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Effect of CYP2B6*6 and CYP2C19*2 genotype on chlorpyrifos metabolism

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Abstract

Chlorpyrifos (CPF) is a widely used organophosphorus (OP) pesticide. CPF is bioactivated by cytochrome P450s (CYPs) to the potent cholinesterase inhibitor chlorpyrifos oxon (CPF-O) or detoxified to 3,5,6-trichloro-2-pyridinol (TCPy). Human CYP2B6 has the highest reported V_{max}/K_m (intrinsic clearance - CL_{int}) for bioactivation while CYP2C19 has the highest reported CL_{int} for detoxification of CPF. In this study, 22 human liver microsomes (HLMs) genotyped for common variants of these enzymes (CYP2B6*6 and CYP2C19*2) were incubated with 10μM and 0.5μM CPF and assayed for metabolite production. While no differences in metabolite production were observed in homozygous CYP2C19*2 HLMs, homozygous CYP2B6*6 specimens produced significantly less CPF-O than wild-type specimens at 10μM (mean 144 and 446 pmol/min/mg, respectively). This correlated with reduced expression of CYP2B6 protein (mean 4.86 and 30.1 pmol/mg, for CYP2B6*6 and *1, respectively). Additionally, CYP2B6*1 and CYP2B6*6 were over-expressed in mammalian COS-1 cells to assess for the first time the impact of the CYP2B6*6 variant on the kinetic parameters of CPF bioactivation. The V_{max} for CYP2B6*6 (1.05×10^5 pmol/min/nmol CYP2B6) was significantly higher than that of CYP2B6*1 (4.13×10^4 pmol/min/nmol CYP2B6) but the K_m values did not differ (1.97 μM for CYP2B6*6 and 1.84 μM for CYP2B6*1) resulting in CL_{int} rates of 53.5 and 22.5 nL/min/nmol CYP2B6 for *6 and *1, respectively. These data suggest that CYP2B6*6 has increased specific activity but reduced capacity to bioactivate CPF in HLMs compared to wild-type due to reduced hepatic protein expression, indicating that individuals with this genotype may be less susceptible to CPF toxicity.

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Conflict of Interest Statement:
Dr. Zanger is named as co-inventor on a patent application directed to the detection of specific CYP2B6 polymorphisms for diagnostic purposes; this entitles him to share in any net income derived from licensing these patent rights under standard academic institutional policies. Drs. Klein and Olson and Ms. Crane declare that they have no conflicts of interest.

Keywords

CYP2B6; CYP2C19; chlorpyrifos; biotransformation

1.1 Introduction

Chlorpyrifos (CPF) is a widely used organophosphorus pesticide and is thought to primarily produce neurotoxic effects via inhibition of acetylcholinesterase (AChE). While acute exposure to CPF is well-known to cause neurotoxicity, less is known about chronic low-level exposure, though there is mounting evidence that neurotoxicity is also the primary endpoint for repeated OP exposures (Jiang et al. 2010; Middlemore-Risher et al. 2010; Rohlman et al. 2011; Salvi et al. 2003; Speed et al. 2011). Although residential use of CPF has been banned in the U.S. since 2001 (U.S. EPA 2000), inhalation and dermal exposures in agricultural communities remain a public health concern due to occupational exposures of pesticide workers and environmental exposures of the residents (Eaton et al. 2008). In addition, CPF is used worldwide with the highest exposures reported in an Egyptian agricultural population (Farahat et al. 2011; Farahat et al. 2010). This population was found to have neurobehavioral deficits when compared to a reference population (Abdel Rasoul et al. 2008; Farahat et al. 2003).

CPF is itself a weak inhibitor of AChE and must be bioactivated to the more potent metabolite, chlorpyrifos oxon (CPF-O) (Figure 1). CPF can also be detoxified to the nontoxic metabolite, 3,5,6-trichloro-2-pyridinol (TCPy). CYP2B6 has the highest reported V_{max}/K_m (intrinsic clearance CL_{int}) (15.6 nL/min/nmol P450) for the bioactivation of CPF to CPF-O, approximately five-fold higher than CYP1A2, the next most active CYP for this reaction (Foxenberg et al. 2007). For the detoxification of CPF to TCPy, CYP2C19 has the highest intrinsic clearance (8.1 nL/min/nmol P450), approximately six-fold higher than the next most active CYP (Foxenberg et al. 2007). However, it should be noted that all CYP enzymes which catalyze CPF biotransformation are capable of both the bioactivation and detoxification reaction at some level though CYP2B6 highly favors the bioactivation pathway while CYP2C19 favors detoxification. The predominant role of these CYP enzymes in CPF metabolism has been consistently demonstrated by both metabolism and inhibitor studies (Buratti et al. 2003; Croom et al. 2010; Foxenberg et al. 2007; Tang et al. 2001). Both enzymes have also been detected in human brain tissue, the target organ of toxicity for CPF (Miksys et al. 2003; Miksys and Tyndale 2002). The other main CYP involved in CPF metabolism is CYP3A4 which, while highly abundant in the liver, has a low affinity for the biotransformation of CPF (K_m 27.2μM, V_{max} 1.20×10^4 pmol/min/nmol P450 for the bioactivation reaction and K_m 33.4μM, V_{max} 1.27×10^4 for the detoxification reaction) and is thought to be the predominant enzyme of the low-affinity component of CPF metabolism, (Croom et al. 2010; Foxenberg et al. 2007).

