Published in final edited form as:

Clin Pharmacol Ther. 2019 January ; 105(1): 142–152. doi:10.1002/cpt.1116.

Common polymorphisms of *CYP2B6* influence stereoselective bupropion disposition

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Abstract

Bupropion hydroxylation is a bioactivation and metabolic pathway, and the standard clinical *CYP2B6* probe. This investigation determined the influence of *CYP2B6* allelic variants on clinical concentrations and metabolism of bupropion enantiomers. Secondary objectives evaluated the influence of *CYP2C19* and P450 oxidoreductase variants. Healthy volunteers in specific cohorts (*CYP2B6**1/*1, *CYP2B6**1/*6, *CYP2B6**6/*6, and also *CYP2B6**4 carriers) received single-dose oral bupropion. Plasma and urine bupropion and hydroxybupropion was quantified. Subjects were also genotyped for *CYP2C19* and P450 oxidoreductase variants. Hydroxylation of both bupropion enantiomers, assessed by plasma hydroxybupropion/bupropion AUC ratios and urine hydroxybupropion formation clearances, was lower in *CYP2B6**6/*6 but not *CYP2B6**1/*6 compared with *CYP2B6**1/*1 genotypes, and numerically greater in *CYP2B6**4 carriers. *CYP2C19* and P450 oxidoreductase variants did not influence bupropion enantiomers hydroxylation or plasma concentrations. Results show that clinical hydroxylation of both bupropion enantiomers was equivalently influenced by *CYP2B6* allelic variation. *CYP2B6* polymorphisms affect S-bupropion bioactivation, which may affect therapeutic outcomes.

Keywords

cytochrome P450 2B6; *CYP2B6*; *CYP2C19*; P450 oxidoreductase; bupropion; stereochemistry; pharmacogenetics

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Author contributions

E.D.K. wrote the manuscript; E.D.K. designed the research; A.C. performed the research; E.D.K. analyzed the data.

Disclosures: The authors declared no competing interests for this work.

Introduction

Bupropion is used for treating major depressive disorder, both initial therapy and relapse prevention, either as monotherapy or in antidepressant combinations.¹ It is also a first-line switch agent for patients intolerant to or non-responsive to selective serotonin reuptake inhibitors. Bupropion is also used for seasonal affective disorder, smoking cessation,² obesity,³ and attention deficit hyperactivity disorder in adults and children.⁴ Bupropion undergoes extensive metabolism, with less than 1% recovered intact in urine, to the three primary metabolites hydroxybupropion (via *t*-butylhydroxylation), and the isomers threohydrobupropion and erythrohydrobupropion (via keto reduction). Hydroxybupropion exposure is approximately an order of magnitude greater than that of parent drug.

Bupropion is used clinically as a racemate. Metabolism *in vitro*⁵ and disposition *in vivo* are stereoselective.^{6–11} Plasma R-bupropion exposures are 2- to 6-fold higher than those of S-bupropion, the apparent oral clearance of S-bupropion exceeds that of R-bupropion, and R,R-hydroxybupropion exposures are 35- to 65-fold higher than S,S hydroxybupropion.^{7–9}

Bupropion hydroxylation is considered to be an important clinical bioactivation pathway, contributing to the antidepressant and smoking-cessation effects of bupropion. Hydroxybupropion is pharmacologically active, inhibiting norepinephrine and dopamine uptake, and antagonizing nicotine and showing antidepressant effects in rodents.^{12–14} Clinically, smoking-cessation and antidepressant effectiveness of bupropion is attributed to hydroxybupropion.^{15–17} These activities are attributed to S,S- rather than R,R-hydroxybupropion, despite much lower plasma concentrations.¹³

Bupropion hydroxylation is also an important catabolic pathway. *In vitro* it is catalyzed predominantly by CYP2B6,¹⁸ and is the standard *in vitro* probe for assessing CYP2B6 activity and drug interactions. Clinically, racemic bupropion hydroxylation has been used to phenotype CYP2B6 activity, polymorphisms, and drug interactions, and is the recommended standard *in vivo* CYP2B6 probe.^{19,20} The *CYP2B6* gene is highly polymorphic, and carriers of allelic variants have altered *in vivo* bupropion metabolism, evidenced by the plasma hydroxybupropion/bupropion AUC ratio, although bupropion clearance is unchanged.^{16,21–25} Hydroxybupropion may also inhibit P450 to a similar or greater extent than the parent drug.²⁶

Bupropion systemic clearance does not depend on CYP2B6 activity because of the low fraction metabolized to hydroxybupropion. However CYP2C19 is of interest regarding bupropion disposition. CYP2C19 does not catalyze *t*-butyl hydroxylation,¹⁸ but contributes to bupropion metabolism through alternate hydroxylation pathways,²⁷ recently identified as aromatic 4'-hydroxylation.²⁸ It is unclear whether CYP2C19 or CYP2B6 contributes more to clinical bupropion metabolism.^{29,30} Nevertheless, CYP2C19 may influence parent drug elimination, as carriers of the reduced activity *CYP2C19**2 allele had slightly higher bupropion steady-state plasma exposure.³¹

Polymorphisms of P450 oxidoreductase (POR), many of which have decreased activity (the extent of which is also CYP isoform-specific), including towards CYP2B6,^{32,33} might also affect bupropion disposition. Recent clinical studies of association between *POR* variants

and CYP2B6 activity have reported increased bupropion metabolism in GG homozygous carriers of the *POR* g.6593A>G polymorphism.^{24,25}

While certain *CYP2B6* allelic variants have altered hydroxylation of racemic bupropion *in vitro*,^{34–36} and *in vivo*,^{16,21–25} the hydroxylation activity of *CYP2B6* variants towards bupropion enantiomers is unknown, as is the influence of *CYP2B6* genetic polymorphisms on *in vivo* bupropion enantiomers hydroxylation and clearance. This investigation determined the influence of *CYP2B6* genetics on *in vivo* bupropion enantiomers metabolism and clearance, specifically the most common and clinically significant variant *CYP2B6**6. A secondary objective was to evaluate other, less common allelic variants, when encountered. Healthy volunteers were genotyped, and then *CYP2B6* genotype cohorts composed to evaluate bupropion disposition. Another secondary objective was to evaluate the influence of *CYP2C19* and *POR* polymorphisms on bupropion enantiomers clinical metabolism and clearance.

