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Comparative pharmacokinetics of the organophosphorus insecticide chlorpyrifos and its major metabolites diethylphosphate, diethylthiophosphate and 3,5,6-trichloro-2-pyridinol in the rat

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Abstract

Chlorpyrifos (CPF) is a commonly used diethylphosphorothionate organophosphorus (OP) insecticide. Diethylphosphate (DEP), diethylthiophosphate (DETP) and 3,5,6-trichloro-2-pyridinol (TCPy) are products of metabolism and of environmental degradation of CPF and are routinely measured in urine as biomarkers of exposure. However, because these same chemicals can result from metabolism or by biodegradation, monitoring total urinary metabolite levels may be reflective of not only an individual's contact with the parent pesticide, but also exposure with the metabolites, which are present in the environment. The objective of the current study was to compare the pharmacokinetics of orally administered DEP, DETP and TCPy with their kinetics following oral dosing with the parent insecticide CPF in the rat. Groups of rats were orally administered CPF, DEP, TCPy or DETP at doses of 140 μmol/kg body weight, and the time-courses of the metabolites were evaluated in blood and urine. Following oral administration, all three metabolites were well absorbed with peak blood concentrations being attained between 1 and 3 h post-dosing. In the case of DEP and TCPy virtually all the administered dose was recovered in the urine by 72 h post-dosing, suggesting negligible, if any, metabolism; whereas with DETP, ∼50% of the orally administered dose was recovered in the urine. The CPF oral dose was likewise rapidly absorbed and metabolized to DEP, TCPy and DETP, with the distribution of metabolites in the urine followed the order: TCPy (22 ± 3 μmol) > DETP (14 ± 2 μmol) > DEP (1.4 ± 0.7 μmol). Based upon the total amount of TCPy detected in the urine a minimum of 63% of the oral CPF dose was absorbed. These studies support the hypotheses that DEP, DETP and TCPy present in the environment can be readily absorbed and eliminated in the urine of rats and potentially humans.

Keywords: Pharmacokinetics; Chlorpyrifos; Diethylthiophosphate; Diethylphosphate; Trichloropyridinol

1. Introduction

Organophosphorus (OP) insecticides constitute a large family of pesticides that are structurally related pentavalent phosphorus acid esters. Diethylphosphorothionates are one of the major sub-classes of OP insecticides, which include a number of commonly used

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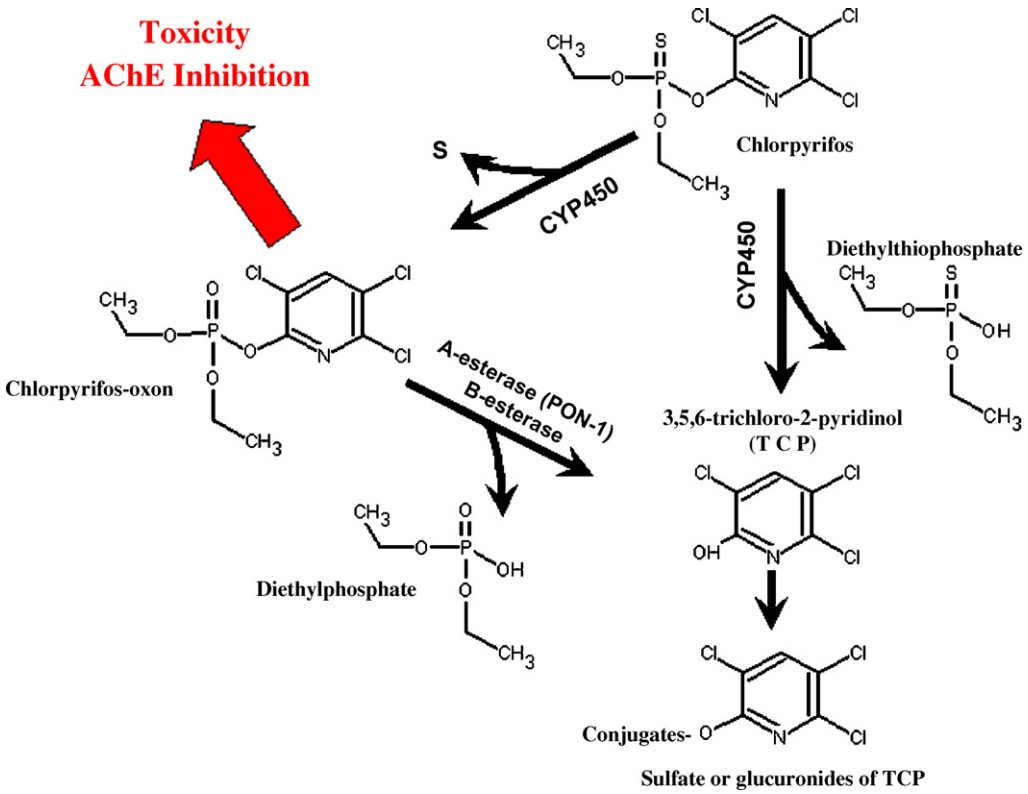

Fig. 1. Metabolic scheme for CYP450 metabolism of chlorpyrifos (CPF) to chlorpyrifos-oxon, 3,5,6-trichloro-2-pyridinol (TCPy) and diethylthiophosphate (DETP). Chlorpyrifos-oxon undergoes further metabolism by both A- and B-esterase to form diethylphosphate (DEP) and TCPy, which can undergo phase II sulfation and glucuronidation.

pesticides, such as chlorpyrifos (CPF). A scheme (Fig. 1) for the metabolism of the insecticide CPF shows that CPF undergoes CYP450-mediated oxidative desulfation or dearylation to form chlorpyrifos-oxon (CPF-oxon; the neurotoxic moiety) or 3,5,6-trichloro-2-pyridinol (TCPy) and diethylthiophosphate (DETP), respectively (Chambers and Chambers, 1989; Ma and Chambers, 1994). Hepatic and extrahepatic A-esterases, such as PON-1, and tissue B-esterases (cholinesterase; ChE) effectively metabolize CPF-oxon forming TCPy and diethylphosphate (DEP). Differences in the ratio of activation to detoxification are associated with chemical-, species-, sex-, and age-dependent sensitivity to OP insecticides (Ma and Chambers, 1994). Dialkylphosphates (DAP), such as DEP and DETP, which are two metabolic products of diethylphosphorothionate insecticides, have long been used as general urinary biomarkers for this class of OP insecticides (Bradway and Shafik, 1977; Hardt and Angerer, 2000; CDC, 2005). For assessing human exposure to many of the OP insecticides and for linking OP exposures in pregnant women to subsequent adverse birth outcomes, the metabolite containing the organic moiety, such as TCPy in the case of CPF, has been used as a more specific urinary biomarker (Nolan et al., 1984; Berkowitz et al., 2004; Eskenazi et al., 2004; CDC, 2005).

