Published in final edited form as:

Toxicology. 2011 July 11; 285(1-2): 57–66. doi:10.1016/j.tox.2011.04.002.

Cytochrome P450-specific human PBPK/PD models for the organophosphorus pesticides: chlorpyrifos and parathion

Robert J Foxenberg^{a,*}, Corie A Ellison^{a,*}, James B Knaak^a, Changxing Ma^b, James R Olson^{a,*}

^aDepartment of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, New York, USA, 14214

^bDepartment of Biostatistics, School of Public Health and Health Professions, University at Buffalo, Buffalo, New York, USA, 14214

Abstract

Organophosphorus pesticides (OPs) remain a potential concern to human health because of their continuing use worldwide. Phosphorothioate OPs like chlorpyrifos and parathion are directly activated and detoxified by various cytochrome P450s (CYPs), with the primary CYPs involved being CYP2B6 and CYP2C19. The goal of the current study was to convert a previously reported human pharmacokinetic and pharmacodynamic (PBPK/PD) model for chlorpyrifos, that used chlorpyrifos metabolism parameters from rat liver, into a human CYP based/age-specific model using recombinant human CYP kinetic parameters (V_{max} , K_m), hepatic CYP content and plasma binding measurements to estimate new values for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition and to use the model as a template for the development of a comparable parathion PBPK/PD model. The human CYP based/age-specific PBPK/PD models were used to simulate single oral exposures of adults (19 yr old) and infants (1 yr) to chlorpyrifos (10,000, 1,000 and 100 $\mu\text{g/kg}$) or parathion (100, 25 and 5 $\mu\text{g/kg}$). Model simulations showed that there is an age dependency in the amount of blood cholinesterase inhibition observed, however additional age-dependent data are needed to further optimize age-specific human PBPK/PD modeling for these OP compounds. PBPK/PD model simulations estimated that a 4-fold increase or decrease in relative CYP2B6 and CYP2C19 content would produce a 9 to 22% inhibition in blood AChE activity following exposure of an adult to chlorpyrifos (1,000 $\mu\text{g/kg}$). Similar model simulation produced an 18 to 22% inhibition in blood AChE activity following exposure of an adult to parathion (25 $\mu\text{g/kg}$). Individuals with greater CYP2B6 content and lower CYP2C19 content were predicted to be most sensitive to both OPs. Changes in hepatic CYP2B6

* Address correspondence to: James R. Olson University at Buffalo, Department of Pharmacology and Toxicology 3435 Main Street, 102 Farber Hall Buffalo, NY 14214 USA. Telephone: (716) 829-2319 Fax: (716) 829-2801 jolson@buffalo.edu.

*These authors contributed equally to this work.

R. J. Foxenberg: rjfoxenberg@yahoo.com

C. A. Ellison: cellison@buffalo.edu

J. B. Knaak: jbknaak@aol.com

C. Ma: cxma@buffalo.edu

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Conflict of Interest statement The authors declare that there are no conflicts of interest.

and CYP2C19 content had more of an influence on cholinesterase inhibition for exposures to chlorpyrifos than parathion, which agrees with previously reported literature that these CYPs are more reaction biased for desulfurization (activation) and dearylation (detoxification) of chlorpyrifos compared to parathion. The data presented here illustrate how PBPK/PD models with human enzyme-specific parameters can assist ongoing risk assessment efforts and aid in the identification of sensitive individuals and populations.

Keywords

Chlorpyrifos; Parathion; Cytochrome P450; Pharmacokinetics; Pharmacodynamics; Pesticide

1. Introduction

Organophosphorus pesticides (OPs) continue to be a potential human health concern due to their continued use worldwide and their potential for human exposure (Alexander et al. 2006; Barr et al. 2005; Farahat et al. 2010; Garabrant et al. 2009; Hines and Deddens 2001). Parathion and chlorpyrifos have often been utilized as model compounds when determining the effects of OPs (Buratti et al. 2003; Carabias Martinez et al. 1992; Neal 1967; Sultatos and Murphy 1983). Figure 1 demonstrates the primary metabolic pathways for the activation and detoxification of parathion and chlorpyrifos and the key human CYPs that activate (desulfation) and detoxify (oxidation) these OPs based on the kinetic data from (Foxenberg et al. 2007). Paraoxon and chlorpyrifos-oxon represent the activated forms of the parent compound while O,O-diethyl phosphate (DEP), O,O-diethylphosphorothionate (DETP), *p*-nitrophenol (PNP), and 3,4,5-trichloropyrindinol (TCPy) are the more readily cleared detoxification products. The active oxon moiety has been shown to irreversibly bind to B-esterases such as butyrylcholinesterase (BuChE), carboxylesterase (CE), and of greatest importance, acetylcholinesterase (AChE) leading to acute OP toxicity (Sultatos 1994). The balance between the formation of activated and detoxified metabolites is influenced by age, gender, CYP enzymes profiles and potentially by CYP enzyme polymorphisms (Atterberry et al. 1997; Dai et al. 2001; Ma and Chambers 1994; Rose et al. 2005). In addition, the A-esterase, paraoxonase 1 (PON1) can metabolically inactivate the active oxon moiety of OPs (Furlong et al. 2005; Reiner et al. 1993; Shih et al. 1998).

Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) models allow researchers to predict the absorption, distribution, metabolism, excretion and associated risks involving exposure to pesticides using biochemical and physiological parameters (Knaak 2004). While non-human parameters are often relied upon to represent humans, these values are not always comparable between species thus limiting usefulness (Karanth and Pope 2000; Timchalk 2004). The quality of the available data governs how well a PBPK/PD model predicts risk (Knaak 2004).

The purpose of this study was to convert a human PBPK/PD model for chlorpyrifos which used rat liver microsomal metabolism kinetic parameters (Timchalk et al. 2002b) to a human CYP based/age-specific model using recombinant human CYP based V_{max} , K_m values (Foxenberg et al. 2007), CYP content and plasma binding measurements to predict new

values for red blood cell (RBC) AChE and plasma BuChE inhibition and to use the model as a template for the development of a parathion PBPK/PD model. Models with human enzyme-specific parameters will assist ongoing risk assessment efforts and aid in the identification of sensitive individuals and populations which are at greatest risk from these pesticides.

2. Materials and Methods

2.1. Materials

Parathion (CAS 56-38-2), paraoxon (CAS 311-45-5), chlorpyrifos (CAS 2921-88-2) and chlorpyrifos-oxon (CAS 5598-15-2) were purchased from ChemService Inc (West Chester, PA). Human serum albumin (HSA, >97%) was purchased from Sigma-Aldrich (St Louis, MO). HPLC grade methanol and acetonitrile were purchased from EMD Chemicals (Gibbstown, NJ).

2.2. OP and Oxon Metabolite Binding to Human Serum Albumin

Parathion, paraoxon, chlorpyrifos and chlorpyrifos oxon protein binding to HSA was assessed using the equilibrium dialysis methodology described in Moon *et al.* (2000). Briefly, cells containing a dialysis membrane (12–14Kd WMCO) allowed for the equilibration of parathion, paraoxon, chlorpyrifos or chlorpyrifos oxon between cell halves containing 0.5ml phosphate buffer (pH 7.4) with either 4% HSA or 3% dextran. Cells were placed on a rotating rod in a water bath (37°C, 50RPM) and allowed to equilibrate for 15min, 1hr and 4hr. Following the addition of two volumes of cold methanol with 0.1% HPO₄ to precipitate either the HSA or dextran, the samples were centrifuged and transferred to HPLC vials for analysis. The OP concentration measured within the dextran fractions represent half the unbound portion which can be compared to the OP starting concentration for determination of the unbound fraction. Reactions were performed in triplicate.

2.3. OP and Metabolite Detection

OPs and their respective metabolites were analyzed by reverse-phase HPLC (C18, 5µm particle size, 25cm × 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 (solvent A) and 94.99% water/5% acetonitrile/0.01% phosphoric acid (solvent B) were utilized in a gradient elution consisting of 40% solvent A / 60% solvent B to 100% solvent A over 30 minutes at a 1ml/min flow rate. Chemical detection was determined at the UV wavelengths of 275nm – parathion and paraoxon, 290nm – chlorpyrifos and chlorpyrifos-oxon.

2.4. Human Hepatic CYP Content Calculator

A literature search for publications reporting CYP content values for human liver microsomes was carried out for each of the active CYPs for OP metabolism (Koukouritaki et al. 2004; Stevens et al. 2003; Wrighton et al. 1990). When only data summaries were published, efforts were made to the contact authors requesting the raw data for each individual hepatic sample from each study. All data points for each CYP reported were combined. CYP content (pmol P450/mg microsomal protein) was plotted against age

(log[days]) using MATLAB (The Mathworks Inc; Natick, MA) to generate curves (Figure 2). The mean of the data was fitted to each plot, as well as the 95% confidence intervals (CI). The equations of each line were Java coded in order to build a web based calculator. The input needed for the calculator is the age (x) and the desired CI. The calculator inputs the age and CI into all of the CYP equations and outputs the average CYP content along with the CI of the mean and the CI of the data. The maximum age allowed by the CYP calculator is 20 years, which is limited by the current available published data. The human hepatic CYP calculator can be accessed at the following website: (<http://wings.buffalo.edu/smb/pmy/resources/Cyp%20Calculator.htm>)

2.5. PBPK/PD Modeling

All model simulations in the current report were conducted using a modified version of the previously reported human PBPK/PD model for chlorpyrifos exposure (Timchalk et al. 2002b). In brief, the Timchalk et al. (2002b) PBPK/PD model describes the disposition of chlorpyrifos, chlorpyrifos-oxon and TCPy and B-esterase inhibition in humans following an oral exposure. Parameters within the human PBPK/PD model reported by Timchalk et al. (2002b) were derived from human data when available and rat data when human data was not available. The Timchalk et al. (2002b) model utilizes several physiologically based pharmacokinetic parameters such as: organ size, blood flow rates, partition coefficients for chlorpyrifos and chlorpyrifos-oxon, metabolic constants for chlorpyrifos metabolism by CYPs (rat liver microsomes) and PON1, and the fraction of chlorpyrifos and chlorpyrifos-oxon bound in blood. To describe the effects of chlorpyrifos exposure on B-esterase enzymes the model utilizes a number of pharmacodynamic parameters such as: enzyme turnover, enzyme activity, enzyme degradation, bimolecular inhibition rate and reactivation rate (Timchalk et al. 2002b). The CYP-mediated metabolism of chlorpyrifos was limited to the liver compartment and was described by data from rat liver microsome studies. Metabolism of chlorpyrifos-oxon by PON1 was limited to the liver and blood compartments and was described by data from rat studies (Timchalk et al. 2002b).