Results

Only 17 *CYP2B6**6/*6 subjects could be identified and studied. Others with rare allelic variants were also evaluated, including two *CYP2B6**5/*5 homozygotes and one *CYP2B6**1/*4 heterozygote and three *CYP2B6**4/*6 heterozygotes.

Bupropion disposition and *CYP2B6* genotypes

Plasma bupropion enantiomer concentrations are shown in Figure 1 for the three major genotype groups (*CYP2B6**1/*1, *CYP2B6**1/*6, *CYP2B6**6/*6), and also for *4 carriers (*CYP2B6**1/*4 and *CYP2B6**4/*6, shown together as *CYP2B6**4/*X) and for *5 homozygotes. Pharmacokinetic parameters are provided in Table 1. Bupropion disposition was stereoselective, as observed previously.^{7,8} The overall R-bupropion/S-bupropion plasma AUC_{0-∞} ratio for all subjects was 2.3 (1.8, 3.1) (median, interquartile range). There were no significant differences between *CYP2B6**1/*1 and *CYP2B6**6 hetero- or homozygotes in bupropion C_{max}, AUC_∞ or Cl/F for either enantiomer or the racemate. For the four *CYP2B6**4/*X subjects, AUC_{0-∞} (ng/ml-hr⁻¹) was 322 ± 98 and 163 ± 74 for R- and S-bupropion respectively, while that for *CYP2B6**1/*1 subjects was 737 ± 214 and 297 ± 123. The R-bupropion/S-bupropion plasma AUC_{0-∞} ratio in *CYP2B6**1/*1, *CYP2B6**1/*6, and *CYP2B6**6/*6 subjects was 2.5 (1.9, 3.4), 2.5 (2.0, 3.3) and 1.9 (1.8, 2.6) and not significantly different between groups. Urine recovery of R- and S-bupropion (% of dose) across all subjects was 1.0 (0.5, 2.7) and 0.3 (0.1, 0.5). There were no significant differences between the major *CYP2B6* genotype groups in bupropion renal clearance (Table 2).

Hydroxybupropion plasma concentrations were highly stereoselective (Figure 1), as observed previously.^{7,8} The plasma R,R-hydroxybupropion/S,S-hydroxybupropion AUC_{0-∞} ratio overall was 24 (17,28). Significant genotypic differences in bupropion metabolism were observed. Bupropion hydroxylation, evaluated by the plasma hydroxybupropion/bupropion AUC ratio, for both enantiomers and the racemate, was significantly lower in *CYP2B6**6 homozygotes compared with *CYP2B6**1 homozygotes (Table 1, Figure S1). Conversely, bupropion hydroxylation was numerically greater in *CYP2B6**4 carriers. For example, median hydroxybupropion/bupropion AUC ratios in *CYP2B6**4/X and

*CYP2B6**1/*1 subjects were 53 (36, 71) and 21 (15, 25) for R,R-hydroxybupropion, 3.4 (2.7, 4.7) and 2.1 (1.6, 2.7) for S,S-hydroxybupropion, and 37 (24, 52) and 15 (10, 18) for total hydroxybupropion. The plasma R,R-hydroxybupropion/S,S-hydroxybupropion AUC_{0-∞} ratio was not different in the *CYP2B6* genotype groups (25 (21, 34), 24 (21, 28) and 18 (16, 26) in *CYP2B6**1/*1, *CYP2B6**1/*6 and *CYP2B6**6/*6 subjects, respectively).

Bupropion hydroxylation was also evaluated using urine excretion data. Overall urine recovery of R,R- and S,S-hydroxybupropion (% of dose) was 5.1 (3.5, 6.6) and 1.6 (1.2, 1.9), respectively. Hydroxybupropion formation clearance for both enantiomers and the racemate was significantly lower in *CYP2B6**6/*6 than *CYP2B6**1/*1 wild types (Table 2, Figure S2). Conversely, bupropion hydroxylation was numerically greater in *CYP2B6**4 carriers. Formation clearances in *CYP2B6**4/X and *CYP2B6**1/*1 subjects, were 8.0 (4.5, 10.9) and 1.3 (0.8, 2.2) for R,R-hydroxybupropion, 4.4 (2.7, 7.0) and 1.2 (0.8, 1.6) for S,S-hydroxybupropion, and 6.9 (3.8, 9.9) and 1.18 (0.83, 1.93) for total hydroxybupropion, respectively. Bupropion hydroxylation, evaluated by either the plasma hydroxybupropion/bupropion AUC ratio or hydroxybupropion formation clearance, was not different from wild-type in *CYP2B6**1/*6 heterozygotes. In contrast to hydroxybupropion formation clearances, the urine hydroxybupropion/bupropion molar ratio was not different in *CYP2B6**6/*6 subjects.

Bupropion disposition and *CYP2C19* genotypes

Plasma bupropion and hydroxybupropion enantiomer concentrations are shown for the *CYP2C19* phenotypes (Figure 2). The distribution of *CYP2C19* phenotypes was a convenience sample, because subjects had been recruited into *CYP2B6* genotype cohorts. *CYP2C19* genetic data were not available for all subjects. The final cohort was 22 normal, 14 intermediate, 4 poor, 14 rapid, and 3 ultrarapid metabolizers. Neither R-bupropion nor S-bupropion plasma C_{max}, AUC_{0-∞}, CL/F, nor elimination t_{1/2} were significantly different between the *CYP2C19* phenotypes, specifically *CYP2C19* normal metabolizers (*CYP2C19**1/*1) and either intermediate (9 *CYP2C19**1/*2, 1 *CYP2C19**1/*3, 4 *CYP2C19**2/*17) or rapid metabolizers (*CYP2C19**1/*17) (Table 3). Results are shown only for the three major phenotype groups for clarity, but were not different in the poor or ultrarapid metabolizers. There were also no significant differences between normal metabolizers and other phenotypes in R,R- or S,S-hydroxybupropion plasma C_{max}, elimination t_{1/2}, AUC, hydroxybupropion/bupropion AUC ratios, or urine R,R- or S,S-hydroxybupropion formation clearances (not shown). Results were not different if grouped by allele. Specifically, the AUC_{0-∞} for *CYP2C9**1 (n=22), *CYP2C9**2 (n=17), and *CYP2C9**17 (n=17) carriers was 620 ± 205, 715 ± 217, and 664 ± 217 hr•ng•ml⁻¹ (P=0.11) for R-bupropion, 275 ± 123, 273 ± 68 and 286 ± 118 hr•ng•ml⁻¹ (P=0.86) for S-bupropion, and 887 ± 291, 986 ± 244, and 952 ± 32 hr•ng•ml⁻¹ (P=0.33) for racemic bupropion.