CYP2B6 is a highly inducible and polymorphic enzyme which metabolizes not only clinically important drugs (e.g., bupropion, cyclophosphamide, efavirenz, propofol, selegiline) but also many environmental chemicals (Hodgson and Rose 2007; Turpeinen et al. 2006; Zanger et al. 2007). Variants of this enzyme have the potential to modify metabolism of CPF in vivo. The most prevalent allelic isoform of CYP2B6 is CYP2B6*6 (15631G>T and 18053 A>G) at a frequency of 20–31% in Caucasian populations and up to 60% in African and other populations (Zanger et al. 2007). The 15631G>T SNP in this variant codes for amino acid change Q172H in exon 4 but the important functional consequence is thought to result from erroneous splicing leading to decreased hepatic expression and activity (Hofmann et al. 2008; Lang et al. 2001). However, the 18053A>G SNP [K262R] also found in CYP2B6*6 and other variants appears to confer increased

substrate turnover and some substrate-dependent alterations in metabolism (Bumpus et al. 2005). Indeed, CYP2B6*6 has been found to confer decreased metabolism of bupropion (Hesse et al. 2004) and efavirenz (for review see (Roca 2008) but not cyclophosphamide (Nakajima et al. 2007; Xie et al. 2006). CYP2C19 is also a polymorphic enzyme with the most common variant being the null variant CYP2C19*2 at a frequency of 12–13% in Caucasians and up to 30% in Asians (Goldstein et al. 1997; Mizutani 2003). Examining the kinetics for CPF metabolism by common CYP variants has the potential to enhance our understanding of variability in human susceptibility to this common pesticide. Kinetic parameters for CPF metabolism can also be used in physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models which attempt to better quantify exposure, transportation, activation, detoxification, and clearance of OP pesticides (Foxenberg et al. 2011; Knaak et al. 2004; Timchalk et al. 2002). PBPK/PD models are dependent on the available kinetic parameters for metabolism, which are often not available and/or consistent and often based on data from studies with rat liver microsomes (for review, see (Knaak et al. 2004). Use of these data can under represent the interindividual variability present in humans. Kinetic data on the CYP-specific metabolism of CPF will inform current CYP specific CPF models by addressing interindividual variability and the impact of CYP2B6 and CYP2C19 genotype on the inhibition of AChE and butyrylcholinesterase (BChE), which serve as biomarkers of neurotoxicity (Foxenberg et al. 2011). A pharmacokinetic/pharmacogenetic model has recently been developed for the optimization of efavirenz therapy in caucasians with the inclusion of known CYP2B6*1/*6 genotype - successfully reducing the initial interindividual variability of the model (Sanchez et al, 2011).

Herein we report the effect of CYP2B6 and CYP2C19 genotype on CPF metabolism in 22 individual human liver microsomes (HLMs) and the effect of CYP2B6 genotype on CPF metabolism in recombinant enzymes. Also reported, for the first time, are the K_m and V_{max} values for bioactivation by CYP2B6*6, the most common genetic variant of CYP2B6. Together with estimates of human hepatic CYP2B6 content, these data can enhance current risk assessment efforts for CPF through assessing the potential impact of CYP2B6*6 and CYP2C19*2 genotype on relative susceptibility to CPF.

2.2 Materials and Methods

2.2 Chemicals

Chlorpyrifos (CAS 2921-88-2), chlorpyrifos-oxon (CAS 5598-15-2), and 3,5,6-trichloro-2-pyridinol (CAS 6515-38-4) were purchased from ChemService Inc. (West Chester, PA). Tetraisopropyl pyrophosphoramide (iso-OMPA; CAS 513-00-8) was of reagent grade and purchased from Sigma-Aldrich (St. Louis, MO). EDTA and $MgCl_2$, methanol, and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ) and were of at least reagent grade quality. Recombinant human CYP2B6 was purchased from BD Gentest (Woburn, MA).

2.3 HLM Characterization

22 individual human liver microsome specimens (HLMs), were selected according to CYP2B6 and CYP2C19 genotype from a collection of liver tissue specimens and corresponding blood samples (n=250) from patients of Caucasian ethnicity undergoing liver surgery at the Campus Virchow, University Medical Center Charité, Humboldt University, Berlin, Germany. The study was approved by the ethics committees of the medical faculties of the Charité, Humboldt University, and of the University of Tuebingen and conducted in accordance with the Declaration of Helsinki. The CYP2B6/CYP2C19 genotype groups (**1*1/*1*1; *6*6/*1*1; *6*6/*1*2; *1*1/*2*2*) did not differ significantly in age (56.3y; 56.8y; 62y; 47.3y, respectively). Gender and medication classification is shown in Table 1.

Genotyping methods have been described before (Blievernicht et al. 2007; Lang et al. 2001; Lang et al. 2004).

2.4 Bupropion hydroxylase activity

HLMs were assayed for the ability to metabolize bupropion to the metabolite OH-Bupropion (bupropion hydroxylase activity), a specific marker of CYP2B6 activity, as previously described (Hofmann et al. 2008). Briefly, 50μg of protein was incubated in a final volume of 0.1mL with a substrate concentration of 50μM. The reaction was initiated with 10μL of a 10-fold concentrated NADPH-regenerating system and terminated after 15 minutes with 20μL 1 N HCl. OH-Bupropion concentration was determined by liquid chromatography-mass spectroscopy with a detection limit of 1pmol as previously described (Richter et al. 2004). Samples were analyzed in duplicate.

2.5 CYP2B6 Content

HLMs were assayed for CYP and total protein content as described previously (Hofmann et al. 2008). Briefly, recombinant CYP2B6 lymphoblast microsomes (BD Biosciences, San Jose, CA) were coanalyzed as a standard on each 10% gel for quantification. CYP2B6 protein was immunodetected with a specific monoclonal mouse anti-human CYP2B6 antibody (BD Biosciences) and quantified by secondary IR Dye 800 labeled goat anti-mouse antibody using the infrared imaging system Odyssey (LI-COR Biosciences GmbH, Bad Homburg, Germany).

