Influence of CYP2B6 Pharmacogenetics on Stereoselective Inhibition and Induction of Bupropion Metabolism by Efavirenz in Healthy Volunteers.

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Abbreviations: HPLC-MS/MS, high performance liquid chromatography/tandem mass spectrometry; DDIs, Drug-Drug Interactions; BUP, bupropion; OHBUP, 4-hydroxybupropion (aka phenylmorpholinol); THBUP, threohydrobupropion; EHBUP, erythrohydrobupropion; R-BUP, R-bupropion; S-BUP, S-bupropion; SS-OHBUP, (2S,3S)-4-hydroxybupropion; RR-OHBUP, (2R-3R)-4-hydroxybupropion; RR-THBUP,
(2R,3R)-threohydrobupropion; SS-THBUP, (2S,3S)- threohydrobupropion; RS-EHBUP, (2R,3S)- erythrohydrobupropion; SR-EHBUP, (2S,3R)- erythrohydrobupropion; NM, normal metabolizer of \(CYP2B6\); IM, intermediate metabolizer of \(CYP2B6\); PM, poor metabolizer of \(CYP2B6\); 11\(^{\text{\beta}}\)-HSD1, 11\(^{\text{\beta}}\)-hydroxysteroid dehydrogenase 1.
Abstract

We investigated the acute and chronic effects of efavirenz, a widely used antiretroviral drug, and CYP2B6 genotypes on the disposition of racemic and stereoisomers of bupropion (BUP) and its active metabolites, 4-hydroxyBUP, threohydroBUP and erythrohydroBUP. The primary objective of this study was to test how multiple processes unique to the efavirenz-CYP2B6 genotype interaction influence the extent of efavirenz-mediated drug-drug interaction (DDI) with the CYP2B6 probe substrate BUP. In a three-phase, sequential, open-label study, healthy volunteers (N=53) were administered a single 100 mg oral dose of BUP alone (control phase), with a single 600 mg oral efavirenz dose (inhibition phase), and after 17-days pretreatment with efavirenz (600 mg/day) (induction phase). Compared to the control phase, we show for the first time that efavirenz significantly decreases (by 11-26%) and chronically increases (by 24-61%) the exposure of hydroxyBUP and its diastereomers, respectively, and the extent of these interactions were CYP2B6 genotype dependent. Chronic efavirenz enhances the elimination of racemic BUP and its enantiomers (by 51-56%) as well as of threo- and erythro-hydroBUP and their diastereomers (by 34-58%), suggesting additional novel mechanisms underlying efavirenz interaction with BUP. The effects of efavirenz and genotypes were nonstereospecific. In conclusion, acute and chronic administration of efavirenz inhibits and induces CYP2B6 activity. Efavirenz-BUP interaction is complex involving time- and CYP2B6 genotype-dependent inhibition and induction of primary and secondary metabolic pathways. Our findings highlight important implications to the safety and efficacy of BUP, study design considerations for
future efavirenz interactions, and individualized drug therapy based on CYP2B6 genotypes.

Significance Statement:
The effects of acute and chronic doses of efavirenz on the disposition of racemic and stereoisomers of BUP and its active metabolites were investigated in healthy volunteers. Efavirenz causes an acute inhibition, but chronic induction of CYP2B6 in a genotype dependent manner. Chronic efavirenz induces BUP reduction and the elimination of BUP active metabolites. Efavirenz’s effects were non-stereospecific. These data reveal novel mechanisms underlying efavirenz DDI with BUP and provide important insights into time- and CYP2B6 genotype dependent DDIs.
Introduction

Efavirenz-based regimens for HIV/AIDS therapy are extensively used worldwide (Gulick et al., 2004), particularly in resource-limited countries (Cluck et al., 2016; WHO, 2021) and millions of stabilized HIV patients continue taking these regimens (Vitoria et al., 2018). However, safe and effective therapy with efavirenz is compromised by narrow therapeutic range (Marzolini et al., 2001), substantial interpatient pharmacokinetic variability (Rotger et al., 2007; Holzinger et al., 2012), and numerous drug-drug interactions (DDIs) that increase the risk for treatment failure and adverse effects of co-administered drugs (BMS, 2017; DHHS, 2022).

CYP2B6 is the principal human hepatic clearance mechanism for efavirenz, with small contributions from other accessory enzymes (Ward et al., 2003; Desta et al., 2007; Belanger et al., 2009; Ogburn et al., 2010). Pharmacogenetic (Desta et al., 2019; Desta et al., 2021) and DDI studies (Cho D-Y, 2016; Desta et al., 2016) have now established the major role CYP2B6 plays in efavirenz clearance and effects.

Efavirenz increases the expression of several drug disposition genes via activation of the constitutive androstane receptor (Faucette et al., 2006; Meyer zu Schwabedissen HE, 2012) and pregnane X receptor (Sharma et al., 2013). Thus, induction of drug metabolism and transport explain many clinically observed efavirenz-mediated DDIs. For example, chronic administration of efavirenz induces CYP2B6 expression (Meyer zu Schwabedissen HE, 2012), thereby enhancing its own metabolism (“autoinduction”) (Zhu et al., 2009; Ngaimisi et al., 2010; Metzger IF, 2013) and the elimination of other CYP2B6 substrates such as methadone (Clarke et al., 2001;
Kharasch ED, 2012) and bupropion (BUP)(Robertson et al., 2008), with large interindividual variability. This variability in autoinduction is mainly dictated by variants in the CYP2B6 gene: no autoinduction and excessive accumulation of efavirenz in poor metabolizer of CYP2B6, and efficient autoinduction in normal and intermediate metabolizers of CYP2B6(Ngaimisi et al., 2011; Metzger IF, 2013; Desta et al., 2019; Desta et al., 2021). The interplay of CYP2B6 genotypes and efavirenz autoinduction is a major determinant of CYP2B6 activity and efavirenz’s exposure, effects and DDIs. For example, poor metabolizers of CYP2B6 were at greater risk for efavirenz inhibition and induction DDI risks and treatment failure for drugs metabolized by enzymes other than CYP2B6 (e.g., contraceptives, lumefantrine, caffeine) (Habtewold et al., 2013; Maganda et al., 2016; Neary et al., 2017; Zakaria and Badhan, 2018; Metzger et al., 2019; Neary et al., 2019; Haas et al., 2020). However, how CYP2B6 genotypes may influence variability in CYP2B6 induction by efavirenz remains unknown. In addition, our previous data show that efavirenz is a potent inhibitor of CYP2B6 in vitro (Xu and Desta, 2013) and the CYP2B6.6 protein is more susceptible to metabolic inhibition than the CYP2B6.1 protein (Xu et al., 2012), but the in vivo relevance of these findings remain unknown.

4-Hydroxylation of BUP (racemic BUP and its enantiomers) is selectively catalyzed by CYP2B6 (Faucette et al., 2000; Hesse et al., 2000; Coles and Kharasch, 2008; Kharasch ED, 2008; Benowitz et al., 2013) and this reaction serves as a valid marker of CYP2B6 activity. Thus, the main aim of this study was to test the hypotheses that acute and chronic administration of efavirenz causes inhibition and induction of
CYP2B6 activity, respectively, as measured by BUP 4-hydroxylation, and CYP2B6 genotypes dictate the extent of the efavirenz-CYP2B6 interaction.

