DMD Fast Forward. Published on November 1, 2006 as doi:10.1124/dmd.106.012427

Human Hepatic Cytochrome P450 –Specific Metabolism of Parathion and Chlorpyrifos

Robert J Foxenberg, Barbara P McGarrigle, James B Knaak, Paul J Kostyniak, James R Olson

University at Buffalo, State University of New York – Dept of Pharmacology and Toxicology

Human CYP –Specific Metabolism of Parathion and Chlorpyrifos

Corresponding Author:
Dr James R Olson
102 Farber Hall
3435 Main St
Buffalo, NY 14214
(716)829-2319
Fax (716)829-2801
jolson@buffalo.edu

Number of Text Pages – 13

Number of Figures – 1

Number of Tables – 1

Number of References – 14

Word Count

Abstract - 250

Introduction - 566

Discussion – 898

Non-standard Abbreviations –

OP – Organophosphorous Pesticides **CYP / P450** – Cytochrome P450

PON1 – Paraoxonase 1 **AChE** – Acetylcholinesterase

PBPK/PD – Physiologically Based Pharmacokinetic / Pharmacodynamic

TCP - 3,4,5-tricholorpyrindinol

PNP – p-nitrophenol

DEP – O,O-diethyl phosphate

DETP - Diethylthiophosphate

Abstract

Organophosphorous pesticides (OPs) remain a potential concern to human health due to their continuing world-wide use. Thiophosphorous OPs, once bio-activated by cytochrome P450s (CYPs), form oxon metabolites which are potent acetylcholinesterase inhibitors. This study investigated the rate of desulfation (activation) and dearylation (detoxification) of parathion and chlorpyrifos in human liver microsomes. In addition, recombinant human CYPs were utilized to quantify for the first time the CYP –specific kinetic variables (K_m and V_{max}) for each compound for future use in refining human PBPK/PD models of OP exposure. CYPs 1A2, 2B6, 2C9, 2C19, 3A4, 3A5, and 3A7 were found to be active to a widely varying degree in parathion metabolism while all, with the exception of CYP2C9, were also found to be active in chlorpyrifos metabolism. CYP2B6 and CYP2C19 demonstrated low K_m and high V_{max} values for the metabolism of both model compounds which supports their role as being the primary enzymes which regulate metabolism at low level human exposures to OPs. With K_m and V_{max} values of 0.61 μM, 4827 pmol/min/nmol P450 and 0.81 μM, 12544 pmol/min/nmol for formation of paraoxon and chlorpyrifos-oxon respectively, CYP2B6 favored the desulfation reaction. CYP2C19 activity favored dearylation with K_m and V_{max} values of 0.60 μM, 2338 pmol/min/nmol P450 and 1.63 μM, 13128 pmol/min/nmol for formation of p-nitrophenol and 3,4,5-tricholorpyrindinol respectively. CYP –specific kinetic parameters for OP metabolism will be utilized with age –dependent hepatic CYP content to enhance PBPK/PD models so that OP exposures can be modeled to protect human health in different age groups.

DMD Fast Forward. Published on November 1, 2006 as DOI: 10.1124/dmd.106.012427
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Organophosphorous pesticides (OPs) are currently the most commonly utilized pesticides in the world with uses ranging from commercial and home use to agricultural application, all for controlling unwanted insect pests (Sultatos, 1994). Bio-activation of thiophosphorous OPs results in formation of oxon metabolites which bind to cholinesterases; both B-esterases which are irreversibly bound by OPs and A-esterases (paraoxonase 1; PON1) which are reversibly bound through hydrolyzing the active oxon metabolite (Sultatos, 1994; Ecobichon, 2001). B-esterase members include butyrylcholinesterase, carboxylesterase, and most importantly for OP action, acetylcholinesterase. Inactivation of acetylcholinesterase (AChE) results in an the accumulation of acetylcholine causing over-stimulation of cholinergic nerves. (Chanda et al., 1997; Ecobichon, 2001).

Activation of OPs has been attributed to the cytochrome P450 (CYP or P450) family of enzymes. CYPs have also been shown to carry out direct detoxification of the parent compounds through a dearylation reaction (Mutch et al., 2003; Poet et al., 2003). Humans, with their ability to bio-activate OPs, primarily through CYPs 1A2, 2B6, 2C19, and 3A4, are particularly sensitive to the actions of OPs (Mutch et al., 1999; Sams et al., 2000; Buratti et al., 2003).