Although DAP and TCPy have been routinely utilized as biomarkers for OP insecticide exposure, OP pesticides can undergo environmental degradation to form these same chemicals. In this regard, Lu et al. (2005) reported the detection of the OP breakdown product DAP in fruit juices, and Morgan et al. (2005) noted higher concentrations (12–29×) of the CPF metabolite TCPy, relative to CPF in solid food samples obtained from homes and day care centers; higher dietary exposures to TCPy may be a confounding factor when attempting to assess dietary exposure to CPF. Hence, due to the environmental stability of the DAP and TCPy, recent research has questioned whether total urinary OP metabolite levels may be reflective of not only an individual's contact with the parent OP pesticide, but also in addition exposure with intact metabolites present in the environment (Barr et al., 2005; Lu et al., 2005; Bradman et al., 2005; Duggan et al., 2003). Thus, measured urinary OP metabolite levels may

represent an exaggerated indicator of an individual's exposure to the parent OP compound (Duggan et al., 2003).

The urinary elimination profiles of the individual metabolites DEP, DETP and TCPy following exposure to CPF have been investigated in both human volunteer studies and in human poison incidences (Nolan et al., 1984; Griffin et al., 1999; Timchalk et al., 2002; Vasilić et al., 1992; Drevenkar et al., 1993; Barr et al., 2004). Bicker et al. (2005) recently evaluated the metabolism and pharmacokinetics of DETP, DEP and TCPy simultaneously in urine samples obtained following an accidental ingestion of a commercial insecticide containing CPF. In addition to the 3 major CPF metabolites (i.e. DETP, DEP and TCPy), 12 additional metabolites of CPF and CPF-oxon were identified. Acid-labile conjugates of TCP appear to be the major urinary conjugate accounting for 10–15% of the total urinary TCP. The more minor metabolites included mono-alkylated CPF and CPF-oxon, as well as a number of mono-dechlorinated CPF and CPF-oxon cysteine and glutathione conjugates. We are unaware of any comparable pharmacokinetic studies in animals, particularly where equal molar doses of CPF and individual metabolites are compared.

There were two key objectives in the current study: (1) to evaluate the pharmacokinetics (absorption, metabolism and elimination) of DEP, TCPy and DETP following *in vivo* oral exposure in the rat and (2) to evaluate and compare the *in vivo* pharmacokinetics of DEP, TCPy and DETP in rats following oral exposure to an equal molar dose of the parent insecticide CPF. We anticipate that the results of these studies will provide a needed quantitative perspective on the pharmacokinetics of ingested pesticide residues relative to the metabolites that are formed from *in vivo* metabolism of the parent OP insecticide.

2. Material 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 AgroSciences (Indianapolis, IN). The DEP (CAS 598-02-7, 98% pure) was purchased from Chem Service Inc. (West Chester, PA). The derivatizing agent (*N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide, (CAS 77377-52-7, MTBSTFA)) was purchased from Sigma–Aldrich (Milwaukee, MI). All other solvents and reagents, including DETP (CAS 5871-17-0, 98% purity) as a potassium salt, were of reagent grade or better and were purchased from Sigma Chemical Company (St. Louis, MO).

2.2. Animals

All procedures using animals were in accordance with protocols established in the NIH/NRC Guide and Use of Laboratory Animals and were reviewed by the Institutional Animal and Care Use Committee (IACUC) of Battelle, Pacific Northwest Division. Male Sprague–Dawley rats were purchased from Charles River Laboratories (Raleigh, NC), were 10 weeks old at the time of use, and were acclimated for ~1 week to laboratory conditions. Prior to their use, the animals were housed in solid-bottom cages with hardwood chips under standard laboratory conditions and given free access to water and food (Purina Rodent Chow). At the time of dosing the mean body weight across all treatment groups ranged from 254 to 309 g (data not shown).

2.3. Dose selection and preparation

Groups of rats (three animals/time-point) were administered a single equal molar dose (140 μmol/kg) of CPF (group I; $n = 21$), DEP (group II; $n = 21$), TCPy (group III; $n = 24$) or DETP (group IV; $n = 24$) via oral gavage (dose volume = 5 mL/kg body weight). For consistency all dose solutions were dissolved in a corn oil matrix, due to the limited aqueous solubility of CPF. The CPF dose was equivalent to ~50 mg CPF/kg of body weight, which did produce some signs of acute toxicity in the rat (CPF LD_{50} ~92 mg/kg). The selection of this high CPF dose was done to optimize the ability to quantify all metabolites in both the blood and urine.

2.4. Pharmacokinetic study design

Rats from all treatment groups were fasted 12 h before dosing and granted access to food 3 h post-dosing. Groups of rats from each treatment group were randomly assigned to the following sample collection times: 1 h, 3 h, 6 h, 12 h, 18 h (groups III and IV only), 24 h, 48 h, and 72 h post-dosing. The animals assigned to the 72 h time-point were housed in metabolism cages immediately after dosing, for collection of urine samples. The urine was collected at 12, 24, 48, and 72 h post-dosing. At the specified time-points, groups of rats were anesthetized with isoflurane, and blood exsanguinations were achieved via cardiac puncture into heparanized syringes; after blood collection the rats were humanely sacrificed, via CO_2 asphyxiation. The blood and urine specimens were stored at −80 °C until analysis.

2.5. Sample processing

For quantitation, matrix (urine and blood from naïve rats) standard curves were prepared alongside each set of samples by spiking the matrix with DEP, DETP, and TCPy. To remove inorganic phosphate from urine, 0.1–0.15 g of $Ca(OH)_2$ was added to samples, and removed via centrifugation before extraction (Blair and Roderick, 1976). To prevent oxidation of DETP to DEP during the derivatization process, 10% (w/v) of sodium metabisulfite ($Na_2S_2O_5$) was added to the urine and blood samples from the DETP treatment (group IV)

as described by Ueyama et al. (2006). All standard curves and samples were initially denatured by adding 0.5 mL of a 3 M HCl solution that was saturated with NaCl. They then underwent three extractions, with each extraction consisting of 0.5–1 mL of extracting solvent (ethyl acetate), followed by 1 min of strong mixing by vortex, and 20 min of centrifugation at $1100 \times g$ at room temperature. The three extracts were combined and dried over anhydrous $MgSO_4$ before being blown down under a gentle stream of inert N_2. Samples and standards were either stored at −80 °C until analysis, or immediately reconstituted and derivatized for analysis by gas chromatography/mass spectrometry (GC/MS). Samples were reconstituted in varying volumes of toluene to place them in the GC/MS linear range (0.1–100 μg/mL, coefficient variable range 0.97–0.99), and incubated with 10 μL of MTBSTFA derivatizing agent at 60 °C for 60 min.

