Toxicology 261 (2009) 47–58

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Comparative chlorpyrifos pharmacokinetics via multiple routes of exposure and vehicles of administration in the adult rat

Jordan Ned Smith[a], James A. Campbell[a], Andrea L. Busby-Hjerpe[a], Sookwang Lee[a], Torka S. Poet[a], Dana B. Barr[b], Charles Timchalk[a,*]

[a] Battelle, Pacific Northwest Division, Richland, WA 99354, USA
[b] National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30341, USA

ARTICLE INFO

Article history:
Received 10 March 2009
Received in revised form 14 April 2009
Accepted 19 April 2009
Available online 3 May 2009

Keywords:
Chlorpyrifos
3,5,6-Trichloro-2-pyridinol
Pharmacokinetics
Trichloropyridinol

ABSTRACT

Chlorpyrifos (CPF) is a commonly used organophosphorus pesticide. A number of toxicity and mechanistic studies have been conducted in animals, where CPF has been administered via a variety of different exposure routes and dosing vehicles. This study compared chlorpyrifos (CPF) pharmacokinetics using oral, intravenous (IV), and subcutaneous (SC) exposure routes and corn oil, saline/Tween 20, and dimethyl sulfoxide (DMSO) as dosing vehicles. Two groups of rats were co-administered target doses (5 mg/kg) of CPF and isotopically labeled CPF (L-CPF). One group was exposed by both oral (CPF) and IV (L-CPF) routes using saline/Tween 20 vehicle; whereas, the second group was exposed by the SC route using two vehicles, corn oil (CPF) and DMSO (L-CPF). A third group was only administered CPF by the oral route in corn oil. For all treatments, blood and urine time course samples were collected and analyzed for 3,5,6-trichloro-2-pyridinol (TCPy), and isotopically labeled 3,5,6-trichloro-2-pyridinol (L-TCPy). Peak TCPy/L-TCPy concentrations in blood (20.2 μmol/l), TCPy/L-TCPy blood AUC (94.9 μmol/l h), and percent of dose excreted in urine (100%) were all highest in rats dosed orally with CPF in saline/Tween 20 and second highest in rats dosed orally with CPF in corn oil. Peak TCPy concentrations in blood were more rapidly obtained after oral administration of CPF in saline/Tween 20 compared to all other dosing scenarios (>1.5 h). These results indicate that orally administered CPF is more extensively metabolized than systemic exposures of CPF (SC and IV), and vehicle of administration also has an effect on absorption rates. Thus, equivalent doses via different routes and/or vehicles of administration could potentially lead to different body burdens of CPF, different rates of bioactivation to CPF-oxon, and different toxic responses. Simulations using a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for CPF are consistent with these possibilities. These results suggest that exposure route and dosing vehicle can substantially impact target tissue dosimetry. This is of particular importance when comparing studies that use varying exposure paradigms, which are then used for extrapolation of risk to humans.

1. Introduction

Pharmacokinetics of xenobiotics and resulting target tissue dosimetry can vary greatly as a function of differing exposure routes and vehicles of administration. Biological response due to any chemical compound is determined by the concentration of that compound at an internal target site. Key processes that govern the disposition of an administered dose to the internal target tissue concentration include absorption, distribution, metabolism, and excretion. These processes and rates of these processes can vary greatly by route and vehicle of administration, thus causing potential discrepancies among various exposure scenarios. Therefore, a robust understanding of internal dosimetry is critical, especially when comparing studies that use varying exposure paradigms on animal models which are then extrapolated to humans for risk assessment purposes. A quantitative understanding of internal dosimetry is even more critical for studies that use animal model exposure scenarios that are of questionable relevance to real-world human exposures and therefore limited utility for assessing risk.

Chlorpyrifos (CPF) is a commonly used OP pesticide that is marketed under trade names including Dursban® and Lorsban® (Dow Chemical Company, Midland, MI, USA). Once in the body, CPF can be metabolized by two different pathways (Fig. 1). Cytochrome P450 (CYP) enzymes oxidize CPF into a phosphooxythiiran

* Corresponding author at: P.O. Box 999, Richland, WA 99354, USA. Tel.: +1 509 376 0434; fax: +1 509 376 9064.
E-mail addresses: jordan.smith@pnl.gov (J.N. Smith), james.campbell@pnl.gov (J.A. Campbell), andrea.busby@pnl.gov (A.L. Busby-Hjerpe), sookwang.lee@pnl.gov (S. Lee), torka.poet@pnl.gov (T.S. Poet), dbarr@cdc.gov (D.B. Barr), charles.timchalk@pnl.gov (C. Timchalk).

0300-483X/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2009.04.041

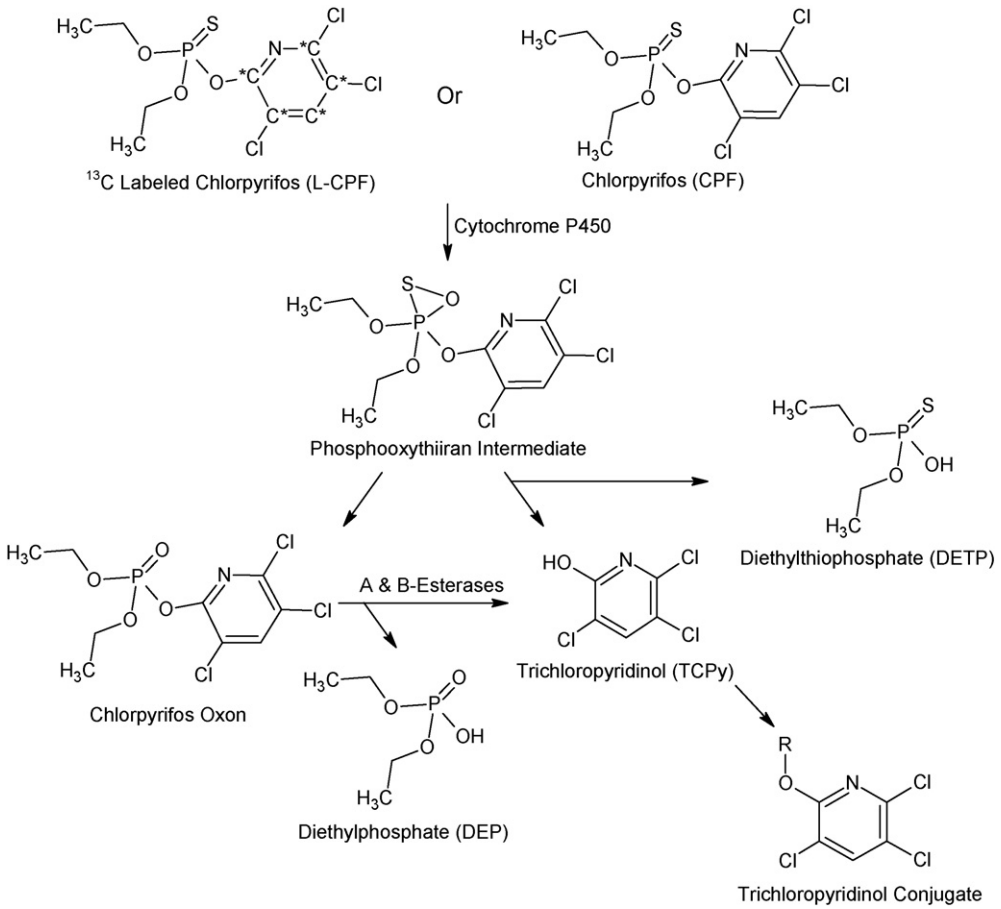

Fig. 1. Metabolism of chlorpyrifos (CPF) and ^{13}C labeled chlorpyrifos (L-CPF). ^{13}C labeled carbons are noted by asterisks in initial parent compounds. For L-CPF, these will follow through to form isotopically labeled phophooxythiiran intermediate, isotopically labeled CPF-oxon, isotopically labeled 3,5,6-trichloro-2-pyridinol (L-TCPy), and L-TCPy conjugates.

intermediate (Kamataki et al., 1976). That intermediate will rearrange to either (1) undergo oxidative desulfation to form chlorpyrifos-oxon (CPF-oxon), the ultimate toxicant that binds to acetylcholinesterase (AChE) (Sultatos, 1994), effectively inhibiting this enzyme, or (2) undergo a dearylation (oxidative ester cleavage) of CPF forming 3,5,6-trichloro-2-pyridinol (TCPy) and diethylthiophosphate (DETP) (Kamataki et al., 1976). Ratios of the two rearrangement products are associated with specific species, gender, age, CYP enzyme profiles, and CYP enzyme polymorphisms (Ma and Chambers, 1994; Dai et al., 2001; Rose et al., 2005). CPF-oxon can be metabolized by hepatic and extrahepatic esterases, such as paraoxonase (PON-1) (Pond et al., 1998), and tissue β-esterases (cholinesterase; ChE) to form TCPy and diethylphosphate (DEP) (Chanda et al., 1997). Excretion is facilitated through urine as DEP, DETP, TCPy or a glucuronide, sulfate, or other conjugate of TCPy (Bakke et al., 1976; Nolan et al., 1984).

There have been numerous studies to evaluate modes and mechanisms of CPF action in animals, in which CPF has been administered via a variety of different exposure routes using different vehicles of administration. Rats have been dosed orally with corn oil (Breslin et al., 1996; Howard et al., 2007; Marty et al., 2007; Moser and Padilla, 1998; Timchalk et al., 2007a; Won et al., 2001) or milk (Marty et al., 2007) as vehicles. A number of rodent studies have bypassed gut absorption administering CPF via subcutaneous (SC) injection with dimethyl sulfoxide (DMSO) (Marty et al., 2007; Slotkin et al., 2006; Whitney et al., 1995) or peanut oil (Karanth et al., 2006; Karanth and Pope, 2003; Whitney et al., 1995). In addition, at least one study has involved exposing rats by intravenous injection (IV) using acetone as the vehicle (Abdel-Rahman et al., 2002). Marty et al. (2007) evaluated the impact of dose, dose-rate, route of exposure, and vehicle on the pharmacokinetics of CPF and TCPy on postnatal day (PND) 5 rats and noted that the pharmacokinetics of CPF and TCPy were clearly impacted by routes and rates of exposure, as well as dosing vehicle. Recently, Carr and Nail (2008) reported that exposing late preweanling rats to CPF using different routes and administration vehicles had also produced differences in pharmacodynamic endpoints at equivalent doses.