Chronic administration of efavirenz decreases racemic BUP exposure by 55%, with evidence of consequent induction of 4-hydroxylation (Robertson et al., 2008). However, the fraction of BUP metabolized via 4-hydroxylation is relatively small (~21%) (Sager et al., 2016) to mediate this large effect of efavirenz on bupropion exposure. It is conceivable that efavirenz reduces BUP exposure primarily via induction of another important elimination pathway, BUP keto-reduction to threo- and erythro-hydroBUP by 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) and/or other carbonyl reductases is another important elimination pathway of BUP (Meyer et al., 2013; Connarn et al., 2015; Bamfo et al., 2022), but this possibility has not been tested clinically. In addition, BUP (a chiral drug) is clinically used as a racemic mixture of R- and S-BUP that also undergo marked stereospecific hydroxylation and keto-reduction (Masters AR, 2016a; Sager et al., 2016; Costa et al., 2019), generating multiple pharmacologically active diastereomers that contribute to BUP’s clinical effect, adverse effects and CYP2D6-dependent DDIs (Silverstone et al., 2008; Sager et al., 2017; Dash et al., 2018; Costa et al., 2019). Yet, the effect of efavirenz on the stereoselective disposition of BUP is not known. Thus, the secondary aim was to test the effect of efavirenz and CYP2B6 genotypes on the disposition of racemic and stereoisomers of BUP and its active metabolites (4-hydroxyBUP, threo- and erythro-hydroBUP).
Methods

Clinical Study Protocol. In this study, healthy volunteers genotyped for variants in the CYP2B6 gene were administered a cocktail of selective probe drugs for CYP2B6 (BUP), CYP2C8 (montelukast), and OATP1B1/3 and BCRP (rosuvastatin) on three occasions: at baseline (control phase); with a single 600 mg dose of efavirenz (acute inhibition); and after treatment with 600 mg/day efavirenz for 17 days (inhibition/induction). In this manuscript, the data describing the effects of efavirenz and CYP2B6 genetic variation on racemic and stereoselective pharmacokinetics of BUP and its metabolites are reported, while details of the rationale for studying CYP2C8, OATP1B1/3 and BCRP and the findings regarding efavirenz’s effects on montelukast and rosuvastatin disposition will be subject to future publications.

Study participants: Healthy male and non-pregnant (and non-breastfeeding) female (n=24) and male (n=29) volunteers (18 to 49 years old), weighing ≥50 kg and within 32% of their ideal body weight and determined to be healthy through pre-enrollment screening that included medical histories, vital signs, and electrocardiography (EKG), demographic variables, and standard laboratory blood and urine tests were enrolled and completed all study phases. Male and female gender is based on self-identification of the subjects during recruitment and may not necessarily correspond with biological female and male as we sex at birth was not established. Complete inclusion and exclusion criteria are provided in Supplemental Table 1. The Indiana University School of Medicine Institutional Review Board (IRB) approved the study. Each subject signed
an IRB approved written informed consent prior to participation in this study after they read the informed consent and the study was carefully explained to them. During the screening, blood (∼15 ml) and urine samples were collected from each subject for routine clinical laboratory tests. An additional ∼10 ml blood sample was obtained from each subject to extract genomic DNA for genotyping purposes. Subjects were evaluated at a single site: the Clinical Research Center of Indiana University's Clinical Translational Sciences Institute Clinical Research Center (ICRC), located at Indiana University Hospital. The trial was registered at http://www.clinicaltrials.gov (ClinicalTrial.gov identifier: NCT02401256).

**Study Design:** This clinical study was carried out as part of a single site, three-phase, sequential, open-label prospective trial that was designed to test the interplay of variants in the CYP2B6 gene and simultaneous autoinhibition/autoinduction of efavirenz metabolism on efavirenz exposure and efavirenz-mediated drug interactions with CYP2B6, CYP2C8, and OATP1B1 substrates. Healthy volunteers genotyped for common and functionally relevant variants in the CYP2B6 gene were administered a cocktail of selective probe drugs for CYP2B6 (100 mg BUP), CYP2C8 (10 mg montelukast), and OATP1B1/3 and BCRP (5 mg rosuvastatin) on three occasions: at baseline (control phase); with a single 600 mg dose of efavirenz (acute inhibition); and after treatment with 600 mg/day efavirenz for 17 days (inhibition/induction). Volunteers who met all eligibility criteria were enrolled and participated in three phases with a total of three inpatient days (phase 1, 2 and 3) and a total of 15 outpatient visits. The study design is depicted in Figure 1. The cocktail probe drugs (bupropion, montelukast and rosuvastatin) were administered as a single and lowest available dose on three occasions and served as their own
controls, with washout periods of 10-, 6- and 14-days, respectively. The washout period was adequate to avoid carryover effects, if any, on mutual interactions or their interaction with efavirenz because each probe exhibits a relatively short elimination half-life. The probe substrates have no common pathway that is catalyzed by the same enzyme or transported by the same drug transporters (montelukast is a CYP2C8 substrate, rosuvastatin is transported by and OATP1B1/3 and BCRP, and bupropion is metabolized by CYP2B6 and carbonyl reductases). Therefore, mutual interactions of the probe substrates are highly unlikely. All study days and visits were performed at the ICRC. We report here the data that relate to the effects of efavirenz and CYP2B6 genetic variation on racemic and stereoselective pharmacokinetics of BUP and its metabolites in this trial.

Phase 1 (day 1, control phase): Subjects arrived at the Clinical Research Center in the morning (about 7 a.m.) after an overnight fast. An intravenous catheter was inserted in one arm for blood collection. A urine pregnancy test (if female) was obtained. Predose blood (∼10 ml) and urine samples were also obtained. Assessment of the subject’s central nervous system (CNS) symptoms were made using the CNS symptom rating questionnaire that has been developed by the NIAID Adult AIDs Clinical Trial group (Clifford et al., 2005), with slight modification. Participants in our prior trial with efavirenz (ClinicalTrial.gov identifier: NCT00668395) recorded two new CNS symptoms (aggressive behavior and irritability) in their home diaries and during inpatient observation after efavirenz administration. Thus, these previously unidentified CNS symptoms were included in the modified CNS symptom rating questionnaire. Then, a cocktail of probe drugs (100 mg BUP, 10 mg montelukast and 5 mg rosuvastatin) was administered with ∼200 ml water (control phase). A standard meal was served 3 h after dosing, and volunteers were otherwise allowed a regular diet. Blood samples (∼10 ml) were collected via the intravenous
catheter (0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, and 24 hours) or peripheral needle stick (48 and 72 hours) for pharmacokinetic evaluation. All urine voided during the 24-hours ICRC stay was collected at intervals of 0 to 12, and 12 to 24 hours for further metabolism studies. In addition, the 24 to 48 hours urine interval was collected at home. Approximately 20 min before each blood sample collection, subjects filled out the questionnaire. As part of safety measures, sitting blood pressure, respiration rate, oxygen saturation (per oximeter), pulse rate, oral temperature (°F), and EKG were recorded every six hours during the 24-hour inpatient stay at the ICRC. All other drug-related side effects were recorded. Subjects were regularly monitored and questioned for any unusual feelings and were requested to report immediately any unusual feelings. Plasma was separated from blood collections by centrifugation at 3,000 rpm. Two 10-ml urine aliquots were saved from each urine collection interval after the total urine collection volume was recorded. Plasma and urine samples were stored at −80°C until analyses.