2.6. Gas chromatography/mass spectrometry conditions

Gas chromatography/mass spectrometry (GC/MS) analyses, for the samples obtained for the single metabolite exposures, DEP-dosed (group II), TCPy-dosed (group III) or DETP-dosed animals (group IV), were performed using a Hewlett Packard (HP) 5973 mass selective detector (MSD) interfaced with a HP model 6890 GC using HP ChemStation software for programming and data analysis. For samples obtained from the CPF-dosed animals (group I), analyses were performed using a HP5972 MSD interfaced with an HP model 5890 GC and HP ChemStation software. For both analyses, spectra were collected in the electron ionization mode at 70 eV with a mass range scanned from 50 to 500 amu. Separation was achieved in splitless mode (initial inlet temperature 250 °C), on a DB5-MS column 30 m × 0.25 mm i.d. × 0.25 μm (J&W Scientific, Folsom, CA) or equivalent, with helium as the carrier gas at a flow rate of 1 mL/min, and the GC oven temperature at 80 °C ramped at 10 °C/min up to a final temperature of 310 °C, and total run time of 24 min. Selected ion monitoring (SIM) was utilized for analysis where increased sensitivity was required. Ions selected for monitoring included DEP: *m/z* 155; TCPy: *m/z* 256 and *m/z* 254 and DETP: *m/z* 171.

2.7. Pharmacokinetic analysis

To estimate pharmacokinetic parameters for DEP, TCPy and DETP such as half-life ($t_{1/2}$) in blood and urine, two- and one-compartment pharmacokinetic models were used depending on the needs to fit the experimental results as previously described (Timchalk et al., 1997). In these models the absorption, elimination and transfer between the central and peripheral compartment are all described with first-order rate constants. Estimates of model parameters were obtained using SIMUSOLV® a computer program containing a numerical integration, optimization and graphical routines. In addition, the two-compartment model rapid and slow disposition rates (α and β) and half-life ($t_{1/2}$) were calculated (Gibaldi and Perrier, 1982). Graphpad Prism® 4 was also used to calculate the area-under-the-curve (AUC) for blood and urine using the trapezoidal rule (Gibaldi and Perrier, 1982).

3. Results

3.1. Dose administration and effects

Groups of male Sprague–Dawley rats were administered equal molar doses of CPF (group I), DEP (group II), TCPy (group III) or DETP (group IV) and the time-course of DEP, DETP, and TCPy were evaluated in both blood and urine (see Table 1). For each metabolite the average molar doses administered to the rats were very comparable and ranged from 34 ± 2 to 38 ± 4 μmol/rat. For those animals administered the CPF dose (36 ± 3 μmol/rat; 140 μmol/kg = 50 mg/kg) observable cholinergic effects were noted in most animals. The acute toxicity was manifested by lethargy, loose stools and soiling, excessive urination, and in several cases mild tremors. In general these effects subsided within 24 h, but one animal in the 72 h treatment group died presumably as a result of the CPF exposure, and was replaced with a spare animal. None of the rats that received equal molar doses of DEP, TCPy or DETP showed any ill-effects.

3.2. Diethylthiophosphate (DEP) pharmacokinetics

The concentrations of DEP in blood and urine following CPF (group I) or DEP (group II) oral administration, respectively, are presented in Table 1, and the pharmacokinetic model fit of DEP in blood is illustrated in Fig. 2A and B, while the time-courses of the DEP in blood and urine from both groups are illustrated in Fig. 3. Following oral administration of DEP (group II) peak blood DEP concentrations of 0.31 ± 0.14 μg/mL were attained within 1 h post-dosing, and the DEP was quantifiable through 24 h post-dosing. The DEP blood pharmacokinetics were biphasic (Fig. 2B) with estimated half-lives ($t_{1/2}$) for the alpha (α, initial) and beta (β, terminal) phases of 0.2 and 52 h, respectively (see Table 2). Peak amounts of DEP in urine (3921 ± 2226 μg) were attained within 12 h post-dosing and the urinary elimination was adequately described by a one-phase exponential decay with an estimated $t_{1/2}$ of ∼13 h (Fig. 3B and Table 2). Following the DEP dose the average total recovery of DEP in the urine through 72 h post-dosing was 32 ± 1 μmol, which was ∼86% of the 37 ± 2 μmol of DEP that was orally administered to the rats assigned to the 72 h post-dosing group (Fig. 6B). This suggests that DEP was

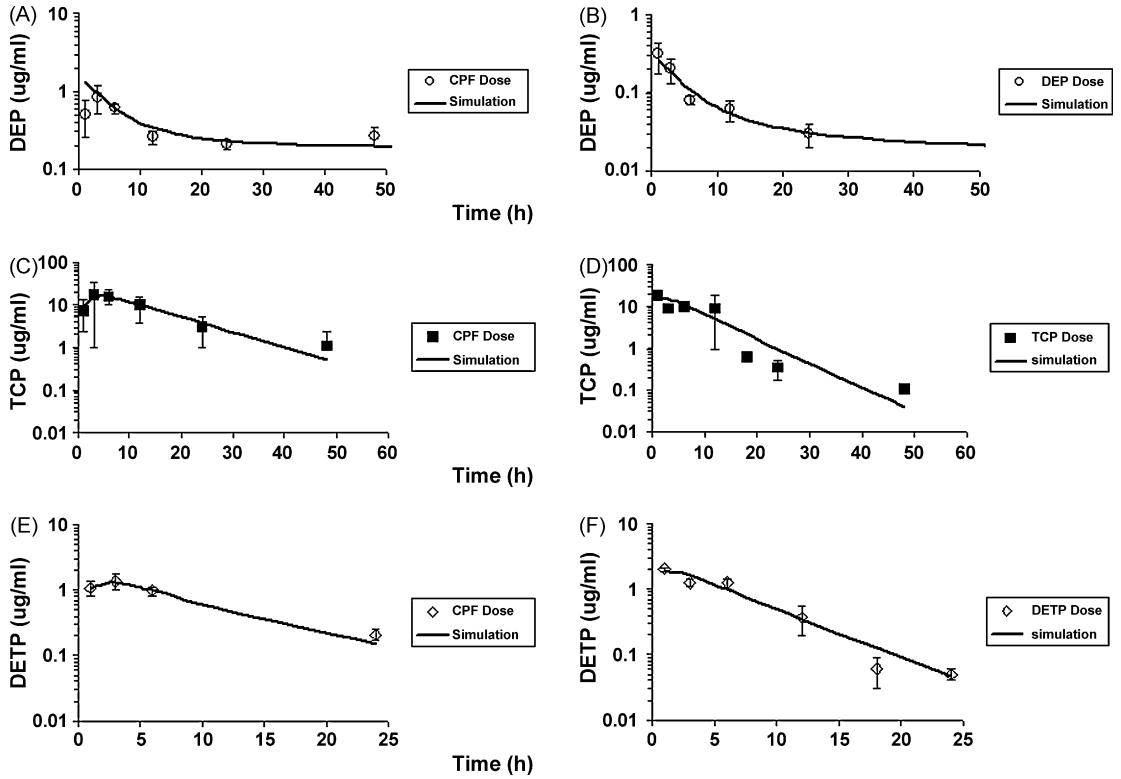

Fig. 2. Blood time-course of chlorpyrifos (CPF) metabolites [diethylphosphate (DEP), trichloropyridinol (TCPy), and diethylthiophosphate (DETP)] in groups of male Sprague–Dawley rats orally administered CPF (A, C and E) or DEP (B), TCPy (D), and DETP (F). The data represents the mean ± S.D. of three animals, and the line is the best fit of the pharmacokinetic models.