2.6 CPF Metabolism Assay

HLMs were incubated with CPF at concentrations greater than (10μM) and less than (0.5μM) the reported K_ms for bioactivation and detoxification of CPF by CYP2B6 (0.8 and 2.1μM) and CYP2C19 (1.2 and 1.6μM), respectively. Incubations with 0.5 mg of protein/mL were carried out in buffer consisting of 100mM Tris-HCL, and 5mM $MgCl_2$, 1mM EDTA to inhibit A-esterases, and 50μM iso-OMPA to inhibit B-esterases at a pH of 7.4. Reactions were initiated with 1mM NADPH and incubated at 37°C for a period of two minutes at a final volume of 200μL and quenched with an equal volume of ice cold methanol with 0.1% phosphoric acid. Reactions were then centrifuged and transferred to HPLC vials for analysis. Production of metabolites chlorpyrifos oxon (CPF-O) and 3,5,6-trichloro-2-pyridinol (TCPy) was measured by reverse phase HPLC with diode array detection as described previously (Foxenberg et al. 2007). Chemical detection was determined at the UV wavelengths of 290nm for CPF and CPF-O and 300nm for TCPy. The minimum level of detection of these compounds was 2.5ng. Assays were conducted in duplicate except where noted as done in singlet due to insufficient total protein content for two assays.

2.7 Transfection

Studies were conducted in COS-1 cells (ATCC, Washington DC). 3×10^6 COS-1 cells were plated onto 10cm dishes two days prior to transfection with cDNA vectors (Lang et al. 2004) for CYP2B6.1 and CYP2B6.6. The sequence of each plasmid was confirmed after transport and storage as well as after amplification for experiments by direct sequencing (Roswell Park Cancer Institute DNA Sequencing Laboratory, Buffalo, NY). One plate was transfected each time with a renilla luciferase reporter plasmid pRL-CMV (Promega, Madison, WI) to monitor relative transfection efficiency between experiments. 24μg of DNA was transfected using 72μL per plate Lipofectamine 2000 (Life Technologies, Carlsbad, CA). Two days post-transfection, cells were washed with phosphate-buffered saline and scraped off the plate. Cells were centrifuged and resuspended in 5mM HEPES buffer (pH 7.4). Cell lysates were homogenized with two strokes of a glass/glass homogenizer.

2.8 Recombinant CYP2B6 Content

The lysate fraction was analyzed for CYP2B6 content via western blot with a rabbit anti-human polyclonal antibody from Enzo Life Sciences (Farmingdale, NY). Recombinant CYP2B6 (BD Gentest, Woburn, AM) was coanalyzed on each gel to generate a standard curve. Horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin was used as the secondary antibody. Immunoreactive proteins were visualized with an enhanced chemiluminescence detection system from Amersham Biosciences (Piscataway, NJ). Relative protein concentration was determined by densitometry and is expressed as pmol/mg.

2.9 CPF Metabolism Assay

Assay was conducted as stated in section 2.6 for HLM specimens with the following changes: cell lysates were incubated with 0.1–100μM CPF and the final protein concentration was 2.0 mg of protein/mL.

2.10 Kinetic and Statistical Analysis

Data were fitted to the Michaelis-Menten equation by non-linear regression analysis using SigmaPlot 11 software (SyStat Software Inc, Chicago, IL) to derive K_m and V_{max} values. The mean and standard error of the mean (SEM) was determined for the K_m and V_{max} value for each allelic isoform. K_m and V_{max} values for CYP2B6*6 were analyzed for statistically significant differences compared to wild type protein using independent samples t-test using SigmaPlot. Genotyped groups of HLMs were compared using independent samples t-test using SPSS (Chicago, IL). Pearson's correlation coefficient was calculated for linear regression analysis (SPSS). In all cases, $p < 0.05$ was considered significant.

3.1 Results

3.2 Characterization of HLMs

Of 22 HLM specimens, 11 were homozygous wild-type CYP2B6 and CYP2C19, five were CYP2B6*6/*6 and homozygous wild-type CYP2C19, two were CYP2B6*6/*6 and CYP2C19*1/*2 and four were homozygous wild-type CYP2B6 and CYP2C19*2/*2. CYP2B6 content ranged from 1.50–100.8 pmol/mg and bupropion hydroxylase activity ranged from 10.5–1205 pmol/min/mg (Table 1). Four specimens were from patients on drugs known to be P450 inducers as noted in Table 1.

3.3 Correlation of CYP2B6 content and bupropion hydroxylation activity

Single donor HLM specimens (n=22) were assayed for CYP2B6 content and bupropion hydroxylation activity. Bupropion hydroxylation is specific for CYP2B6 and is used as an in vitro probe for CYP2B6 catalytic activity (Faucette et al. 2000; Loboz et al. 2006). To test the reliability of this assay in our sample set, bupropion hydroxylation activity (measured as pmol/min/mg OH-Bupropion formation) was compared to total CYP2B6 content (pmol/mg). Bupropion hydroxylation activity and CYP2B6 content were well-correlated ($r^2 = 0.86$, $p<0.01$) in HLM samples (Figure 2).

3.4 Correlation of CYP2B6 content with CPF metabolism by HLMs

Production of toxic metabolite CPF-O and nontoxic metabolite TCPy was measured at 10μM CPF and at 0.5μM CPF for 22 characterized HLM specimens. The rate of CPF-O formation from CPF (10μM) varied widely in these specimens from 0 (below the limit of detection) to 1738 pmol/min/mg, while TCPy formation was less variable with a range of 72.5 to 456.4 pmol/min/mg. The range of CPF-O formation at 0.5μM CPF was 0 to 127.7 pmol/min/mg while for TCPy it was 0 to 184.5 pmol/min/mg. At 10μM CPF, CYP2B6

content was correlated with the rate of CPF-O formation ($r = 0.76$, $p<0.01$) and also correlated to a lesser extent with TCPy formation ($r = 0.62$, $p<0.01$) (Figure 3A&3C). At 0.5μM CPF, CYP2B6 content was neither correlated with CPF-O formation ($r^2 = 0.33$) nor with TCPy formation ($r^2 = 0.30$) (Figure 3B&3D). Specimens with undetectable metabolite formation were removed from analyses. Every sample except one produced detectable levels of CPF-O at 10μM CPF and 10/22 produced detectable levels of CPF-O at 0.5μM CPF. All samples produced detectable levels of TCPy at 10μM CPF while 16/22 produced detectable levels of TCPy at 0.5μM CPF (Figure 4).