Phase 2 (day 11, inhibition phase): Subjects were readmitted to the ICRC for a second time after an overnight fast and underwent the same procedures as in phase 1 except the following differences. First, a cocktail of selective probe drugs was administered 1 hour after dosing with a single 600 mg oral dose of efavirenz to minimize interference of efavirenz with the absorption of the cocktail drugs (or vice versa) and blood samples were collected at 30 min and 1 hour. Second, additional 96- and 120-hour blood samples were collected after efavirenz dosing (compared to phase 1) to better capture the long elimination half-life of efavirenz, while sampling for 72 hours in phase 1 (control phase) covers more than 3-4 times the termination elimination half-life of the probe substrates and deemed sufficient.
Phase 3 (inhibition and induction phase): Immediately after the 120-hour blood sampling of phase 2 (day 16), home efavirenz treatment started on the same day that evening and subjects continued taking efavirenz (600 mg oral dose) every evening for 16 consecutive days (16 to 32 days). On day 16, subjects were supplied with a dosing diary to record the date and time of efavirenz ingestion and any side effects they might experience. Then, volunteers were readmitted to the Clinical Research Center for a third time in the morning of day 33 after an overnight fast. A second urine pregnancy test was performed. A cocktail of selective probe drugs was given 1 hour after the administration of the last dose of efavirenz (600 mg). All other procedures were identical to those in phase 2. The total duration of the study was 38 days. On day 38, an exit exam was performed consisting of a repeat of the screening laboratory tests (including blood and urine tests). The dosing diary and medication bottles with leftover efavirenz (if any) were collected and counted for assessment of compliance.

Quantification of BUP and its metabolites in plasma.

**Chemicals and reagents:** Racemic-, R- and S-BUP (BUP), racemic hydroxyBUP (OHBUP), (2R,3R)-OHBUP (RR-OHBUP), (2S, 3S)-OHBUP (SS-OHBUP), racemic erythrohydroBUP (EHBUP), racemic threohydroBUP (THBUP), (1R,2R)-THBUP (RR-THBUP), and (1S,2S)-THBUP (SS-THBUP) were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). Optically pure standards for racemic EHBUP [(1R,2S)-EHBUP (RS-EHBUP), (1S,2R)-EHBUP (SR-EHBUP)] were not commercially available, thus we used racemic EHBUP. Characterization of
diastereomers of EHBUP has been described previously (Gufford BT, 2016; Masters AR, 2016a; Masters AR, 2016b). Nevirapine (internal standard) was supplied through the National Institutes of Health AIDS Research and Reference Reagent Program (Germantown, MD). Acetaminophen, which was used as an internal standard in samples from 15 subjects, was purchased from Sigma Aldrich Chemical Co. (St. Louis, MO). Laboratory water was prepared for liquid chromatography-tandem mass spectrometry (LC-MS/MS) applications using a Nanopure Infinity UV system (Barnstees/ Thermolyne, Dubuque, IA). Glycine was purchased from Sigma-Aldrich (St. Louis, MO). Plasma from human whole blood (tri-K EDTA, male, drug-free, non-smoker) for standard and quality control preparations was purchased from Biological Specialty Corp. (Colmar, PA). Methanol, acetic acid, ethyl acetate, ammonium bicarbonate, and ammonium hydroxide, sodium hydroxide were purchased from Fisher Scientific Company LLC (Hanover Park, IL). All the other solvents and chemicals were purchased from Fishers Scientific (Hampton, NH) and were of high-performance liquid chromatography/mass spectrometry (LC/MS/MS) grade or higher.

High Performance Liquid-Chromatography Tandem Mass Spectrometry (LC-MS/MS) Method Development: Plasma BUP and its metabolites as well as the corresponding R- and S- BUP, R,R- and S,S- hydroxyBUP, R,R- and S,S- threo-hydroBUP, (1R, 2S) and (2S, 1R) erythro-hydroBUP from 15 subjects were initially quantified using a validated LC-MS/MS (5500 QTRAP® AB Sciex, Framingham, MA) as previously reported by our group (Masters AR, 2016a). Briefly, 50 µL of plasma was utilized for the assay with acetaminophen added as the internal standard, followed by liquid-liquid
extraction with ethyl acetate. Separation of all analytes was achieved using a Lux Cellulose-3 chiral column, 250 x 4.6mm, 3 micron (Phenomenex, Torrance, CA). The assay details are as listed in our previous publication (Masters AR, 2016a).

However, due to the prolonged separation time required for this method (Masters AR, 2016a), we developed a new LC-MS/MS method with better chromatographic resolution of the stereoisomers and short retention times of BUP and its metabolites. In brief, an API 3200 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization (ESI) source and coupled to a high-performance liquid chromatography system consisting of two LC-20AD pumps, SIL-20AHT UFLC autosampler, DGU-20A3 degasser, and a CBM-20A system controller (Shimadzu, Columbia, MD) was used. Chromatographic separation was achieved using an AMP column (150 X 4.6 mm i.d.; 3-µm particle size; Phenomenex, Torrance, CA) and mobile phase consisting of methanol (mobile phase B) and 5mM ammonium bicarbonate (pH 11 adjusted by ammonium hydroxide, mobile phase A) using the following gradient: initial conditions of 60% mobile phase B between 0.01-3 minutes was changed to 75% mobile phase B at 3.01 minute followed by a linear gradient to 95% mobile phase B between 3.01 and 12 minutes, then re-equilibrated to initial conditions at 12.01 minutes and continued until 13 minutes using a total flow rate of 0.8 ml/min.

Data acquisition and processing were performed using Analyst software (version 1.5.1, AB SCIEX). Analyte concentrations were quantified using Analyst software by interpolation from matrix-matched calibration curves and quality controls with dynamic assay ranges of 0.1–1000 ng/ml for all analytes. All data were collected in positive ion mode. Parameters were set according to standard drugs flow injection analysis results.
Matrix-matched calibration curves were generated to directly quantify BUP and all metabolites using the available standards with a dynamic assay range of 0.1-1000 ng/ml. The responses of the analytes were optimized at a source temperature of 600°C, under unit resolution for quadrupole 1 and 3. In addition, the analytes were given a dwell time of 100 msec and a settling time of 10 msec. The ion spray voltage was 5500 V, and the interface heater was on. Optimal gas pressures for all the analytes were: collision gas medium, curtain gas 25, ion source gas (1) 35, and ion source gas (2) 40.

Multiple reaction monitoring was used to measure Q1/Q3 transitions for: BUP, R- and S-BUP at 240.1/184.0; OHBUP, RR- and SS-OHBUP at 255.9/238.0; EHBUP, RS- and SR-EHBUP as well as THBUP, RR- and SS-THBUP at 241.9/168.0; and nevirapine (internal standard) at 267.5/226.1. This new LC-MS/MS method was used to quantify plasma samples from the remaining 38 subjects.

**Plasma liquid-liquid extraction:** An aliquot of each plasma sample (200 µL) was transferred to a culture tube with screw cap and 25 µl of 500 ng/ml nevirapine was added as internal standard. After vortex mix, 200µL Glycine/NaOH (pH 11.3) followed by 6 mL ethyl acetate was added for liquid-liquid extraction. The sample was shaken for 15 minutes and then centrifuged at 3600 rpm at 4 °C for 10 minutes. The resulting supernatant was transferred to a test tube and dried under a vacuum. The dried sample was reconstituted in 50 µL methanol and 10 µL was injected into the LC-MS/MS system. Calibration curves were generated by addition of known concentrations of the analytes (0 to 300 ng/ml) into blank human plasma. After the addition of internal standards, standards were extracted as described above. The standard curves were linear over the range of 1 to 300 ng/ml. Inter- and intra-day assay accuracy for the new
LC/MS/MS method was evaluated using Quantitate software. Standard and quality control samples were deemed acceptable if within 20% and 10% of the nominal value, respectively, while the precision was greater than >90% (% CV<10). The accuracy and precision of the old assay was described in our earlier publication (Masters AR, 2016a).

