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Bioactivation of chlorpyrifos by CYP2B6 variants

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Abstract

1. Chlorpyrifos (CPF), an organophosphorus (OP) pesticide, is bioactivated by cytochrome P450s (CYPs) to the active metabolite chlorpyrifos oxon (CPF-O). Given that human CYP2B6 has the highest intrinsic clearance (CL_{int}) for CPF bioactivation, CYP2B6 polymorphisms may impact human susceptibility to CPF at real world environmental and occupational CPF exposure levels.
2. CYP2B6.4, .5, .7, and .18 were over-expressed in mammalian COS-1 cells to assess the impact of CYP2B6 variants on the K_m and V_{max} for bioactivation of CPF. Cell lysates were incubated with CPF (0–100 μ M) and the production of CPF-O was measured via HPLC analysis. CYP2B6 content was determined by western blot.
3. CYP2B6.18 had neither detectable protein nor activity levels. The V_{max} value for each remaining variant was significantly higher than wild-type (CYP2B6.1, V_{max} 4.13×10^4 pmol/min/nmol CYP2B6), with CYP2B6.4, .5, and .7 having V_{max} values of 4.52×10^5 , 1.82×10^5 , and 9.60×10^4 pmol/min/nmol CYP2B6, respectively. The K_m values for these variants ranged from 0.39–1.09 μ M and were not significantly different from wild-type. All active variants examined had significantly higher CL_{int} than CYP2B6.1.
4. Variants of CYP2B6 have altered capacity to bioactivate CPF and may affect individual susceptibility by altering the V_{max} for CPF-O formation.

Keywords

biotransformation; toxicokinetics; pesticide metabolism; cytochrome P450; polymorphism

Introduction

Chlorpyrifos (CPF) belongs to the diethylphosphorothionate subclass of organophosphorus (OP) pesticides. Though residential use of CPF has been banned in the U.S. since 2001 (U.S. EPA 2000), CPF is still used in agriculture and is in heavy use worldwide. Like other pesticides in this class, the parent compound has limited toxicity and must be bioactivated by a desulfation reaction to the active metabolite chlorpyrifos oxon (CPF-O). The production of the nontoxic CPF-specific metabolite 3,5,6-trichloro-2-pyridinol (TCPy) and non-specific dialkylphosphates are formed as a result of the detoxification of CPF by human

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Declaration of Interest:

The authors report no declarations of interest.

cytochrome P450 enzymes (CYPs) and of CPF-O by serum paraoxonase 1 (PON1) (Figure 1). Different CYPs mediate each major pathway for CPF metabolism so that varying CYP content and/or CYP genetic variants may influence the balance between the detoxification and bioactivation of CPF. Based on the low reported K_m (0.81 μM) and high $V_{\max}$ (12,544 pmol/min/nmol P450), CYP2B6 is the main CYP enzyme responsible for the bioactivation of CPF while CYP2C19 is primarily responsible for the detoxification of CPF (Foxenberg *et al.*, 2007). This specificity has been found consistently in additional CPF metabolism studies (Sams *et al.*, 2004; Mutch and Williams, 2006; Croom *et al.*, 2010). In contrast, CYP3A4 is a low affinity enzyme for CPF metabolism (27.3 μM) and equally participates in bioactivation and detoxification reactions (Foxenberg *et al.*, 2007). Thus, individual variability in CYP3A4 may have a limited impact on the balance between CPF bioactivation and detoxification.

CYP2B6 is a highly polymorphic enzyme and genetic variants of CYP2B6 may be one of the factors contributing to individual susceptibility to CPF toxicity. In addition, CYP2B6 is expressed in the brain, the target organ of CPF toxicity, primarily in neurons and in astrocytes (Miksys *et al.*, 2003). It is not known how genetic variants of CYP2B6 affect the kinetics of CPF metabolism compared to wild-type enzyme and kinetic parameters can be used to inform risk assessment efforts for CPF exposure. Current human risk assessment efforts rely heavily on rat metabolism data and do not account for the degree of interindividual variability present in human populations. By understanding factors contributing to interindividual variability in the metabolism of CPF and other environmental chemicals, the most vulnerable populations can be identified and protected.