With the exception of Marty et al. (2007) and Carr and Nail (2008) studies, which focused on evaluating neonatal pharmacokinetic and pharmacodynamic endpoints, there have not been any systematic studies that evaluated the impact of different exposure scenarios or dosing vehicles on CPF pharmacokinetics in adult rats. Without a robust understanding of internal CPF dosimetry, there is uncertainty in deciphering biological mode and mechanisms of CPF action, as well as extrapolating those modes and mechanisms to humans, especially from studies that used routes of exposure not relevant in a human risk assessment standpoint, such as SC injection.

Therefore, the objective of this study was to compare CPF pharmacokinetics using various exposure routes and vehicles of administration, with focus on those routes and vehicles that have been previously utilized, to provide a better understanding of internal CPF dosimetry. To accomplish this object, rats were simultaneously co-exposed to CPF and isotopically labeled CPF (L-CPF), in which five ^{13}C carbons have been substituted for the ^{12}C carbons around the TCPy ring, creating a mass difference of 5 amu. Pharmacokinetically, these two compounds behave identically *in vivo*; however, differences in mass allowed for differentiation of

each compound analytically in biological matrices using mass spectrometry.

Three *in vivo* pharmacokinetic studies were completed to compare pharmacokinetics of various routes and vehicles of exposure using these two forms of CPF. First, CPF and L-CPF were co-administered either orally (CPF) or intravenously (L-CPF) in a saline/Tween 20 solution in the same animals. This study was designed to evaluate oral bioavailability and gut/first pass metabolism of CPF relative to an IV exposure, which would omit absorption and the first pass metabolism. Secondly, CPF and L-CPF were co-exposed at equivalent doses via SC injection using corn oil (CPF) and DMSO (L-CPF) as two separate vehicles. CPF pharmacokinetics of these two vehicles were compared to one another to evaluate vehicle of administration differences. Finally, CPF was administered orally in a corn oil vehicle; which could then be evaluated against orally administered CPF in saline/Tween 20 to provide insight on the impact of corn oil on the bioavailabililty and metabolism of CPF. For comparison, data from these *in vivo* studies were modeled using compartmental pharmacokinetic analysis. In addition, a previously published and validated physiologically based pharmacokinetics/pharmacodynamic (PBPK/PD) model for CPF (Timchalk et al., 2002) was updated and modified to accommodate SC exposures. This model was then used to compare the pharmacokinetic time course and pharmacodynamic response for CPF in blood and tissues following oral and SC exposures. Results from these studies will elucidate potential uncertainty in CPF pharmacokinetics associated with differing routes and vehicles of administration and also exploit computational modeling as a tool to link these varying routes and vehicles as well as a potential tool to better extrapolate results from this and previous studies to humans.

2. Materials and methods

2.1. Chemicals

Chlorpyrifos (CAS #2921-88-2, 99% pure) and TCPy (CAS #6515-38-4, 99% pure) were kindly provided by Dow Agro-Sciences (Indianapolis, IN, USA). Chlorpyrifos labeled with five ^{13}C on the TCPy ring (L-CPF, 355.6 amu) was obtained from the Centers for Disease Control and Prevention (CDC) (Atlanta, GA, USA). All other chemicals including polyoxyethylene sorbitan monolaurate (Tween 20, CAS #9005-64-5), N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA, CAS #77377-52-7), dimethyl sulfoxide (DMSO, CAS #67-68-5), and other general laboratory chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) and were reagent grade or better.

2.2. Animals

For all studies, male Sprague–Dawley rats (215–275 g) with jugular vein cannulae were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA). Rats were housed in glass metabolism cages designed for collection of urine and feces. Rats were fed Purina Certified Rodent Chow® 5002 (Purina Mills, St. Louis, MO, USA) *ad libitum*, with the exception that it was withdrawn 12 h prior to dosing and returned 3 h post-dosing. Water was available *ad libitum* throughout the duration of the study. Blood (100–200 μl) was collected through the jugular vein cannula at 0, 5 min, 0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 3 h, 6 h, 8 h, 12 h, 24 h, and 48 h post-dosing and was replaced with the appropriate volume of physiological saline. Urine was collected continuously, and sample collections were accumulated for 0–12, 12–24, and 24–48 h post-dosing. Rats were euthanized at 48 h post-dosing, except for the oral exposure via corn oil rats, which were euthanized at 72 h.

2.3. Dosing

Groups of rats (5/treatment) were dosed with 5 mg/kg CPF in saline (+5% Tween 20) by oral gavage using 5 ml/kg dose volume, and 5 mg/kg L-CPF in saline (+5% Tween 20) by intravenous (IV) tail vein injection using 1 ml/kg dose volume. A second group of rats (5/treatment) were dosed with 5 mg/kg CPF in corn oil by SC injection, and 5 mg/kg L-CPF in DMSO also by SC injection using 1 ml/kg dosing volume for both vehicles. Separate SC injection sites were located slightly posterior and ventral to the shoulders of the rat, bilaterally symmetrical from one another. For all animals that were co-exposed, CPF was administered concurrently with minimal time (~1–3 min) between administrations. A third group of rats (5/treatment) were dosed with 5 mg/kg CPF in corn oil by oral gavage at 5 ml/kg dose volume.

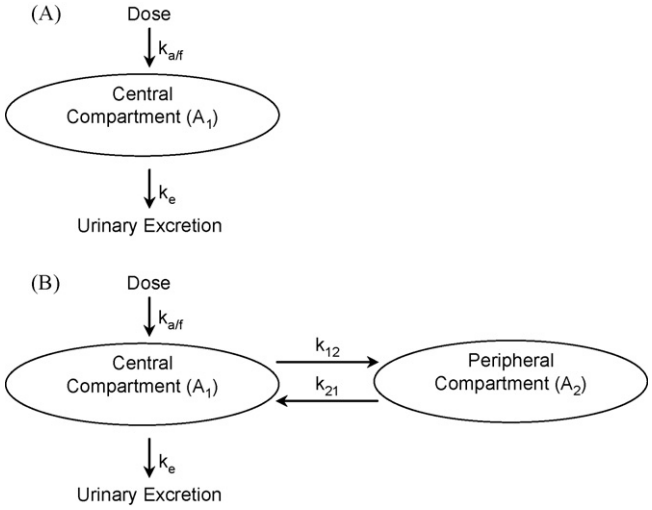

Fig. 2. Schematics of a one-compartment (A) and two-compartment (B) pharmacokinetic model for 3,5,6-trichloro-2-pyridinol (TCPy) or isotopically labeled TCPy (L-TCPy) formed after dosing with chlorpyrifos (CPF) or isotopically labeled CPF (L-CPF).

2.4. Analytical methods

To extract TCPy (198.4 amu), and 3,5,6-trichloro-2-pyridinol with five ^{13}C (L-TCPy, from metabolized L-CPF, 203.4 amu, Fig. 1) from biological matrices, a method similar to that of Brzak et al. (1998) was utilized. In brief, blood samples and aliquots of urine samples were first acidified with 3 M HCl saturated with NaCl. Samples were extracted three times with ethyl acetate, dried with anhydrous Na_2SO_4, and evaporated under a gentle stream of inert N_2. Once all solvent was evaporated, samples were reconstituted in toluene and derivatized using MTBSTFA. Separate aliquots of urine samples were subjected to an acid hydrolysis prior to the extraction protocol to quantify not only free TCPy and L-TCPy (as extraction without acid hydrolysis), but also the conjugated TCPy and L-TCPy from phase II metabolism (sulfate and glutathione conjugates). Urine aliquots were hydrolyzed by adding concentrated HCl and heating to 80 °C for 1 h prior to extraction. The acid hydrolyzed and extracted urine specimens were subsequently handled similarly to other biological samples.

Gas chromatography–mass spectrometry (GC–MS) was used to quantify analytes post derivatization using a Hewlett-Packard (HP) (Wilmington, DE, USA) 5973B mass selective detector (MSD) in electron ionization mode interfaced with HP model 6890 GC using HP ChemStation software for programming and data analysis. Separation was achieved using a 30 m × 0.25 mm i.d × 0.25 μm film thickness RTX-5MS column (Restek, Bellefonte, PA, USA). Helium was used as the carrier gas at a constant flow rate of 6 ml/min. The inlet temperature was 210 °C, and the oven temperature initiated at 80 °C, maintained for 3 min, ramped to 310 °C at a rate of 15 °C/min, and held at 310 °C for 3 min. Selected ion monitoring (SIM) was used for analysis for increased sensitivity. Ions selected for monitoring included 254 and 256 *m*/*z* for derivatized TCPy as well as 259 and 261 *m*/*z* for derivatized L-TCPy. This method allowed for a quantification limit of approximately 0.12 μmol/l of TCPy and L-TCPy. Concentrations of CPF in dose solutions were verified using this same GC–MS method.

2.5. Pharmacokinetic analysis

Previous studies have shown that CPF and CPF-oxon are difficult to detect in blood and are not excreted into urine, thus the analytical and pharmacokinetic analysis focused on TCPy and L-TCPy (Nolan et al., 1984; Timchalk et al., 2007a, 2002). To estimate pharmacokinetic parameters, either a one or two-compartment pharmacokinetic model was fit to blood data from the various *in vivo* studies. Rats dosed orally with CPF (saline/Tween 20) required a two-compartment model to obtain an adequate fit. Model structures were as follows:

One-compartment pharmacokinetic model (Fig. 2A):

$$A_0 = \text{Dose} \times e^{-k_{a/f} \times t} \quad (1)$$

$$\frac{dA_1}{dt} = (k_{a/f} \times A_0) - (k_e \times A_1) \quad (2)$$

$$CA_1 = \frac{A_1}{V_1} \quad (3)$$

Two-compartment pharmacokinetic model (Fig. 2B):

$$A_0 = \text{Dose} \times e^{-k_{a/f} \times t} \quad (4)$$

$$\frac{dA_1}{dt} = (k_{a/f} \times A_0) - (k_e \times A_1) - (k_{12} \times A_1) + (k_{21} \times A_2) \quad (5)$$

Table 1
Optimized parameters used for simulations with a pharmacokinetic and pharmacodynamic (PBPK/PD) model of chlorpyrifos (CPF).