**CYP2B6 Genotyping**

Genomic DNA was extracted at the Indiana University Clinical Translational Sciences Laboratory from whole blood using standard protocol. DNA CYP2B6 genotyping for rs3745274 (516G>T, Q172H), rs28399499 (983T>C, I328T), and rs2279343 (785A>G, K262R) was performed by use of the pre-developed TaqMan Assay-Reagents Allelic Discrimination Kits (rs3745274 and rs28399499) according to the supplier's instructions (Applied Biosystems, Foster City, CA) or by use of a custom TaqMan Genotyping Assay after first amplifying exon 5 with primers 5′-CTCTCTCCCTGTGACCTGCTA-3′ (forward) and 5′-CTCCCTCTGTCTTTTCATTCTGTC-3′ (reverse) (Integrated DNA Technologies, Coralville, IA) (rs2279343) as described by our previous publications (Burgess et al., 2017; Robarge et al., 2017; Metzger et al., 2019). Polymerase chain reaction (PCR) was performed on BioRad iCycler and QuantStudio 12K Flex real-time PCR instruments. CYP2B6 star allele designations were assigned in accordance with the Pharmacogene Variation (Pharmvar) Consortium [https://www.pharmvar.org/gene/CYP2B6](https://www.pharmvar.org/gene/CYP2B6). Genotype groups considered for the analysis were normal metabolizer (NM) (*1/*1 genotype, n = 19), intermediate metabolizer (IM) (*1/*6 genotype, n = 27), and poor metabolizer (PM) (*6/*6* genotype, n = 6).

**Pharmacokinetic Analysis**
Non-compartmental analysis of data was performed using Phoenix® WinNonlin® (version 7.0, Pharsight Corp., Cary, NC). Pharmacokinetic outcomes for analysis included: $K_{el}$ (elimination rate constant), $t_{1/2}$ (half-life, $t_{1/2} = 0.693/K_{el}$), $C_{max}$ (maximal plasma concentration), $t_{max}$ (time of maximal plasma concentration), $AUC_{0-\infty}$ (area under the plasma concentration time curve from zero to infinity), apparent volume of distribution ($V_d/F$) was calculated by apparent oral clearance/$K_{el}$, and apparent oral clearance (calculated by $(CL/F) = \text{dose}/\text{AUC}$). The terminal elimination rate constant ($\lambda_z$) was estimated by linear regression of the terminal portion of the log-transformed concentration-time profile using at least three data points. The terminal half-life ($t_{1/2}$) was calculated as $\ln(2)/\lambda_z$. The $C_{max}$, $t_{max}$, and last measured concentration ($C_{last}$) were recovered directly from the concentration-time profile. Area under the curve from time zero to $C_{last}$ ($AUC_{0-last}$) was determined using the trapezoidal rule with linear up/log down interpolation. The AUC from time zero to infinity ($AUC_{0-\infty}$) was calculated as the sum of $AUC_{0-last}$ and the ratio of $C_{last}$ to $\lambda_z$. $AUC_{0-\infty}$ values reported for analytes where the extrapolation percentage was more than 30% are denoted accordingly. Statistical data comparisons between the pharmacokinetic outcomes were evaluated using the two one-sided testing procedure according to the 2001 FDA Guidance to Industry on Statistical Approaches to Establishing Bioequivalence (FDA, 2001). This approach is based on testing whether the 90% confidence interval for the ratio of the averages (population geometric means) of the pharmacokinetic measures (average bioequivalence) were comparable between the treatment groups, i.e., within 80-125% for the ratio of treatment averages. A p-value <0.05 was considered statistically significant.
Results

The enrollment report is depicted in Supplemental Figure 1 and shows that 53 subjects completed the entire study phase, and 17 subjects partially completed the study phases. Data from those participants who fully completed all phases of the study are presented and the demographic characteristics of these participants are listed in Supplemental Table 2.

Effect of EFV on the disposition of racemic BUP and its metabolites.

Racemic BUP and metabolite concentration-time profiles and the corresponding pharmacokinetic parameters derived (n=53 healthy volunteers) following a single oral dose of BUP (100 mg) given alone (control phase) or 1 hour after a single oral efavirenz (600 mg) dose (inhibition phase) or following 17-day treatment with efavirenz (induction phase) are presented in Figure 2. In Table 1, the corresponding pharmacokinetic parameters [geometric mean and 90% confidence interval (CI)] in the control, inhibition, and induction phases as well as the geometric mean ratios (GMR) in percent with 90% CI for the inhibition phase (inhibition/control) and induction phase (induction/control) are shown.

In the inhibition phase, GMR values for the exposure (C_{max}, AUC_{0-24} and AUC_{0-∞}) of racemic OHBUP was slightly but significantly decreased (by 11% to 25%) when compared to the control phase; no difference was noted in t_{1/2}. The 90% CI of the GMR values for any of the pharmacokinetic parameters of BUP, EHBUP and THBUP were
within the no-effect boundaries (80% – 125%) except for a slight increase in THBUP (Figure 2; Table 1).

Conversely, the GMR values for $\text{AUC}_{0-24}$ and $C_{\text{max}}$ of OHBUP was significantly increased (by 25% and 46%, respectively) and $t_{1/2}$ was significantly shortened (by 22%) in the induction phase compared with the control phase. No difference was observed regarding $\text{AUC}_{0-\infty}$. In contrast, the GMR values for the plasma exposure ($\text{AUC}_{0-24}$, $\text{AUC}_{0-\infty}$ and $C_{\text{max}}$) of BUP, THBUP, and EHBUP in the induction phase were all significantly lower compared to the control phase (by 20% to 55%), and half-lives were shortened except that of BUP which did not change significantly (Figure 2; Table 1).

**Effect of EFV on stereoselective disposition of BUP and its metabolites.**

Concentration-time profiles and the corresponding pharmacokinetic parameters ($n=53$ healthy volunteers) of stereoisomers of BUP and its metabolites following the control, inhibition, and induction phases are shown in Figure 3. In Table 2, the corresponding pharmacokinetic parameters of all phases as well as the GMR in percent with 90% CI for the inhibition phase (inhibition/control) and induction phase (induction/control) are shown.

In the inhibition phase, the GMR values for $\text{AUC}_{0-24}$, $\text{AUC}_{0-\infty}$, and $C_{\text{max}}$ of both SS- and RR-OHBUP were modest but significantly lower (by 11-26%) compared to BUP alone, without difference in their elimination half-lives. In contrast, the GMR values for SS-THBUP exposure ($\text{AUC}_{0-24}$, $\text{AUC}_{0-\infty}$ and $C_{\text{max}}$) after a single dose of efavirenz was modest but significantly higher (by 18-19%), while the $t_{1/2}$ was prolonged (by 35%), compared to control phase (Figure 3 and Table 2). The pharmacokinetic parameters of
S- and R-BUP, SR- and RS-EHBUP, and RR-THBUP in the inhibition phase were not significantly affected compared to the control phase, except for a slight effect on t_{1/2} of RR-THBUP. In the induction phase, the 90% CI of the GMR values for (AUC_{0-24}, and C_{max}) of SS- and RR-OHBUP was outside the no-effect boundaries and significantly increased (by 24-61%) and the terminal elimination half-lives shortened (by 17-25%) compared to the control phase; AUC_{0-\infty} values were within the no effect range (Figure 3; Table 2). In contrast, GMR values for AUC_{0-24}, AUC_{0-\infty} and C_{max} of S- and R-BUP, SR- and RS- EHBUP, and RR- and SS-THBUP in the induction phase were significantly decreased (by 43-66%, 24-49%, and 17-58%, respectively) compared to the control phase. The half-lives of SR-EHBUP, RS- EHBUP, and RR-THBUP were significantly shortened in the induction phase except that of SS-THBUP which was significantly prolonged (by 33%) compared to the control phase; the half-lives of R-and S-BUP were within the no-effect boundaries (Figure 3; Table 2).