The refined CYP-specific human PBPK/PD model for chlorpyrifos replaces rat liver metabolic parameters with parameters for human metabolism of 1) chlorpyrifos to dimethylthiophosphate and TCPy; 2) chlorpyrifos to chlorpyrifos oxon; and 3) chlorpyrifos to dimethyl phosphate and TCPy. The three metabolic reactions are incorporated into the code of the modified model (Table 1) using Eq. 1

$$\begin{aligned} \text{RAM} = & [V_{\max 1}(S) / K_{m1} + S] + [V_{\max 2}(S) / K_{m2} + S] \\ & + [V_{\max 3}(S) / K_{m3} + S] + \\ & [V_{\max 4}(S) / K_{m4} + S] + [V_{\max 5}(S) / K_{m5} + S] + [V_{\max 6}(S) / K_{m6} + S] \end{aligned} \quad \text{Eq. (1)}$$

where “RAM” is the rate of metabolism; “ $V_{\max}$ ” ($\mu\text{moles/hr/kg bw}$) and “ K_m ” (μM) are the *in vivo* kinetic parameters for the activation or detoxification pathways for each respective CYP, represented as “1” – “6” for human CYPs 1A2, 2B6, 2C19, 3A4, 3A5 and 3A7; “S” is the blood concentration of the OP parent compound. The *in vivo* human kinetic parameters ($V_{\max}$ and K_m) were obtained from the *in vitro* human kinetic parameters of Foxenberg *et al.* (2007) using Eq 2 to extrapolate the *in vitro* $V_{\max}$ values (pmol/min/nmol P450) to *in vivo* values ($\mu\text{moles/hr/kg bw}$):

$$V_{\max(in vivo)} = \frac{(\text{CYP content} \times V_{\max(in vivo)} \times 60 \times \text{mic. pro.} \times \text{liver wt})}{1.0E6} \quad \text{Eq. (2)}$$

where “CYP content” is the amount of CYP isoform in human liver microsomal protein (pmol/mg microsomal protein); “60” is the number of minutes per hour (60min/hr); “mic. pro.” is the amount of microsomal protein in human liver (mg protein/g liver); and “liver wt.” is the weight of the liver (g/kg bw). The CYP content for each CYP isoform was determined using the human hepatic CYP calculator (Table 2). Model simulations and calculations were performed using an adult liver weight of 25.7 grams of liver per kg body weight (Brown et al. 1997; Davies and Morris 1993) and an infant liver weight of 37 grams of liver per kg body weight (Barret et al. 2001; Haddad et al. 2001). Simulations and calculations for adults and infants assumed the microsomal content of the liver to be 30 mg of microsomal protein per g of liver (Barter et al. 2007; Lipscomb et al. 1998; Wilson et al. 2003). Other organs of the body were scaled for the adult and infant based on percentage of body weight.

The CYP-specific chlorpyrifos model was used as a template for the parathion model (Table 2). Model parameters, such as CYP-specific kinetic values for pesticide metabolism ($V_{\max}$ and K_m), protein binding, bimolecular rate constants and molecular weights, were adjusted to reflect the OP (parathion). For parathion metabolism, kinetic parameters ($V_{\max}$ and K_m) for CYP2C9 were also included.

PBPK/PD model simulations were performed for a single oral exposure to chlorpyrifos or parathion and the resulting percent inhibition of BuChE and AChE in blood was recorded as the model output. Model simulations for BuChE and AChE inhibition following a single exposure to chlorpyrifos (10,000, 1,000 and 100 $\mu\text{g/kg}$) or parathion (100, 25 and 5 $\mu\text{g/kg}$) were conducted for a 1yr old (10 kg bw) and a 19yr old (70 kg bw). The chlorpyrifos dose range was selected because it would produce a similar magnitude of BuChE and AChE inhibition as that recently reported for Egyptian agricultural workers highly exposed to chlorpyrifos (Farahat et al. 2011). A lower dose range was used for parathion since it is more potent than chlorpyrifos (reviewed in Vidair 2004). Model simulations for an adult exposed to chlorpyrifos or parathion were also performed where CYP2B6 and/or CYP2C19 protein content was increased or decreased by 4-fold and/or CYP2C19 content was omitted, as reflected in the null phenotype for CYP2C19*2.

3. Results

3.1. CYP-Specific Kinetics and Human Hepatic Microsomal CYP Content

Timchalk *et al.* (2002b) published a human PBPK/PD model for chlorpyrifos exposure which included kinetic parameters for chlorpyrifos metabolism generated by rat hepatic microsomes. In the current study, rat microsomal metabolism was replaced with recombinant human CYP base $V_{\max}$ and K_m values (Table 1) (Foxenberg et al. 2007). Included CYPs were those identified as participating in the active metabolism of chlorpyrifos or parathion. In combination, the activity of the individual CYPs within the PBPK/PD model equates the activity of intact human liver microsomes.