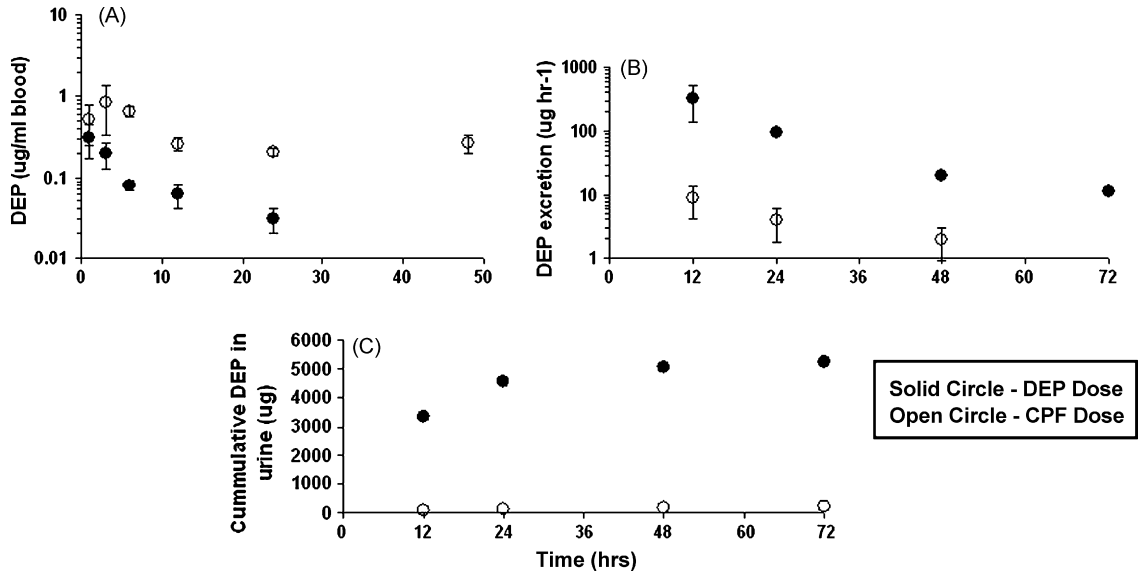

Fig. 3. Time-course of diethylphosphate (DEP) in blood (A), urine (B), and cumulative amounts of DEP in urine (C) following oral administration of 140 μmol/kg of DEP (solid circles) or chlorpyrifos (CPF) (open circles) to male Sprague–Dawley rats. Data represents mean ± S.D. of three animals per time-point.

Table 1
Concentration of chlorpyrifos (CPF; group I), diethylphosphate (DEP) trichloropyridinol (TCPy) and diethylthiophosphate (DETP) in blood (μg/mL) or the metabolite amounts in urine following oral administration of either DEP (group II), trichloropyridinol (TCPy; group III), or diethylthiophosphate (DETP; group IV) to male Sprague–Dawley rats

Time post-dosing (h)	Blood metabolites (μg/mL) (mean ± S.D.)			Urine metabolites (μg) (mean ± S.D.)		
	DEP	TCPy	DETP	DEP	TCPy	DETP
Group I[a]						
1	0.52 ± 0.26	7.5 ± 5.2	1.08 ± 0.26	–	–	–
3	0.85 ± 0.34	18 ± 17	1.37 ± 0.33	–	–	–
6	0.61 ± 0.10	16 ± 6.0	0.97 ± 0.16	–	–	–
12	0.26 ± 0.05	9.9 ± 6.2	–	107 ± 57	710 ± 325	616 ± 68
24	0.21 ± 0.03	3.1 ± 2.1	0.21 ± 0.04*	47 ± 26	772 ± 462	269 ± 102
48	0.27 ± 0.07	1.1 ± 1.3	ND	47 ± 25	1948 ± 693	775 ± 225
72	ND	ND	ND	9.0 ± 3.1	156 ± 56	123 ± 34
Groups II, III, and IV[b]						
1	0.31 ± 0.14	18 ± 2.8	1.99 ± 0.13	–	–	–
3	0.20 ± 0.07	9.4 ± 1.7	1.26 ± 0.14	–	–	–
6	0.08 ± 0.01	9.9 ± 0.8	1.23 ± 0.21	–	–	–
12	0.06 ± 0.02*	9.4 ± 8.5	0.37 ± 0.18	3921 ± 2226*	3048 ± 1230	2105 ± 1481
18	–	0.62 ± 0.13*	0.06 ± 0.03	–	–	–
24	0.03 ± 0.01	0.35 ± 0.18*	0.05 ± 0.01	1256 ± 241	2723 ± 647	1428 ± 1451
48	ND	0.11 ± 0.00*	ND	492 ± 392	1131 ± 196	210 ± 155
72	ND	0.11 ± 0.01	ND	271 ± 96*	697 ± 100	ND

Values are mean ± S.D. for three animals per time-point, * indicates $n = 2$ due to a sample loss. ND, non-detected; (–) no sample.
[a] CPF (~140 μmol/kg body weight, ~50 mg/kg body weight). Overall average dose: CPF 36.0 ± 2.5 μmol/rat ($n = 21$ animals).
[b] DEP, TCPy, and DETP (140 μmol/kg). Overall average dose: DEP, 37.0 ± 1.9 μmol/rat ($n = 21$ animals); TCPy, 38.0 ± 4.0 μmol/rat ($n = 24$ animals); DETP, 34.3 ± 2.0 μmol/rat ($n = 24$ animals).

well absorbed orally and undergoes negligible, if any, metabolism.

Following the CPF oral dose (group I), DEP was likewise rapidly detected in the blood with peak levels of 0.85 ± 0.34 μg/mL attained by 3 h post-dosing (Table 1). The DEP was detectable in the blood through 48 h post-dosing and the biphasic blood time-course had an estimated $t_{1/2}$ of 0.2 and 118 h for the α and β phase, respectively (Fig. 2A and Table 2). Peak urinary DEP (107 ± 57 μg) was achieved by 12 h post-dosing, with an estimated $t_{1/2}$ of 14 h (one-phase exponential decay) (Fig. 3B and Table 2).