**Effect of EFV and CYP2B6 genotypes on the disposition of racemic BUP and its metabolites.**

Concentration-time profiles of hydroxyBUP for in the control, inhibition, and induction phase [n=52 total; 19 normal (NM), 27 intermediate (IM), 6 poor metabolizers PM] are shown in Figure 4. The corresponding pharmacokinetic parameters [geometric mean and 95% confidence interval (CI)] in the control, inhibition, and induction phases as well as the geometric mean ratios (GMR) in percent with 90% CI for the inhibition phase (inhibition/control) and induction phase (induction/control) are listed in Table 3.
CYP2B6 genotypes were associated with OHBUP exposure in a gene-dose effect manner: highest in NM and lowest in PM irrespective of the treatment phase (Figure 4, Table 3). The plasma exposure of OHBUP was significantly lower in PM than in NM and IM metabolizers (Figure 4, Table 3). In the induction phase, the exposure of BUP, EHBUP, and THBUP was significantly higher in PM than NM of CYP2B6 (Table 3).

In the inhibition phase, GMR values for OHBUP C\textsubscript{max} and AUC\textsubscript{0-24} were significantly lower (by 16.7% to 28.4%) in NM and IM metabolizers of CYP2B6 but not in PM (Figure 4 inset: Table 3). The 90% CI of GMR values for the other pharmacokinetic parameters of OHBUP as well as those of S- and R-BUP, SR- and RS-EHBUP, and RR- and SS-THBUP were within the no effect boundaries except for AUC\textsubscript{0-24} of BUP in NM, AUC\textsubscript{0-∞} of OHBUP in IM metabolizer and t\textsubscript{1/2} of THBUP which were outside the boundaries (Table 3). In the induction phase, GMR values for C\textsubscript{max} and AUC\textsubscript{0-24} of OHBUP were significantly increased (by 40-60% and 20-40%, respectively) in NM and IM of CYP2B6 only; t\textsubscript{1/2} was shortened in intermediate metabolizer of CYP2B6 (Figure 4; Table 3). The 90% CI of the GMR values in the induction phase compared to the control phase were outside the no effect range (significantly lower GMR values) for: C\textsubscript{max}, AUC\textsubscript{0-24} and AUC\textsubscript{0-∞} of BUP in NM and IM (by 45.4% to 77%); C\textsubscript{max} and AUC\textsubscript{0-24} of EHBUP (by 24.6% to 51.3%) and THBUP (by 19.5% to 53.8%) in NM and IM; AUC\textsubscript{0-∞} of EHBUP and THBUP (by a range of 28.5% to 53.8%) NM, IM and PM; t\textsubscript{1/2} of EHBUP (NM, IM, and PM) and THBUP (NM and IM) (Figure 4; Table 3).

Plasma metabolic ratios (OHBUP/BUP) versus time profiles (0-120 hours, upper panel; 0-24 hours lower panel) are illustrated in Suppl Fig. 2. Substantially higher MRs during the induction phase was observed in NM and IM of CYP2B6 compared to the control.
and inhibition phase, while the metabolic ratios during the inhibition phase overlapped with that of control phase. The metabolic ratios in PM of CYP2B6 were substantially lower than in normal and intermediate metabolizers. The ratios overlapped among treatment phases (control, inhibition, and induction) (Suppl Fig. 2)

**Effect of EFV stratified by CYP2B6 genotypes on the stereoselective disposition of BUP and its metabolites.** The effect of efavirenz and CYP2B6-genotypes on the pharmacokinetic parameters of stereoisomers of BUP and its metabolites following control, inhibition, and induction phases) (n=52 total; 19 normal, NM; 27 intermediate, IM; and 6 poor metabolizers, PM, of CYP2B6) was determined. CYP2B6 genotype-dependent geometric mean concentration-time profiles of RR- and SS-OHBUP is presented as a representative plot in Figure 5. The pharmacokinetic parameters [geometric mean and 95% confidence interval (CI)] in the control, inhibition, and induction phases as well as the geometric mean ratios (GMR) in percent with 90% CI for the inhibition phase (inhibition/control) and induction phase (induction/control) were determined for each genotype group (Table 4).

In the inhibition phase, the GMR values were slightly but significantly lower for the: C\text{max} and AUC\textsubscript{0-24} of RR- and SS-OHBUP (range by 14.2-28.5%) in NM and IM of CYP2B6; AUC\textsubscript{0-24} of SS- and RR-OHBUP (IM); C\text{max}, and AUC\textsubscript{0-24} of BUP (NM); and AUC\textsubscript{0-24} (NM), AUC\textsubscript{0-∞} and t\textsubscript{1/2} (both in IM) of RR-THBUP. In contrast, the GMR values were higher (range by 14-33%) for SS-THBUP C\text{max} (IM), AUC\textsubscript{0-24} (IM and PM), AUC\textsubscript{0-∞} (NM, IM, and PM); t\textsubscript{1/2} was shorter by 28-49% (NM and IM) (Table 4). Other pharmacokinetic parameters of the analytes remained within the no-effect boundaries in the inhibition phase versus the control phase (Table 4). In the induction phase, the 90% CI of GMR
values was outside the no-effect boundaries (the GMR values were significantly lower by 48-77% and 37-77%, respectively) for S-BUP and R-BUP $C_{\text{max}}$, AUC$_{0-24}$, and AUC$_{0-\infty}$ (NM and IM but not in PM of CYP2B6) (Table 4). Conversely, the GMR values for RR- and SS-OHBUP $C_{\text{max}}$ and AUC$_{0-24}$ were significantly increased (by 21-66%) in NM and IM of CYP2B6, while $t_{1/2}$ values for RR-OHBUP (NM and IM) and SS-OHBUP (IM) was significantly shortened (Table 4). Lower GMR values were observed in the induction phase for $C_{\text{max}}$ and AUC$_{0-24}$ of RS- and SR-EHBUP and RR- and SS-THBUP (NM and IM); AUC$_{0-\infty}$ of SR-EHBUP and RR-THBUP (all genotypes) and RS-EHBUP and SS-THBUP (NM and IM). The $t_{1/2}$ of SR- and RS-EHBUP (IM and PM) and RR-THBUP (NM and IM) was shorter. In contrast, the GMR values for $t_{1/2}$ of SS-THBUP was increased significantly in NM and IMs of CYP2B6.

In the Suppl Fig 3: a) metabolic ratios (OHBUP/BUP) SS- and RR-OHBUP versus time profiles are illustrated and indicate substantially higher MRs during the induction phase in normal and intermediate metabolizers; b) MRs during the inhibition phase overlapped with that of control phase for both SS- and RR-OHBUP; and c) MRs were substantially lower in poor metabolizer compared to normal and intermediate metabolizers. No difference in MRs was observed among the control, inhibition, and induction phases in poor metabolizer.

The $C_{\text{max}}$ and AUC$_{0-24}$ metabolic ratios (OHBUP/BUP, SS-OHBUP/S-BUP, and RR-OHBUP/R-BUP) in each genotype (normal, intermediate, and poor metabolizer) and in each treatment phases (control, inhibition, and induction phases) were determined as a measure of CYP2B6 activity (Figure 6). Bars and error bars denote the geometric mean ratio and upper limits of the 95% confidence interval, respectively. The MRs (both $C_{\text{max}}$
and AUC₀-2₄) were altered in gene dose effect manner (i.e., higher in NM followed by IM and then PM of CYP₂B₆) consistently in all treatment phases (control, inhibition, and induction); CYP₂B₆ activity was not induced by efavirenz in poor metabolizers.