Parameter	Value	Units
k_m hepatic CPF to CPF-oxon[A]	2.25	μmol/l
k_m hepatic CPF to TCPy[B]	3.50	μmol/l
k_{as}: first order oral absorption rate	0.04	h^{-1}
k_e: first order TCPy elimination rate	0.23	h^{-1}
v_{diss}: volume of distribution of TCPy compartment	0.12	l
V_{max} brain CPF to CPF-oxon	3.5E-04	μmol/h/kg
k_m brain CPF to CPF-oxon	79.40	μmol/l
k_d brain AChE[C] degradation rate	3.0E-03	h^{-1}
k_d blood AChE degradation rate	3.0E-03	h^{-1}
Corn oil k_{sc}: first order transfer rate from SC compartment to mixed blood	0.34	h^{-1}
Corn oil k_{sp}: first order transfer rate from the SC compartment to peripheral SC compartment	1.46	h^{-1}
Corn oil k_{ps}: first order transfer rate from the peripheral SC compartment to SC compartment	0.04	h^{-1}
DMSO k_{sc}: first order transfer rate from SC compartment to mixed blood	0.03	h^{-1}
DMSO k_{sp}: first order transfer rate from the SC compartment to peripheral SC compartment	0.07	h^{-1}
DMSO k_{ps}: first order transfer rate from the peripheral SC compartment to SC compartment	0.03	h^{-1}

[A]Chlorpyrifos-oxon (CPF-oxon); [B]3,5,6-Trichloro-2-pyridinol (TCPy); [C]Acetylcholinesterase (AChE).

$$\frac{dA_2}{dt} = (k_{12} \times A_1) - (k_{21} \times A_2) \quad (6)$$

$$CA_1 = \frac{A_1}{V_1} \quad (7)$$

"A_0", "A_1", and "A_2" represent the amount (μmol) of TCPy or L-TCPy in the absorption/formation, central, and peripheral compartments, respectively. "Dose" is the molar amount of CPF or L-CPF administered, thus it is the maximal molar equivalent dose of TCPy or L-TCPy available for disposition. The rate constants "$k_{a/f}$", "k_e", "k_{12}", and "k_{21}" are first-order transfer rates (h^{-1}) among compartments. "V_1" represents the volume of distribution (l) for the central compartment. "CA_1" is the concentration (μmol/l) of TCPy or L-TCPy in the central compartment (blood), and "t" is time (h). Estimates of model parameters were obtained using the Nelder-Mead algorithm in acslX Version 2.4.2.1 software (AEgis Technologies Group, Inc., Huntsville, AL, USA).

Area under the curve (AUC) was calculated for blood data using the trapezoidal rule (Gibaldi and Perrier, 1982). For analyte concentrations in blood which included analytical data that was not quantifiable, the Kaplan-Meier method was used for calculating descriptive statistics (Helsel, 2005). Maximum TCPy or L-TCPy concentration in blood were statistically analyzed for all *in vivo* studies using a one-way analysis of variance (ANOVA) coupled with a Tukey–Kramer *post hoc* test for multiple comparisons. All statistical tests used an α value of 0.05. Software used to statistically analyze data was "R: A language and environment for statistical computing" Version 2.7.1 (R, 2004).

A PBPK/PD model for CPF (Timchalk et al., 2002) was used to simulate CPF concentrations in blood, brain, and fat. The PBPK/PD model was modified to accommodate SC dose administration and was then used to simulate oral and SC dosing using both corn oil and DMSO (SC only) as dosing vehicles. Updated PBPK/PD model parameters based on the initial estimates (Timchalk et al., 2002) were used, including fractional oral absorption (Timchalk et al., 2007b); CPF and CPF-oxon partitioning coefficients (Lowe et al., 2009); fraction of CPF and CPF-oxon bound in blood (Timchalk et al., 2007b); metabolic coefficients (Poet et al., 2003; Timchalk and Poet, 2008); and enzyme degradation, reactivation, and aging rates (Timchalk et al., 2007b). With these new parameter estimates, other parameters were refit including k_m for hepatic conversion of CPF to CPF-oxon, k_m for hepatic conversion of CPF to TCPy, the first-order absorption constant from the stomach, k_e for TCPy elimination, and volume of distribution for TCPy elimination (Table 1). Brain metabolism of CPF to CPF-oxon was added as a Michaelis–Menten process (Chambers and Chambers, 1989). The V_{max} (0.00035 μmol/h/kg) for this metabolic rate was scaled to fit the *in vivo* system and the k_m (79.4 μmol/l) was fit using data from Timchalk et al. (2005). Enzyme degradation rates were estimated to better fit experimental data that extended beyond 24 h post-dosing (Table 1). Ongoing modeling efforts are underway to more fully evaluate the appropriateness of these decreased enzyme degradation rates to fit plasma and brain ChE activity data and will be reported in subsequent communications. Additionally, a two-compartment system was added to the PBPK/PD model to simulate CPF movement from the SC dosing depot to the mixed blood compartment. First-order transfer rate constants were optimized among these compartments using concentrations of TCPy in blood and urine from the *in vivo* SC exposures in both corn oil and DMSO vehicles as well as brain ChE inhibition after SC exposure where applicable (Karanth et al., 2006; Karanth and Pope, 2003). The model structure for the SC absorption is as follows (Fig. 3):

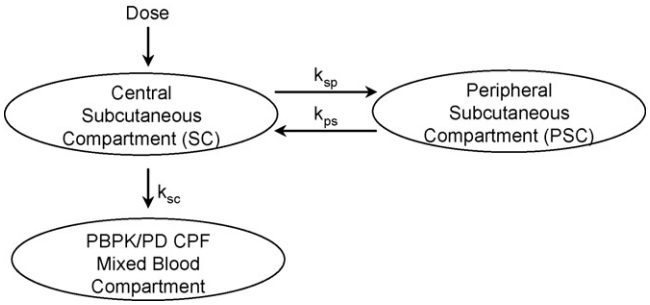

Fig. 3. Schematic of subcutaneous dose incorporation into a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model of chlorpyrifos (CPF).

$$\frac{dSC}{dt} = \text{Dose} - (k_{sc} \times SC) - (k_{sp} \times SC) + (k_{ps} \times PSC) \quad (8)$$

$$\frac{dPSC}{dt} = (k_{sp} \times SC) - (k_{ps} \times PSC) \quad (9)$$

$$\frac{dB}{dt} = (k_{sc} \times SC) \quad (10)$$

"SC" and "PSC" are amounts of CPF or isotopically labeled CPF (L-CPF) (μmol) in the central and peripheral subcutaneous compartments, respectively. "B" is the incorporation of CPF or L-CPF from the subcutaneous compartment into the mixed blood compartment of the PBPK/PD. "Dose" is the molar amount of CPF or L-CPF administered. "k_{sc}", "k_{sp}", and "k_{ps}" are first order transfer rates (μmol/h) among compartments. "t" is time (h).

3. Results

For all treatment groups the targeted CPF dose was 5 mg/kg of body weight. Based upon GC–MS analysis, verified doses ranged from 3.49 mg/kg (oral gavage in corn oil) to 5.85 mg/kg (SC in corn oil) (Table 2). None of the rats displayed overt toxicity following exposure to CPF or L-CPF from any of the dosing scenarios.

Following exposure to CPF or L-CPF, TCPy or L-TCPy was detected in the majority of blood samples analyzed. Peak TCPy/L-TCPy levels in blood (C_{max}) were highest (20.2 μmol/l) following oral dosing of CPF in saline/Tween 20 ($p < 0.018$) (Table 2). Maximum TCPy concentration in blood from orally administrated CPF in corn oil (11.3 μmol/l) was significantly lower than that of CPF administered orally in saline/Tween 20 ($p = 0.018$), but also significantly higher than all systemic (IV and SC) dosing scenarios ($p < 0.016$) ranging from 1.8 to 2.8 μmol/l. There were no other significant differences

Table 2
Pharmacokinetic parameters of blood levels of 3,5,6-trichloro-2-pyridinol (TCPy) or isotopically labeled TCPy (L-TCPy) from administration of chlorpyrifos (CPF) or isotopically labeled CPF (L-CPF), respectively, using different routes and vehicles of administration.

Administration Route	Vehicle	Dose (mg/kg)	C_{max} (μmol/l)	T_{max} (h)	AUC (0.48) (μmol/l h)
Oral Gavage	Saline/Tween 20	4.15	20.24[A]	0.5	94.91
Oral Gavage	Corn Oil	3.49	11.33[B]	6.0	81.85
Intravenous injection	Saline/Tween 20	4.05	2.50[C]	8.0	68.69
Subcutaneous injection	Corn Oil	5.85	2.80[C]	2.0	43.21
Subcutaneous injection	DMSO	5.08	1.83[C]	12.0	49.53

Superscript capital letters indicate statistical groupings determined by a one-way analysis of variance (ANOVA) coupled with a Tukey–Kramer *post hoc* test for multiple comparisons.