In comparing the DEP blood concentrations and the amount excreted in the urine following equal molar doses of CPF (group I) or DEP (group II) it is clear that animals dosed with DEP had lower blood concentrations and substantially greater amounts of DEP excreted in the urine than in those animals dosed with CPF. The shapes of the blood time-courses were fairly similar although the DEP from the CPF dose group had a doubling of the β phase (118 h) relative to the DEP dose group (52 h) (Fig. 3A and Table 2). Of importance to note, in comparing the DEP versus the CPF dose groups, the total amount of DEP excreted in the urine (Table 1 and Fig. 3C) was substantially different (5940 μg versus 210 μg, respectively) and a comparison of the urinary AUC (2725 μmol h^{-1} and 61,183 μmol h^{-1}, respectively), indicates that the amount of DEP excreted in the urine for the CPF dose was only 4% of the amount detected following an equal molar dose of DEP. Yet the $t_{1/2}$ for the urinary excretion of DEP was very comparable (13–14 h) for both groups (Fig. 3B and Table 2).

3.3. 3,5,6-Trichloro-2-pyridinol (TCPy) pharmacokinetics

The concentrations of TCPy in blood and urine following CPF (group I) or TCPy (group III) oral administration, are presented in Table 1. The pharmacokinetic model simulation of the blood TCPy time-course is illustrated in Fig. 2C and D, while the time-course of the TCPy in blood and urine from both groups are illustrated in Fig. 4. Following oral administration of TCPy (group III) peak blood TCPy concentrations of 18 ± 2.8 μg/mL were rapidly attained within 1 h post-dosing, and the TCPy was quantifiable through 72 h post-dosing. The TCPy blood pharmacokinetics had a terminal half-life ($t_{1/2\beta}$) estimate of 5.1 h (see Fig. 2D and Table 2). Peak amounts of TCPy in urine (3048 ± 1230 μg) were attained within 12 h post-dosing.

Table 2
Calculated area-under-the-concentration (AUC) for diethylphosphate (DEP), diethylthiophosphate (DETP) and trichloropyridinol (TCPy) in male Sprague–Dawley rats orally administered chlorpyrifos (CPF; group I), DEP (group II), TCPy (group III) or DETP (group IV) at equal molar doses

Metabolites	AUC (blood: μmol mL^{-1} h^{-1} or urine: μmol h^{-1})[a]							
	Group I (CPF dose)		Group II (DEP dose)		Group III (TCPy dose)		Group IV (DETP dose)	
	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine
DEP	14.7 ($t_{1/2\alpha}$, 0.2 h; $t_{1/2\beta}$, 118 h)	2,725 ($t_{1/2}$ 13.9 h)	1.88 ($t_{1/2\alpha}$, 0.2 h; $t_{1/2\beta}$, 52 h)	61,183 ($t_{1/2}$ 12.6 h)	N/A	N/A	N/A	N/A
DETP	16.7 ($t_{1/2\alpha}$, 5.4 h; $t_{1/2\beta}$, 31 h)	28,614 ($t_{1/2}$ 21.7 h)	N/A	N/A	N/A	N/A	13.4 ($t_{1/2}$ 4.2 h)	40,861 ($t_{1/2}$ 8 h)
TCPy	280 ($t_{1/2\alpha}$, 0.3 h; $t_{1/2\beta}$, 8.5 h)	66,784 ($t_{1/2}$ 20.6 h;)	N/A	N/A	153 ($t_{1/2\alpha}$, 0.3 h; $t_{1/2\beta}$, 5.1 h)	102,806 ($t_{1/2}$ 17.3 h)	N/A	N/A

N/A, not applicable.
[a] AUC calculations did not extrapolate to infinity.

The average total recovery of TCPy in the urine through 72 h post-dosing was 38 ± 10 μmol, which was very comparable to the 38 ± 4 μmol of TCPy that was orally administered to the rats (Fig. 6B). This suggests that TCPy was well absorbed orally and undergoes negligible, if any, metabolism.

Following the CPF oral dose (group I), TCPy was likewise rapidly detected in the blood with peak levels of 18 ± 17 μg/mL attained by 3 h post-dosing. In contrast to the group III results, the TCPy was detectable in the blood only through 48 h post-dosing and the blood time-course had estimated terminal $t_{1/2\beta}$ of 8.5 h. Near peak urinary TCPy concentrations were attained by 12 h (710 ± 325 μg) and were maintained at elevated level through 48 h post-dosing (1948 ± 693 μg).

In comparing the TCPy blood concentrations and the amount excreted in the urine following equal molar doses of TCPy (group III) or CPF (group I) it is clear that the peak concentrations of TCPy in the blood were very comparable, although the TCPy dosed blood concentrations decreased faster than the CPF dose group (Fig. 4A). Consistent with this observation was the finding that the urinary TCPy time-course (Fig. 4B) and cumulative concentration (Fig. 4C) for the TCPy (group III) were slightly higher than the urinary TCPy concentrations following the CPF dose (group I). As illustrated in Fig. 6A, urinary TCPy (22 ± 3 μmol) accounted for ∼63% of the orally administered CPF dose (35 ± 2 μmol). In this regard, no attempt was made to quantify the extent of TCPy conjugation (i.e. sulfation and glucuronidation) following either the CPF or TCPy doses. Although the overall recovery of TCPy was different following the CPF or TCPy doses; in general, the shapes of the blood and urinary time-courses were fairly similar with comparable $t_{1/2}$ for the blood β phase (5–9 h) and urinary excretion rate (17–21 h).

3.4. Diethylthiophosphate (DETP) pharmacokinetics

Following oral administration of DETP (group IV) peak blood DETP concentrations of 2.0 ± 0.1 μg/mL were rapidly attained within 1 h post-dosing, and the DETP was quantifiable through 24 h post-dosing (Table 1). The DETP blood pharmacokinetics had a terminal phase half-life ($t_{1/2}$) of 4.2 h (Fig. 2F and Table 2). Peak amounts of DETP in urine (2105 ± 1481 μg) were attained within 12 h post-dosing and the estimated urinary $t_{1/2}$ was ∼8 h. The average total recovery of DETP in the urine through 48 h post-dosing was 22 ± 5 μmol of DETP, which was ∼65% of the 34 ± 2 μmol of DETP that was orally administered to the rats (Fig. 6B). The

