Fasted
Vehicle:	0.5% HPMC / 0.1% Tween 80		0.5% HPMC / 0.1% Tween 80		0.5% HPMC / 0.1% Tween 80		Solution
Method of Administration:	Oral		Oral		Oral		Oral
Dose (mg/kg):	30		30		10		30 mg/subject
Radionuclide:	[^{14}C]		[^{14}C]		[^{14}C]		[^{14}C]
Specific Activity (μCi/mg):	5.13		5.13		4.88		2.49
Assay:	Liquid scintillation counting		Liquid scintillation counting		Liquid scintillation counting		Liquid scintillation counting
Report No.:	2012N145873		2012N143605		2013N174861		2013N179556
Location in CTD (Module):	m4.2.2.5		m4.2.2.5		m4.2.2.5		m5.3
Number of Animals/(M/F):	4M	3M (BDC)	3M	3M (BDC)	3M	1M[b] (BDC)	6M
Sample	**Mean Percent of Administered Dose Recovered**						
Urine	0.81	0.42	0.31	0.33	11.1	14.7	26.8
Feces	94.0	93.5	94.5	89.1	78.7	54.6	58.5
Bile	NA	1.79	NA	1.6	NA	14.5	NA
Total[a]	94.8	96.4	94.9	91.1	90.1	84.1	85.3

Key: Data shown are means, except for BDC monkeys as noted.
a = Includes, as appropriate, radioactivity recovered in cage rinses/washes/wipes/debris GI tract and residual carcass.
b = Values are from a single animal, as two of the cannulated monkeys on study did not produce sufficient bile samples to provide reliable data.
BDC = Bile duct cannulated. NA = Not applicable.

14. PHARMACOKINETICS: EXCRETION INTO BILE

Data pertaining to the excretion of CAB in bile are presented in Table 13.1 above.

15. PHARMACOKINETICS: DRUG-DRUG INTERACTIONS

No studies appropriate to this category have been performed with CAB.

16. PHARMACOKINETICS: OTHER

No studies appropriate to this category have been performed with CAB.