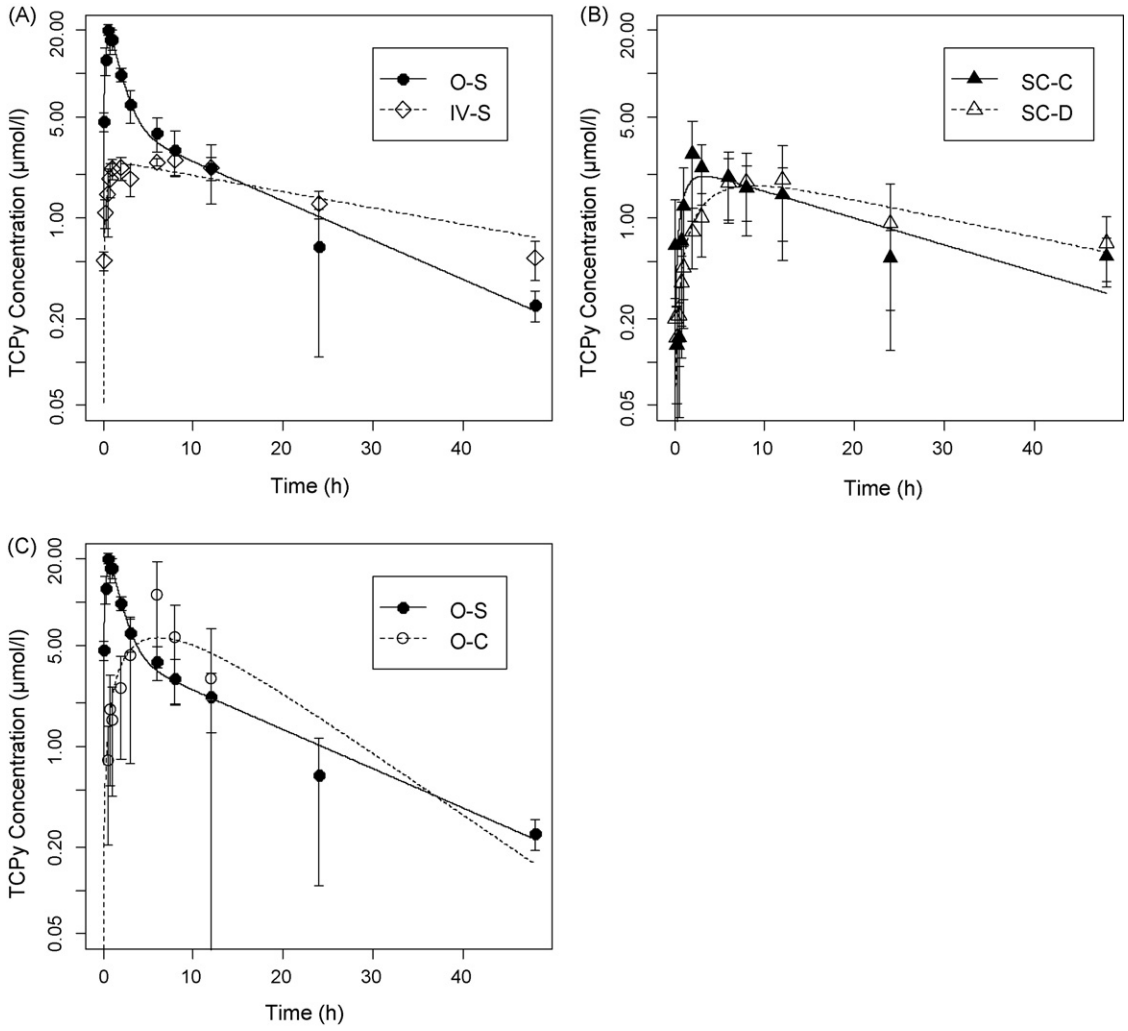

Fig. 4. Mean (with SD error bars) 3,5,6-trichloro-2-pyridinol (TCPy) or isotopically labeled TCPy (L-TCPy) concentrations in blood of rats after exposure to chlorpyrifos (CPF) or isotopically labeled CPF, respectively, via oral gavage in saline/Tween 20 (O-S), intravenous exposure in saline/Tween 20 (IV-S), subcutaneous injection (SC) in corn oil (SC-C), SC in dimethyl sulfoxide (DMSO) (SC-D), and oral gavage in corn oil (O-C). Lines are the respective compartmental pharmacokinetic model fits to the experimental data.

in peak TCPy levels in blood among the remaining dosing scenarios ($p > 0.99$).

Consistent with the high C_{max} following oral administration in saline/tween 20, the time to peak concentration (T_{max}) was rapid (0.5 h); whereas, for all other treatment groups the T_{max} ranged from 2 to 12 h (Table 2). Calculated AUC of TCPy/L-TCPy in the blood ranged from 82 to 95 μmol/l h, for the two oral routes of administration, nearly double those of the two SC routes (43–50 μmol/l h). The IV route of administration yielded an AUC value between those from oral and SC administrations (69 μmol/l h).

TCPy blood pharmacokinetics were biphasic after exposure to CPF via oral gavage (saline/Tween 20), thus a two-compartment model was used to obtain an adequate fit for these data (Fig. 4). Whereas, one-compartment pharmacokinetic models were used for TCPy blood pharmacokinetic fits of the other dosing scenarios.

TCPy/L-TCPy was detected in all urinary samples including those that did and did not undergo acid hydrolysis (Fig. 5). After 48 h, total acid labile TCPy/L-TCPy excreted ranged from 1.47 to 3.54 μmol for all dosing scenarios. These values accounted for 100% of the oral CPF molar dose, 81% of the CPF molar dose for IV route, and only 39–44% of the molar dose from SC routes, regardless of vehicle (Table 4). Total unconjugated TCPy/L-TCPy excreted ranged from 0.22 to 0.64 μmol for all dosing scenarios, accounting for 11.8 to 30.3% of total TCPy/L-TCPy excreted. There were no differences in percent of TCPy conjugation among dosing scenarios.

Blood TCPy half-lives (including alpha (α, initial) and beta (β, terminal) phases from the two-compartment model) ranged from 0.5 to 9.7 h for the oral routes of administration (Table 3). Other systemic dosing scenarios had longer blood TCPy half-lives (16.1–26.7 h). Urinary half-lives were similar for the two oral

Table 3
Pharmacokinetic parameters of compartmental models fit to blood levels of 3,5,6-trichloro-2-pyridinol (TCPy) or isotopically labeled TCPy (L-TCPy) from administration of chlorpyrifos (CPF) or isotopically labeled CPF (L-CPF), respectively.

Administration Route	Vehicle	Pharmacokinetic model	$k_{a/f}$ (μmol/h)	k_e (μmol/h)	k_{12} (μmol/h)	k_{21} (μmol/h)	V_1 (l)	Half-life (h)
Oral gavage	Saline/Tween 20	Two-compartment	2.69	0.29	0.45	0.19	0.11	α: 0.50, β: 9.69
Oral gavage	Corn oil	One-compartment	0.25	0.10			0.21	7.02
Intravenous injection	Saline/Tween 20	One-compartment	2.10	0.03			1.21	26.65
Subcutaneous injection	Corn oil	One-compartment	1.00	0.04			1.67	16.19
Subcutaneous injection	DMSO	One-compartment	0.29	0.03			1.65	23.20

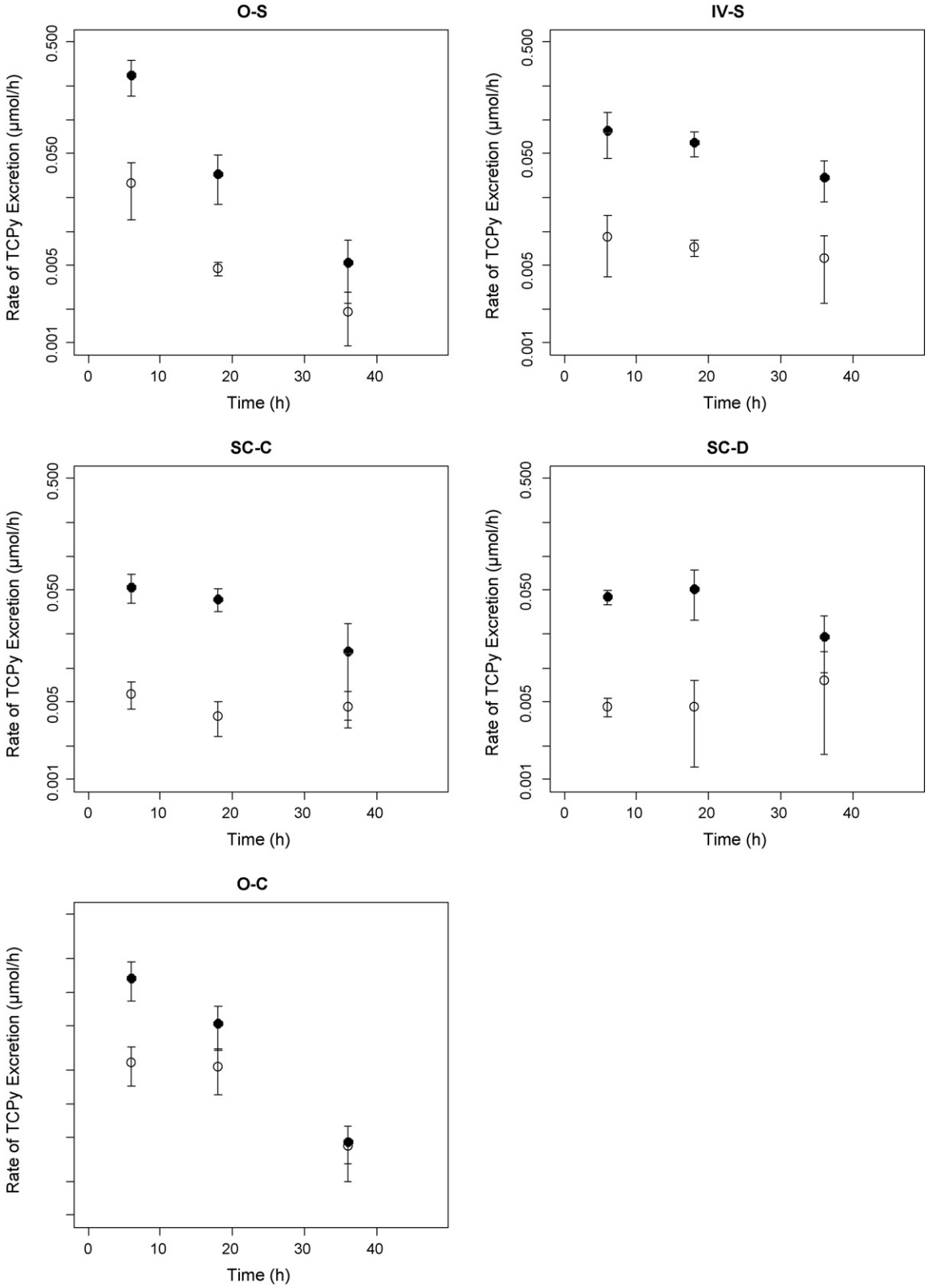

Fig. 5. Mean (with SD error bars) rate of 3,5,6-trichloro-2-pyridinol (TCPy) or isotopically labeled TCPy (L-TCPy) excretion in urine by rats after exposure to chlorpyrifos (CPF) or isotopically labeled CPF, respectively, via oral gavage in saline/Tween 20 (O-S), intravenous exposure in saline/Tween 20 (IV-S), subcutaneous injection (SC) in corn oil (SC-C), SC in dimethyl sulfoxide (DMSO) (SC-D), and oral gavage in corn oil (O-C). Acid labile TCPy/L-TCPy is the total TCPy/L-TCPy in urine (closed circles), and free TCPy/L-TCPy is the unconjugated TCPy/L-TCPy in urine (open circles).