Bupropion disposition and *POR* genotypes

Plasma bupropion and hydroxybupropion enantiomer concentrations are shown in Figure 3 for the *POR* genotypes. The distribution of *POR* genotypes was a convenience sample, because subjects had been recruited into *CYP2B6* genotype cohorts. *POR* genetic data were not available for all subjects. Cohorts included 31 *POR**1/*1, 22 *POR**1/*28, and 7

*POR*28/*28* subjects. No *POR*5* or *POR*8* subjects were identified. Compared with wild-type *POR*1/*1*, neither the *POR*28* heterozygotes or homozygotes had any difference in R- or S-bupropion plasma $C_{\max}$, $AUC_{0-\infty}$, CL/F, elimination $t_{1/2}$, renal clearance, or R,R- or S,S-hydroxybupropion plasma $C_{\max}$, elimination $t_{1/2}$, or AUC (Table 4). There were also no differences in plasma hydroxybupropion/bupropion AUC ratios, or urine R,R- or S,S-hydroxybupropion formation clearances. Subjects were also genotyped for the intronic variant *g.6593 A>G*, which does not have an assigned allele. Genotype groups were 28 *g.6593AA*, 26 *g.6593AG*, and 9 *g.6593GG* subjects. There were no significant differences in R- or S-bupropion plasma $C_{\max}$, $AUC_{0-\infty}$, CL/F, elimination $t_{1/2}$, renal clearance, or R,R- or S,S-hydroxybupropion plasma $C_{\max}$, elimination $t_{1/2}$, or AUC. There were also no differences in plasma hydroxybupropion/bupropion AUC ratios or urine R,R- or S,S-hydroxybupropion formation clearances between *g.6593AA*, *g.6593AG*, or *g.6593GG* genotypes (Table 4).

Discussion

The major finding of this investigation is that clinical *t*-butyl hydroxylation of both bupropion enantiomers was equivalently influenced by *CYP2B6* polymorphisms. Specifically, both the plasma R,R-hydroxybupropion/R-bupropion and S,S-hydroxybupropion/S-bupropion AUC ratios, and the R,R- and S,S-hydroxybupropion formation clearances, were significantly lower than wild-type controls in *CYP2B6*6/*6*, but not *CYP2B6*1/*6* genotypes. Racemic hydroxybupropion/bupropion plasma AUC ratios and urine hydroxybupropion formation clearances were also significantly lower than wild-type controls in *CYP2B6*6/*6*, but not *CYP2B6*1/*6* genotypes. Although not statistically analyzed due to the small sample size, plasma bupropion concentrations were lower, hydroxybupropion concentrations were higher, hydroxybupropion/bupropion AUC ratios and urine hydroxybupropion formation clearances were numerically greater in *CYP2B6*4* carriers vs *CYP2B6*1* controls, for both enantiomers. In contrast to bupropion hydroxylation, R- or S-bupropion disposition was not influenced by *CYP2B6* genotype.

CYP2B6 genetic effects on *in vivo* hydroxylation of both R- and S-bupropion are consistent with prior observation that cDNA-expressed and human liver microsomal *CYP2B6.1* protein metabolized both enantiomers, and stereoselectively.⁵ The *in vitro* hydroxylation of bupropion enantiomers by expressed *CYP2B6* allelic variants has not been reported, however, thus the present *in vivo* findings cannot be compared with *in vitro* data. The equivalent influence of *CYP2B6* polymorphisms on clinical R- vs S-bupropion hydroxylation is different than the greater influence of *CYP2B6* induction on R- vs S-bupropion hydroxylation, based on plasma metabolite/parent ratios.^{7,8} For example, rifampin induction increased the plasma S,S-hydroxybupropion/S-bupropion AUC ratio 2.3-fold, and the R,R-hydroxybupropion/R-bupropion AUC ratio 1.2-fold, while effects on the formation clearances of R,R- vs S,S-hydroxybupropion were similar. Ritonavir induction increased the plasma S,S-hydroxybupropion/S-bupropion AUC ratio 1.2-fold while the R,R-hydroxybupropion/R-bupropion AUC ratio was unchanged, and R,R- and S,S-hydroxybupropion formation clearances were equally increased. As discussed, below, it is possible that these apparent differences in plasma metabolite/parent AUC ratios may be related more to R,R- and S,S-hydroxybupropion disposition, than to true stereoselective

differences in effects of altered CYP2B6 activity, whether due to *CYP2B6* polymorphisms or drug interactions.

Enantioselective effects of *CYP2B6* polymorphisms can be compared with previous studies using achiral analysis, and which mostly evaluated *CYP2B6**6. The present investigation found a gene-dose effect of the *6 allele to reduce hydroxylation of both bupropion enantiomers, based on both plasma (hydroxybupropion/bupropion AUC ratio) and urine (hydroxybupropion formation clearance) data (Tables 1 and 2). Previous plasma racemic hydroxybupropion/bupropion AUC ratios in *CYP2B6**1/*1, *1/*6, and *6/*6 genotypes were (mean) 8, 6 and 5,²¹ 17, 10 and 9,²⁴ and 16, 9 and 8,²⁵ after single-dose immediate release bupropion. At steady state, ratios were 41, 22, and 16 in the three genotype groups,¹⁶ and were less in *6 carriers than wild types (13 vs 17).²³ The present investigation also found increased bupropion hydroxylation in *CYP2B6**4 carriers, with median hydroxybupropion/bupropion ratios in *CYP2B6**1/*1 and *CYP2B6**4/X subjects of 15 and 37 for total hydroxybupropion, 21 and 53 for R,R-hydroxybupropion, and 2.1 and 3.4 for S,S-hydroxybupropion. An influence of *CYP2B6**4 was also previously observed, with total hydroxybupropion/bupropion ratios of 8 and 14 in *CYP2B6**1/*1 and *CYP2B6**1/*4 genotypes, respectively.²¹ Although it has been speculated that *CYP2B6* genetic variability may contribute to differences in the R/S-bupropion plasma AUC ratio,²⁹ this did not differ significantly between *CYP2B6**1/*1, *CYP2B6**1/*6, and *CYP2B6**6/*6 subjects.