Lang *et al.*, (2001) identified nine point mutations of CYP2B6 in a Caucasian population including the most common genetic variant, CYP2B6.6, which has been recently examined by our group (Crane *et al.*, 2012), as well as CYP2B6.4, .5, and .7 which are examined in this study. (A full listing of currently identified CYP2B6 alleles can be found at <http://www.cypalleles.ki.se/cyp2b6.htm>). These variants have been examined with CYP2B6 probe substrates including bupropion, S-mephenytoin, and 7-ethoxy-4-trifluoromethylcoumarin. CYP2B6.4 (785A>G) has been found to have increased 7-ethoxy-4-trifluoromethylcoumarin *O*-deethylation activity (Jinno *et al.*, 2003) and bupropion hydroxylation activity relative to wild-type (Lang *et al.*, 2001; Kirchheiner *et al.*, 2003). CYP2B6.5 (1459C>T) and CYP2B6.7 (516G>T, 785A>G, 1459C>T) were both found to have increased 7-ethoxy-4-trifluoromethylcoumarin *O*-deethylation activity (Jinno *et al.*, 2003) but decreased activity towards S-mephenytoin and decreased hepatic expression relative to wild type (Lang *et al.*, 2001). CYP2B6.18 (983T>C), identified by Klein *et al.* (2005), had decreased bupropion hydroxylation activity compared to wild type and protein could not be detected in a heterologous system. It is clear that variant effects on metabolism may have a substrate dependent effect on metabolism that cannot be predicted based on ability to metabolize probe substrates.

In this study we transiently transfected mammalian COS-1 cells with cDNA vectors of CYP2B6.4, CYP2B6.5, CYP2B6.7, and CYP2B6.18 in order to characterize the effects of these alleles on CPF metabolism and to generate K_m and $V_{\max}$ parameters for each CYP2B6 allele.

Materials and Methods

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 pyrophosphoramidate (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 with reductase and b5 was purchased from BD Gentest (Woburn, MA).

Transfection of cDNA vectors

cDNA vectors (a generous gift from Dr. Ulrich Zanger - Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany - Lang *et al.*, 2004) for CYP2B6.4, CYP2B6.5, CYP2B6.7, and CYP2B6.18 were transfected into COS-1 cells (ATCC, Washington, DC). Briefly, cells were plated onto 10cm dishes at a density of 3 million cells per plate two days prior to transfection. One plate was transfected each time with a renilla luciferase reporter plasmid pRL-CMV (Promega, Madison, WI) to monitor relative transfection efficiency between experiments. Two days post-transfection, cells were washed with phosphate-buffered saline (PBS) and scraped off the plate. Cells were centrifuged and resuspended in 5mM HEPES buffer (pH 7.4) and homogenized with two strokes of a glass/glass homogenizer.

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).

Determination of CYP2B6 content in transfected COS-1 cells

The cell lysate fraction was analyzed for CYP2B6 content via western blot as described previously with a rabbit anti-human polyclonal antibody for CYP2B6 from Enzo Life Sciences (Farmingdale, NY). Recombinant CYP2B6 (BD Gentest, Woburn, AM) was only used to generate a standard curve and not for metabolism studies. Each Western blot contained a standard curve consisting of five different concentrations of recombinant commercial CYP2B6 ranging from 0.01 – 0.2pmol, which was used to quantify CYP2B6 content of lysates from COS-1 cells. Relative CYP2B6 protein concentration was determined by densitometry (Crane et al, 2012).

CPF Metabolism

COS-1 cell lysates were incubated with 0.1 μM – 100 μM CPF at 37°C for an incubation period of 2 minutes at a final protein concentration of 2mg/mL. Preliminary time course studies were run to determine 2 minutes as the optimal incubation time and within the linear timeframe of metabolite production. No exogenous cytochrome P450 reductase or cytochrome b5 was added. Briefly, incubations were carried out in glass tubes to limit substrate binding in buffer consisting of 100mM Tris-HCL, and 5mM MgCl_2 , 1mM EDTA to inhibit A-esterases, and 50 μM iso-OMPA to inhibit B-esterases at a pH of 7.4. Reactions were initiated with 1mM NADPH at a final volume of 200 μL and quenched with an equal volume of ice cold methanol with 0.1% phosphoric acid. Production of metabolites chlorpyrifos oxon (CPF-O) and 3,5,6-trichloro-2-pyridinol (TCPy) were measured by reverse phase HPLC with diode array detection as described previously (Foxenberg *et al.*, 2007). Experiments were conducted 3–4 times with different COS-1 expressed CYP2B6 lysate preparations for each genotype. Reported data for CYP2B6.1 and CYP2B6.6 were generated under the same experimental conditions (Crane *et al.*, 2012).