Parathion and chlorpyrifos often have been utilized as model compounds with the research base for parathion spanning decades (for review see Knaak *et al.* (2004)). Although parathion use is banned within the USA, it is still used worldwide due to its potency, effectiveness, and low cost. Chlorpyrifos is available for use in the U.S. and is found in formulations carrying brand names such as Dursban® and Lorsban® and is often the insecticide of choice when it comes to cockroach control. Paraoxon and chlorpyrifos-oxon represent the activated forms of parathion and chlorpyrifos respectively while O,O-diethyl phosphate (DEP)

and p-nitrophenol (PNP), from parathion, or DEP and 3,4,5-trichloropyrindinol (TCP), from chlorpyrifos, represent the more readily cleared detoxification products. The balance between activation and detoxification of OPs determines their risk to humans.

Physiologically based pharmacokinetic / pharmacodynamic (PBPK/PD) models attempt to better quantify exposure, transportation, activation, detoxification, and clearance of OPs in order to determine a safe levels of human exposure (Timchalk et al., 2002; Knaak, 2004). PBPK/PD models are dependent on the available kinetic parameters for metabolism which are often not available and/or consistent (for review see Knaak *et al.* (2004)).

PBPK/PD models which utilize previously published kinetic data for OP metabolism generated from mouse, rat, or human liver microsomes have proven to be unstable, non-reproducible, and under-represent inter-individual variability (Knaak, 2004). To circumvent this issue, modifying PBPK/PD models to use individual CYP activities combined with their respective hepatic CYP content may prove to be more reproducible and stable. In order to have reliable modeling, accurate and complete kinetic data need to be generated. Kinetic data (K_m and V_{max}) on the metabolism of OPs by specific CYPs along with CYP –specific content (pmol P450 / mg microsomal protein) will generate a PBPK/PD model that can better adjust for age, sex, genetic polymorphisms, or other factors which may alter CYP content and activity which in turn contribute directly to the risk OPs pose to individuals.

We report in this study the identification of specific human CYPs that mediate parathion and chlorpyrifos metabolism, as well as report for the first time their respective K_m and V_{max} values for activation and detoxification. These CYP –specific kinetic parameters can then be linked to specific hepatic CYP content values as a function of age for future inclusion in PBPK/PD models.

DMD Fast Forward. Published on November 1, 2006 as DOI: 10.1124/dmd.106.012427
This article has not been copyedited and formatted. The final version may differ from this version.

Materials and Methods

2.1 Materials

Parathion (CAS 56-38-2), paraoxon (CAS 311-45-5), chlorpyrifos (CAS 2921-88-2), chlorpyrifos-oxon (CAS 5598-15-2), 3,5,6-trichloro-2-pyridinol (CAS 6515-38-4), and diethylthiophosphate (CAS 119-12-0) were purchased from ChemService Inc (West Chester, PA). p-nitrophenol (CAS 100-02-7) and tetraisopropyl pyrophosphoramide (iso-OMPA; CAS 513-00-8) were of reagent grade and purchased from Sigma-Aldrich (St Louis, MO). EDTA and $MgCl_2$ were from JT Baker (Phillipsburg, NJ) and were of at least reagent grade quality. Methanol and Acetonitrile (EMD Chemicals; Gibbstown, NJ) were HPLC grade as well. Recombinant human Cytochrome P450s (1A1, 1A2, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5, 3A7) and characterized human hepatic microsomes (pooled and single donor) were purchased from Gentest (BD Biosciences; Bedford, MA).

2.2 Experimental Conditions

OP stock solutions were prepared in methanol and stored at -20^{0}C when not in use. Incubations with either human liver microsomes (0.5mg protein / ml final concentration) or recombinant CYPs (0.03 to 0.06nmol P450 final concentration) were carried out in buffer (100mM Tris-HCL, and 5mM $MgCl_2$, 1mM EDTA and 50μM iso-OMPA (pH 7.4 at 37^{0}C) in a final volume of 0.5ml. Reactions were initiated with the addition of 1mM NADPH and incubated for 2 minutes at 37^{0}C. EDTA was included to inhibit A-esterases while iso-OMPA inhibited B-esterases (Reiner et al., 1993). The reaction was quenched with 1 volume of cold methanol with 0.1% phosphoric acid, centrifuged, and the supernatant was transferred to HPLC vials and capped for analysis. All reactions were carried out in replicate (n=3-4) for statistical purposes.