A secondary finding was that *CYP2C19* polymorphisms did not influence bupropion enantiomers disposition. Current understanding of CYP2C19 relevance in bupropion metabolism and elimination is evolving, and less characterized than CYP2B6. The fraction of bupropion metabolized clinically by CYP2C19 was predicted to be only one-third that by CYP2B6.²⁹ CYP2C19 does not catalyze *t*-butyl hydroxylation to hydroxybupropion *in vitro*,¹⁸ and *CYP2C19* genotype did not affect plasma hydroxybupropion/bupropion AUC ratios *in vivo*.³¹ CYP2C19 does however catalyze bupropion aromatic 4'-hydroxylation,^{27,28,30} and secondary 4'-hydroxylation of the primary metabolites erythrohydrobupropion and threohydrobupropion.^{28,29} Little is known about the influence of *CYP2C19* variants on bupropion disposition. Oral bupropion (racemate) systemic clearance was unaffected by *CYP2C19* genotype.²¹ In contrast, a small (1.15-fold) but statistically significantly greater racemic bupropion plasma AUC was reported in *CYP2C19**2 loss of function allele carriers, but there was no difference in *CYP2C19**17 gain of function carriers.³¹ The present investigation found no difference in bupropion enantiomers AUC between *CYP2C19* normal and either intermediate or rapid metabolizers, or between allele types. These results, albeit with small subject numbers, do not support the conclusion that *CYP2C19* polymorphisms influence bupropion plasma concentrations.

Another secondary finding was that *POR* polymorphisms did not influence bupropion enantiomers disposition. Neither *POR**28 homozygotes, nor *g*.6593GG genotypes, had altered hydroxylation of either bupropion enantiomer, assessed by both plasma hydroxybupropion/bupropion AUC ratios and urine hydroxybupropion formation clearances. Results for *POR**28 are congruent with previous observations.^{24,25} Results for the *g*.6593A>G polymorphism contrast with previous studies, which found greater bupropion

hydroxylation in *g.6593GG* genotypes.^{24,25} Reasons for the differences are not apparent, but could be related to ethnic differences in the study populations, as well as small sample sizes.

The intersection of stereochemistry and pharmacogenetics for CYP2B6 substrates is relatively unexplored. Common CYP2B6 substrates, which are chiral and used clinically as racemates include bupropion, methadone, ketamine, cyclophosphamide and ifosfamide, while nevirapine is achiral and efavirenz and artemether are single enantiomers. Ketamine N-demethylation by CYP2B6 is stereoselective, and both enantiomers are metabolized *in vitro* less by cDNA-expressed CYP2B6.6 than CYP2B6.1 and in liver microsomes from humans carrying the *CYP2B6*6* allele.³⁷ Clinical ketamine enantiomers metabolism was found not to differ in *CYP2B6*6* carriers compared with *CYP2B6*1/*1* genotypes,³⁸ while another study found diminished (racemate) metabolism, although genetic effects could not be distinguished from age effects.³⁹ Methadone *in vitro* is N-demethylated stereoselectively by CYP2B6, and both enantiomers are metabolized less and more efficiently, respectively, by expressed CYP2B6.6 and CYP2B6.4 compared with CYP2B6.1.^{40,41} Clinical methadone metabolism and clearance are decreased and increased respectively, in *CYP2B6*6/*6* genotypes and *CYP2B6*4* carriers.⁴² In contrast, metabolism of racemic bupropion is less by both CYP2B6.4 and CYP2B6.6,^{35,43} as is racemic ifosfamide, which is metabolized more efficiently by CYP2B6.7 and CYP2B6.9.⁴⁴ Ifosfamide metabolism by CYP2B6.1 is enantioselective,⁴⁵ but stereoselectivity with *CYP2B6* variants is unknown. Cyclophosphamide hydroxylation by CYP2B6.6 was greater than CYP2B6.1 *in vitro*, but this was not apparent clinically.^{46–48} The influence of *CYP2B6* polymorphisms and stereochemistry on human ifosfamide and cyclophosphamide metabolism is unknown. Available data show that stereoselectivity and genetic influences on disposition of bupropion and methadone are similar, with *CYP2B6*6* and *CYP2B6*4* associated with decreased and increased metabolism, respectively, and both enantiomers are affected, *in vitro* and *in vivo*.

One clinical implication of this investigation attends to bupropion pharmacotherapy. Bupropion hydroxylation is a bioactivation pathway, and hydroxybupropion, specifically S,S-hydroxybupropion, is considered to contribute to bupropion l antidepressant and anti-smoking effects.^{12,13,15–17} Higher plasma racemic hydroxybupropion concentrations, related to *CYP2B6* genetic variation, were associated with better smoking-cessation outcomes,¹⁶ and better improvement in depression.¹⁷ This investigation shows that genetic differences in *CYP2B6* influence S-bupropion metabolism and S,S-hydroxybupropion formation and thus could affect clinical outcomes.^{15,16}

Another clinical implication attends more broadly to *CYP2B6* pharmacogenetics. Questions continue regarding the best *in vivo* CYP2B6 probe. Racemic bupropion, and more specifically racemic bupropion hydroxylation, is the standard *in vivo* probe for CYP2B6 activity, polymorphisms and drug interactions, and is recommended by regulatory agencies.^{19,20} Bupropion clearance and plasma AUC do not reflect CYP2B6 activity because hydroxylation is a minor route of elimination. More commonly used is the plasma hydroxybupropion/bupropion AUC ratio, but this is confounded because racemic hydroxybupropion is elimination rather than formation-rate limited. Urine metrics, such as hydroxybupropion formation clearance and the hydroxybupropion/bupropion molar ratio, do reflect CYP2B6 induction,^{7,8} but there are few other evaluations of these metrics, such as