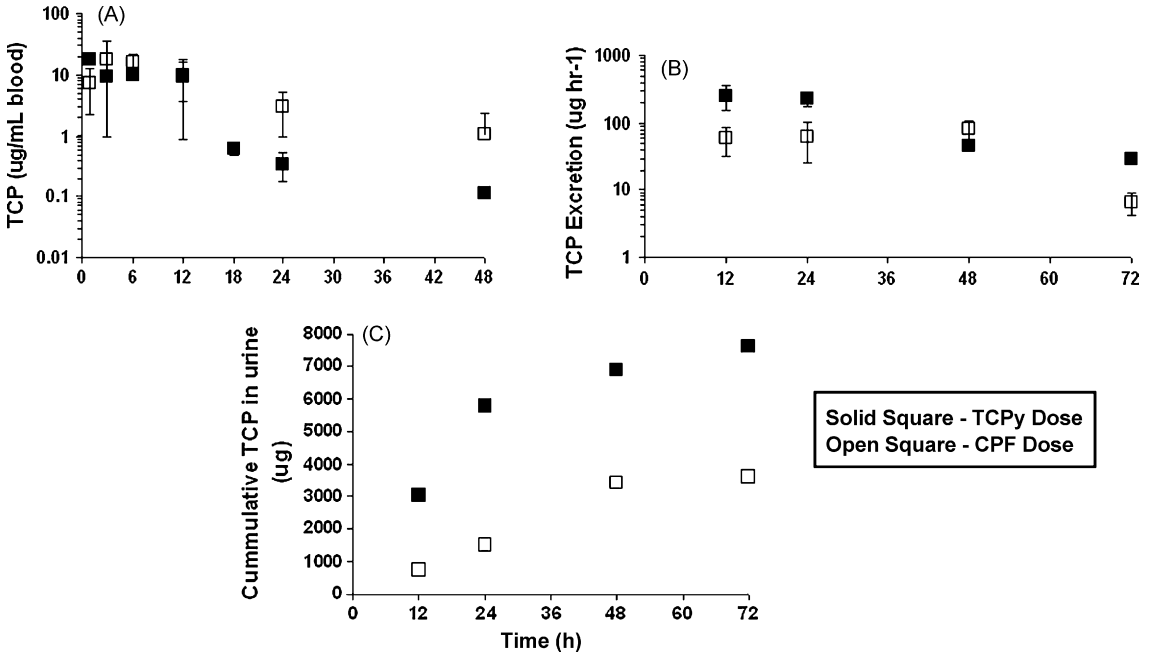

Fig. 4. Time-course of trichloropyridinol (TCPy) in blood (A), urine (B), and cumulative amounts of TCPy in urine (C) following oral administration of 140 μmol/kg of TCPy (solid square) or chlorpyrifos (CPF) (open square) to male Sprague–Dawley rats. Data represents mean ± S.D. of three animals per time-point.

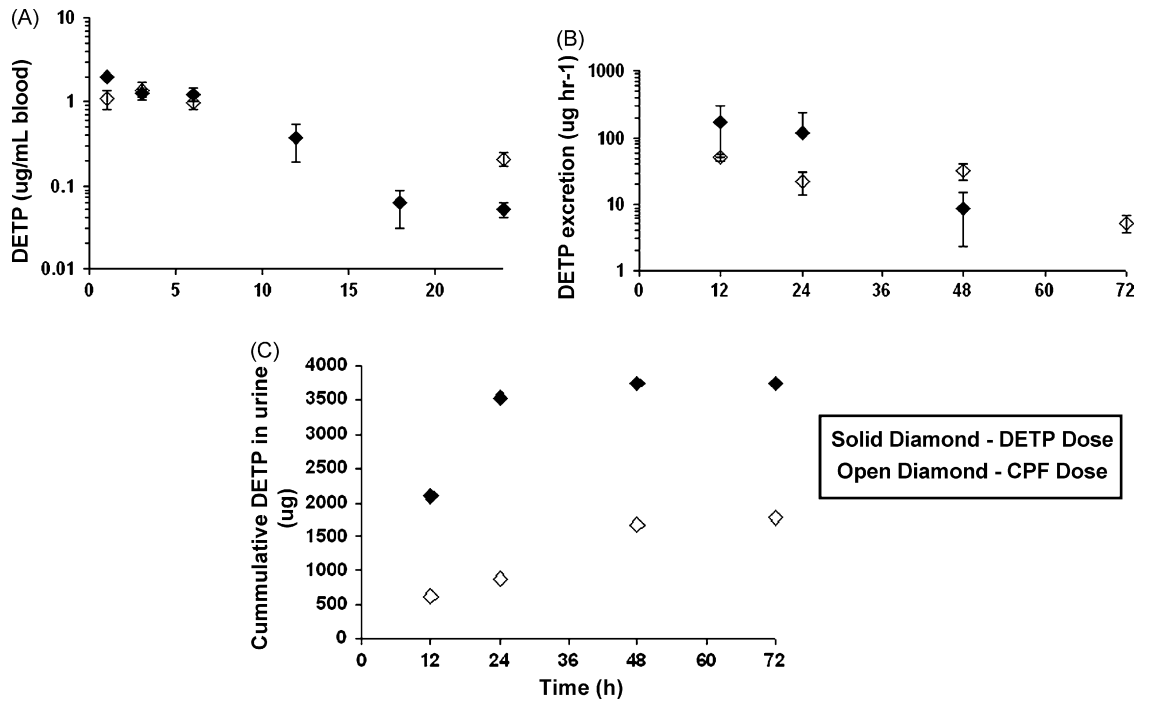

Fig. 5. Time-course of diethylthiophosphate (DETP) in blood (A), urine (B), and cumulative amounts of DETP in urine (C) following oral administration of 140 μmol/kg of DETP (solid diamond) or chlorpyrifos (CPF) (open diamond) to male Sprague–Dawley rats. Data represents mean ± S.D. of three animals per time-point.

incomplete recovery of DETP in the urine may suggest that the DETP was not completely absorbed, and/or the DETP was eliminated from the body by other pathways (i.e. additional metabolism or alternative excretion route).

Following the CPF oral dose (group I), DETP was likewise rapidly detected in the blood with peak levels of 1.4 ± 0.3 μg/mL attained by 3 h post-dosing (Table 1 and Fig. 2E). The DETP was detectable in the blood through 24 h post-dosing and the blood time-course had an estimated $t_{1/2\beta}$ of 31 h (Table 2). Peak urinary DETP concentrations following the CPF dose were attained by 12 h (616 ± 68 μg) and the urinary elimination $t_{1/2}$ was ~22 h.

A comparison of the DETP blood concentrations and urinary excretion rates following equal molar doses of DETP (group IV) or CPF (group I) indicates that the peak blood DETP concentrations, were very comparable (Fig. 5A and B and Table 1). However, differences were noted in the terminal blood time-course (4 h versus 31 h), urinary excretion rates (8 h versus 21 h) and the cumulative amounts of DETP (3743 μg versus 1783 μg) quantified in the urine when comparing the DETP versus CPF dose groups.