3.5 Impact of genotype on CPF metabolism by HLMs

CYP2B6 protein content in CYP2B6*6/*6 (4.86 ± 1.28 pmol/mg - mean ± SEM) HLM specimens was significantly lower than in CYP2B6*1/*1 wild type specimens (30.1 ± 7.19 pmol/mg) (Figure 5A). Similarly, CYP2B6*6/*6 specimens had a significant, albeit less dramatic decrease in the rate of formation of CPF-O at 10μM CPF relative to CYP2B6*1/*1 wild type specimens with means and SEMs of 144 ± 40.9 and 446 ± 109 pmol/min/mg, respectively (Figure 5B). Differences between genotypes remained significant when the analysis was repeated with omission of the potential outlier wild-type point (specimen with the highest CPF-O activity). The mean with this point removed for wild type CYP2B6 specimens was 353 ± 62.2 pmol/min/mg. In addition, the specimen that produced undetectable levels of CPF-O was a CYP2B6*6/*6 specimen. When production of CPF-O was standardized to nmol of CYP2B6 protein at 10μM CPF the mean rate was $1.79 \times 10^4 \pm 2.38 \times 10^3$ pmol/min/nmol CYP2B6 for CYP2B6 homozygous wild-type specimens and $4.44 \times 10^4 \pm 1.92 \times 10^4$ pmol/min/nmol CYP2B6 for CYP2B6*6/*6 specimens with no statistically significant difference between the two (Figure 5C). Mean rates of TCPy formation at 10μM CPF for homozygous wild-type CYP2B6 were 253 ± 23.8 pmol/min per mg total protein or $1.45 \times 10^4 \pm 2.79 \times 10^3$ pmol/min per nmol of CYP2B6 and for CYP2B6*6/*6 were 207 ± 32.8 pmol/min/mg or $6.04 \times 10^4 \pm 1.55 \times 10^4$ pmol/min/nmol CYP2B6 (Figures 5D&E). When the data is expressed per mg of total protein, CYP2B6*6/*6 variant specimens produce significantly less CPF-O metabolite. However, there is no significant difference between the two groups on a per nmol CYP2B6 basis, indicating that differences in CPF-O production are likely attributable to the lower overall CYP2B6 protein content of CYP2B6*6/*6 HLMs. Similar results were found at 0.5μM CPF, with the formation of CPF-O being significantly less for CYP2B6*6/*6 variants than CYP2B6*1/*1 specimens when expressed as pmol/min/mg (data not shown).

Of the 15 homozygous wild-type CYP2B6 HLMs, 10/15 produced more CPF-O metabolite than TCPy (expressed as pmol/min/mg) while only 1/6 CYP2B6*6/*6 HLMs produced more CPF-O than TCPy at 10μM CPF. At 0.5 μM CPF, of the 12 wild-type specimens that produced detectable levels of both CPF metabolites, only three produced greater amounts of CPF-O than TCPy while all four CY2B6*6/*6 HLMs with detectable metabolite production produced more TCPy.

No significant differences in the formation of CPF-O or TCPy were found between homozygous CYP2C19 wild type specimens and CYP2C19*2/*2 specimens at 10μM CPF (Figure 6A&B). The two heterozygous CYP2C19 samples were not included in this analysis. Means and SEM for homozygous wild-type CYP2C19 were 342 ± 107 pmol CPF-O/min/mg and 236 ± 23.4 pmol TCPy/min/mg. Means for CYP2C19*2 specimens were 419 ± 130 pmol CPF-O/min/mg and 251 ± 32.5 pmol TCPy/min/mg. No significant differences in metabolite formation were found between the two genotype groups at 0.5μM CPF (data not shown).

3.6 CPF metabolism by recombinant CYP2B6*1 and CYP2B6*6

CYP2B6*1 and CYP2B6*6 were over-expressed in mammalian COS-1 cells. Three different cell lysate preparations for each isoform were incubated with 0.1–100μM CPF to assess the kinetics for CPF metabolism for each genotype. CYP2B6 content of each preparation was determined by western blot. The rate of CPF metabolism exhibited Michaelis-Menten kinetics with the *1 and *6 genotypes exhibiting similar K_m values of 1.84 ± 0.80 and 1.97 ± 0.97μM, respectively (Figure 7). The V_{max} for CYP2B6*1 was found to be $4.13 \times 10^4 \pm 0.38 \times 10^4$ pmol/min/nmol CYP2B6 (Figure 7A). The V_{max} for CYP2B6*6 was determined to be $1.05 \times 10^5 \pm 0.12 \times 10^5$ pmol/min/nmol CYP2B6 (Figure 7B). While K_m values were not significantly different between the two recombinant CYP2B6 isoforms the V_{max} of CYP2B6*6 was significantly higher compared to that of CYP2B6*1. CL_{int} rates (53.5 and 22.5 nL/min/nmol for CYP2B6*6 and *1, respectively), were not significantly different.