CYP2B6 genetic influences. Urine racemic hydroxybupropion formation clearances differed more than plasma hydroxybupropion/bupropion AUC ratios in response to *CYP2B6* induction,^{7,8} and *CYP2B6* genotypes (Tables 1 and 2). Recognition that bupropion hydroxylation is stereoselective,⁵ led to the clinical evaluation of bupropion enantiomers hydroxylation to probe *CYP2B6*.^{7,8} Because S,S-hydroxybupropion was formation rate-limited while R,R-hydroxybupropion and the racemate were elimination-rate limited, S,S-hydroxybupropion formation clearance appeared to be an improved measure of bupropion hydroxylation and *CYP2B6* activity. After *CYP2B6* induction by rifampin and ritonavir, plasma hydroxybupropion/bupropion AUC ratios were increased more for S,S- than R,R-hydroxybupropion, although the formation clearances of both isomers equivalently reflected *CYP2B6* induction.^{7,8} In the present investigation the hydroxybupropion/bupropion plasma AUC ratios of the isomers differed comparably in *CYP2B6**6 homozygotes, as did the S,S- and R,R-hydroxybupropion formation clearances. Based on *in vitro* data predicting that the fraction of bupropion metabolized to hydroxybupropion was 3-fold greater for R- than S-bupropion, despite more efficient hydroxylation of S- than R-bupropion, it was suggested that R,R-hydroxybupropion formation clearance or R,R-hydroxybupropion/R-bupropion metabolic ratios may be a more sensitive and better probe of *CYP2B6* activity than S,S-hydroxybupropion/S-bupropion formation clearance or metabolic ratios.²⁹ This is not consistent with the present and previous data.^{7,8} It is also unclear whether R,R-hydroxybupropion/R-bupropion formation clearance or metabolic ratios would improve on those of the racemate, since the plasma AUC for R,R-hydroxybupropion constitutes 95% of the total hydroxybupropion AUC. One of the greater unknowns, is the reason why plasma R,R- and S,S-hydroxybupropion concentrations are so different.^{7,8,29} Alternative *CYP2B6* probes have also been suggested, such as 8-hydroxylation of efavirenz,⁴⁹ whose metabolism *in vitro* was considered to be a better predictor of clinical drug interactions than bupropion.

In summary, this investigation shows that *CYP2B6* genotype influenced the hydroxylation of both R- and S-bupropion without affecting bupropion plasma concentrations or clearance. *CYP2B6**6 homozygotes had decreased hydroxylation, and *CYP2B6**4 carriers had increased hydroxylation. *CYP2B6* genetic polymorphism had equivalent effects on R- and S-bupropion hydroxylation. Neither the *CYP2C19* nor *POR* genetic variants studied affected bupropion hydroxylation or clearance.

Methods

Study population and protocol

The investigation was registered () and was approved by the Washington University in St. Louis Institutional Review Board, and all subjects provided written informed consent. Subjects were normal healthy volunteers (smokers or nonsmokers) age 18–50 yr in good general health within 30% of ideal body weight (body mass index <33), had no history of hepatic or renal disease, use of prescription or non-prescription medications, herbals or foods known to be metabolized by or affect *CYP2B6* activity. Pregnant or nursing females were not eligible. Potential subjects were genotyped for single nucleotide polymorphisms (SNPs) of *CYP2B6* 516G>T (rs3745274), 785A>G (rs2279343), 983T>C (rs28399499), and

1459C>T (rs3211371) as described previously.⁴² SNP analysis permitted identification of *CYP2B6* *1, *4 (785A>G), *5 (1459C>T), *6 (516G>T, 785A>G), *7 (516G>T, 785A>G, 1459C>T), *9 (516G>T), *16 (785A>G, 983T>C), and *18 (983T>C) alleles. Genotyping results then were used to invite subjects to create target cohorts of twenty subjects each with *CYP2B6**1/*1, *CYP2B6**1/*6 and *CYP2B6**6/*6 genotypes. A 30% difference between groups in hydroxybupropion/bupropion AUC ratio was considered clinically significant. To detect a 30% difference between *CYP2B6* genotypes, with 30% variability, $\beta=0.8$, and $\alpha=0.05$, would require 17 subjects per group. The target was 20 per group. In addition, subjects of other rare *CYP2B6* genotypes coincidentally identified were also studied.

Enrolled subjects were also genotyped for *CYP2C19* 681G>A (rs4244285), 636G>A (rs4986893) and -806C>T (rs12248560); and also for *POR* 859G>C (rs121912974), Y181D 541T>G (rs72552771), A503V 1508C>T (rs1057868), and intron *g*.6593 A>G (rs2868177). SNP analysis permitted identification of *CYP2C19* *2 (681G>A), *3 (636G>A), and *17 (-806 C>T) alleles. *CYP2C19* variants were grouped into normal, intermediate, poor, rapid, and ultrarapid metabolizer phenotypes. SNPs also identified *POR* *5 (859G>C), *8 (541T>G), and *28 (A503V) alleles, as well as the *g*.6593 A>G polymorphism.

A total of 63 subjects (33 male, 30 female; 42 Caucasians, 9 African-Americans, 10 Asians, 2 other/unknown), 29 ± 8 yr, 74 ± 13 kg, were studied. Detailed demographic data are provided in Table S1.

Subjects were instructed to refrain from: 1) oranges, grapefruit or apples or their juices for 5d before and throughout the study period, 2) non-study medications (including over the counter and/or herbal), for 3d prior to the study day without prior approval, 3) alcohol for 48 hr prior to and during the study day, 4) caffeine-containing beverages on the study day, 5) food/liquids after midnight the day prior to bupropion administration.