Table 4
Amounts of 3,5,6-trichloro-2-pyridinol (TCPy) or isotopically labeled TCPy (L-TCPy) excreted in urine following administration of chlorpyrifos (CPF) or isotopically labeled CPF (L-CPF), respectively, using different routes and vehicles of administration.

Administration route	Vehicle	Total TCPy/L-TCPy amount at 48 h (μmol)	Dose excreted at 48 h (%)	Free TCPy/L-TCPy amount at 48 h (μmol)	Urinary half-life (h)
Oral gavage	Saline/Tween 20	3.54	100	0.37	4.10
Oral gavage	Corn Oil	2.33	100	0.64	8.49
Intravenous injection	Saline/Tween 20	2.45	81	0.33	26.85
Subcutaneous injection	Corn Oil	1.47	39	0.22	21.28
Subcutaneous injection	DMSO	1.58	44	0.29	36.73

routes of administration (terminal phase) ranging from 4.1 to 8.5 h (Table 4). Whereas, the two SC and IV routes also had similar urinary half lives, ranging from 21.2 to 36.7 h, which was also consistent with the observed TCPy blood time course in these same treatment groups.

Blood and urinary data for each dosing scenario correlated as the relative magnitude for blood TCPy AUCs (amount of CPF that has been absorbed, metabolized, and excreted) from each different dosing scenario was similar to the relative magnitude of the fractional molar dose excreted in the urine [oral (saline/Tween 20) ≥ oral (corn oil) > IV > SC (DMSO) ≥ SC (corn oil)]. Blood and urinary half lives were similar, especially those calculated from the one-compartment pharmacokinetic models, and the urinary half-life of CPF dosed orally in saline/Tween 20 was between the α and β phase half-lives of the two-compartment pharmacokinetic model.

PBPK/PD model simulations were fit to blood and urinary time course TCPy data from CPF exposed orally (corn oil), SC (corn oil), and SC (DMSO) (Fig. 6). The peripheral SC compartment allowed for an adequate fit to both blood and urinary TCPy time course data. Data from the SC dose in corn oil required larger first-order transfer rates into the blood (k_{sc}: 0.34 and 0.03 h^{-1}, respectively) and the peripheral SC compartment (k_{sp}: 1.46 and 0.07 h^{-1}, respectively) than did data from SC dose in DMSO (Table 1). The transfer rates from the peripheral SC compartment to the central SC compartment were similar (k_{ps}: 0.04 and 0.03 h^{-1}, respectively).

4. Discussion

A number of toxicity and pharmacokinetic studies have been conducted, in which CPF has been administered using different exposure routes and administration vehicles (Breslin et al., 1996; Howard et al., 2007; Karanth et al., 2006; Marty et al., 2007; Moser and Padilla, 1998; Slotkin et al., 2006; Timchalk et al., 2007a; Whitney et al., 1995; Karanth and Pope, 2003; Won et al., 2001). The objective of this study was to quantitatively evaluate CPF pharmacokinetics of these different dosing paradigms using co-exposure to CPF and L-CPF, permitting concurrent analysis of pharmacokinetic differences in a single animal and, thus, decreased variability.

As demonstrated in this investigation, CPF and L-CPF are rapidly metabolized since TCPy and L-TCPy were detected in blood within 5 min of dosing for all scenarios and a substantial fraction (15–85%) of the administered dose was detected in the urine by 12 h post-dosing in urine samples from all treatment groups. The isotopic label on CPF did not alter the pharmacokinetics, and rapid metabolism was expected (Timchalk et al., 2007a).

Peak TCPy concentrations in blood, TCPy blood AUC, and percent of dose excreted in urine were all highest in rats dosed orally with CPF in saline/Tween 20 and second highest in rats dosed orally with CPF in corn oil. This suggests a first pass effect where the majority of CPF metabolism occurs in the liver (both activation and detoxification pathways) (Ma and Chambers, 1994). Hence, these results are very consistent with the hypothesis that orally administered CPF is rapidly absorbed and extensively metabolized in the gut (Poet et al., 2003) and liver prior to distribution throughout the body. In this regard, it is expected that CPF would undergo greater metabolism following an oral or dietary exposure than when directly administered systemically (i.e. IV, SC), where 25% of the cardiac output flows to the liver in the rat (Gearhart et al., 1990). Differences in peak TCPy concentrations in blood, TCPy blood AUC, and percent dose excreted in urine following the oral versus IV and SC dosing are entirely consistent with the important role of first pass CPF metabolism in the gut and liver.

Orally administered CPF in saline/Tween 20 yielded not only the highest peak TCPy concentrations, but those peak concentrations appeared the quickest as well. Time of maximum TCPy concentration (0.5 h) was also much earlier than what has previously been observed with orally exposed CPF. Timchalk et al. (2007a) used a corn oil vehicle with a larger dose of CPF (50 mg/kg) than this study and reported a time of maximum TCPy concentration at ~3 h. Marty et al. (2007) observed a time of maximum concentration at 4 h, using both corn oil and milk as vehicles (equivalent times of max concentration) in young rats (5 postnatal days of age). In this study, CPF dosed in corn oil was much slower to produce peak TCPy than CPF dosed in saline/Tween 20, with peak TCPy concentrations occurring at 6 h, indicating that corn oil functioned to delay the absorption/metabolism of CPF. This is consistent with previous studies that reported a more rapid pharmacokinetic absorption using aqueous vs. corn-oil vehicles with halogenated hydrocarbons (Staats et al., 1991; Withey et al., 1983).

When exposed to CPF by SC injection, time to peak TCPy concentrations was much quicker with corn oil vehicle than that of DMSO (2 h vs. 12 h). Marty et al. (2007) also observed a slow time to peak TCPy concentrations in blood (6 h) after dosing SC with CPF in DMSO; and using radiotracers, observed a depot of ^{14}C-activity (presumably CPF) at the injection site. Marty et al. (2007) hypothesized that DMSO leaves the CPF at this injection site, as DMSO is rapidly distributed throughout the body. They further suggest that this creates a slower release of CPF from the depot, and thus, slower metabolism to TCPy. The AUC of TCPy concentrations in blood from CPF administered SC in DMSO was 52 and 61% of those same AUCs from CPF administered orally in saline/Tween 20 and corn oil, respectively. This indicates that not all of the CPF had been absorbed and/or metabolized from the SC dose in DMSO. Urinary data further supports this as after 48 h, 44% of the original molar dose of L-CPF administered as a SC dose in DMSO had been eliminated in the urine as conjugated or free L-TCPy, while both oral doses had the entire molar equivalent dose eliminated by 48 h. Likewise, SC injection of CPF in corn oil had a similarly low AUC of TCPy concentration in the blood (45% of that from orally administered CPF in saline/Tween 20), and 39% of the urinary dose had been eliminated by 48 h. This suggests that like CPF administered in DMSO, CPF administered in corn oil was not all absorbed and/or metabolized by 48 h, suggesting that a similar depot may exist at the injection site following SC administration in corn oil.

The majority of CPF and L-CPF were excreted in urine as conjugated TCPy and L-TCPy and ranged from 70 to 88% for all *in vivo* administrations of CPF or L-CPF. This is consistent with others who have seen 88% of urinary TCPy as conjugated following an oral dose