4.1 Discussion

Current efforts in risk assessment often rely heavily on animal data which does not accurately reflect human metabolism or human interindividual variability. The understanding of human variability is critical to accurate models of risk assessment, including PBPK/PD models. Using 22 single donor HLMs, we determined that 21/22 samples formed detectable levels of CPF-O and all formed detectable levels of TCPy at a concentration of 10μM CPF. Roughly half (10/22) formed detectable levels of CPF-O at 0.5μM CPF and most (16/22) formed detectable levels of TCPy at the lower concentration. The high correlation of the rate of CPF-O production with HLM CYP2B6 content at 10μM CPF suggests that CYP2B6 is the dominant P450 enzyme at relatively low concentrations for bioactivation of CPF which agrees with previous studies indicating that the metabolism of CPF is mediated by both low and high-affinity enzymes with CYP2B6 and CYP2C19 being the most likely high-affinity candidates for the biotransformation of CPF (Croom et al. 2010; Foxenberg et al. 2007; Mutch and Williams 2006; Sams et al. 2004; Tang et al. 2001). More TCPy than CPF-O production and less correlation between CPF-O and CYP2B6 content was found in previous studies using CPF concentrations of 20μM and 100μM which also suggests that CPF may be metabolized by lower affinity enzymes such as CYP3A4 at higher concentrations (Croom et al. 2010). Importantly, our data also suggest that bioactivation of CPF to CPF-O may outpace detoxification to TCPy at low exposure levels that are relevant to occupational and environmental exposures (Farahat et al. 2011). The majority of CYP2B6*1/*1 HLM specimens (10/15) favored the bioactivation reaction over the detoxification reaction at 10μM CPF, though not at 0.5μM CPF (3/12). Unlike at higher concentrations of CPF (> 20μM), low concentrations of CPF that are above the K_m of CYP2B6 may result in the domination of the bioactivation pathway and production of the toxic metabolite. CYP2B6*6/*6 HLM specimens, on the other hand, favored the detoxification reaction at both 0.5μM and 10μM of CPF. This is an indication of the potential for CYP2B6 genotype to alter the balance between the two major pathways of CPF metabolism.

When comparing CYP2B6*1/*1 and CYP2B6*6/*6 HLM specimens, statistical differences were found in both total CYP2B6 protein content as well as formation of the toxic metabolite CPF-O at both 10μM and 0.5μM CPF. This is consistent with the proposed mechanism of the CYP2B6*6 polymorphism which is thought to result in decreased hepatic expression due to erroneous splicing (Hofmann et al. 2008). No statistical differences in metabolite formation were found when comparing CYP2C19*1/*1 and CYP2C19*2/*2 HLM specimens. It is surprising that CYP2C19*2 homozygous samples did not produce less TCPy on average when compared to wild type specimens given that CYP2C19*2 is a null phenotype although the availability of CYP2C19*2 homozygous sample group was very

small (n=4). It is likely that other CYPs in HLMs such as CYP3A4, 3A5, and 1A2 can compensate in the detoxification of CPF in the absence of CYP2C19, even at low concentrations of CPF. Despite the small sample size, an advantage of this study was the use of mostly homozygous samples, allowing us to more easily compare genotype effects which are more difficult to detect in a mix of heterozygous and homozygous samples.

The K_ms found here for recombinant CYP2B6*1 and *6 (1.84 vs. 1.97μM, respectively) are very close to that of 0.8μM determined for commercially available recombinant CYP2B6*1 by Foxenberg et al., (2007). While the K_ms did not differ, CYP2B6*6 had a significantly higher V_{max} than the wild-type enzyme. Despite the differences in V_{max} values, the *in vitro* data generated in this study as well as previous literature reports for the metabolism of other substrates by CYP2B6*6 suggest that this slightly higher V_{max} does not compensate for the decreased hepatic expression of CYP2B6*6. Results from characterized HLMs and these data indicate that the reduced rate of bioactivation of CPF in HLMs with the CYP2B6*6 genotype is due to reduced protein expression and not in different kinetic properties of the two isoforms.

4.2 Conclusions

Bioactivation of CPF in HLMs is decreased in CYP2B6*6/*6 specimens as compared to CYP2B6*1/*1 specimens and this decrease in metabolism corresponds to a decrease in protein expression in CYP2B6*6 HLMs. No change in the detoxification of CPF was seen in CYP2C19*2/*2 specimens, although the N was small (4). Kinetics of human recombinant CYP2B6*1 and CYP2B6*6 indicate that while CYP2B6*6 has a higher V_{max} than wild-type, there is no significant difference in K_m or CL_{int}. Together, these data suggest that the CYP2B6*6 allele is a protein with similar kinetics but decreased expression, leading to decreased bioactivation of CPF to CPF-O and possibly conferring decreased susceptibility to CPF.

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Nonstandard abbreviations used in this manuscript

CPF	chlorpyrifos
CYP	cytochrome P450
OP	organophosphorus
CPF-O	chlorpyrifos oxon
TCPy	3,5,6-trichloro-2-pyridinol
HLM	human liver microsome
AChE	acetylcholinesterase
BChE	butyrylcholinesterase

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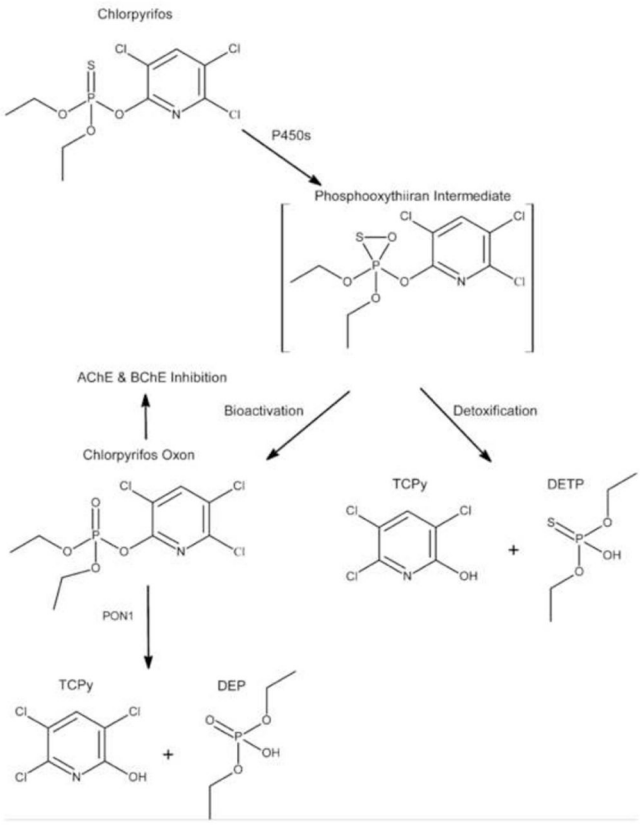

Figure 1.