The study design was a single-center, open-label, single-session protocol. A peripheral IV catheter was inserted in an arm for blood sampling. Subjects received 150 mg oral racemic bupropion with 200cc water, then a standard breakfast and lunch 3 and 5 hr after bupropion administration, respectively, and free access to food and water thereafter. Venous blood was sampled for 73 hr after bupropion, centrifuged, and plasma stored at -80°C . Continuous 24hr urine samples were collected for 3d and stored at -80°C . Plasma and urine R- and S-bupropion and R,R- and S,S-hydroxybupropion concentrations were measured by HPLC-tandem mass spectrometry as described previously.⁵⁰ Interday coefficients of variation were 7, 6, and 5% and 7, 5, and 5% for 1, 10 and 100 ng/ml R- and S- bupropion, respectively, and 9, 5, and 6% and 7, 5, and 6% for 5, 50, and 500 ng/ml R,R- and S,S-hydroxybupropion in plasma, and 9, 7, and 6% and 6, 6, and 7% for 10, 100 and 1000 ng/ml R- and S-bupropion, respectively, and 6, 7, and 8% and 7, 7, and 5% for 5, 50, and 500 ng/ml R,R- and S,S-hydroxybupropion in urine.

Data and statistical analysis

Pharmacokinetic data were analyzed using noncompartmental methods (Phoenix, Pharsight Corp, Mountain View, CA), assuming complete absorption, as described previously.^{7,8} Differences between *CYP2B6* genotypes for pharmacokinetic parameters, and major

CYP2C19 phenotypes and *POR* variants were analyzed using one-way analysis of variance followed by the Student-Newman-Keuls test for multiple comparisons (Sigmaplot 12.5, Systat Software, Inc, San Jose, CA). Non-normal data were log transformed for analysis, but reported as the non-transformed results. Statistical significance was assigned at $P < 0.05$. Formal comparison of other *CYP2B6* allelic variants to *CYP2B6**1/*1 subjects, and comparison of *CYP2C19* poor and ultrarapid metabolizer phenotypes to regular metabolizers was not performed due to the small subject numbers studied. Normally distributed data are reported as the mean $\pm$ SD, others are reported as the median and interquartile range.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank Jennifer Parchomski, RN, and Jane Blood, RN, Department of Anesthesiology, Washington University in St. Louis (St. Louis, MO) for their excellent clinical research assistance, and Chris Sawyer and Richard Head, PhD, of the Genome Technology Access Center and Department of Genetics at Washington University in St. Louis School of Medicine (St. Louis, MO) for genomic analysis. The Center is partially supported by NCI Cancer Center Support Grant P30CA91842 to the Siteman Cancer Center (St. Louis, MO) and by ICTS/CTSA Grant# UL1TR000448 to Washington University in St. Louis (St. Louis, MO) from the National Center for Research Resources (NCRR), a component of the National Institutes of Health.

Funding: Supported by National Institutes of Health grant R01-DA14211 and by FDA grant U01-FD004899 (to Dr. Kharasch), and by National Institutes of Health grant UL1TR000448 to the Washington University in St. Louis Institute of Clinical and Translational Sciences.

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STUDY HIGHLIGHTS

What is the current knowledge on the topic?

Bupropion hydroxylation is a bioactivation pathway, CYP2B6 probe, and is stereoselective. The *CYP2B6* gene is highly polymorphic, with some variant allele products catalyzing altered racemic bupropion hydroxylation activity.

What question did this study address?

The influence of *CYP2B6* allelic variants, as well as *CYP2C19* and P450 oxidoreductase variants, on the clinical plasma concentrations of bupropion enantiomers and bupropion hydroxylation.

What does this study add to our knowledge?

*CYP2B6**6/*6 genotypes and *CYP2B6**6/*4 carriers had decreased and increased hydroxylation, respectively, of both bupropion enantiomers, based on plasma hydroxybupropion/bupropion AUC ratios and urine hydroxybupropion formation clearance. *CYP2C19* and P450 oxidoreductase variants had no effect on plasma concentrations or hydroxylation.

How might this change clinical pharmacology or translational science?

CYP2B6 variants may affect bupropion bioactivation to the active metabolite S,S-hydroxybupropion. Results may influence use of bupropion hydroxylation as a CYP2B6 probe.

Figure 1.

Influence of *CYP2B6* genotype on bupropion disposition. Subjects received 150 mg oral immediate release bupropion. Shown are plasma concentrations of (A) R-bupropion, (B) R,R-hydroxybupropion, (C) S-bupropion, (D) S,S-hydroxybupropion, (E) RS-bupropion, (F) total hydroxybupropion. Each data point is the mean $\pm$ SD. Some SD values are omitted for clarity. Genotype cohorts were *CYP2B6**1/*1, *CYP2B6**1/*6, *CYP2B6**6/*6, *CYP2B6**4/*X (with results for one *CYP2B6**1/*4 and three *CYP2B6**4/*6 subjects combined) and *CYP2B6**5/*5.

Figure 2.

Influence of *CYP2C19* genotype on bupropion disposition. Subjects received 150 mg oral immediate release bupropion. Shown are plasma concentrations of (A) R-bupropion, (B) R,R-hydroxybupropion, (C) S-bupropion, (D) S,S-hydroxybupropion, (E) RS-bupropion, (F) total hydroxybupropion. Each data point is the mean $\pm$ SD. Some SD values are omitted for clarity. Subjects whose results are shown in Figure 1 were also genotyped for the *CYP2C19**2, *3, and *17 alleles, and were grouped into normal, intermediate, poor, rapid, and ultrarapid metabolizer phenotypes. *CYP2C19* genetic data were not available for all subjects.

Figure 3.

Influence of P450 oxidoreductase (*POR*) variants on bupropion disposition. Subjects received 150 mg oral immediate release bupropion. Shown are plasma concentrations of (A) R-bupropion, (B) R,R-hydroxybupropion, (C) S-bupropion, (D) S,S-hydroxybupropion, (E) RS-bupropion, (F) total hydroxybupropion. Each data point is the mean $\pm$ SD. Some SD values are omitted for clarity. Subjects whose results are shown in Figure 1 were also genotyped for the *POR**5, *8, and *28 alleles, as well as the g.6593 A>G polymorphism. *POR* genetic data were not available for all subjects.