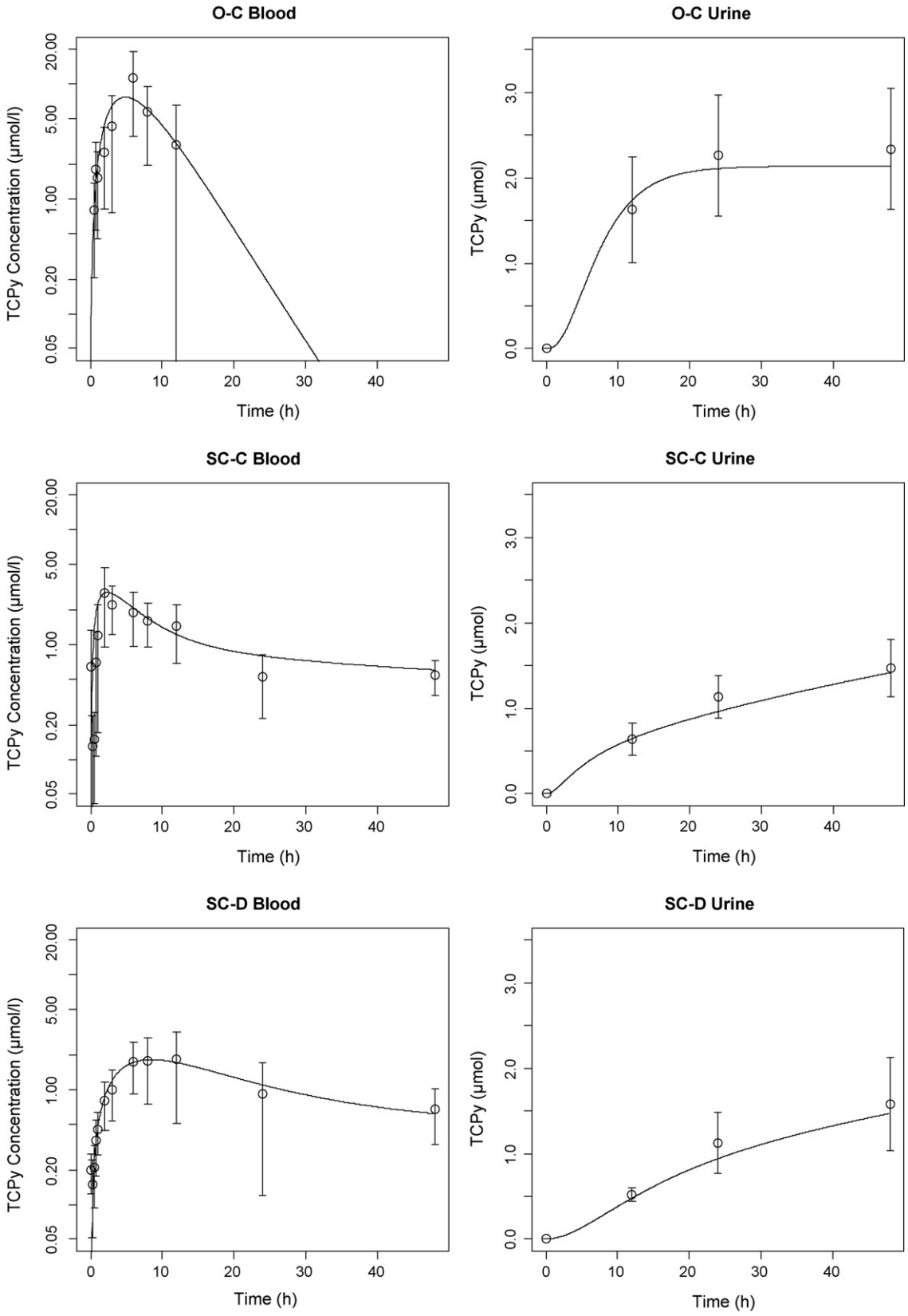

Fig. 6. Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model fits of mean (with SD bars) 3,5,6-trichloro-2-pyridinol (TCPy) concentrations (μmol/l) in blood and cumulative amount of TCPy (μmol) excreted in urine from rats exposed to chlorpyrifos dosed orally in corn oil (O-C), subcutaneously in corn oil (SC-C), and subcutaneously in dimethyl sulfoxide (DMSO) (SC-D).

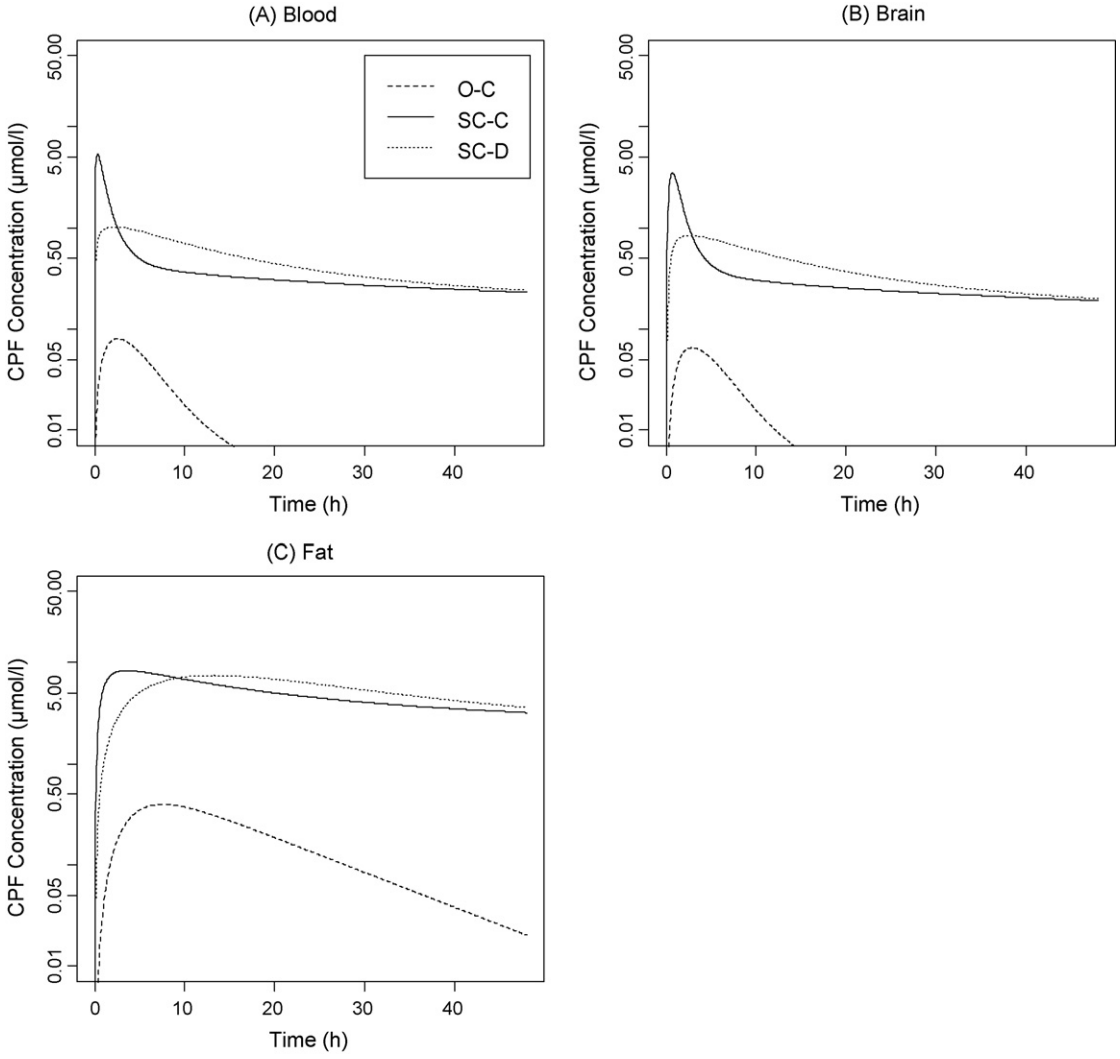

Fig. 7. Chlorpyrifos (CPF) concentrations (μmol/l) from physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model simulations of 5 mg/kg of CPF dosed in 250 g rats orally in corn oil (O-C), subcutaneously in corn oil (SC-C), or subcutaneously in dimethyl sulfoxide (DMSO) (SC-D) in blood, brain, and fat compartments.

of CPF to rats (Bakke et al., 1976). Specific conjugates have been identified as mostly glucuronide, sulfate, or glycoside conjugates of TCPy (Bakke et al., 1976; Nolan et al., 1984). In this study, there was no attempt to characterize the specific conjugates.

Regardless of vehicle, orally administered CPF underwent much more extensive metabolism than CPF administered by other routes investigated, probably due to increased gut and first pass metabolism of the oral dose as well as the slow release from the potential depot of SC doses at the injection site. This suggests that the body burden of CPF following exposure via IV or SC routes could potentially lead to higher systemic CPF concentrations than an equivalent dose via an oral/dietary exposure. To further assess this hypothesis, a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for CPF (Timchalk et al., 2002) was used to simulate CPF concentrations in blood, brain, and fat. As previously noted, the PBPK/PD model was updated and modified to accommodate brain metabolism of CPF to CPF-oxon and SC dose administration.

PBPK/PD model simulations of equivalent CPF doses (5 mg/kg) following SC injection (corn oil and DMSO) were compared with results following oral administration in corn oil (Fig. 7). Relative to oral corn oil simulations, and SC in corn oil or DMSO resulted in elevated peak CPF concentrations in blood (67- and 13-fold, respectively), brain (50- and 12-fold, respectively), and fat (21- and 19-fold, respectively). These altered levels of parent compound are substantial and could potentially lead to drastically different biological responses than would be expected following oral or dietary exposures.

The PBPK/PD model was also used to simulate the extent and duration of ChE inhibition in blood and brain and the corresponding levels of CPF-oxon at equivalent CPF doses (5 mg/kg) following SC injection (corn oil and DMSO) relative to oral administration in corn oil (Fig. 8). Model simulations of equivalent CPF doses administered orally in corn oil and SC injection in both corn oil and DMSO show equivalent and limited maximum inhibition of ChE activity in brain (94–95% ChE activity); however, SC administration in corn oil and DMSO greatly prolonged inhibition of ChE activity in the brain relative to oral exposure (time of 50% recovery: 7- and 6-fold, respectively). Whereas in blood, SC administration in corn oil and DMSO decreased the maximum inhibition of ChE activity in blood plasma (1.6- and 1.5-fold, respectively) relative to oral administration, and the recovery following the SC administration was likewise prolonged. Based upon our well established understanding of OP pesticide pharmacokinetics and pharmacodynamics, the greater degree of inhibition of ChE activity in plasma from an oral dose is due to increased metabolism of orally administered CPF not only to TCPy, but to CPF-oxon, where increased levels of CPF-oxon would lead to greater degrees of ChE inhibition (Sultatos, 2007). In this