In order to model the age-dependency of hepatic CYP content, a search was conducted with the aim of identifying reports which assessed human hepatic CYP content (pmol/mg microsomal protein) as a function of age for the CYP isoforms active in OP metabolism. CYP-specific content was plotted against subject age to determine the average CYP content with 95% CI as a function of age for each isoform (Figure 2). Equations which were generated by plotting the content of individual CYPs as a function of age were utilized for the creation of the CYP content calculator (<http://wings.buffalo.edu/smbs/pmy/resources/Cyp%20Calculator.htm>). The estimated average hepatic content of individual CYPs for a 1-year old and a 19-year old is shown in Table 2.

3.2. OP and Metabolite Binding to Human Serum Albumin

Equilibrium dialysis was utilized to determine the extent of protein binding for parathion, paraoxon, chlorpyrifos and chlorpyrifos oxon in an attempt to improve on these parameters which are needed for PBPK/PD modeling. At equilibrium (60 min), parathion had a greater affinity for HSA (~94% bound) than paraoxon (~60% bound) (Table 3). Based on these data, the free fraction of parathion and paraoxon in human blood was estimated to be 6 and 40%, respectively. Under the experimental conditions, chlorpyrifos and chlorpyrifos-oxon consistently bound to the reaction apparatus thus preventing us from determining protein binding for these compounds. Thus, in the revised human PBPK/PD model, the free fraction of chlorpyrifos and chlorpyrifos oxon in human blood was estimated to be 3 and 2% based on Timchalk *et al.* (2002b).

3.3. Human PBPK/PD Model Simulations

In model simulations, the human CYP-specific kinetics for pesticide metabolism (Table 1) were amended within the chlorpyrifos model framework published by Timchalk *et al.* (2002b). Separately, all chemical-specific parameters were also modified to create a human CYP-specific PBPK/PD model for parathion exposures. Utilizing the human hepatic CYP-specific content values listed in Table 2 along with changes in other relevant physiological parameters, the time course of BuChE and AChE inhibition was assessed between infants (1yr old) and adults (19 yrs old) exposed to a single oral dose of chlorpyrifos or parathion (Figure 3). The maximum inhibition of ChE activity occurred ~8 hrs after oral exposure to chlorpyrifos and ~10 hrs after oral exposure to parathion. Compared to adults, infants showed less susceptibility to cholinesterase inhibition following chlorpyrifos exposure. A similar result was seen following parathion exposure; however the magnitude in the difference between infants and adults was smaller than that observed following chlorpyrifos exposure.

CYP2B6 and CYP2C19 are the two most active CYPs involved in chlorpyrifos and parathion metabolism (Foxenberg et al. 2007). CYP2B6 and CYP2C19 have several different polymorphic variants which can potentially alter protein expression and/or catalytic activity of the enzymes (Demorais et al. 1994; Lang et al. 2001). Model simulations were performed where the microsomal content of these two CYPs were either increased or decreased by 4-fold singly or in combination to determine the potential affect on blood cholinesterase inhibition. The 4-fold change in CYP2B6 and CYP2C19 content used here is within the inter-individual variability range for these CYPs (reviewed in Bozina et al. 2009;

Zanger et al. 2007). Additional simulations were performed for CYP2C19 where enzyme activity was also omitted, thus mimicking the null allele as seen in the CYP2C19*2 genotype (Demorais et al. 1994). Table 4 represents model simulations for a single oral exposure to chlorpyrifos. Using the average CYP content (Table 2) a 19-year old exposed orally to 1,000 µg/kg demonstrated 16.9% RBC AChE inhibition (Trial 1). Decreasing CYP2B6 content 4-fold while increasing CYP2C19 content 4-fold decreased AChE inhibition to 9.0% (Trial 7) for the same chlorpyrifos exposure. Alternatively, increasing CYP2B6 content 4-fold while omitting CYP2C19 increased AChE inhibition to 22.4% (Trial 10). Table 5 represents comparable model simulations for a single oral exposure to parathion. Using the average CYP content (Table 2) a 19-year old exposed orally to 25 µg/kg demonstrated a 20.8% inhibition in RBC AChE activity (Trial 1). Decreasing CYP2B6 content 4-fold while omitting CYP2C19 content decreased AChE inhibition to 18.4% (Trial 8). Alternatively, increasing CYP2B6 content 4-fold while omitting CYP2C19 content increased AChE inhibition to 22.3% (Trial 10).

4. Discussion

4.1. Refining the Human PBPK/PD Model for Chlorpyrifos Exposure

The original publication for the PBPK/PD models for chlorpyrifos exposure included a model for rats as well as one for humans (Timchalk et al. 2002b). Several investigations have contributed toward the refinement of the PBPK/PD model for chlorpyrifos exposure, with the primary focus being on the rat PBPK/PD model (Lee et al. 2010; Lowe et al. 2009; Marty et al. 2007; Smith et al. 2009; Timchalk et al. 2007). The current study converted the human PBPK/PD model for chlorpyrifos, based on chlorpyrifos metabolism parameters from rat liver (Timchalk et al. 2002b), into a human CYP based/age-specific model and predicted new values for RBC AChE and plasma BuChE inhibition and used the model as a template for the development of a comparable parathion PBPK/PD model.