The total amount of metabolites recovered as TCPy, DETP and DEP in the urine following oral administration of CPF or individual metabolites are presented in Fig. 6. In the current study the overall average dose of CPF administered to all rats was 36 ± 2.5 μmol/rat, and of the recovered metabolites TCPy was the highest at 22 ± 3 μmol (62%); whereas, the metabolites DETP and DEP accounted for 14 ± 2 μmol (40%) and 1.4 ± 1 μmol (4%), respectively (Fig. 6A). The overall average oral doses of TCPy, DETP and DEP were 38 ± 4, 34 ± 2, and 37 ± 2 μmol/rat, respectively. All of the orally administered TCPy (38 ± 10 μmol) was recovered in the urine; whereas ~65% (22 ± 5 μmol) of the DETP dose and ~86% (32 ± 1 μmol) of the DEP dose were recovered (Fig. 6B).

4. Discussion

The current study was designed to facilitate direct comparison of the CPF metabolite pharmacokinetics resulting from exposure to either the parent insecticide or the individual metabolites when administered orally at equal molar doses. These results provide a quantitative perspective on the potential confounding contribution of ingested CPF degradates (i.e. metabolites) which are also currently used to estimate exposure. The focus was on TCPy, DETP and DEP since these represent the major CPF metabolites currently used for biological monitoring (Bradway and Shafik, 1977; Nolan et al., 1984; Hardt and Angerer, 2000; CDC, 2005). Previous pharmacokinetic studies in both rats and humans have demonstrated that CPF and its neurotoxic metabolite CPF-oxon are difficult to detect in blood and are not excreted into the urine (Nolan et al., 1984; Timchalk et al., 2002). Although detection of CPF in blood represents a definitive biomarker of exposure it has not been extensively utilized as a biomarker due to the challenges associated with low level analytical quantification. However, it is important to note that sophisticated high resolution mass spectrometry analytical methods have been used to quantify low concentrations of CPF (pg/g) in human blood from a biomonitoring/epidemiology study to access non-occupational exposure to insecticides (Barr et al., 2002; Whyatt et al., 2003, 2005).

The current study involved a serial sacrifice design, where groups of rats were terminated at differing time-

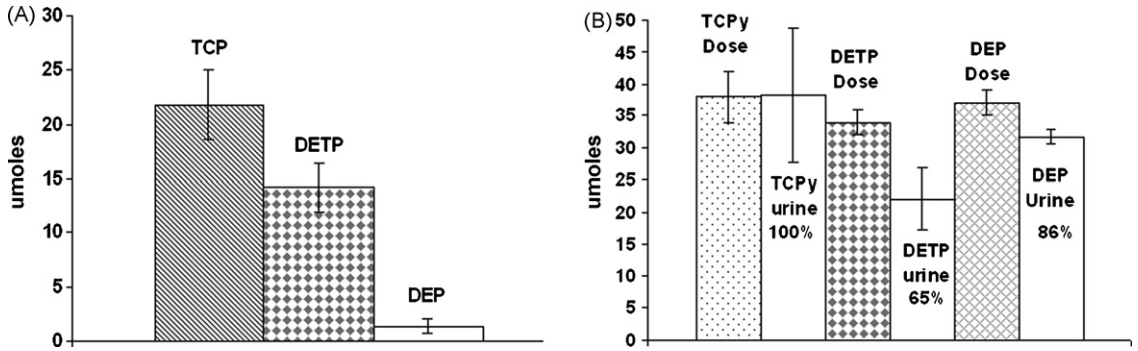

Fig. 6. (A) Mass balance (μmol) of the chlorpyrifos (CPF) metabolites trichloropyridinol (TCPy), diethylthiophosphate (DETP), and diethylphosphate (DEP) measured in urine at 72 h post-dosing. The CPF was orally administered to groups of rats at a dose of 140 μmol/kg of body weight. (B) Mass balance (μmol) for the TCPy, DETP and DEP doses recovered in the urine following oral administration of each metabolite at a dose of 140 μmol/kg of body weight. The data represents the mean ± S.D. of three animals. The % value represents the relative amount of the orally administered analyte quantified in the urine.

points and the blood was taken and analyzed for CPF metabolites. The extent of variability reported for some of the data points (see Table 1) for blood metabolite levels is most likely a reflection of this experimental design. It is anticipated that tighter data could be obtained by doing repeated measures on a fewer number of animals; nonetheless, the experimental findings are consistent with the known metabolism of CPF. The current study demonstrated that CPF is rapidly metabolized to the metabolites TCPy, DEP and DETP since peak blood levels were detected within 3 h of oral administration and peak urinary excretion rates for all metabolites occurred by 12 h post-dosing. Based on the amount of metabolites recovered in the urine, quantitatively the urinary excretion of metabolites followed the order: TCPy > DETP ≫ DEP; and based on the total amount of TCPy detected in the urine a minimum of ∼63% of the orally administered CPF dose was absorbed. The observed metabolic trend is consistent with previous *in vitro* and *in vivo* CPF metabolism studies (Ma and Chambers, 1994; Tang et al., 2001; Timchalk et al., 2002, 2006). In addition, the limited oral absorption of CPF has been previously reported in both rats (∼80% oral absorption) and humans (20–70% oral absorption), and was clearly dependent upon the formulation of the dose (Timchalk et al., 2002).

Peak blood concentrations of TCPy and DETP were very consistent following either a CPF dose or equal molar doses of the individual metabolites (see Table 1 and Fig. 2), Although the amounts of TCPy and DETP excreted in the urine were lower following the CPF dose versus direct exposure to the metabolites, the urinary excretion kinetics were remarkably similar; suggesting that once these metabolites are formed their pharmacokinetics are readily predicted from the kinetics of the individual metabolites.

In contrast, the pharmacokinetics of DEP was dramatically different depending upon whether the DEP was a product of CPF metabolism or resulted from direct exposure to DEP. As previously noted, a comparison of the DEP blood concentrations and the amount excreted in the urine indicated that the concentrations of DEP in the blood was clearly lower while the amount excreted in the urine was substantially higher for the DEP dose group than for CPF. This observed difference between the DEP kinetics may be related to differences in the amount of DEP–ChE enzyme complex formed after a CPF versus DEP exposure. As is illustrated in Fig. 1, CPF is metabolized by CYP450 to CPF-oxon which has a very high affinity for binding with AChE as well as other ChE [i.e. butyrylcholinesterase (BuChE) or carboxylesterase (CaE)] and readily complexes with the enzymes (Ma and Chambers, 1994; Timchalk, 2006). Once bound to the ChE, the leaving group (TCPy in the case of CPF) is rapidly hydrolyzed from the enzyme; however, the phosphorylated enzyme is highly stable, with the DEP–ChE complex having a potential $t_{1/2}$ of several days (Timchalk, 2006). The longer $t_{1/2}$ for the DEP blood β phase (118 h versus 52 h) in those animals dosed with CPF versus DEP, respectively, is consistent with the DEP from CPF-oxon binding with ChE and being released slowly over time. Since ChE enzymes are well distributed in all tissues, including blood, a substantial percentage of the DEP resulting from the CPF-oxon phosphorylation of ChE will be associated with the enzyme active sites throughout the body; hence, contributing to the observed differences in DEP pharmacokinetics.