2.3 Metabolite Detection

OPs and their respective metabolites were analyzed by reverse-phase HPLC (C18, 5μM particle size, 25cm x 4.6mm I.D; Supelco; St Louis, MO) utilizing a Hewitt-Packard Model 1100 HPLC with Model 1046A diode-array detector (Santa Clarita, CA). Methanol (Buffer A) and 94.99%water/5%acetonitrile/0.01%phosphoric acid (Buffer B) were utilized in a gradient elution consisting of 40% Buffer A / 60% Buffer B to 100% Buffer A over 30 minutes at a 1ml/min flow rate. Chemical detection was determined at the UV wavelengths of 275nm – parathion and paraoxon, 320nm – PNP, 290nm –chlorpyrifos and chlorpyrifos-oxon, 300nm- TCP, 320nm – DETP. The minimum level of detection of these compounds was 2.5ng.

2.4 Kinetic Calculations

V_{max} and K_m were determined by nonlinear regression analysis (Enzyme Kinetics module of SigmaPlot (SyStat Software Inc, V9.01) of hyperbolic plots (i.e., velocity vs [S]) obeying Michaelis-Menten Kinetics. OP parent compound concentration (μM) was set as the independent variable while rate of product formation (pmol/min/nmol P450 or pmol/min/mg protein) was the dependent variable. The kinetic module handles multiple data sets and determines average values along with standard deviations from multiple experiments.

DMD Fast Forward. Published on November 1, 2006 as DOI: 10.1124/dmd.106.012427
This article has not been copyedited and formatted. The final version may differ from this version.

Results

The major metabolites detected via HPLC analysis were paraoxon and PNP from parathion and chlorpyrifos-oxon and TCP from chlorpyrifos. Parathion and chlorpyrifos metabolism was initially assessed utilizing a commercially available sample of pooled human liver microsomes constituted from 15 liver donors. K_m and V_{max} values for paraoxon and PNP formation were 17.7 μM, 790 pmol/min/mg protein and 25.1 μM, 588 pmol/min/mg protein, respectively. K_m and V_{max} values for chlorpyrifos oxon and TCP formation were 2.5 μM, 346 pmol/min/mg protein and 16.2 μM, 453 pmol/min/mg protein, respectively.

Since the use of pooled human liver microsomes did not reflect the inter-individual variability in OP metabolism, multiple single donor hepatic microsomal specimens were assayed. As expected, the rate and amount of OP product formation varied for each donor. While multiple single donors were assayed, one sample, SD101, demonstrated K_m and V_{max} values for paraoxon and PNP formation of 8.7 μM, 731 pmol/min/mg protein and 15.2 μM, 630 pmol/min/mg protein, respectively. K_m and V_{max} values for chlorpyrifos-oxon and TCP formation were 4.5 μM, 611 pmol/min/mg protein and 6.6 μM, 705 pmol/min/mg protein, respectively. In contrast, for SD112, K_m and V_{max} values for paraoxon and PNP formation were 39.1 μM, 850 pmol/min/mg protein and 55.9 μM, 683 pmol/min/mg protein, respectively. K_m and V_{max} values for chlorpyrifos-oxon and TCP formation in this specimen were 24.7 μM, 490 pmol/min/mg protein and 27.1 μM, 402 pmol/min/mg protein, respectively. Gentest reported the CYP -specific enzyme activities for SD101 as 2.2, 0.34, 0.37, and 4.2 nmol/min/mg protein for CYPs 1A2, 2B6, 2C19, and 3A4, respectively. In contrast, CYP -specific enzyme activities for SD112 were 0.89, 0.11, 0.059, and 8.9 nmol/min/mg protein for CYPs 1A2, 2B6, 2C19, and 3A4, respectively.

DMD Fast Forward. Published on November 1, 2006 as DOI: 10.1124/dmd.106.012427
This article has not been copyedited and formatted. The final version may differ from this version.

Studies with individual recombinant human CYPs were also conducted to identify specific CYPs that bio-transform these OPs and generate CYP –specific kinetic data to predict the CYPs which will play a prominent role *in vivo*. CYPs 1A2, 2B6, 2C9, 2C19, 3A4, 3A5, and 3A7 were found to metabolize parathion while no detectable metabolites were produced with CYPs 1A1, 2D6, or 2E1. Figure 1 illustrates Michaelis-Menton plots for three of the most metabolically active CYPs (2B6, 2C19, 3A4) for chlorpyrifos although CYPs 1A2, 3A5, and 3A7 were also found to be capable of limited metabolism of chlorpyrifos. The Hill equation was also used for determining the kinetics of CYP3A4 in figure 1 (plot not shown) due to more than 1 mole of substrate being bound to the enzyme ($n > 1$), however both equations resulted in the same K_m and V_{max} values.