The refined human PBPK/PD models utilized human CYP-specific kinetic parameters (K_m and V_{max}) for the metabolism of each OP (Table 1). Our working assumption is that enzyme activity remains constant for various age groups, but that the hepatic microsomal content of specific CYPs is age-dependent (Table 2).

The CYP content calculator was designed to provide age-specific estimates of the average hepatic content of specific CYPs in individuals from 1 to 20 years of age along with a range of confidence intervals for the estimates (<http://wings.buffalo.edu/smbbs/pmy/resources/Cyp%20Calculator.htm>). A secondary function of the calculator is to provide a depository of age-specific CYP content data as it becomes available and shared. The calculator can be expanded to include additional CYPs and a wider age group. In the event additional data is identified or contributed, the CYP content calculator will be updated.

The Timchalk *et al.* (2002b) human model for chlorpyrifos estimated the unbound or free fraction of chlorpyrifos and chlorpyrifos oxon in human blood at 3% and 2%, respectively; representing the fraction of the internal dose which is both metabolically and toxicologically active. Experimental efforts to refine these estimates were unsuccessful, but experimentally

derived estimates of 6 and 40% were determined for the free or unbound fraction of parathion and paraoxon in human blood, respectively (Table 3).

4.2. Age Dependency of PBPK/PD Model Simulations for Chlorpyrifos and Parathion

Multiple experimental model scenarios were performed to assess the contributions of adding the refined human CYP-specific parameters within the model framework. In the age-specific models, the CYP content calculator was used to estimate the CYP content for a 1-year old as well as a 19-year old. For simulations which compared the effect of age on BuChE/AChE inhibition demonstrated that inhibition is less in the 1-year old than in the 19-year old. This effect could be partly due to the decreased CYP2B6 levels in the 1-year old which would favor detoxification over activation of the OPs. While this difference has been observed, this model has not altered additional potential age-related differences such as variation in PON1 activity which could mask or contradict these results. There is functional evidence that PON1 activity is not consistent over age; however, protein activity and content has not been quantitatively addressed (Cole et al. 2003). A decrease in PON1 activity in infants could decrease the clearance rates of the activated oxon metabolite and thus increase cholinesterase inhibition. Additionally, this model has not account for age-related differences in the amount of microsomal protein in the liver. Preliminary evidence into this age-related difference suggests that microsomal protein per gram of liver weight increases from birth to approximately 28 years of age followed by a gradual decrease in older age (Barter et al. 2008), however, additional studies are needed to confirm these preliminary observations. Current age-specific outputs from the refined PBPK/PD models are also limited by the critical lack of data on the localized, age-specific, activation and detoxification of OPs in the brain, which is the primary target of the neurotoxicity of these agents. Furthermore, the refined models are also limited by the minimal data available for any age-specific differences related to AChE, the target enzyme for both of these OPs. The absence of these age-specific data results in incomplete analyses within the refined model from an age-specific standpoint and highlights the importance of generating more human based age-specific data which can be used in human PBPK/PD modeling.

4.3. Influence of Hepatic CYP Content on PBPK/PD Simulations

Few human exposure studies have used the Timchalk *et al.* (2002b) human PBPK/PD model to compare real-world chlorpyrifos exposures with model simulations. A recent study reported a good consistency between model simulations and biomarkers of chlorpyrifos exposure (urinary 3,5,6-trichloro-2-pyridinol) and effect (plasma BuChE) from chlorpyrifos manufacture workers (Garabrant et al. 2009); however, for some individuals' BuChE inhibition was noticeably different from the human PBPK/PD model predictions. Differences between model simulations and human exposure data may be a result of genetic variability. Since CYP2B6 and CYP2C19 are the most active CYPs in chlorpyrifos and parathion metabolism (Foxenberg et al. 2007), it is plausible that increased or decreased CYP2B6 or CYP2C19 expression and/or activity may alter OP metabolism which could potentially effect BuChE/AChE activity. Unlike the original Timchalk *et al.* (2002b) PBPK/PD model which relies on rat microsomal metabolism of OPs, our refined PBPK/PD model includes human CYP-specific metabolism thus providing a means to account for variability in individual CYPs.

Model simulations were performed which represented a 4-fold increase or decrease in CYP2B6 and/or CYP2C19 content or a null allele for CYP2C19 to predict the effect on BuChE or AChE inhibition. Physiologically, it is feasible to alter hepatic CYP protein content to an even greater extent. Studies using immunochemical detection methods have reported large variability in the expression levels of CYP2B6 in human liver samples with reported ranges from <1 – 148 pmol/mg microsomal protein (Croom et al. 2009; Ekins et al. 1998; Hanna et al. 2000; Hesse et al. 2000; Lamba et al. 2003; Lang et al. 2001; Stresser and Kupfer 1999). Similar to hepatic CYP2B6 content, there is substantial interindividual variability in hepatic CYP2C19 content with ranges from <1 – 49 pmol/mg microsomal protein (BD Gentest, <http://www.bdbiosciences.com>; Koukouritaki et al. 2004). Model simulations were also performed with no active CYP2C19 protein to simulate the CYP2C19*2 genotype, which has a relatively high prevalence (~20%) in several populations (reviewed in Bozina et al. 2009).