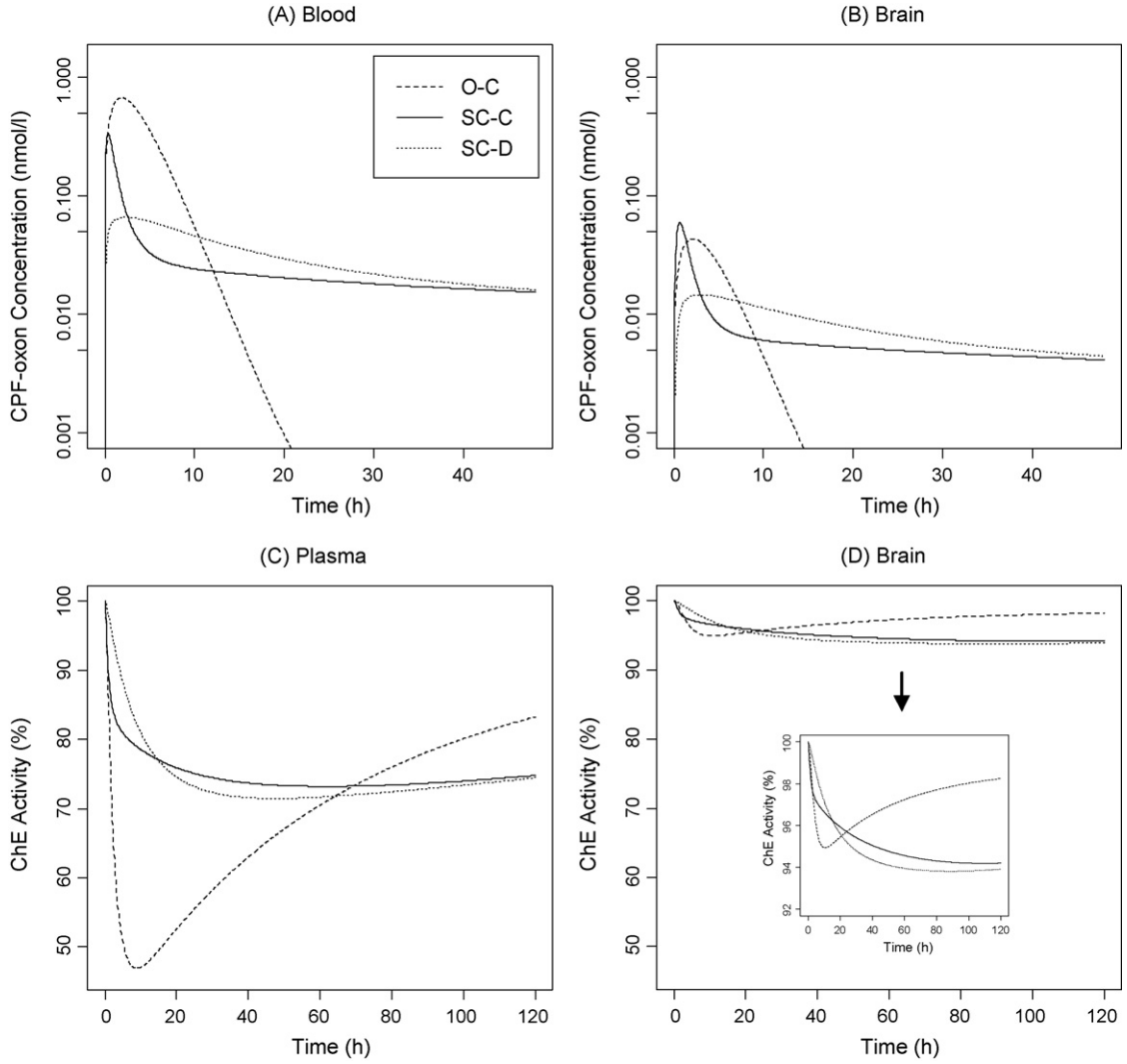

Fig. 8. Chlorpyrifos-oxon (CPF-oxon) concentrations (nmol/l) from physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model simulations of 5 mg/kg of CPF dosed in 250 g rats orally in corn oil (O-C), subcutaneously in corn oil (SC-C), or subcutaneously in dimethyl sulfoxide (DMSO) (SC-D) in blood (A) and brain (B) compartments. Cholinesterase (ChE) activity (percent of control) from the same PBPK/PD model simulations are included in plasma (C) and brain (D) compartments.

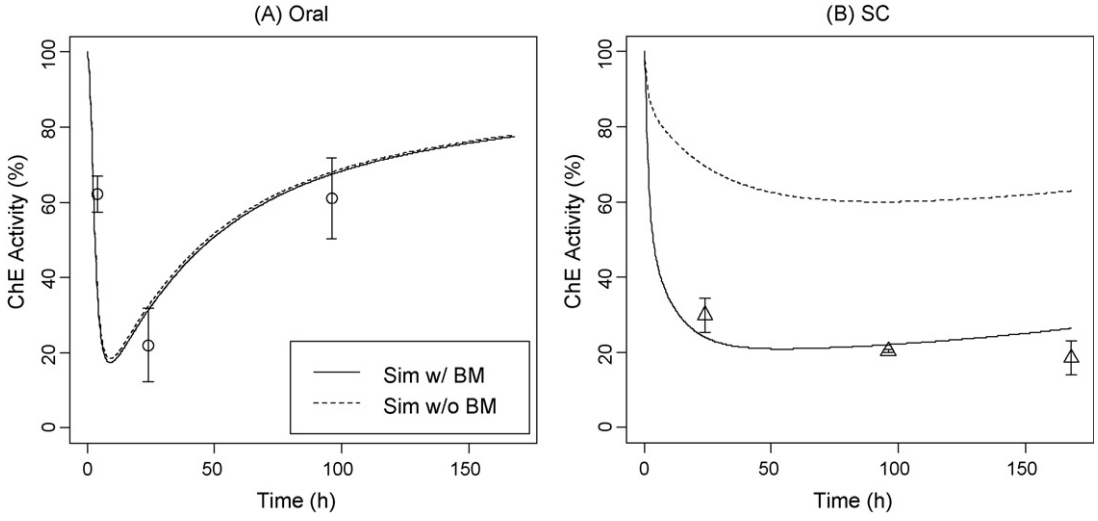

Fig. 9. Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model simulations brain cholinesterase activity (percent of control) with and with out brain metabolism (BM) of 68 mg/kg dosed orally in corn oil (A) (Won et al., 2001) and 84 mg/kg dosed subcutaneously (SC) in peanut oil (B) (Karanth et al., 2006). Data from Won et al. (2001) (circles) and Karanth et al. (2006) (triangles) are means with SEM bars.

regard, model simulations of CPF-oxon pharmacokinetics following oral or SC administration are consistent with the extent and duration of ChE inhibition in the blood (Fig. 8). Likewise, the prolonged degree of inhibited ChE activity in brain from a SC dose is due to a greater sequestering of CPF in brain tissue, slow release of CPF from the injection site depot, and, most importantly, localized brain CPF metabolism to CPF-oxon (Chambers and Chambers, 1989). These significant route dependent differences bring into question the relevance of using exposure routes (i.e. SC) that result in unique pharmacokinetics that are not of relevance to human exposures.

Using repeated dosing via oral gavage in corn oil and SC injection in DMSO, administered to developing rats, Carr and Nail (2008) observed increased ChE inhibition in brain (36.5–45.6 and 27.3–35.1% ChE activity, respectively) and plasma (35.3–40 and 19.4% ChE activity, respectively) due to the SC administration when using 1 ml/kg dose volume of DMSO. While the experimental observation of increased brain ChE inhibition due to SC dose is consistent with the single dose PBPK/PD model simulations conducted for adult rats, the experimental observation of greater plasma ChE inhibition from a CPF SC dose in DMSO than an oral dose in corn oil is inconsistent. Differences between simulations and experimental data (Carr and Nail, 2008) could exist due to many physiological differences between developing and adult rats such as dynamically changing body and organ weights, enzyme turnover rates, relative organ volumes, and amount of lipid tissue. Future PBPK/PD modeling studies will focus on exploiting the PBPK/PD preweanling model (Timchalk et al., 2007b) to help discern what age-dependent parameters may contribute to differences in ChE response.

To further illustrate differences in pharmacodynamic response by dosing paradigm, PBPK/PD simulations were completed for two previously published studies. Won et al. (2001) dosed CPF to rats at 68 mg/kg orally in corn oil and measured ChE activity in brain (Fig. 9A). Karanth et al. (2006) dosed CPF to rats at 84 mg/kg SC in peanut oil and measured brain ChE (Fig. 9B). Overall, the PBPK/PD model reasonably simulates these experimental results, which are consistent with our previous model simulations (Figs. 7 and 8), where SC administration results in a more prolonged inhibition of brain ChE relative to oral exposure. In these simulations, we have also further evaluated the importance of localized brain metabolism. Although we previously acknowledged the potential for localized brain metabolism (Chambers and Chambers, 1989) in our initial PBPK/PD model development, brain CYP450 metabolic activation had little if any impact on target tissue dosimetry (Timchalk et al., 2002). This is further illustrated (Fig. 9A) where the extent of brain ChE inhibition was not impacted by brain metabolism following oral exposure. However, the potential importance of localized metabolism following SC administration is substantial (Fig. 9B). In the absence of brain metabolic activation (dashed line), the PBPK/PD model substantially under-predicts the extent of brain ChE inhibition. However, by including a low level of CYP450 metabolic activation of CPF to CPF-oxon, the extent and duration of brain ChE inhibition is more reasonably simulated. These simulations suggests that low level (V_{max} = 0.00035 μmol/h/kg) localized brain CPF metabolism can dramatic increase brain ChE inhibition from SC administrations (79% vs. 40% maximum ChE inhibition with and with out brain metabolism, respectively) due to the high levels of parent compound being sequestered in the brain. Based on these results, additional metabolism studies may be warranted to more fully characterize the CYP450 metabolic parameters for CPF activation and detoxification in the brain. Additionally, there is evidence of PON-1 activity in brain, which is not accounted for in this model (Rodrigo et al., 2001). This could reduce the amount of CPF-oxon predicted by the model and efforts are ongoing to discern. Nonetheless, these finding do suggest that localized metabolism, even at very low levels, may be important for evaluating target tissue dosimetry, where the metabolic capacity of the liver has been bypassed.