Figure 1 illustrates that the active CYPs varied in both their overall activity and affinity (V_{max} and K_m) values, but also with regard to which reaction was favored, desulfation or dearylation. Table 1 summarizes the K_m, V_{max}, and V_{max}/K_m, or intrinsic clearance (Cl_{int}), for parathion and chlorpyrifos. CYP2B6 and CYP2C19 have the greatest affinity for parathion metabolism having K_m values of less than 1.0 μM for both desulfation and dearylation. Higher V_{max} values and slightly higher K_m values for parathion are observed for CYP1A2, while CYP3A4 has the greatest V_{max}, but much higher K_m values (>30 μM) for both reactions. CYP2B6 also shows a higher affinity and activity for chlorpyrifos metabolism to chlorpyrifos-oxon with a K_m value of 0.81 μM and V_{max} of 12544 pmol/min/nmol P450. In contrast, CYP2C19 has a high affinity and activity for TCP formation (dearylation) with a K_m of 1.63 μM and a V_{max} of 13128 pmol/min/nmol P450. As with parathion, CYP3A4 has a high V_{max} for chlorpyrifos metabolism but also a high K_m (>27 μM) for both reactions.

DMD Fast Forward. Published on November 1, 2006 as DOI: 10.1124/dmd.106.012427
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

This study investigated the activation and detoxification of parathion and chlorpyrifos, two model OPs, in human liver microsomes. In addition, recombinant human CYPs were utilized to quantify the CYP –specific kinetic variables for each compound in order to ultimately refine human PBPK/PD models of OP exposure. This data will be used to modify a PBPK/PD model for human chlorpyrifos exposure which utilizes kinetic parameters derived from rat hepatic microsomal studies (Timchalk et al., 2002).

While several studies have assessed the *in vitro* metabolism of OPs by human liver microsomes, the kinetic values can vary widely due to the marked variability of CYP content and activity in procured human specimens and differences in incubation conditions and analytical methodology (for review see Knaak *et al* (2004)). Differences in experimental methods include: detection of only oxon metabolites, the use of excessive substrate concentrations (supersaturated conditions), and non-physiological pH ranges. Complications and extensive variability in the procurement of human liver specimens for *in vitro* studies is a major concern. Often it is not possible to limit warm-ischemic time in liver tissue obtained from organ donors. It is necessary to limit warm-ischemic time prior to proper cryostorage in order to have accurate measures of enzyme activity. Furthermore, exposure to drugs and other substances which modulate enzyme activity are also often not controlled for in specimens obtained from tissue donors. Thus, there remains considerable uncertainty regarding whether variability in metabolism kinetics reflects true age and genetic variability or artifact of human liver specimen procurement and storage. The metabolism of selected OPs has also been assessed using specific recombinant human CYPs, however, these earlier studies did not report kinetic parameters (K_m, V_{max}) for all reactions (Sams et al., 2000; Tang et al., 2001; Mutch et al., 2003; Sams et al., 2004).

DMD Fast Forward. Published on November 1, 2006 as DOI: 10.1124/dmd.106.012427
This article has not been copyedited and formatted. The final version may differ from this version.

In the present study, pooled human liver microsomes demonstrated the ability to metabolize both parathion and chlorpyrifos. Kinetic parameters for the two model OPs varied as did the ratio of dearylation / desulfation products. While the results represent an average K_m and V_{max} values utilizing 9 substrate concentrations, the results from pooled microsomes did not estimate the inter-individual variability which occurs within a population.

Inter-individual variability in hepatic metabolism was illustrated in reactions conducted with characterized single donor human liver microsomes. Marked differences in K_m and V_{max} values for OP metabolism exist and may be due to variable levels of various CYPs within a given microsomal sample from a given donor liver. The higher K_m values for specimen SD112 and the high K_m values for CYP3A4 (Table 1) suggests that CYP3A4 is playing a primary role in the metabolism of the model OPs by this sample of human liver microsomes. The relative activities of CYP1A2, CYP2B6 and CYP2C19 were less while CYP3A4 activity was higher in SD112 compared to SD101, supporting the dominant role of CYP3A4 in the metabolism of the two model OPs in SD112.