Although a number of studies (Nolan et al., 1984; Vasilić et al., 1992; Drevenkar et al., 1993; Griffin et al., 1999; Timchalk et al., 2002, 2006) have reported on the metabolism of CPF to TCPy and DAP metabolites, Bicker et al. (2005) recently provided the most detailed assessment of human CPF metabolism and reported for the first time on the toxicokinetics of the three major metabolites (simultaneously) in an adult (59 years old) female who was acutely intoxicated with an insecticide formulation containing CPF (∼20–25% as a hydrocarbon mixture). The major metabolites were identified as TCPy, DETP and DEP, which are consistent with the metabolites quantified in the rat in the current study. However, twelve additional metabolites including a number of glucuronide and glutathione conjugates, as well as dechlorinated metabolites were noted. A number of studies have reported that following oral exposure to CPF in rats that TCPy was also excreted in the urine as sulfate or glucuronide conjugates (Bakke et al., 1976; Smith et al., 1967; Nolan et al., 1987). In the current study, analytical conditions were not optimized to ensure full hydrolysis of TCPy conjugates and no attempt was made to detect TCPy conjugates by GC/MS; however, it is conceivable that some acid-labile conjugates of TCPy could have been released due to the initial acid treatment of the blood and urine samples (see Section 2.5). We have reported that following the TCPy oral dose that all the orally administered TCPy was accounted for in the urine. However, it is feasible that additional conjugates of TCPy could also have been formed following the CPF oral dose, since only 62% of the dose was accounted for as TCP in the urine. Additional explanations for the lower recovery of TCPy in the urine following the CPF oral dose may also be due to first-pass gut metabolism of CPF (Poet et al., 2003) and/or incomplete oral absorption (Timchalk et al., 2002; Nolan et al., 1984). Nonetheless,

the overall mass balance suggested that TCPy, DETP and DEP accounted for the majority of the recovered dose in the rat.

As we have noted, following a single dose of 50 mg CPF/kg of body weight the relative percentages of TCPy, DETP and DEP excreted in the urine were ~62, ~40 and ~4%, respectively, suggesting that TCPy and DETP were the predominant urinary metabolites in the rat. Vasilić et al. (1992) reported on the urinary excretion rates of DEP and DETP in three persons who were self-poisoned by CPF. Similar to the findings in the current study, and as previously reported for controlled human exposures to CPF (Nolan et al., 1984), no parent CPF was detectable in the urine even following exposure to acutely (i.e. high) toxic doses. However, there does appear to be potential quantitative difference in the amounts of DEP and DETP excreted in the urine between rats and humans. In human CPF poison victims the amount of DEP and DETP excreted in the urine was very comparable (54% versus 45%); whereas in rats, urinary DEP was less than 5% of the recovered dose. In humans, the DEP and DETP pharmacokinetics were also very consistent and reported to be biphasic with α and β phase half-lives ranging from ~5–6 to 67–104 h, respectively. Although these results do suggest a potential species-specific difference with regard to DEP urinary excretion, the results may be confounded by the oxime treatments that were utilized in human poison victims to rapidly reactivate AChE activity. Reactivation would result in the release of the DEP from the ChE active site resulting in increased blood and urine levels of DEP. Although there is no direct evidence for this effect, future experiments in rats could readily evaluate the impact of oxime reactivation on DEP pharmacokinetics.

As we previously noted a number of studies have reported the detection of the OP breakdown product DAP in fruit juices or the measurement of relatively higher levels (12–29×) of the CPF metabolite TCPy, relative to the parent insecticide, in solid food samples obtained from homes and day care centers, suggesting the potential for alternative metabolite exposure sources (Lu et al., 2005; Morgan et al., 2005; Wilson et al., 2003). Lu et al. (2005) further reported the results from a two-dimensional Monte Carlo computational model analysis to determine whether DAPs detected in the urine could account for the amount of OP insecticide measured in both the environment and in the diet of children. Their model output clearly showed that the observed urinary DAP metabolites were consistently higher than the model predicted values. The current study in rats provides quantitative experimental evidence that both the DAP metabolites and TCPy are readily absorbed orally. Assuming similar human absorption of these metabolites, then the excessive amounts of DAP in human urine (Lu et al., 2005) would be consistent with absorption of these metabolite as residues from food.

An important consideration is that in the current study conditions were optimized for quantifying the CPF metabolites in blood and urine by administering CPF at a relatively high dose (50 mg/kg). As previously noted this dose did result in acute signs of cholinergic toxicity. Although the current study provides important results characterizing individual metabolite pharmacokinetics, there is still an unanswered question as to whether administration at lower environmentally relevant doses would result in a different pharmacokinetic profile. In this regard, future studies are planned utilizing a co-exposure design (CPF + metabolites) and will employ isotopically labeled CPF to enable us to discern the low dose pharmacokinetics of CPF and key metabolites.

In light of the confounding impact of metabolite residues on the determination of OP insecticide dosimetry, we should question if modifications in biomonitoring strategies can be implemented to more effectively enable the use of both DAP and TCPy as quantitative biomarkers for exposures. A potentially more useful biomonitoring strategy could include an approach that measures OP insecticides as well as their urinary metabolites (i.e. the organic moieties leaving groups and DAPs) in not only urine samples, but also in relevant environmental media and in a person's diet (Lu et al., 2005). As suggested by Needham (2005) the quantitation of CPF in blood, although technically challenging, represents the most relevant biomarker of exposure; while concurrently measuring blood and urinary TCPy and DAP within the same subject will provide a higher degree of confidence in estimating systemic dosimetry. In this regard, computational dosimetry models could then be used to establish and predict the ratio of major urinary metabolites (i.e. DEP, DETP and TCPy), and subsequently used to discern alternative exposure sources. Finally, additional OP biomarkers are needed that are only associated with direct exposure to the parent insecticide CPF or its toxic metabolite (i.e. CPF-oxon); these would be extremely useful and in effect eliminate any confounding influence resulting from secondary metabolite residue exposures.

In conclusion, the current study supports the hypotheses that DEP, DETP and TCPy present in the environment can be readily absorbed and eliminated in the urine of rats. Assuming similar pharmacokinetics in humans, it is plausible that total urinary CPF metabolite levels may be reflective of not only an individual's contact with the parent OP pesticide, but also exposure with intact metabolites present in the environment.

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