Overall, these studies confirm that different routes of exposure and vehicles of administration can have significant impacts on CPF pharmacokinetics and pharmacodynamics. Thus, care must be taken when comparing studies that use different dosing scenarios, and internal dosimetry should be taken into consideration when designing and interpreting experiments. In this regard, computational models, such as a PBPK/PD model, can be utilized as a tool to address uncertainty in comparing results from different dosing scenarios.

In conclusion, oral CPF administration in saline/Tween 20 resulted in the highest peak concentration of TCPy, which also occurred most rapidly. SC administration with a corn oil vehicle led to faster absorption/metabolism of CPF than did a SC administration in a DMSO vehicle. The majority of CPF from all dosing regiments was excreted in urine as a conjugated form of TCPy. These results indicate that orally administered CPF is more extensively metabolized than systemic exposures of CPF (SC and IV), and vehicle of administration also has an effect on absorption rates. Thus, equivalent doses via different routes and/or vehicles of administration could potentially lead to different body burdens of CPF, different rates of bioactivation to CPF-oxon, and different toxic responses. These results will be useful for elucidating differences regarding potential uncertainty in pharmacokinetics and potential adverse effects associated with different routes and vehicles of CPF administration.

Conflict of interest

Drs. Charles Timchalk and Torka Poet have received funding from The Dow Chemical Company, the manufacturer of chlorpyrifos, to conduct research. The Dow Chemical Company had no input on the current research.

Acknowledgements

This publication was supported by funding from Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health (CDC/NIOSH) grants R01 OH008173, R01 OH003629, and AGR05FED40077.02. Findings in this study were those of the authors, and do not necessarily reflect the official opinion of the CDC/NIOSH.

References

Abdel-Rahman, A.A., Blumenthal, G.M., Abou-Donia, S.A., Ali, F.A., Abdel-Monem, A.E., Abou-Donia, M.B., 2002. Pharmacokinetic profile and placental transfer of a single intravenous injection of [(14)C]chlorpyrifos in pregnant rats. Arch. Toxicol. 76, 452–459.

Bakke, J.E., Feil, V.J., Price, C.E., 1976. Rat urinary metabolites from O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate. J. Environ. Sci. Health B 11, 225–230.

Breslin, W.J., Liberacki, A.B., Dittenber, D.A., Quast, J.F., 1996. Evaluation of the developmental and reproductive toxicity of chlorpyrifos in the rat. Fundam. Appl. Toxicol. 29, 119–130.

Brzak, K.A., Harms, D.W., Bartels, M.J., Nolan, R.J., 1998. Determination of chlorpyrifos, chlorpyrifos oxon, and 3,5,6-trichloro-2-pyridinol in rat and human blood. J. Anal. Toxicol. 22, 203–210.

Carr, R.L., Nail, C.A., 2008. Effect of different administration paradigms on cholinesterase inhibition following repeated chlorpyrifos exposure in late preweanling rats. Toxicol. Sci. 106, 186–192.

Chambers, J.E., Chambers, H.W., 1989. Oxidative desulfuration of chlorpyrifos, chlorpyrifos-methyl, and leptophos by rat brain and liver. J. Biochem. Toxicol. 4, 201–203.

Chanda, S.M., Mortensen, S.R., Moser, V.C., Padilla, S., 1997. Tissue-specific effects of chlorpyrifos on carboxylesterase and cholinesterase activity in adult rats: an in vitro and in vivo comparison. Fundam. Appl. Toxicol. 38, 148–157.

Dai, D., Tang, J., Rose, R., Hodgson, E., Bienstock, R.J., Mohrenweiser, H.W., Goldstein, J.A., 2001. Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. J. Pharmacol. Exp. Ther. 299, 825–831.

Gearhart, J.M., Jepson, G.W., Clewell III, H.J., Andersen, M.E., Conolly, R.B., 1990. Physiologically based pharmacokinetic and pharmacodynamic model for the inhibition of acetylcholinesterase by diisopropylfluorophosphate. Toxicol. Appl. Pharmacol. 106, 295–310.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics. M. Dekker, New York.

Helsel, D.R., 2005. More than obvious: better methods for interpreting nondetect data. Environ. Sci. Technol. 39, 419A–423A.

Howard, M.D., Mirajkar, N., Karanth, S., Pope, C.N., 2007. Comparative effects of oral chlorpyrifos exposure on cholinesterase activity and muscarinic receptor binding in neonatal and adult rat heart. Toxicology 238, 157–165.

Kamataki, T., Lee Lin, M.C., Belcher, D.H., Neal, R.A., 1976. Studies of the metabolism of parathion with an apparently homogeneous preparation of rabbit liver cytochrome P-450. Drug. Metab. Dispos. 4, 180–189.

Karanth, S., Pope, C., 2003. Age-related effects of chlorpyrifos and parathion on acetylcholine synthesis in rat striatum. Neurotoxicol. Teratol. 25, 599–606.

Karanth, S., Liu, J., Mirajkar, N., Pope, C., 2006. Effects of acute chlorpyrifos exposure on in vivo acetylcholine accumulation in rat striatum. Toxicol. Appl. Pharmacol. 216, 150–156.

Lowe, E.R., Poet, T.S., Rick, D.L., Marty, M.S., Mattson, J.L., Timchalk, C., Bartels, M.J., 2009. The effect of plasma lipids on the pharmacokinetics of chlorpyrifos and the impact on interpretation of blood biomonitoring data. Toxicol. Sci. 108, 258–272.

Ma, T., Chambers, J.E., 1994. Kinetic parameters of desulfuration and dearylation of parathion and chlorpyrifos by rat liver microsomes. Food Chem. Toxicol. 32, 763–767.

Marty, M.S., Domoradzki, J.Y., Hansen, S.C., Timchalk, C., Bartels, M.J., Mattsson, J.L., 2007. The effect of route, vehicle, and divided doses on the pharmacokinetics of chlorpyrifos and its metabolite trichloropyridinol in neonatal sprague-dawley rats. Toxicol. Sci. 100, 360–373.

Moser, V.C., Padilla, S., 1998. Age-and gender-related differences in the time course of behavioral and biochemical effects produced by oral chlorpyrifos in rats. Toxicol. Appl. Pharmacol. 149, 107–119.

Nolan, R.J., Rick, D.L., Freshour, N.L., Saunders, J.H., 1984. Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol. Appl. Pharmacol. 73, 8–15.

Poet, T.S., Wu, H., Kousba, A.A., Timchalk, C., 2003. In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon. Toxicol. Sci. 72, 193–200.

Pond, A.L., Chambers, H.W., Coyne, C.P., Chambers, J.E., 1998. Purification of two rat hepatic proteins with A-esterase activity toward chlorpyrifos-oxon and paraoxon. J. Pharmacol. Exp. Ther. 286, 1404–1411.

R Foundation for Statistical Computing, 2004. R: A Language and Environment for Statistical Computing. Vienna, Austria.

Rodrigo, L., Hernández, A.F., López-Caballero, J.J., Gil, F., Pla, A., 2001. Immunohistochemical evidence for the expression and induction of paraoxonase in rat liver, kidney, lung and brain tissue. Implications for its physiological role. Chem. Biol. Interact. 137, 123–137.

Rose, R.L., Tang, J., Choi, J., Cao, Y., Usmani, A., Cherrington, N., Hodgson, E., 2005. Pesticide metabolism in humans, including polymorphisms. Scand. J. Work Environ. Health 31 (Suppl. 1), 156–163 (discussion 119–122).

Slotkin, T.A., Levin, E.D., Seidler, F.J., 2006. Comparative developmental neurotoxicity of organophosphate insecticides: effects on brain development are separable from systemic toxicity. Environ. Health Perspect. 114, 746–751.

Staats, D.A., Fisher, J.W., Connolly, R.B., 1991. Gastrointestinal absorption of xenobiotics in physiologically based pharmacokinetic models. A two-compartment description. Drug Metab. Dispos. 19, 144–148.

Sultatos, L.G., 1994. Mammalian toxicology of organophosphorus pesticides. J. Toxicol. Environ. Health 43, 271–289.

Sultatos, L.G., 2007. Concentration-dependent binding of chlorpyrifos oxon to acetylcholinesterase. Toxicol. Sci. 100, 128–135.

Timchalk, C., Poet, T.S., 2008. Development of a physiologically based pharmacokinetic and pharmacodynamic model to determine dosimetry and cholinesterase inhibition for a binary mixture of chlorpyrifos and diazinon in the rat. Neurotoxicology 29, 428–443.

Timchalk, C., Nolan, R.J., Mendrala, A.L., Dittenber, D.A., Brzak, K.A., Mattsson, J.L., 2002. A Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol. Sci. 66, 34–53.

Timchalk, C., Poet, T.S., Hinman, M.N., Busby, A.L., Kousba, A.A., 2005. Pharmacokinetic and pharmacodynamic interaction for a binary mixture of chlorpyrifos and diazinon in the rat. Toxicol. Appl. Pharmacol. 205, 31–42.

Timchalk, C., Busby, A., Campbell, J.A., Needham, L.L., Barr, D.B., 2007a. Comparative pharmacokinetics of the organophosphorus insecticide chlorpyrifos and its major metabolites diethylphosphate, diethylthiophosphate and 3,5,6-trichloro-2-pyridinol in the rat. Toxicology 237, 145–157.

Timchalk, C., Kousba, A.A., Poet, T.S., 2007b. An age-dependent physiologically based pharmacokinetic/pharmacodynamic model for the organophosphorus insecticide chlorpyrifos in the preweanling rat. Toxicol. Sci. 98, 348–365.

Whitney, K.D., Seidler, F.J., Slotkin, T.A., 1995. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. Toxicol. Appl. Pharmacol. 134, 53–62.

Withey, J.R., Collins, B.T., Collins, P.G., 1983. Effect of vehicle on the pharmacokinetics and uptake of four halogenated hydrocarbons from the gastrointestinal tract of the rat. J. Appl. Toxicol. 3, 249–253.

Won, Y.K., Liu, J., Olivier Jr., K., Zheng, Q., Pope, C.N., 2001. Age-related effects of chlorpyrifos on acetylcholine release in rat brain. Neurotoxicology 22, 39–48.