Table 1.Pharmacokinetic parameters for plasma bupropion and hydroxybupropion in *CYP2B6* genotypes

	<i>CYP2B6</i> *1/*1 (n=21)	<i>CYP2B6</i> *1/*6 (n=20)	<i>CYP2B6</i> *6/*6 (n=17)	All subjects (n=64)
<u>bupropion</u>				
C _{max} (ng/ml)	170 (119, 209)	145 (113, 184)	149 (123, 195)	148 (114, 194)
AUC _{0-∞} (hr•ng•ml ⁻¹)	1035 ± 295	853 ± 232	970 ± 251	933 ± 288
CL/F (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	37 ± 11	41 ± 14	39 ± 10	42 ± 17
Elimination t _{1/2} (h)	25 (23, 27)	22 (18, 28)	22 (17, 30)	23 (19, 28)
<u>R-bupropion</u>				
C _{max} (ng/ml)	130 (91, 175)	102 (82, 139)	106 (84, 130)	108 (84, 145)
AUC _{0-∞} (ng •h •ml ⁻¹)	737 ± 214	610 ± 185	652 ± 173	657 ± 215
CL/F (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	26 (19, 31)	28 (24, 34)	29 (24, 37)	27 (22, 34)
Elimination t _{1/2} (h)	24 (19, 26)	22 (19, 28)	19 (17, 25)	22 (18, 26)
<u>S-bupropion</u>				
C _{max} (ng/ml)	30 (24, 60)	35 (26, 49)	52 (36, 65)	36 (25, 55)
AUC _{0-∞} (hr•ng•ml ⁻¹)	297 ± 123	248 ± 79	324 ± 116	279 ± 111
CL/F (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	64 (45, 86)	69 (49, 99)	55 (43, 81)	67 (49, 86)
Elimination t _{1/2} (h)	26 (13, 26)	25 (23, 26)	26 (25, 27)	26 (24, 26)
<u>hydroxybupropion</u>				
C _{max} (ng/ml)	370 (268, 419)	283 (210, 344)*	234 (157, 282)*	296 (230, 400)
Elimination t _{1/2} (hr)	24 (23, 34)	21 (17, 33)	28 (22, 30)	24 (20, 30)
AUC ₀₋₇₂ hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	13.5 (9.8, 15.5)	10.9 (8.4, 13.0)	9.2 (5.4, 12.7)*	11.7 (8.4, 15.2)
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	15.1 (10.4, 18.3)	12.2 (8.8, 15.1)	10.2 (6.1, 15.3)*	13.2 (9.1, 18.0)
<u>R,R-hydroxybupropion</u>				
C _{max} (ng/ml)	336 (252, 401)	276 (195, 311)*	220 (147, 261)*	278 (217, 385)
Elimination t _{1/2} (hr)	24 (23, 35)	21 (17, 33)	28 (23, 30)	24 (20, 30)
AUC ₀₋₇₂ hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	18.0 (13.1, 20.4)	14.6 (10.4, 20.0)	14.0 (7.9, 17.7)*	15.7 (11.5, 20.6)
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	20.8 (15.1, 24.9)	15.9 (11.9, 23.5)	15.7 (8.5, 20.8)*	27.1 (12.0, 24.8)
<u>S,S-hydroxybupropion</u>				
C _{max} (ng/ml)	21 (17, 29)	18 (11, 23)	16 (17, 24)	19 (14, 26)
Elimination t _{1/2} (h)	23 ± 6	24 ± 9	25 ± 8	23 ± 8
AUC ₀₋₇₂ hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	2.1 (1.6, 2.6)	1.6 (1.3, 2.3)	1.6 (1.0, 2.0)*	1.9 (1.5, 2.4)
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	2.1 (1.6, 2.7)	1.8 (1.3, 2.3)	1.6 (1.0, 2.0)*	1.9 (1.5, 2.3)

C_{max}, maximum concentration; T_{max}, time to maximum concentration; AUC₀₋₇₂ area under concentration-time curve from 0 to 72 hr; AUC_{0-∞}, area under concentration-time curve extrapolated to infinity; CL/F, apparent oral clearance; t_{1/2}, elimination half-life. Results are the mean ± SD or median (25th, 75th quartile)

* Significantly different from *CYP2B6**1/*1 (p<0.05).

Results are not shown for 3 *CYP2B6**4/*6, 1 *CYP2B6**1/*4 and 2 *CYP2B6**5/*5 subjects

Table 2.Pharmacokinetic parameters for urine bupropion and hydroxybupropion in *CYP2B6* genotypes

	<i>CYP2B6</i> *1/*1 (n=21)	<i>CYP2B6</i> *1/*6 (n=19)	<i>CYP2B6</i> *6/*6 (n=17)	All subjects (n=64)
Apparent renal or formation clearance ($\text{ml}^{-1} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)				
bupropion	0.23 (0.14, 0.54)	0.37 (0.14, 0.67)	0.28 (0.07, 0.62)	0.34 (0.12, 0.55)
R-bupropion	0.27 (0.14, 0.60)	0.41 (0.15, 0.85)	0.36 (0.07, 0.72)	0.36 (0.14, 0.70)
S-bupropion	0.16 (0.12, 0.28)	0.25 (0.10, 0.38)	0.17 (0.07, 0.30)	0.20 (0.10, 0.32)
hydroxybupropion	1.18 (0.83, 1.93)	1.29 (0.86, 1.92)	0.70 (0.32, 1.39)*	1.27 (0.75, 1.92)
R,R-hydroxybupropion	1.31 (0.77, 2.17)	1.36 (0.95, 1.95)	0.67 (0.32, 1.54)*	1.36 (0.71, 2.05)
S,S-hydroxybupropion	1.15 (0.75, 1.56)	1.02 (0.70, 1.57)	0.64 (0.28, 1.12)*	1.01 (0.67, 1.57)
0–73 hr hydroxybupropion/bupropion molar ratio				
Hydroxybupropion / bupropion	4.4 (2.3, 14.6)	3.5 (1.9, 8.2)	2.7 (1.9, 5.2)	3.7 (2.2, 9.1)
R,R-hydroxybupropion / R-bupropion	4.4 (2.1, 14.6)	3.3 (1.7, 8.4)	2.7 (1.7, 5.2)	3.5 (1.9, 8.8)
S,S-hydroxybupropion / S-bupropion	5.4 (3.1, 14.1)	4.3 (2.6, 8.4)	3.8 (2.3, 6.3)	5.0 (3.0, 10.8)

Results are the median (25th, 75th quartile)* Significantly different from *CYP2B6**1/*1 ($p < 0.05$).