Illustrates the major pathways of metabolism for chlorpyrifos (CPF). CYP2B6 has the highest CL_{int} for the bioactivation pathway while CYP2C19 has the highest CL_{int} for the detoxification pathway. TCPy (3,5,6-trichloro-2-pyridinol) is a metabolite specific to CPF. DETP (o,o-diethylphosphorothionate) and DEP (o,o-diethylphosphate) are non-specific metabolites of CPF. PON1 (paraoxonase1) is an alpha esterase.

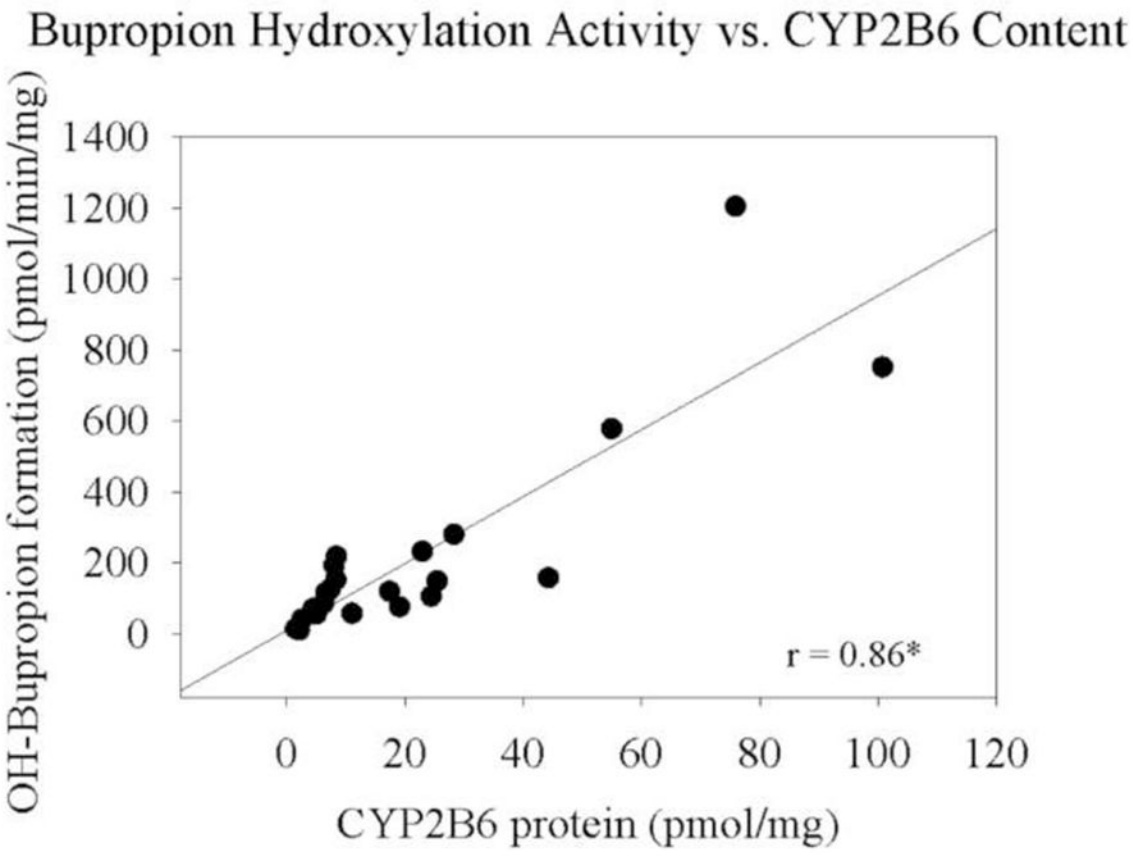

Figure 2.
Relationship between bupropion metabolism (OH-bupropion formation, pmol/min/mg) and CYP2B6 content (pmol/mg) in HLMs. Each point represents an individual specimen.
* indicates statistical significance at $p < 0.01$.

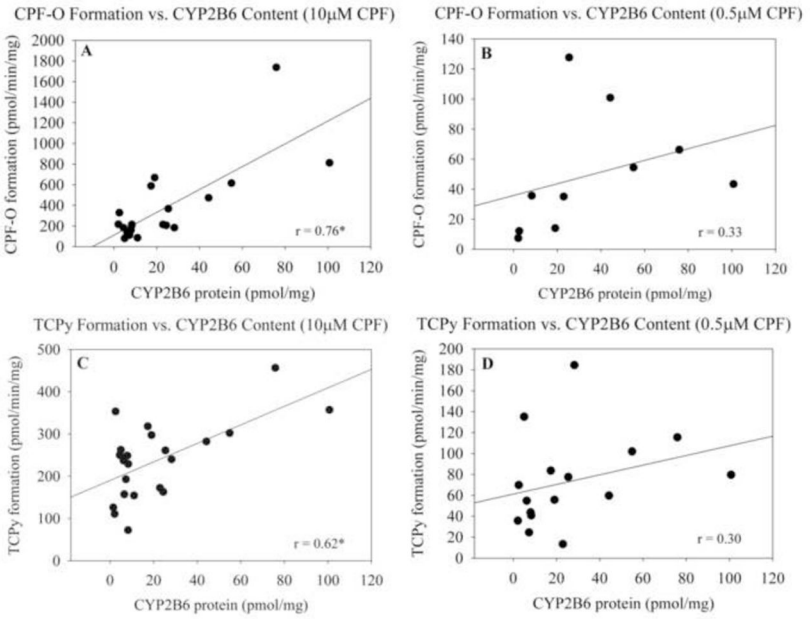

Figure 3.