Statistics/Kinetic Plots

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 CYP2B6.4, CYP2B6.5 and CYP2B6.7 were analyzed for statistically significant differences compared to wild type protein using ANOVA with Dunnett's post-hoc analysis in SigmaPlot. CL_{int} (V_{max}/K_m) values were determined based on individual V_{max} and K_m kinetic parameters for each experiment for each of the variants and wild type CYP2B6. In all cases, $p < 0.05$ was considered significant.

Results

CYP2B6 cDNA of CYP2B6.4, .5, .7, and .18 were expressed in mammalian COS-1 cells. The expression level of wild-type and variant CYP2B6 protein in COS-1 cells was assessed by western blot (Figure 2). CYP2B6.18 protein was not detected by this method. CYP levels in COS-1 cell lysates expressing CYP2B6.1, .4, .5, and .7 were 9.89 ± 7.78 , 4.75 ± 3.64 , 0.69 ± 0.23 , and 1.86 ± 0.89 pmol/mg protein, respectively.

In vitro kinetics for the bioactivation of CPF by recombinant CYP2B6 genetic variants were determined for four alleles that are known to occur at relevant frequencies (Table 1). Bioactivation was measured by production of the active metabolite, CPF-O, via HPLC analysis and the results from 3–4 separate experiments were plotted (Figure 3). The detoxification product, TCPy, was detected at inadequate levels to determine the kinetics for the detoxification reaction. The *in vitro* kinetics for bioactivation of CPF by CYP2B6.1 (wild-type) and CYP2B6.6 were also recently determined by our group and presented again here for comparison purposes (Crane et al, 2012).

As expected, no metabolism of CPF was detected for CYP2B6.18. The V_{max} values for CYP2B6.4, .5, and .7 were significantly greater than wild-type CYP2B6.1 while the K_m for each variant was not significantly different from wild-type enzyme (Table 2). CYP2B6.4, .5, and .7 all exhibited intrinsic clearance values significantly greater than wild-type, with CYP2B6.4 having the highest CL_{int} (414 ± 17.2 compared to wild-type $26.4 \pm 11.3 \mu\text{L}/\text{min}/\text{nmol}$ CYP2B6, respectively). CYP2B6.6 had a significantly higher V_{max} ($1.05 \times 10^5 \pm 1.16 \times 10^4$ pmol/min/nmol CYP2B6) than CYP2B6.1 but the CL_{int} ($61.1 \pm 8.14 \mu\text{L}/\text{min}/\text{nmol}$ CYP2B6) did not differ from wild type (Table 2).

Discussion

CPF is a public health concern due to widespread, worldwide use with great potential for human exposure. Its status as a pro-poison indicates that balance between bioactivation and detoxification reactions may directly impact the relative toxicity associated with a given exposure. Due to the involvement of different CYP enzymes in bioactivation and detoxification reactions, this balance may be altered depending on both individual enzyme expression and genetic variability. The current literature confirms the importance of CYP2B6 in the bioactivation of CPF although few kinetic studies have been done *in vitro* (Table 3). Variants of CYP2B6 have the potential to shift the balance of CPF metabolism and thus are likely biomarkers of susceptibility. Furthermore, CYP2B6 may be particularly relevant at occupational and environmental exposures levels at which low affinity enzymes such as CYP3A4 would not play a large role in CPF metabolism.

CYP2B6.4, .5, and .7 were successfully expressed in mammalian COS-1 cells and all variant forms, particularly CYP2B6.5, had decreased expression in this heterologous system compared to CYP2B6.1, but the difference was not significant due to the wide range of variability in expression between experiments. Expression levels of variants may be lower than wild-type due to unstable conformation of the protein or altered folding efficiency. Altered transfection efficiency of the plasmids may also play a role. CYP2B6.18 protein

could not be detected in this system at all, which agrees with previous reports that recombinant CYP2B6.18 has no activity towards artemether, another CYP2B6 substrate (Honda *et al.*, 2011) and has undetectable expression in heterologous systems (Klein *et al.*, 2005). Together, these data are suggestive of a null variant.