When altering the hepatic content of CYP2B6 and CYP2C19, more substantial changes in cholinesterase inhibition were observed following exposure to chlorpyrifos compared to parathion, which can be explained by the CYP-specific metabolism of these compounds. CYP2B6 and CYP2C19 are the most active CYPs involved in chlorpyrifos desulfurization (activation) and dearylation (detoxification), respectively, but are less reaction biased in parathion metabolism (Foxenberg et al. 2007). Therefore, changes in CYP2B6 and CYP2C19 will have a greater influence on the metabolic activation and detoxification of chlorpyrifos and the ensuing cholinesterase inhibition compared to that seen following a parathion exposure. As evident by the ranges of cholinesterase inhibition which could be created by simulations which altered CYP content of the two most active CYPs in OP metabolism, polymorphisms or other factors which increase or decrease enzyme content and/or activity can potentially alter OP toxicity. Further investigation into whether this phenomenon is physiologically representative at relevant occupational exposures should be addressed.

Although, the current PBPK/PD model simulations demonstrate the potential contribution of hepatic CYP content on inter-individual variability of cholinesterase inhibition, it is important to emphasize that CYPs are one of several enzyme systems involved in the metabolism of chlorpyrifos and parathion. There is functional evidence that human PON1 protein and PON1 activity towards chlorpyrifos-oxon and paraoxon varies with genotype (Furlong et al. 2010; Povey 2010). Timchalk *et al.* (2002a) used Monte Carlo analysis to show that PON1 genotype could alter simulated brain concentrations of chlorpyrifosoxon at higher chlorpyrifos exposures (0.05–5 mg/kg) suggesting that PON1 genotype may be an important determinant in chlorpyrifos toxicity. The effect of PON1 genotype on pesticide worker health has been examined by multiple groups with conflicting conclusions being made about the importance of PON1 for worker health (Albers et al. 2010; Hernandez et al. 2003; Prabhavathy Das and Jamil 2009). As more human kinetic data becomes available for PON1 activity towards chlorpyrifos-oxon and paraoxon, the PBPK/PD model should be further updated to assess the effects of genetic variability of PON1 alone and in combination with CYP variability on cholinesterase inhibition.

4.4. Conclusions

This study has demonstrated that human PBPK/PD models which utilize CYP-specific content and OP-specific metabolic activity can account for age-dependent differences in CYP content and potential genetic differences in content and/or activity between individuals. Individuals with polymorphisms within the coding region of either CYP2B6 or CYP2C19 could be more or less sensitive to the toxicity of OPs. Further confirmation of this observation can be addressed in populations exposed to OPs by measuring biomarkers of exposure and effect (e.g. cholinesterase activity). Continuing efforts to revise and improve PBPK/PD models will help protect human health and aid in the identification of sensitive individuals and populations which are at the greatest risk for OP toxicity.

Acknowledgements

This work was supported by funding from the Environmental Protection Agency Science to Achieve Results (US EPA STAR, grant # R-83068301) and the National Institute of Environmental Health Sciences (NIEHS, grant #ES016308). Corie A. Ellison was supported by a Research Supplement to Promote Diversity in Health-Related Research from the NIEHS (ES016308-02S). The content is solely the authors' responsibility and does not necessarily represent official views of the US EPA or the NIEHS.

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Figure 1.

Bioactivation and detoxification pathways for parathion and chlorpyrifos. Panel (A) illustrates the primary human CYPs that biotransform parathion to the active paraoxon, and detoxification products p-nitrophenol (PNP) and diethylphosphorothionate (Foxenberg et al. 2007). Paraoxon undergoes further metabolism by A-esterases to form diethylphosphate and PNP. Panel (B) illustrates the primary human CYPs that biotransform chlorpyrifos to the active chlorpyrifos oxon, and the detoxification products 3,5,6-trichloro-2-pyridinol (TCPy) and diethylphosphorothionate (Foxenberg et al. 2007). Chlorpyrifos oxon undergoes further metabolism by A-esterases to form diethylphosphate and TCPy.

Figure 2.

Human hepatic CYP content (pmol/mg microsomal protein) for CYP2C9, CYP2C19, CYP2E1, CYP3A4, CYP3A5 and CYP3A7 plotted against age. For each individual plot, the center of the three lines is the average CYP content while the upper and lower two lines represent the 95% confidence intervals. Equations generated from plots are utilized in the human hepatic CYP content calculator (<http://wings.buffalo.edu/smb/pmy/resources/Cyp%20Calculator.htm>).

Figure 3. Human PBPK/PD model simulations of the time course for AChE (solid line) and BuChE (dashed line) inhibition following a single oral exposure to chlorpyrifos or parathion in infants and adults. Average hepatic CYP content for the two ages (Table 2) and protein binding values for chlorpyrifos (Timchalk et al. 2002b) and parathion (Table 3) were utilized.

Table 1

A comparison of metabolic parameters for PBPK/PD models published by Timchalk *et al.* (2002) and the refined human CYP-specific models.