Biotransformation of CPF to CPF-O (A&B) and TCPy (C&D) (pmol/min/mg) by HLMs relative to CYP2B6 content (pmol/mg) at 10μM CPF and at 0.5μM CPF. Specimens producing non-detectable levels of metabolite were not included in the analysis. * indicates statistical significance at $p < 0.01$.

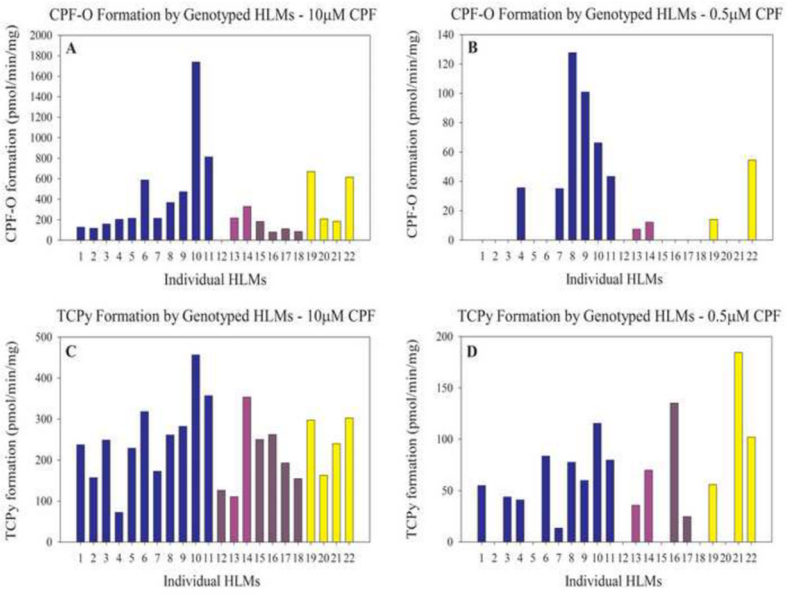

Figure 4.

Biotransformation of CPF to CPF-O (A&B) and TCPy (C&D) (pmol/min/mg) by individual, genotyped HLMs at 10μM CPF and at 0.5μM CPF. Each bar represents an individual HLM assayed in duplicate except samples 3, 6, 7, 17, and 19, which were done in singlet. Specimens 1–11 are CYP2B6*1/*1 and CYP2C19*1/*1, specimens 12 & 15–18 are CYP2B6*6/*6 and CYP2C19*1/*1, specimens 13 & 14 are CYP2B6*6/*6 and CYP2C19*1/*2, and specimens 19–22 are CYP2B6*1/*1 and CYP2C19*2/*2. A color version of this figure is available in the online version of this manuscript.

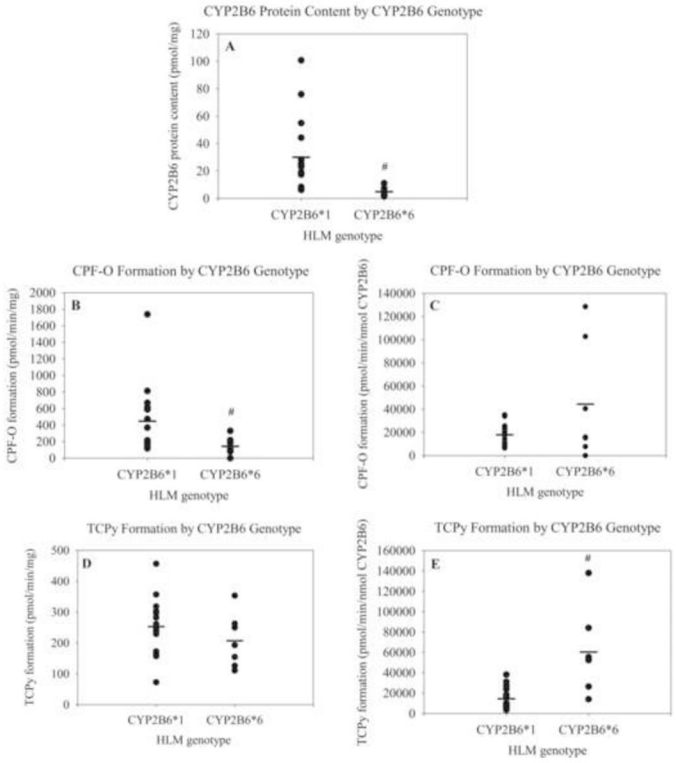

Figure 5.

(A) CYP2B6 protein content (pmol/mg) of individual HLM specimens by CYP2B6 genotype. Each dark circle represents an individual HLM and the mean is represented by a black line. CPF-O formation by CYP2B6 genotype groups expressed as pmol/min/mg (B) or in pmol/min/nmol CYP2B6 (C). TCPy formation by CYP2B6 genotype groups expressed as pmol/min/mg (D) or in pmol/min/nmol CYP2B6 (E). # indicates statistical significance at $p < 0.05$.