CYP2B6 is responsible for the metabolism of 2–13% of clinically relevant drugs and a panoply of environmental xenobiotics (for review see Hodgson and Rose, 2007; Wang and Tompkins, 2008; Mo *et al.*, 2009). CYP2B6 genetic variation has been shown to impact susceptibility to disease (Berkoz and Yalin, 2009) and therapeutic efficacy and toxicity, particularly with the chemotherapeutic drug cyclophosphamide and the non-nucleoside reverse transcriptase inhibitor efavirenz (Gatanaga *et al.*, 2007; Nakajima *et al.*, 2007; Viljoen *et al.*, 2011; Wyen *et al.*, 2011) and remains an active area of investigation. To our knowledge, no previous studies have addressed the effects of these CYP2B6 variants on the kinetics of CPF metabolism and there is only limited information available on other P450 polymorphisms (Tang *et al.*, 2001). Some SNPs found in these genetic variants have been found to be substrate specific in action so that it is not possible to predict the effect of genetic variants based on known substrates. The impact of genetic variants of CYP2B6 on CPF has never been examined, although CYP2B6 is regarded as the primary enzyme for bioactivation at relevant human exposures. While CYP3A4 is the most abundant CYP in the human liver and is capable of metabolizing many pesticides, it is a low affinity enzyme for these compounds while CYP2B6 is a very efficient xenobiotic metabolizing enzyme for several pesticides including azinphos-methyl (K_m 1.0 μ M), parathion (K_m 1.9 μ M), and chlorpyrifos (K_m 0.8 μ M) (Buratti *et al.*, 2003; Foxenberg *et al.*, 2007). This is particularly important for occupational and environmental CPF exposures, which occur at levels favoring metabolism by CYP2B6, a high affinity enzyme.

In addition, estimates of human kinetic parameters are needed for physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models. PBPK/PD models provide means to quantify the impact of genotype on the endpoint of ChE inhibition and relative susceptibility. However, available models are currently unable to fully take into account interindividual susceptibility, partially due to lack of human kinetic data for common variant genotypes although a CYP-specific human PBPK/PD model for CPF was recently reported (Foxenberg *et al.*, 2011).

With the exception of CYP2B6.18, the prevalent variants studied have essentially the same K_m s for CPF but higher V_{max} s when compared to the wild-type enzyme. CYP2B6.4, .5, and .7 all had significantly higher intrinsic clearance than CYP2B6.1 with CYP2B6.4 having the highest CL_{int} . The A785G SNP of CYP2B6.4 (also found in CYP2B6.6 and .7) tends to be substrate specific in that it has been found to have an unchanged K_m for 7-ethoxy-4-(trifluoromethyl)coumarin (7-EFC) but an increased turnover, while turnover of bupropion was decreased compared to CYP2B6.1 (Jinno *et al.*, 2003; Wang *et al.*, 2006). Hepatic expression of CYP2B6.4 in human liver microsomes has been so far inconclusive for this variant though levels tended to be lower than wild type, as in our expression system (Lang *et al.*, 2001; Desta *et al.*, 2007). It is interesting that all variants examined in this study had significantly increased activity towards the bioactivation of CPF as a substrate *in vitro*. In human liver specimens, the C1459T SNP, possessed by both CYP2B6.5 and CYP2B6.7, conferred decreased activity towards S-mephenytoin and decreased protein expression, though the difference in expression was not significant (Lang *et al.*, 2001). CYP2B6.5 in a later study was confirmed to be expressed in human liver microsomes at much lower levels than CYP2B6.1 (Desta *et al.*, 2007) though no significant alteration in bupropion metabolism was observed *in vivo* (Kirchheiner *et al.*, 2003).

Our data suggest that these CYP2B6 variants confer altered kinetics towards CPF bioactivation. It would be anticipated that *in vivo* CYP2B6.18 carriers would be at a lower risk of CPF toxicity than wild-type carriers while CYP2B6.4, .5, and .7 carriers would be at a higher risk due to increased production of toxic metabolite CPF-O. CYP2B6.6, as determined previously, had a significantly increased $V_{\max}$ but similar CL_{int} to wild-type enzyme (Crane et al., 2012). Future studies will be conducted in order to determine the impact of protein expression on CPF bioactivation by these CYP2B6 variants. In particular, studies conducted in genotyped human liver microsomes are needed to address the potentially different kinetics of these CYP2B6 variants in the presence of the other, competing, CYP enzymes. Differences in expression level among the variants may also be characterized through these experiments.