To better address the limitations of using human liver microsomes for establishing kinetic values, OP metabolism was investigated with recombinant human CYPs (summarized in Table 1). As expected, since each CYP isozyme has substrate binding sites that differ, each enzyme demonstrated different K_m and V_{max} values along with different ratios of product formation. While CYP3A4 showed the greatest V_{max} value for parathion, this enzymes lack of specificity resulted in the highest K_m value which minimizes the role of CYP3A4 in metabolism at lower OP exposures. CYP2B6 and 2C19 have the lowest K_m values for parathion and chlorpyrifos supporting the major role these forms play in metabolism at low level real-world exposures.

Due to the low K_m values of CYP2B6, its activity is of interest. CYP2B6 activity can be expressed through its V_{max}/K_m values of 7.87, 2.45, 15.56, and 0.74 for the formations of paraoxon, PNP, chlorpyrifos-oxon, and TCP respectively (Table 1). Thus, CYP2B6 has greater activity in the formation of the oxon, and thus may be more important in assessing risk as it preferentially forms the toxic compound over the direct detoxification of the OPs at low level exposures. Conversely, CYP2C19 has the highest V_{max}/K_m in the formation of PNP and TCP, and thus plays a prominent role in the dearylation (detoxification) reaction.

Current PBPK/PD models for OPs utilize kinetic values from rat liver microsomal metabolism studies that do not reflect human enzymes. The building of kinetic models that use human CYP –specific kinetic parameters and specific hepatic CYP content should prove to be more accurate and more easily modified to address factors such as gender, age, or polymorphisms which may affect CYP protein expression and activity. Human hepatic CYP levels are know to vary across different age groups (Tateishi et al., 1997). Additionally, polymorphism in CYPs 2B6 and 2C19 are known to alter protein expression and/or activity, and thus could potentially serve as biomarkers of susceptibility to these agents. Current PBPK/PD models which vary parameters based on body weight treat infants as small adults and may not be accurate or protective of the most sensitive segment of the population. As OPs are the most widely used class of pesticides in the world, safe levels of exposure need to be set to protect the most sensitive individuals. Determining human CYP –specific kinetic parameters for OP metabolism is the first step in constructing more refined models which will include age – dependent CYP content to better assess risk associated with OP exposures in infants, children, or other sensitive sub groups.

References

Buratti FM, Volpe MT, Meneguz A, Vittozzi L and Testai E (2003) CYP-specific bioactivation of four organophosphorothioate pesticides by human liver microsomes. *Toxicology & Applied Pharmacology* **186:**143-154.

Chanda SM, Mortensen SR, Moser VC and Padilla S (1997) Tissue-specific effects of chlorpyrifos on carboxylesterase and cholinesterase activity in adult rats: an in vitro and in vivo comparison. *Fundamental & Applied Toxicology* **38:**148-157.

Ecobichon DJ (2001) *Toxic Effects of Pesticides*. McGraw-Hill, New York City.

Knaak JD, C. Power, F. Thompson, C. Blancato, J. (2004) Physicochemical and Biological Data for the Development of Predictive Organophosphorus Pesticide QSARs and PBPK/PD Models for Human Risk Assessment. *Critical Reviews in Toxicology* **34:**143-207.

Mutch E, Blain PG and Williams FM (1999) The role of metabolism in determining susceptibility to parathion toxicity in man. *Toxicology Letters* **107:**177-187.

Mutch E, Daly AK, Leathart JB, Blain PG and Williams FM (2003) Do multiple cytochrome P450 isoforms contribute to parathion metabolism in man? *Archives of Toxicology* **77:**313-320.

Poet TS, Wu H, Kousba AA and Timchalk C (2003) In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon. *Toxicological Sciences* **72:**193-200.

Reiner E, Pavkovic E, Radic Z and Simeon V (1993) Differentiation of esterases reacting with organophosphorus compounds. *Chemico-Biological Interactions* **87:**77-83.

Sams C, Cocker J and Lennard MS (2004) Biotransformation of chlorpyrifos and diazinon by human liver microsomes and recombinant human cytochrome P450s (CYP). *Xenobiotica* **34:**861-873.

Sams C, Mason HJ and Rawbone R (2000) Evidence for the activation of organophosphate pesticides by cytochromes P450 3A4 and 2D6 in human liver microsomes. *Toxicology Letters* **116:**217-221.

Sultatos LG (1994) Mammalian toxicology of organophosphorus pesticides. *Journal of Toxicology & Environmental Health* **43:**271-289.