In Supplemental Figure 4, the ratios of R-BUP/S-BUP, RR-OHBUP/SS-OHBUP, RR-THBUP/SS-THBUP, and SR-EHBUP/RS-EHBUP ratios (0-120 hour and 0-24 hour) are presented. The effect of efavirenz on the disposition of BUP and its metabolites was nonstereospecific as shown by the overlapping ratios of each corresponding stereoisomer pair among the treatment phases ((control, inhibition, and induction phases) (Suppl Figure 4)). The ratios also remained within each CYP₂B₆ genotype group among the treatment phases (data not shown). Although a slight difference in the disposition of SS- and RR-THBUP was observed during the inhibition phase, the effect was too small to reveal stereospecific interaction.
Discussion

Efavirenz (a substrate, inducer, and inhibitor of CYP2B6) is hypothesized to influence CYP2B6 activity, efavirenz exposure and DDI risks with other substrates of CYP2B6 and those drugs metabolized by enzymes other than CYP2B6 via complex time- and CYP2B6 genotype-dependent mechanisms. In this study, we determined the effects of a single dose and multiple doses of efavirenz as well as CYP2B6 genotypes on the complex disposition of racemic and stereoisomers of BUP and its active metabolites. We have shown previously that efavirenz is a strong inhibitor of CYP2B6 activity in vitro (Xu and Desta, 2013). The current findings provide the first in vivo evidence that acute administration of efavirenz inhibits CYP2B6 activity in a genotype dependent manner as reflected by significantly lower exposure of OHBUP and its diastereomers in NM and IM of CYP2B6 but not in PM of CYP2B6. These data contrast with our previous in vitro data showing that the CYP2B6*6 allele is more susceptible to inhibition by voriconazole than CYP2B6*1/*1 (Xu et al., 2012). This discrepancy could be due to differences in the substrate (efavirenz) and inhibitor (voriconazole) used in vitro (Xu et al., 2012) compared to the in vivo substrate (BUP) and inhibitor (efavirenz) (current data). Alternatively, the in vitro data are simply poor predictors of in vivo outcomes. In contrast, the exposure of THBUP and SS-THBUP (but not that of RR-THBUP) was increased during the inhibition phase, and this effect was greater in PM and/or IM of CYP2B6. Efavirenz is a strong inhibitor of enzymes (e.g., UGT2B7 and CYP2C19) (Belanger et al., 2009; Ji et al., 2012; Xu and Desta, 2013) catalyzing further metabolism of SS-THBUP (Zhu et al., 2014; Gufford BT, 2016; Sager et al., 2016) and may inhibit the elimination of SS-THBUP in a concentration dependent manner.
Together, acute administration of efavirenz inhibits CYP2B6 and the metabolism of SS-THBUP in CYP2B6 genotype dependent manner. This acute inhibition is offset by a net induction during chronic dosing with efavirenz (see below), although the short-term safety of CYP2B6 substrates with narrow therapeutic range may be altered when efavirenz-based HIV therapy is added to patients stabilized on CYP2B6 substrates.

Our data show that chronic treatment with efavirenz strikingly reduced the exposure of racemic-, S- and R-BUP, while the exposure of racemic OHBUP and its diastereomers as well as the respective metabolite/parent ratios were significantly increased. The chronic effect of efavirenz on racemic BUP and OHBUP confirm previous findings (Robertson et al., 2008). However, ours is the first to demonstrate substantial effect on stereoisomers of BUP and OHBUP and their metabolic ratios, providing important mechanistic insights. Chronic efavirenz activates the constitutive androstane receptor (Faucette et al., 2006; Meyer zu Schwabedissen HE, 2012) and pregnane X receptor (Sharma et al., 2013) and thereby increases the expression of several drug disposition genes including CYP2B6 (Meyer zu Schwabedissen HE, 2012; Sharma et al., 2013). Although simultaneous acute inhibition/chronic induction of CYP2B6 may occur with chronic administration of efavirenz, the net average effect was induction. The extent of CYP2B6 induction by efavirenz exhibited extensive variability among individuals. We demonstrated for the first time that this variability is dictated by CYP2B6 genotypes. Compared to the control phase, significantly higher exposure of OHBUP, its diastereomers and the respective metabolic ratios was observed after pretreatment with efavirenz in NM>IM, with no (or marginal) difference in PM of CYP2B6. The direction of CYP2B6 genotype dependent interaction mirrored interplay between CYP2B6
genotypes and efavirenz autoinduction (Ngaimisi et al., 2011; Metzger IF, 2013; Desta et al., 2019; Desta et al., 2021). Other inducers such as rifampin (Li et al., 2010), carbamazepine (Zhu et al., 2009), and sodium ferulate (Gao et al., 2013) also appear to induce this enzyme in a CYP2B6 genotype dependent manner, suggesting that this phenomenon may not be unique to efavirenz alone and thus the data may be generalizable to other DDIs mediated by CYP2B6 induction. Together, chronic administration of efavirenz may alter safety or efficacy of CYP2B6 substrates by enhancing bioactivation (e.g., BUP 4-hydroxylation) (present data; (Robertson et al., 2008)) or systemic clearance (e.g., methadone) (Kharasch et al., 2012) in NMs and IMs but not in PMs of CYP2B6.

The mechanism by which efavirenz causes large decrease in the exposure of BUP and its enantiomers is unlikely due to induction of CYP2B6 mediated BUP 4-hydroxylation as the fraction metabolized by CYP2B6 under basal conditions is small ( ~21%) (Sager et al., 2016). This is further supported by the lack of meaningful effect of modulators of CYP2B6 on BUP systemic clearance despite their marked effect on OHBUP exposure (present data from the inhibition phase; (Eum et al., 2022)). Our recent data (Bamfo et al., 2022) and findings from other authors (Sager et al., 2016) support that BUP reduction, particularly to THBUP and SS-THBUP, represents the major clearance mechanism of BUP and its enantiomers in vitro. Therefore, we speculate that efavirenz’s chronic effect on the systemic elimination of BUP and its enantiomers is primarily via induction of one or more enzymes that catalyze BUP reduction, typically 11β-HSD1 and/or aldo-keto-reductase located in the liver and intestine (Meyer et al., 2013; Connarn et al., 2015; Bamfo et al., 2022). The extent of decrease in exposure of
racemic-, R- and S-BUP by efavirenz was greater for NM and IMs compared to PMs of CYP2B6, reflecting a potential increase in fraction metabolized via 4-hydroxylation during induction in NM and IM but not PM of CYP2B6. Induction of the reductive pathways of BUP by efavirenz have not been reported before and this finding may reveal novel efavirenz DDI mechanisms.

Unlike the exposure of OHBUP and its diastereomers, we noted a substantial reduction in the exposure of THBUP and EHBUP and their diastereomers following chronic efavirenz. A similar observation was observed with another inducer carbamazepine (Ketter et al., 1995). Since a substantial decrease in the clearance of BUP was shown with efavirenz and carbamazepine, inhibition of BUP reductive pathways cannot explain the significantly reduced exposure of the reductive BUP metabolites. Instead, efavirenz likely causes induction of secondary metabolism of BUP reductive metabolites. All BUP metabolites (OHBUP, THBUP and EHBUP) and their stereoisomers undergo conjugation via UGTs (mainly UGT1A9 and UGT2B7)(Gufford BT, 2016). Additionally, THBUP and EHBUP undergo 4-hydroxylation via CYP2C19 (Zhu et al., 2014; Sager et al., 2016). These enzymes (UGTs and CYP2C19) are inducible by efavirenz in vivo(Cho et al., 2011; Michaud V, 2012). Our data showing a more rapid terminal elimination of racemic and stereoisomers of OHBUP (following the early increase in maximal plasma concentrations), THBUP and EHBUP as well as the substantial reduction in exposure of BUP reductive metabolites by chronic efavirenz provide support for induction of secondary metabolic pathways of BUP. Of note, the elimination rate appears to be higher than the formation rate for the reductive metabolites, probably due to induction via both CYP2C19(Zhu et al., 2014; Sager et al., 2016) and UGTs(Gufford BT, 2016),
while the formation rate OHBUP appears to be greater than its elimination indicating a
greater rate of induction of CYP2B6 than the rate of elimination via UGTs (Gufford BT, 2016).