Timchalk <i>et al.</i> (2002) Chlorpyrifos				
Parameter	Rat	Human	CYP-specific Human Model for Chlorpyrifos	CYP-specific Human Model for Parathion
CYP Activation (liver)				
K _{m1} (μmol/l)	2.86 ^a	2.86 ^a	CYP specific parameters used [V _{max} ^b ;K _m ^b ; CYP content ^c]	CYP specific parameters used [V _{max} ^b ;K _m ^b ; CYP content ^c]
V _{max} C ₁ (μmol/h/kg)	80 ^a	80 ^a		
CYP1A2 Vmax (pmol/min/nmol P450)	-	-	1193	6131
CYP1A2 Km (μmol/l)	-	-	0.38	1.63
CYP2B6 Vmax (pmol/min/nmol P450)	-	-	12544	4827
CYP2B6 Km (μmol/l)	-	-	0.81	0.61
CYP2C9 Vmax (pmol/min/nmol P450)	-	-	-	1140
CYP2C9 Km (μmol/l)	-	-	-	9.78
CYP2C19 Vmax (pmol/min/nmol P450)	-	-	2470	4470
CYP2C19 Km (μmol/l)	-	-	1.23	0.56
CYP3A4 Vmax (pmol/min/nmol P450)	-	-	11946	14009
CYP3A4 Km (μmol/l)	-	-	27.3	65.5
CYP3A5 Vmax (pmol/min/nmol P450)	-	-	2569	2020
CYP3A5 Km (μmol/l)	-	-	16.6	43.2
CYP3A7 Vmax (pmol/min/nmol P450)	-	-	794	-
CYP3A7 Km (μmol/l)	-	-	34	-
CYP Detoxification (liver)				
K _{m2} (μmol/l)	24 ^a	24 ^a	CYP specific parameters used [V _{max} ^b ;K _m ^b ; CYP content ^c]	CYP specific parameters used [V _{max} ^b ;K _m ^b ; CYP content ^c]
V _{max} C ₂ (μmol/h/kg)	273 ^a	273 ^a		
CYP1A2 Vmax (pmol/min/nmol P450)	-	-	892	5656
CYP1A2 Km (μmol/l)	-	-	0.63	2.15
CYP2B6 Vmax (pmol/min/nmol P450)	-	-	1545	1804
CYP2B6 Km (μmol/l)	-	-	2.09	0.74
CYP2C9 Vmax (pmol/min/nmol P450)	-	-	-	742
CYP2C9 Km (μmol/l)	-	-	-	12.1
CYP2C19 Vmax (pmol/min/nmol P450)	-	-	13128	2223
CYP2C19 Km (μmol/l)	-	-	1.63	0.6
CYP3A4 Vmax (pmol/min/nmol P450)	-	-	12667	15738
CYP3A4 Km (μmol/l)	-	-	33.4	31.2
CYP3A5 Vmax (pmol/min/nmol P450)	-	-	2141	1175
CYP3A5 Km (μmol/l)	-	-	23.9	68.2
CYP3A7 Vmax (pmol/min/nmol P450)	-	-	-	1739
CYP3A7 Km (μmol/l)	-	-	-	37.3
PON1 Detoxification of oxon (liver)				

Timchalk <i>et al.</i> (2002) Chlorpyrifos				
Parameter	Rat	Human	CYP-specific Human Model for Chlorpyrifos	CYP-specific Human Model for Parathion
K_{m3} ($\mu\text{mol/l}$)	240 ^d	240 ^d	240 ^d	240 ^d
$V_{\max}C_3$ ($\mu\text{mol/h/kg}$)	74421 ^d	74421 ^d	74421 ^d	74421 ^d
PON1 Detoxification of oxon (blood)				
K_{m4} ($\mu\text{mol/l}$)	250 ^d	250 ^d	250 ^d	250 ^d
$V_{\max}C_4$ ($\mu\text{mol/h/kg}$)	57003 ^d	57003 ^d	57003 ^d	57003 ^d

^a (Ma and Chambers 1994),

^b (Foxenberg et al. 2007),

^c determined using human hepatic CYP calculator,

^d (Mortensen et al. 1996)

Table 2

The resulting output for human hepatic CYP content for a 1 year old and 19 year old using the human hepatic CYP content calculator.

CYP	<u>Estimated average hepatic CYP Content (pmol/mg microsome)</u>	
	1yr old	19yr old
1A2	3.27 ^a	24.93 ^a
2B6	2.65 ^a	19.36 ^a
2C9	17.23 ^b	14.97 ^b
2C19	10.30 ^b	14.15 ^b
3A4	18.48 ^b	67.55 ^b
3A5	3.84 ^b	5.38 ^b
3A7	15.21 ^b	N/A ^b

^a Average value (Tateishi et al. 1997), not calculated.

^b Determined using the CYP content calculator (<http://wings.buffalo.edu/smbs/pmy/resources/Cyp%20Calculator.htm>).

Table 3

Time and concentration dependency for protein binding of parathion and paraoxon to 4% human serum albumin utilizing equilibrium dialysis.

	Percent of unbound compound ^a		
	15min	60min	240min
Parathion			
50μM	2.6 ± 0.1	5.6 ± 0.7	6.2 ± 0.1
25μM	1.4 ± 2.4	6.1 ± 0.3	6.8 ± 0.2
Paraoxon			
50μM	24.3 ± 4.0	41.6 ± 3.5	40.0 ± 5.2
25μM	18.2 ± 4.0	37.6 ± 3.7	39.1 ± 1.1
10μM	25.7 ± 3.1	41.7 ± 11.3	43.8 ± 3.7

^aN=3 for each reaction. Values represent the mean ± SD.