Tang J, Cao Y, Rose RL, Brimfield AA, Dai D, Goldstein JA and Hodgson E (2001) Metabolism of chlorpyrifos by human cytochrome P450 isoforms and human, mouse, and rat liver microsomes. *Drug Metabolism & Disposition* **29:**1201-1204.

Tateishi T, Nakura H, Asoh M, Watanabe M, Tanaka M, Kumai T, Takashima S, Imaoka S, Funae Y, Yabusaki Y, Kamataki T and Kobayashi S (1997) A comparison of hepatic cytochrome P450 protein expression between infancy and postinfancy. *Life Sciences* **61:**2567-2574.

Timchalk C, Nolan RJ, Mendrala AL, Dittenber DA, Brzak KA and Mattsson JL (2002) A Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. *Toxicological Sciences* **66:**34-53.

Footnotes

Supported by US EPA STAR grant R-83068301

Part of this work was present at the 2006 Society of Toxicology Annual meeting, San Diego, CA. *The Toxicologist* (supplement to Toxicological Sciences). 90: S-1, March 2006.

Reprint requests;

Dr James R Olson
102 Farber Hall
3435 Main St
Buffalo, NY 14214

DMD Fast Forward. Published on November 1, 2006 as DOI: 10.1124/dmd.106.012427
This article has not been copyedited and formatted. The final version may differ from this version.

Legends for Figures

Figure 1 – Michaelis-Menton plots are shown for chlorpyrifos metabolism by recombinant human CYP2B6 (**A** and **B**), CYP 2C19 (**C** and **D**) or CYP3A4 (**E** and **F**). Values represent the mean ± S.E.M of 3 experiments for CYP2B6 and CYP3A4 and 4 experiments for CYP2C19.

Table 1 Parathion and Chlorpyrifos Metabolism by Recombinant Human P450s

	Paraoxon Formation			PNP Formation		
Cytochrome P450	**Km (uM)**	**Vmax (pmol/min/nmol P450)**	**Vmax/Km Cl_{int}**	**Km (uM)**	**Vmax (pmol/min/nmol P450)**	**Vmax/Km Cl_{int}**
1A2[a]	1.63 ± 0.13	6131 ± 90.1	3.755	2.15 ± 0.16	5656 ± 83.9	2.637
2B6[b]	0.61 ± 0.08	4827 ± 134	7.875	0.74 ± 0.10	1804 ± 46.4	2.447
2C9[a]	9.78 ± 1.63	1140 ± 47.7	0.117	12.1 ± 4.01	742 ± 63.3	0.061
2C19[a]	0.56 ± 0.04	4879 ± 73.7	8.705	0.60 ± 0.09	2338 ± 65.4	3.872
3A4[a]	65.5 ± 6.83	14009 ± 767	0.214	31.2 ± 2.40	15738 ± 488	0.504
3A5[b]	43.2 ± 27.1	2020 ± 540	0.047	68.2 ± 121	1175 ± 1039	0.017
3A7[a]				37.3 ± 10.6	1739 ± 201	0.047
	Chlorpyrifos Oxon Formation			**TCP Formation**		
Cytochrome P450	**Km (uM)**	**Vmax (pmol/min/nmol P450)**	**Vmax/Km Cl_{int}**	**Km (uM)**	**Vmax (pmol/min/nmol P450)**	**Vmax/Km Cl_{int}**
1A2[c]	0.38 ± 0.53	1193 ± 143	3.131	0.63 ± .33	892 ± 51.7	1.416
2B6[a]	0.81 ± 0.12	12544 ± 418	15.560	2.09 ± 0.79	1545 ± 132	0.740
2C19[b]	1.23 ± 1.20	2470 ± 485	2.013	1.63 ± 1.09	13128 ± 2296	8.075
3A4[a]	27.3 ± 5.04	11946 ± 941	0.437	33.4 ± 10.0	12667 ± 1758	0.379
3A5[a]	16.6 ± 2.25	2569 ± 122	0.155	23.9 ± 9.87	2141 ± 354	0.090
3A7[a]	34.0 ± 24.0	794 ± 252	0.023			

Table 1 – Kinetic values for the metabolism of parathion and chlorpyrifos by recombinant human P450s. Values represent the mean ± SEM of [a]n=3, [b]n=4, [c]n=6 experiments.

DMD Fast Forward. Published on November 1, 2006 as DOI: 10.1124/dmd.106.012427
This article has not been copyedited and formatted. The final version may differ from this version.

FIGURE 1