Our data from both the inhibition and induction phases provide no evidence that the
effects of efavirenz and CYP2B6 on the disposition of BUP and its metabolites is
stereospecific (Suppl Figure 4). Stereoselective induction of OHBUP by rifampin was
suggested previously (Xu et al., 2007; Kharasch ED, 2008). Although there appears to
be a crosstalk, the relative activation of CAR and PXR by efavirenz and rifampin in the
liver and gut appear to differ (Faucette S, 2006; Meyer zu Schwabedissen HE, 2012).
Thus, it is possible that efavirenz and rifampin differ in their ability to induce the complex
primary and/or secondary elimination pathways or other BUP disposition pathways.

In summary, efavirenz exhibits complex, time- and CYP2B6 genotype-dependent
interactions with BUP disposition. Acute and chronic administration of efavirenz inhibits
and induces CYP2B6 activity, respectively, and the extent of these effects were
CYP2B6 genotype dependent. Chronic efavirenz increases the rate of elimination of 1)
BUP and its enantiomers, predominantly via induction of BUP reductive pathways, and
2) all BUP metabolites and their diastereomers via induction of sequential metabolic
pathways. The effects of efavirenz and CYP2B6 genotypes on the disposition of BUP
and its metabolites were nonstereoselective. Overall, these findings provide important
mechanistic and clinical insights. First, these data reveal additional new mechanisms
underlying efavirenz DDIs with BUP. Second, 4-hydroxylation of racemic BUP and its
enantiomers represents an acceptable, though complex, in vivo probe for ascertaining
the effect of genetic and nongenetic factors dictating CYP2B6 activity at basal and
induced conditions. Third, altered metabolic patterns may have implications for the safe and optimal use of BUP when prescribed with inducers given that BUP’s in vivo effects, toxicity and DDI with CYP2D6 are mediated by BUP and its active metabolites (Silverstone et al., 2008; Sager et al., 2017; Dash et al., 2018; Costa et al., 2019). Lastly, the complex time- and genotype-dependent DDI mechanisms observed in this study highlight important study design considerations when characterizing future additional efavirenz DDIs.
Authorship Contributions

**Participated in research design:** Zeruesenay Desta, Brandon T. Gufford

**Conducted experiments:** Eric A. Benson, Andrea R. Masters, Jessica Bo Li Lu, Ingrid F. Metzger

**Contributed new reagents or analytical tools:** Andrea R. Masters

**Performed data analysis:** Ingrid F. Metzger, Nadia O. Bamfo, Zeruesenay Desta, Brandon T. Gufford

**Wrote or contributed to the writing of the manuscript:** Zeruesenay Desta, Jessica Bo Li Lu, Brandon T. Gufford, Nadia O. Bamfo, Ingrid F. Metzger, Andrea R. Masters
References


metabolic pathways and cytochrome P450 (CYP) 2A6 as the principal catalyst of efavirenz 7-hydroxylation. *Drug Metab Dispos* **38:**1218-1229.


Footnotes

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c. No author has an actual or perceived conflict of interest with the contents of this article.
**Figure Legends**

**Figure 1.** Clinical Study Design. *Bupropion (100 mg) given as part of a probe drug cocktail that included montelukast and rosvastatin.

**Figure 2.** Racemic bupropion (BUP) and metabolites concentration-time profiles (n=53 healthy volunteers) following a single oral dose of bupropion (100 mg) given alone (Control) or 1 hour after a single oral efavirenz (600 mg) dose (Inhibition) or following 17-day treatment with efavirenz (Induction). Symbols and error bars denote geometric means and the limits of the 95% confidence interval, respectively. Inset, represent concentrations up to 24-hour post dosing. OHBUP, 4-hydroxyBUP; THBUP, threohydroBUP; and EHBUP, erythrohydroBUP.

**Figure 3.** Stereoselective bupropion (BUP) and metabolite concentration-time profiles (n=53 healthy volunteers) following a single oral dose of bupropion (100 mg) given alone (Control) or 1 hour after a single oral efavirenz (600 mg) dose (Inhibition) or following 17-day treatment with efavirenz (Induction). Symbols and error bars denote geometric means and the limits of the 95% confidence interval, respectively. Inset, represent concentrations up to 24-hour post dosing. RR- and SS-OHBUP, RR- and SS-hydroxyBUP; RR- and SS-THBUP, RR- and SS-threohydroBUP; and SR- and RS-EHBUP, SR- and RS-erythrohydroBUP.

**Figure 4.** CYP2B6 genotype-dependent geometric mean concentration-time profiles of racemic hydroxybupropion (OHBUP) following a single oral dose of bupropion (100 mg) given alone (Control) or 1 hour after a single oral efavirenz (600 mg) dose (Inhibition) or
following 17-day treatment with efavirenz (Induction) (n=53 total) in extensive
(CYP2B6*1/*1, n=20), intermediate (CYP2B6*1/6, n=27) and poor (CYP2B6*6/*6, n=6)
metabolizers. Upper, concentrations up to 120 hours post dosing; and below,
concentrations up to 24 hours post dosing. Symbols and error bars denote geometric
means and the limits of the 95% confidence interval, respectively.

**Figure 5.** CYP2B6 genotype-dependent geometric mean concentration-time profiles of
R,R- and S,S-hydroxyBUP (OHBUP) following a single oral dose of bupropion (100 mg)
given alone (Control) or 1 hour after a single oral efavirenz (600 mg) dose (Inhibition) or
following 17-day treatment with efavirenz (Induction) (n=52 total) in extensive
(CYP2B6*1/*1, n=20), intermediate (CYP2B6*1/6, n=27) and poor (CYP2B6*6/*6, n=6)
metabolizers. Inset, represent concentrations up to 24-hour post dosing. Upper panel,
RR-OHBUP; and lower panel, SS-OHBUP. Symbols and error bars denote geometric
means and the limits of the 95% confidence interval, respectively.

**Figure 6.** Genotype-dependent metabolite: parent ratios (n=53) of AUC$_{0-24}$ and C$_{\text{max}}$ in
20 normal metabolizers (NM), 27 intermediate metabolizers (IM) and 6 poor
metabolizers (PM) of CYP2B6. AUC$_{0-24}$ and C$_{\text{max}}$ ratios, respectively, for R,R-
hydroxyBUP (R, R-OHBUP)/R-bupropion (BUP) (A, B); for S, S-hydroxyBUP (S,S-
OHBUP)/S-bupropion (S-BUP) (C, D); and for racemic hydroxyBUP
(OHBUP)/bupropion (BUP) (E, F). Bars and error bars denote the geometric mean ratio
and upper limits of the 95% confidence interval, respectively.
### Table 1. Pharmacokinetic parameters of racemic bupropion and its metabolites.