Table 3.Pharmacokinetic parameters for plasma bupropion and hydroxybupropion in *CYP2C19* phenotypes

	<i>CYP2C19</i> NM (n=22)	<i>CYP2C19</i> IM (n=14)	<i>CYP2C19</i> RM (n=14)	All subjects (n=57)
<u>Bupropion</u>				
C _{max} (ng/ml)	134 (108, 187)	146 (120, 193)	160 (111, 202)	148 (114, 193)
AUC _{0-∞} (hr•ng•ml ⁻¹)	887 ± 291	964 ± 255	912 ± 316	943 ± 282
CL/F (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	38 (30, 51)	38 (29, 44)	37 (34, 45)	37 (30, 45)
Elimination t _{1/2} (h)	23 (20, 27)	21 (18, 25)	23 (20, 27)	23 (20, 27)
<u>R-bupropion</u>				
C _{max} (ng/ml)	99 (80, 136)	108 (91, 164)	127 (84, 148)	108 (85, 146)
AUC _{0-∞} (ng •h •ml ⁻¹)	620 ± 205	716 ± 231	629 ± 204	668 ± 213
CL/F (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	27 (23, 34)	27 (19, 32)	28 (23, 34)	27 (22, 32)
Elimination t _{1/2} (h)	21 (18, 25)	21 (17, 26)	22 (17, 25)	22 (18, 25)
<u>S-bupropion</u>				
C _{max} (ng/ml)	36 (16, 55)	28 (20, 50)	49 (24, 60)	36 (25, 53)
AUC _{0-∞} (hr•ng•ml ⁻¹)	275 ± 126	249 ± 53	280 ± 125	277 ± 104
CL/F (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	69 (44, 90)	71 (62, 87)	56 (51, 85)	65 (49, 86)
Elimination t _{1/2} (h)	26 (24, 27)	25 (13, 26)	25 (19, 27)	26 (24, 27)
<u>Hydroxybupropion</u>				
C _{max} (ng/ml)	295 (230, 378)	325 (259, 402)	238 (184, 352)	293 (233, 381)
Elimination t _{1/2} (hr)	23 (18, 32)	26 (22, 34)	23 (21, 27)	24 (20, 30)
AUC ₀₋₇₂ hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	11.6 (8.6, 16.7)	12.9 (10.2, 15.2)	9.2 (6.9, 14.9)	11.7 (8.5, 14.8)
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	12.9 (9.7, 20.0)	14.2 (11.9, 17.0)	9.8 (7.1, 16.4)	13.6 (9.1, 17.9)
<u>R,R-hydroxybupropion</u>				
C _{max} (ng/ml)	278 (219, 348)	300 (245, 382)	225 (174, 332)	277 (220, 363)
Elimination t _{1/2} (hr)	23 (18, 33)	27 (22, 35)	23 (20, 27)	24 (21, 30)
AUC ₀₋₇₂ hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	15.7 (12.8, 23.0)	16.8 (13.2, 20.7)	12.6 (9.2, 19.8)	15.7 (11.5, 20.3)
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	17.0 (13.2, 27.7)	18.5 (15.9, 24.0)	14.0 (9.5, 22.1)	17.1 (12.8, 24.2)
<u>S,S-hydroxybupropion</u>				
C _{max} (ng/ml)	19 (15, 23)	21 (15, 24)	17 (10, 23)	19 (14, 24)
Elimination t _{1/2} (h)	21 ± 8	24 ± 7	23 ± 8	23 ± 8
AUC ₀₋₇₂ hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	2.0 (1.5, 2.5)	1.9 (1.6, 2.3)	1.5 (1.0, 2.1)	1.9 (1.5, 2.3)
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	2.1 (1.5, 2.7)	2.0 (1.8, 2.3)	1.5 (0.9, 2.6)	1.9 (1.5, 2.3)

C_{max}, maximum concentration; T_{max}, time to maximum concentration; AUC₀₋₇₂ area under concentration-time curve from 0 to 72 hr; AUC_{0-∞}, area under concentration-time curve extrapolated to infinity; CL/F, apparent oral clearance; t_{1/2}, elimination half-life. Results are the mean ± SD or median (25th, 75th quartile).

Results are not shown for 3 UM and 4 PM

Table 4.Pharmacokinetic parameters for hydroxybupropion in *POR* genotypes

	<i>POR</i> *1/*1 (n=31)	<i>POR</i> *1/*28 (n=22)	<i>POR</i> *28/*28 (n=7)
<u>R,R-hydroxybupropion</u>			
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	17.3 (11.7, 28.5)	17.6 (11.9, 23.0)	16.6 (12.6, 25.8)
formation clearance (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	1.2 (0.7, 1.9)	1.7 (1.0, 2.2)	0.8 (0.7, 2.9)
<u>S,S-hydroxybupropion</u>			
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	1.9 (1.7, 2.1)	2.0 (1.5, 2.6)	1.9 (1.4, 2.5)
formation clearance (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	1.1 (0.6, 1.6)	1.0 (0.8, 1.7)	0.7 (0.7, 1.6)
	<i>g</i> .6593AA (n=28)	<i>g</i> .6593AG (n=27)	<i>g</i> .6593GG (n=9)
<u>R,R-hydroxybupropion</u>			
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	16.9 (12.1, 25.1)	16.5 (11.6, 24.8)	20.8 (15.1, 35.9)
formation clearance (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	1.1 (0.7, 1.9)	1.4 (0.9, 2.3)	1.5 (0.9, 2.4)
<u>S,S-hydroxybupropion</u>			
AUC _{0-∞} hydroxybupropion/bupropion (hr•ng•ml ⁻¹)	1.9 (1.4, 2.3)	2.0 (1.5, 2.6)	2.1 (1.4, 2.7)
formation clearance (ml ⁻¹ •kg ⁻¹ •min ⁻¹)	0.8 (0.7, 1.4)	1.2 (0.7, 1.8)	0.9 (0.4, 1.4)

Results are the mean ± SD or median (25th, 75th quartile)