Table 4

Effect of varying hepatic CYP content on human PBPK/PD model simulations for a 19yr old following a single oral exposure to chlorpyrifos.

Chlorpyrifos Trials for a 19yr old (70 kg bw)				
	CYP Content Relative to Average in Table 2^a	Oral Dose (µg/kg)	Percent BuChE Inhibition (peak inhibition b)	Percent RBC AChE Inhibition (peak inhibition b)
Trial 1	Average	10,000	100	83.2
		1,000	99.0	16.9
		100	47.6	1.74
Trial 2	1/4 CYP2B6	10,000	99.9	72.1
		1,000	98.1	12.9
		100	39.0	1.33
Trial 3	4X CYP2B6	10,000	100	90.5
		1,000	99.4	20.9
		100	55.2	2.17
Trial 4	1/4 CYP2C19	10,000	100	86.7
		1,000	99.3	19.3
		100	52.4	2.00
Trial 5	4X CYP2C19	10,000	99.9	73.1
		1,000	97.8	12.2
		100	36.8	1.24
Trial 6	4X CYP2B6 1/4 CYP2C19	10,000	100	91.6
		1,000	99.5	22.0
		100	57.2	2.30
Trial 7	1/4 CYP2B6 4X CYP2C19	10,000	99.9	60.4
		1,000	95.1	8.96
		100	28.5	0.90
Trial 8	1/4 CYP2B6 Null CYP2C19	10,000	100	80.0
		1,000	99.1	17.1
		100	48.8	1.80
Trial 9	Null CYP2C19	10,000	100	87.9
		1,000	99.4	20.3
		100	54.3	2.12
Trial 10	4X CYP2B6 Null CYP2C19	10,000	100	91.9
		1,000	99.5	22.4
		100	57.9	2.35

^aModel simulations for altered CYP2B6 and CYP2C19 content were conducted to simulate interindividual differences within the main CYPs responsible for chlorpyrifos metabolism. CYP2B6 and/or CYP2C19 hepatic concentrations were increased or decreased 4-fold from average levels (see table 2 for average values), which is within the normal degree of interindividual variability. Model simulations were also conducted with null CYP2C19 to simulate the CYP2C19*2 genotype. The remaining hepatic CYP content was held constant at levels of a typical 19yr old (see table 2). The free or unbound fractions of chlorpyrifos and chlorpyrifos oxon in human blood were 3 and 2% (Timchalk et al. 2002).

^bThe peak inhibition in BuChE and AChE activity following chlorpyrifos exposure was generally between 4 and 9 hours post exposure.

Table 5

Effect of varying hepatic CYP content on human PBPK/PD model simulations for a 19yr old following a single oral exposure to parathion.

Parathion Trials for a 19yr old (70 kg bw)				
CYP Content Relative to Average in Table 2 ^a		Oral Dose (µg/kg)	Percent BuChE Inhibition (peak inhibition ^b)	Percent RBC AChE Inhibition (peak inhibition ^b)
Trial 1	Average	100	79.3	59.0
		25	33.0	20.8
		5	7.65	4.56
Trial 2	1/4 CYP2B6	100	77.7	57.2
		25	31.4	19.8
		5	7.29	4.32
Trial 3	4X CYP2B6	100	81.6	61.7
		25	35.1	22.3
		5	8.23	4.92
Trial 4	1/4 CYP2C19	100	78.8	58.3
		25	32.6	20.4
		5	7.53	4.47
Trial 5	4X CYP2C19	100	80.2	60.1
		25	33.8	21.3
		5	7.85	4.70
Trial 6	4X CYP2B6 1/4 CYP2C19	100	81.7	61.7
		25	35.2	22.3
		5	8.24	4.93
Trial 7	1/4 CYP2B6 4X CYP2C19	100	79.6	59.4
		25	33.3	21.0
		5	7.71	4.61
Trial 8	1/4 CYP2B6 Null CYP2C19	100	75.3	54.4
		25	29.8	18.4
		5	6.78	3.99
Trial 9	Null CYP2C19	100	78.5	58.0
		25	32.4	20.3
		5	7.47	4.43
Trial 10	4X CYP2B6 Null CYP2C19	100	81.7	61.8
		25	35.2	22.3
		5	8.25	4.94

^aModel simulations for altered CYP2B6 and CYP2C19 content were conducted to simulate interindividual differences within the main CYPs responsible for parathion metabolism. CYP2B6 and/or CYP2C19 hepatic concentrations were increased or decreased 4-fold from average levels (see table 2 for average values), which is within the normal degree of interindividual variability. Model simulations were also conducted with null CYP2C19 to simulate the CYP2C19*2 genotype. The remaining hepatic CYP content was held constant at levels of a typical 19yr old (see table 2). Data for equilibrium dialysis with 4% human serum albumin (Table 3) was used to estimate the free, unbound fraction of parathion and paraoxon in human blood (6 and 40%, respectively).

^bThe peak inhibition in BuChE and AChE activity following parathion exposure was generally between 10 and 12 hours post exposure.