<table>
<thead>
<tr>
<th></th>
<th>Geometric Mean [90% CI]</th>
<th>Geometric Mean Ratio % [90% CI]</th>
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<tr>
<td></td>
<td>Control Phase</td>
<td>Inhibition Phase</td>
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<tr>
<td><strong>Bupropion</strong></td>
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<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (nM*h)</td>
<td>4160 [3740-4620]</td>
<td>4140 [3680-4660]</td>
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<tr>
<td><strong>Hydroxybupropion</strong></td>
<td></td>
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<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (nM*h)</td>
<td>47600 [41600-54300]</td>
<td>42400 [36800-48800]</td>
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<tr>
<td><strong>Erythrohydrobupropion</strong></td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (nM)</td>
<td>107 [97.2-117]</td>
<td>105 [94.9-117]</td>
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<tr>
<td><strong>Threohydrobupropion</strong></td>
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<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>35.4 [32.2-39]</td>
<td>33 [30.3-36]</td>
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</table>

Area under the plasma concentration versus time curve (AUC), maximal plasma concentration (C<sub>max</sub>), time to reach C<sub>max</sub> (T<sub>max</sub>), and terminal elimination half-life (t<sub>1/2</sub>) determined via non-compartmental analysis of untransformed data. Ratios and corresponding confidence intervals calculated using Phoenix WinNonlin (v7.0); confidence intervals excluding 100% considered statistically significant. AUC<sub>0-48</sub>, area under the concentration-time curve from 0 to 48 hours; AUC<sub>0-∞</sub>, AUC from 0 to infinite time.
Table 2. Stereoselective Bupropion Pharmacokinetic Outcomes.

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<th>Geometric Mean [90% CI]</th>
<th>Geometric Mean Ratio % [90% CI]</th>
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<tr>
<td></td>
<td>Control Phase</td>
<td>Inhibition Phase</td>
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<tr>
<td>(R)-bupropion</td>
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<tr>
<td>(S)-bupropion</td>
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<tr>
<td>(R, R)-hydroxybupropion</td>
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<tr>
<td>AUC$_{0-\infty}$ (nM*h)</td>
<td>34800 [31900-37900]</td>
<td>31100 [28300-34100]</td>
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<tr>
<td>(S, S)-hydroxybupropion</td>
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<tr>
<td>C$_{\text{max}}$ (nM)</td>
<td>98.2 [87.5-110]</td>
<td>78.5 [68.8-89.6]</td>
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<tr>
<td>Substance</td>
<td>$t_{1/2}$ (h)</td>
<td>AUC$_{0-24}$ (nM*h)</td>
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Area under the plasma concentration versus time curve (AUC), maximal plasma concentration ($C_{max}$), time to reach $C_{max}$ ($T_{max}$), and terminal elimination half-life ($t_{1/2}$) determined via non-compartmental analysis of untransformed data. Ratios and corresponding confidence intervals calculated using Phoenix WinNonlin (v7.0); confidence intervals excluding 100% considered statistically significant. AUC$_{0-48}$, area under the concentration-time curve from 0 to 48 hours; AUC$_{0-\infty}$, AUC from 0 to infinite time.
Table 3. Pharmacokinetic parameters of racemic bupropion and its metabolites.

<table>
<thead>
<tr>
<th></th>
<th>Control Phase</th>
<th>Inhibition Phase</th>
<th>Induction Phase</th>
<th>Inhibition/Control</th>
<th>Induction/Control</th>
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<tr>
<td><strong>Bupropion</strong></td>
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<tr>
<td>AUC_{0-24} (nM*h)</td>
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<tr>
<td>PM</td>
<td>3480 [2550-4770]</td>
<td>4210 [2570-6900]</td>
<td>2910 [1740-4860]</td>
<td>121 [93.6-156]</td>
<td>83.5 [64.6-108]</td>
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<tr>
<td>AUC_{0-∞} (nM*h)</td>
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<td>70.9 [64.2-78.3]</td>
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**Erythrohydrobupropion**

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**Threohydrobupropion**

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<td>7640 [5770-10100]</td>
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<td>21700 [17800-26500]</td>
<td>10900 [8760-13600]</td>
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<td>30.6 [25.6-36.4]</td>
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<td>112 [94.8-133]</td>
<td>84.7 [71.6-100]</td>
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Area under the plasma concentration versus time curve (AUC), maximal plasma concentration ($C_{\text{max}}$), time to reach $C_{\text{max}}$ ($T_{\text{max}}$), and terminal elimination half-life ($t_{1/2}$) determined via non-compartmental analysis of untransformed data. Ratios and corresponding confidence intervals calculated using Phoenix WinNonlin (v7.0); confidence intervals excluding 100% considered statistically significant. $\text{AUC}_{0-48}$, area under the concentration-time curve from 0 to 48 hours; $\text{AUC}_{0-\infty}$, AUC from 0 to infinite time.

Table 4. Genotype-dependent pharmacokinetic drug-drug interaction outcomes

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<th>Inhibition Phase</th>
<th>Induction Phase</th>
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<td>885 [661-1190]</td>
<td>84.6 [75.9-94.4]</td>
<td>32.9 [29.5-36.7]</td>
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<td>2720 [2310-3200]</td>
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<td>2830 [2170-3680]</td>
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<td>t(_{1/2}) (h)</td>
<td>NM 14.1 [11.7-16.9]</td>
<td>17.9 [10.7-29.8]</td>
<td>14.2 [7.82-25.8]</td>
</tr>
<tr>
<td><strong>(S, S)-threo</strong></td>
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<tr>
<td>AUC(_{0-24}) (nM*h)</td>
<td>NM 3270 [2310-4620]</td>
<td>3270 [2310-4620]</td>
<td>3720 [2650-5230]</td>
</tr>
<tr>
<td></td>
<td>IM 3460 [2630-4540]</td>
<td>3460 [2630-4540]</td>
<td>3720 [2650-5230]</td>
</tr>
</tbody>
</table>

**Note:** This article has not been copyedited and formatted. The final version may differ from this version.
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<th>PM</th>
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<tbody>
<tr>
<td><strong>AUC_{0-∞}</strong> (nM*h)</td>
<td>3910 [2200-6980]</td>
<td>5090 [3020-8580]</td>
<td>3850 [2120-6970]</td>
<td>130 [101-167]</td>
</tr>
<tr>
<td><strong>t_{1/2} (h)</strong></td>
<td>4700 [2520-8750]</td>
<td>6230 [3530-11000]</td>
<td>4340 [2350-8010]</td>
<td>133 [103-170]</td>
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Area under the plasma concentration versus time curve (AUC), maximal plasma concentration (C_{max}), time to reach C_{max} (T_{max}), and terminal elimination half-life (t_{1/2}) determined via non-compartmental analysis of untransformed data. Ratios and corresponding confidence intervals calculated using Phoenix WinNonlin (v7.0); confidence intervals excluding 100% considered statistically significant. AUC_{0-48}, area under the concentration-time curve from 0 to 48 hours; AUC_{0-∞}, AUC from 0 to infinite time.
Fig. 1

Screening and Enrollment

Control

Bupropion (100 mg)*

Inhibition

Bupropion (100 mg)*
Efavirenz (600 mg)

Efavirenz 600 mg daily x 17 days

Induction

Bupropion (100 mg)*
Efavirenz (600 mg)

Intensive sampling (0-24 hours)

Single blood draw (48, 72, 96, 120 hours)
Fig. 5

- Control
- Inhibition
- Induction

Concentration (nM)

Time (hrs)

*1*/1 RR-OHBUP
*1*/6 RR-OHBUP
*6*/6 RR-OHBUP

*1*/1 SS-OHBUP
*1*/6 SS-OHBUP
*6*/6 SS-OHBUP