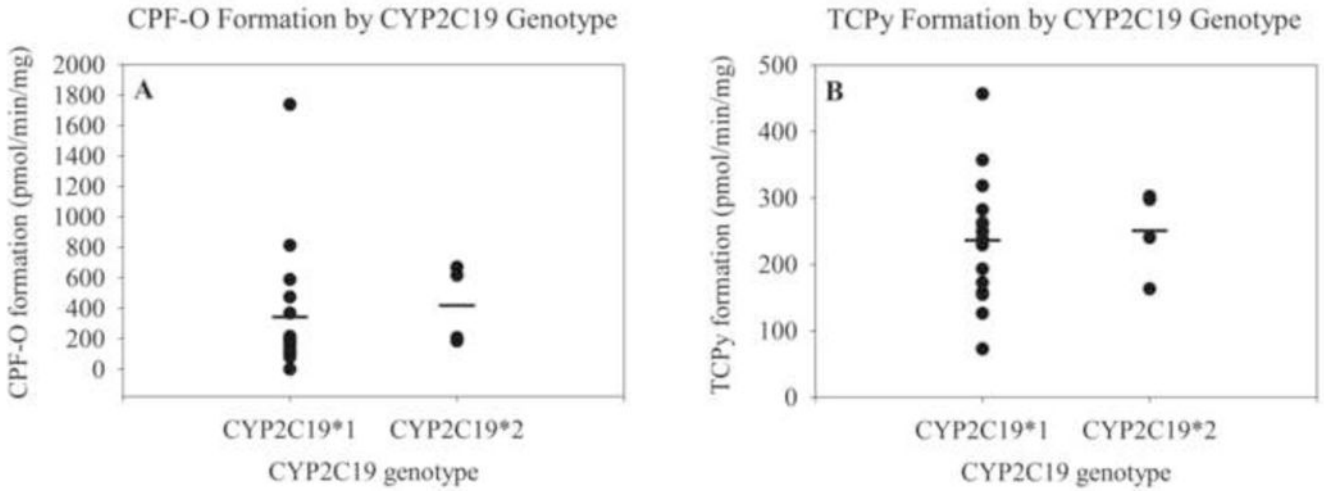

Figure 6.

CPF-O formation (A) or TCPy formation (B) by HLMs according to CYP2C19 genotype expressed as pmol/min/mg. Each dark circle represents an individual HLM and the mean is represented by a black line. The two heterozygous CYP2C19 samples were not included in this analysis.

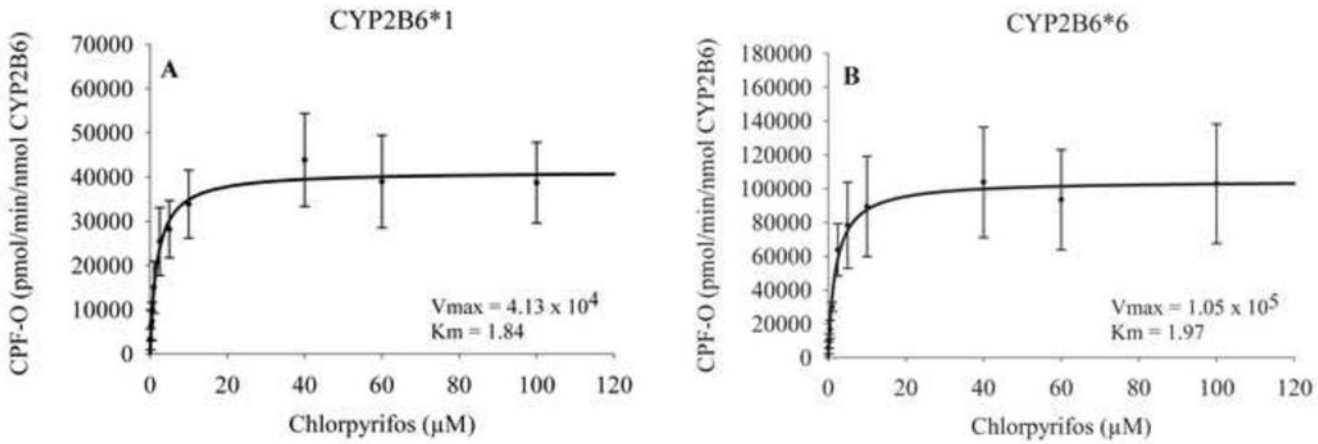

Figure 7.

Michaelis-Menton plot for the biotransformation of CPF to CPF-O by recombinant CYP2B6*1 (A) and CYP2B6*6 (B). Formation of metabolite is expressed as pmol/min/nmol CYP2B6. Graphs are the composite of three separate experiments and error bars represent standard error of the mean.

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Table 1

Genotype and Phenotype Characteristics of Human Liver Microsome Specimens

HLM ID	Drug exposure	Gender	CYP2B6 Genotype	CYP2C19 Genotype	CYP2B6 (pmol/mg)	Bupropion Hydroxylation Activity (pmol/min/mg OH-Bupropion formation)
1	Yes	female	*1/*1	*1/*1	6.21	86.22
2	P450 inducer	male	*1/*1	*1/*1	6.59	115.66
3	Yes	female	*1/*1	*1/*1	8.00	194.13
4	Yes	male	*1/*1	*1/*1	8.30	152.30
5	Yes	female	*1/*1	*1/*1	8.40	218.73
6	No	female	*1/*1	*1/*1	17.36	119.60
7	Yes	male	*1/*1	*1/*1	22.94	232.46
8	Yes	male	*1/*1	*1/*1	25.43	148.65
9	Yes	male	*1/*1	*1/*1	44.26	158.33
10	No	male	*1/*1	*1/*1	75.91	1204.93
11	N/A	male	*1/*1	*1/*1	100.76	752.41
12	P450 inducer	male	*6/*6	*1/*1	1.50	14.70
13	No	female	*6/*6	*1/*2	2.11	10.53
14	Yes	male	*6/*6	*1/*2	2.56	41.36
15	No	female	*6/*6	*1/*1	4.51	71.66
16	P450 inducer	female	*6/*6	*1/*1	5.00	56.18
17	No	male	*6/*6	*1/*1	7.30	124.26
18	No	female	*6/*6	*1/*1	11.04	57.76
19	Yes	male	*1/*1	*2/*2	19.05	76.40
20	Yes	female	*1/*1	*2/*2	24.45	105.88
21	P450 inducer	male	*1/*1	*2/*2	28.27	280.67
22	No	male	*1/*1	*2/*2	54.95	578.60

Genotype and phenotype characteristics of the 22 individual HLMs used. Original ID numbers were changed to a simple numbering system. Drug exposure indicates whether liver donors were exposed to known P450 inducers (omeprazole, pantoprazole, budenosid); the other patients received either no drugs or drugs not known to induce P450 prior to surgery; N/A., not available.