Besides genetically determined CYP2B6 protein expression and activity, other major factors which can modulate susceptibility include protein induction and inhibition, CYP2C19 and CYP3A4 content and polymorphisms, and diet. CYP2B6 is an inducible enzyme under the regulation of the pregnane X-receptor (PXR) and the constitutively active receptor (CAR) (Sueyoshi *et al.*, 1999; Goodwin *et al.*, 2001). Several substrates of CYP2B6 are also good inducers (Gervot *et al.*, 1999; Burk *et al.*, 2005; Faucette *et al.*, 2007). Among the most potent inhibitors are clopidogrel and ticlopidine (Walsky *et al.*, 2006) and CPF has been found to inhibit CYP2B6 as well (Joo *et al.*, 2007). However, despite myriad factors that determine the end metabolic result, successful attempts in adjusting therapeutic doses based on CYP2B6 genotype alone have occurred (Gatanaga *et al.*, 2007). With information about genetic variants of pesticide metabolism, it may be possible to identify and protect vulnerable agricultural worker and pesticide manufacturer populations from the toxic effects of these chemicals based on CYP2B6 genotype.

Conclusion

The objective of the current work was to determine if variants of CYP2B6 altered the kinetics of biotransformation of CPF. Recombinant CYP2B6.4, .5, .7, and .18 were incubated with CPF and CPF-O production was monitored. CYP2B6.18 had no activity while CYP2B6.4, .5, and .7 has an increased $V_{\max}$ and CL_{int} compared to wild-type CYP2B6.1. These results suggest that the variants studied alter catalytic activity of CYP2B6 towards CPF bioactivation which in turn suggests that individuals harboring these variants may have altered susceptibility to environmental or occupational exposures to CPF. Future studies will determine the effects of these variants on hepatic CYP2B6 expression.

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Figure 1.

Primary pathways for metabolism of CPF in humans (Crane et al, 2012). 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. AChE and BChE are acetylcholinesterase and butyrylcholinesterase, respectively, and are both inhibited by CPF-O. PON1 (paraoxonase1) is an alpha esterase.

Figure 2.

Representative Western blots for the determination of CYP2B6 content in lysate preparations. The standard curve lanes refer to pmol of commercial CYP2B6 standard. CYP2B6.1 and CYP2B6.4 samples refer to CYP2B6 transfected COS-1 cell lysate of known protein concentration (5 μ g and 10 μ g, respectively). Similar experiments were conducted for each CYP2B6 variant and for each lysate preparation used for metabolism studies.

Figure 3.

The relationship between metabolite formation (CPF-O) and substrate concentration (CPF) is shown for the bioactivation of chlorpyrifos to CPF-O by CYP2B6.4 (A), CYP2B6.5 (B), and CYP2B6.7 (C) expressed in COS-1 cells. SigmaPlot derived kinetic parameters according to Michaelis-Menten kinetics; the mean $\pm$ SD of three (CYP2B6.4 & .7) or four (CYP2B6.5) separate experiments is shown.

Table 1

Reported frequencies of CYP2B6 variants in Caucasian and African/African-American populations

CYP2B6 Variant	Frequency (%)	Gene Mutations	Protein (AA changes)
CYP2B6.1	48–61	None	None
CYP2B6.4	2.7–8.7	A785G	Lys262Arg
CYP2B6.5	6.2–14	C1459T	Arg487Cys
CYP2B6.6	20–31	G516T, A785G	Gln172His, Lys262Arg
CYP2B6.7	0–1.9	G516T, A785G, C1459T	Gln172His, Lys262Arg, Arg487Cys
CYP2B6.18	0–7.5	T983C	Ile328Thr

CYP2B6.1, .4, .5, .6, and .7 frequency is given for caucasians (Kirchheiner *et al.*, 2003; Lang *et al.*, 2004). CYP2B6.18 is absent in Caucasians and Asians but found in up to 7.5% of African and African-American populations (Mehlotra *et al.*, 2007).

Table 2

Kinetic parameters for the bioactivation of chlorpyrifos to CPF-O by CYP2B6 variants expressed in COS-1 cells

Variant	K _m (μM)	V _{max} (pmol/min/nmol CYP2B6)	V _{max} /K _m (nL/min/nmol CYP2B6)
CYP2B6.1	1.84 ± 0.80	4.13 × 10 ⁴ ± 3.85 × 10 ³	26.4 ± 11.3
CYP2B6.4	1.09 ± 0.21	4.52 × 10 ⁵ ± 1.71 × 10 ⁴ *	414 ± 17.2 *
CYP2B6.5	0.80 ± 0.48	1.82 × 10 ⁵ ± 2.11 × 10 ⁴ *	229 ± 57.8 *
CYP2B6.6//	1.97 ± 0.97	1.05 × 10 ⁵ ± 1.16 × 10 ⁴ *	61.1 ± 8.14
CYP2B6.7	0.39 ± 0.14	9.60 × 10 ⁴ ± 6.10 × 10 ³ *	207 ± 57.1 *
CYP2B6.18	ND	ND	ND

V_{max} and K_m values represent the mean ± SD of 3–4 separate experiments as determined by SigmaPlot. V_{max}/K_m (intrinsic clearance) values represent the mean ± SD of 3–4 separate experiments, based on the individual V_{max} and K_m parameters for each experiment with wild-type (CYP2B6*1) and each genetic variant.

* Kinetic parameters for CYP2B6 variants that are significantly different than CYP2B6.1 using ANOVA with Dunnett's post-hoc analysis (*p* < 0.05)

// reported previously (Crane et. al, 2012).

ND – no metabolite detected.

Table 3
Summary of current literature findings describing CPF biotransformation by human recombinant CYPs or human liver microsomes

Study	Protein & CPF Concentration	Primary CYPs involved in bioactivation of CPF	Bioactivation Kinetics	Primary CYPs involved in detoxification of CPF	Detoxification Kinetics
Recombinant CYPs					
Tang et al. 2001	Recombinant (human lymphoblast except 2C19-E Coli) 100µM	2B6>3A4>1A2, 2C9 and 2C19 (2C19*5,*6,*8 not active)	N/A	2C19>2C9>3A4>2B6>1A2 2C19>1>*8>*6 and *5	N/A
Buratti et al. 2002a	Commercial recombinant 25µM & 100µM	At both concentrations 2B6>3A4, 1A2>2C19	N/A	25µM 2C19>3A4, 2B6>1A2 100µM 2C19>3A4>2B6>1A2	N/A
Sams et al. 2004	Commercial Recombinant 12.5µM & 100µM	At both concentrations: 2B6>1A2>2C19>2A6, 2C9, 2D6, and 3A4	N/A	12.5µM 2C19>2C9 100µM 2C19>2B6, 2C9, 2D6>1A2	N/A
Mutch et al. 2006	Commercial Recombinant 100µM	2D6>3A5>2B6>3A4>2C19> 2C8>1A2	N/A	2C19>2D6>3A5>3A4>2B6>2C8>1A2	N/A
Foxenberg et al. 2007	Commercial Recombinant 0.5–60µM	2B6>1A2>2C19> 3A4>3A5>3A7	CL _{int} (nL/min/nmol CYP) 2B6 – 15.6 1A2 – 3.1 2C19 – 2.0	2C19>1A2>2B6> 3A5>3A5	CL _{int} (nL/min/nmol CYP) 2C19 – 8.1 1A2 – 1.4 2B6 – 0.7
Croom et al. 2010	Commercial Recombinant 100µM	2B6>2C9>3A4>2C19	N/A	2C19>3A4>2C9>2B6	N/A
Crane et al. 2012	Recombinant (COS-1) 0.5–100µM	N/A	CL _{int} (nL/min/nmol CYP) 2B6*1 – 26.4 2B6*6 – 61.1	N/A	N/A
Human Liver Microsomes					
Tang et al. 2001	Pooled (10 donor) 2–100µM	N/A	CL _{int} (µL/min/mg) Pooled: 0.01	N/A	CL _{int} (µL/min/mg) Pooled: 0.05
Buratti et al., 2002b	Pooled (5 donor) and individual 0.2–10µM Correlation study: 5µM	2B6>3A4>1A2	CL _{int} (µL/min/mg) Individual: 0.91 Pooled: 0.52	N/A	N/A
Mutch et al. 2006	Individual 100µM	None Significantly Correlated	N/A	3A4/5, 2C8, 2C19, 1A1/2	N/A
Croom et al., 2010	Individual 20µM & 100µM	20µM 2B6>2C8>2C9, 3A4/5 100µM 3A4/5>2B6, 2C8>2C19	N/A	20µM 3A4/5>2B6>2C8, 2C19 100µM 3A4/5>2C19>2B6>2C8	N/A

Study	Protein & CPF Concentration	Primary CYPs involved in bioactivation of CPF	Bioactivation Kinetics	Primary CYPs involved in detoxification of CPF	Detoxification Kinetics
Smith et al., 2011	Individual 20–781.25µM	Correlation with V_{max} 3A4/5>2B6,2C8 Correlation with CL_{int} 2B6>3A4/5>2D6	CL_{int} (µL/min/mg) 0.10–25.80	Correlation with V_{max} 3A4/5 Correlation with CL_{int} 2C19	CL_{int} (µL/min/mg) 1.42–43.67